

THE RELATION BETWEEN DEGREE OF INJURY AND RATE OF REGENERATION—ADDITIONAL OBSER- VATIONS AND GENERAL DISCUSSION¹

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In some recent papers² I have given the results of experiments which show an increase in rate of regeneration of an organ with increase in degree of injury to the individual. This relation does not keep up indefinitely but sooner or later an optimum degree of injury is reached beyond which additional injury results in a decrease in rate. In some cases the optimum is comparatively high, in others, comparatively low.

Realizing the many factors that control rate of regeneration and the difficulties encountered in attempting to eliminate all but one of them I have extended the experiments with two points in view, (1) the more careful control of the accessory factors, and (2) the inclusion of as many different forms as possible. The new results together with a summary of the old, make up the subject matter of the present paper.

GENERAL METHODS AND SOURCES OF ERROR

Since in any particular case the rate of regeneration is determined by a large number of factors, many of which give marked changes in rate with slight changes in the factors, success in obtaining the pure effect of degree of injury is dependent upon the completeness of elimination of the other factors. The principal method employed was chosen with this point in view. It consists of a study of *the change in rate of regeneration from a particular level when other parts of the individual are subjected to different degrees of injury*. In the present paper by effect of

¹ Contributions from the Zoölogical Laboratory of Indiana University. No. 109.

² See Bibliography.

degree of injury is meant the effect of additional injuries to the individual. Special emphasis needs to be laid on this fact to avoid confusion in interpretation. A comparison of the rate of regeneration from different levels of a particular organ is not of direct value in connection with this problem because difference in level involves a great many factors not connected with degree of injury to the individual as a whole. The local conditions at different levels are obviously different and these differences have no necessary connection with degree of injury. Furthermore level cannot be assumed to be a measure of degree of injury, especially in the case of long, slender organs.

The lack of knowledge of the several subsidiary factors influencing the rate of regeneration makes it impossible to compare the changes in rate within single individuals. In every case it is necessary to compare the rate in different groups of individuals, each group being as nearly like the others as possible except in the degree of injury. The success of the method is dependent upon the similarity between the different groups in all respects except the factor under consideration.

The principal sources of error arise in connection with the following factors:

- 1 Age.
- 2 Periodic physiological changes.
- 3 Character of the laboratory history.
- 4 Changes in rate during the regeneration period.
- 5 Level of the cut.
- 6 Successive regenerations.
- 7 Temperature.
- 8 Food.
- 9 Differences in manipulation.
- 10 Departure of the living conditions from the optimum.
- 11 The relation of the degree of injury to the optimum degree.
- 12 Individual variation.

The general methods employed in the elimination of these sources of error are based upon the attempt to make every two

compared groups with different degrees of injury as nearly alike in all other respects as possible. Each of the disturbing factors is taken up in turn.

1 *Age.* It is necessary to have the individuals of two compared groups of the same age, of the same brood if possible, because it is known that rate of regeneration changes with age. According to general belief rate decreases with age but exceptions to this rule are not unknown.³

2 *Periodic physiological changes.* In the Arthropods in general the outer covering is cast off at intervals and it has been shown that growth and regeneration have a very intimate relation to this habit. In Crustacea especially it has been determined that the time of the operation with respect to the molting period is of importance.⁴ In experiments on Crustacea it is therefore necessary to take special pains in making the molting habit the center for adjustment of the compared groups. Notwithstanding the greatest care taken it is not possible to wholly eliminate the molt as a disturbing factor. The data dealing with animals having a molting habit are separated from those dealing with animals not having a molting habit.

3 *Character of laboratory history.* It is known that animals transferred from their natural environment to the laboratory show changes in their behavior. These changes are usually associated with a general decrease in vitality. It is therefore necessary to have the compared groups with identical laboratory histories.

4 *Changes in rate during the regeneration period.* Following an operation the rate of regeneration is not the same for the whole time up to the completion of the organ. In the frog tadpole and probably in other forms it is at first slow, then increases rapidly to a maximum, then decreases, rapidly at first, and then more and more slowly to zero at the time of completion of the regeneration.⁵ It is necessary in our present experiments to compare the rates in identical periods only.

5 *The level of the cut.* The rate of regeneration varies with

³ See this journal, vol. vii, no. 3.

⁴ Emmel, 1904.

⁵ See this journal, vol. vii, no. 3.

the level of the cut. In the frog tadpole's tail Ellis has shown that the amount of regeneration in length except for the first few days following an operation is directly proportional to the length removed.⁶ Special care must therefore be taken to have the levels from which regeneration is to be compared as nearly identical as possible.

6 *Successive regenerations.* In a number of cases⁷ it has been shown that the rate of regeneration changes with successive removal. It is therefore necessary to make comparisons for the effect of degree of injury between such groups only as have first regenerations of the organ in question. Second and succeeding regenerations may also be compared but the danger from disturbing factors is greater than in the first.

7 *Temperature* has a very direct effect upon growth and regeneration and it must be carefully controlled. It is probable that in some cases at least the influence of temperature upon rate of regeneration is more marked even than upon rate of ordinary growth.

8 *Food.* Morgan has shown for salamanders that abundance of food has very little if any influence upon rate of regeneration in length of the tails.⁸ In a study of the frog tadpole Miss Durbin has however found that change in quantity or in character of food influences the rate.⁹ In the present experiments compared sets were fed alike. A source of error that could not be controlled is due to the fact that individuals with the greater injury are frequently handicapped in catching or manipulating their food. When present this error probably favors the animals with the lesser degree of injury.

9 *Differences in manipulation.* The extra handling to which individuals with the greater degree of injury are subjected may in itself be harmful apart from the removal of the appendages. This is especially true of aquatic animals that must be operated upon in the air. It was frequently seen that individuals with the

⁶ See this journal, vol. vii, no. 3.

⁷ See this journal, vol. vii, no. 3.

⁸ Morgan, 1906. The physiology of regeneration.

⁹ See this journal, vol. vii, no. 3.

greater injury did not recover their ordinary activities as soon as those with the lesser injury.

10 *Departure of the living conditions from the optimum.* If the two compared groups are exactly alike in original choice of animals and in treatment there is still a source of error that must not be neglected. The object in view is the determination of the effect under optimum living conditions. If the living conditions are poor it is evident that one group may suffer more than the other. Under poor conditions such as foul water, presence of pathogenic organisms, insufficient food, etc., it is natural to suppose that an individual with a greater degree of injury is likely to suffer much more than one with a lesser injury. The same may be said of differences inherent in the experiment, such as loss of blood, nervous shock, and the greater difficulty experienced by individuals with the greater injury in seizing and handling their food. Therefore under identical treatment of the two compared groups the series with greater injury is at a disadvantage whenever there is any departure from optimum living conditions.

11 *Relation of the degree of injury to the optimum degree.* It is obviously true that with a degree of injury so great that the animal is barely able to survive the operation a rapid rate of regeneration cannot be expected. Accordingly near the upper limits of degree of injury a decrease in rate is found. The general question therefore resolves itself into the location of the optimum degree of injury. Is it coincident with a very low, a very high or with a medium degree of injury? If we compare two groups of animals with different degrees of injury both of which are near the upper limit of degree the result will be different from that obtained near the lower limit of injury. Furthermore if the lower injury is below and the upper injury above the optimum there may be no difference in rate of regeneration. In most cases the present experiments deal with medium degrees of injury.

12 *Individual variation.* In most experiments differences classed as individual variations are due not to any inherent differences in the individuals themselves but to uncontrolled differences in treatment or in age of the animals. The principal sources of

error have been removed in the present experiments in the ways indicated above. The individual differences that remain are partly at least due to inherent differences. This source of error can be eliminated only by the use of a large number of individuals in each group. In the following data after throwing out all cases coming into conflict with the disturbing factors given above the number remaining for comparison is sufficient in some cases while in others it is unfortunately insufficient for conclusive results.

The data for each experiment are treated separately. New observations are given in full and the results of former observations in brief. The data are presented in two divisions, the first including animals without and the second those with a molting habit.

EXPERIMENTS ON ANIMALS WITHOUT A MOLTING HABIT

Animals without a molting habit, as compared with those possessing this habit have a distinct advantage due to the absence of this complicated factor. They however have in most cases the disadvantage of less constancy in the level of the injury from which regeneration takes place. The level of the cut greatly influences the rate of regeneration and special attention was therefore paid in all non-molting cases to its elimination as a source of error.

1 *The Opercula of Hydroides dianthus*¹⁰

The Serpulid worm, *Hydroides dianthus*, has a large, so-called functional, operculum on one side of the head and a small rudimentary operculum on the other side. Regeneration of the small operculum when it alone is removed is compared with its regeneration when the other or functional operculum is removed at the same time. In the first instance a new, small operculum gradually grows out from the cut surface. In the second instance the small operculum rapidly regenerates into a large one in case the func-

¹⁰ Roux's *Archiv*, 1902 and *Journal Exp. Zööl.*, 1905, pp. 18-77.

tional operculum has been removed at the proper level. Thus with an additional injury to the individual the regenerating bud produces an organ larger in size and different in character from the original one. In my former papers I was concerned merely with the final results of the operations. A reference to my original notes however shows that there is a striking advantage in favor of the greater injury not only in the final size but also in the rate at which the material of the new operculum is proliferated. The same result was found in other species of Serpulids with dissimilar opercula such as *Hydroides pectinata*, *H. uncinata*, *Serpula vermicularis*, *Apomatus ampullifera*, etc.

2 *Rapidity of Differentiation of the Opercula in Apomatus ampullifera*¹¹

The branchiæ of the Serpulid *Apomatus ampullifera* are arranged in two semi-circular groups one on each side of the mouth. One of the branchiæ has a large terminal bulb which serves as the functional operculum. The corresponding branchia of the opposite side has a slight enlargement. Both circlets of branchiæ are readily thrown off at a breaking joint near their bases. In fact it is scarcely possible to operate on the animals without producing this autotomy. When the animal is otherwise uninjured the two circlets of branchiæ are regenerated as described below. The two opercular branchiæ begin to show the terminal enlargements comparatively late in regeneration being at first wholly similar to the other branchiæ. When however the greater part of the posterior region of the body is removed at the same time the two branchial circlets begin to regenerate from the breaking joints as before but the opercular enlargements appear at the very beginning of regeneration. In this case an additional injury to the individual produces an acceleration of the rate of differentiation of the opercula.

The observations on the opercula of the Serpulid worms as well as simultaneous observations on the chelæ of the Crustacea

¹¹ *Journal Exp. Zool.*, 1905, pp. 77-80.

mentioned below point to the view that additional injury in these cases at least results in an acceleration of the rate of regeneration from a particular level. They naturally led to a general investigation of the effect of the degree of injury upon the rate of regeneration.

3 *The Arms of the Brittle-star,*¹² *Ophioglypha lacertosa*

A comparison was made of the rate of regeneration of individual arms from cuts at their bases when one, two, three, four and all five arms were removed. Care was taken to make the level of injury the same throughout. Nine individuals were used in each of the five groups and no food was given throughout the experiment. Measurements of the lengths of the regenerating arms were made 22, 33 and 46 days after the operation. The data are given in Table I and Figs. 2 and 3 of the *Journal of Experimental Zoölogy*, vol. ii, no. 1.

Notwithstanding the complications due to the age factor and the large degree of individual variation it is seen that there is *an increase in the rate of regeneration of an arm as we pass from the cases with a smaller to those with a greater number of removed arms*. The series with all five arms removed is excepted in this statement because the animals in this lot in every instance died or showed evidences of decay before the completion of the experiment. The difference between the series with four and that with only one arm removed is very evident. The intermediate cases with two and three arms removed show the same trend though somewhat obscured by individual variation.

The general results obtained with the arms of the brittle-star therefore confirm the suggestion given by the opercula of the Serpulids that an increase in degree of injury to the individual leads to an increase in rate of regeneration from a particular level. The results suggest also that this is not true indefinitely but that beyond a certain optimum degree of injury further injury is followed by a decline in the rate. When all five arms are removed

¹² *Biological Bulletin*, 1903, and *Journal Exp. Zööl.*, 1905, pp. 7-77.

at their bases the degree of injury is evidently beyond the optimum. A point has been reached at which the injury is rapidly or gradually fatal in all cases and the rate of regeneration in those surviving for a time is slow. The rate of regeneration thus increases up to the optimum which is reached when four arms are removed. Beyond the optimum the rate slows down.

4 *The Oral Arms of Cassiopea xamachana*¹³

The rate of regeneration of a single oral arm in each of the following cases was determined:

- a One arm removed at its base.
- b Two arms removed at their bases.
- c Four arms removed at their bases.
- d Six arms removed at their bases.
- e All eight arms removed at their bases.

In the last four cases the averages of the rates of regeneration of the individual arms were taken. As in the case of the arms of the brittle-star there is a complication due to the age or size factor and to individual variation. *The general result is however clear* that there is an increase in rate of regeneration up to an optimum degree of injury beyond which further injury causes a decrease in rate. In this experiment the removal of four to six arms seems to constitute the most favorable degree of injury. When four arms are removed the rate is not as great as with six removed but the difference is so small that it may not be significant. On both sides of the optimum of four or six arms however there is a marked decline. The average specific amount of regeneration, i. e., the rate per unit of disk diameter, was 0.058 for a single removed arm, 0.112 for each of two removed arms, 0.144 for each of four removed arms, 0.157 for each of six, and 0.117 for each of eight. The data showing the considerable individual variation are given in Table I of the paper on regeneration in *Cassiopea* (Journal of Exp. Zool., vol. v, no. 2, 1907, p. 267).

¹³ Journal Exp. Zool., 1907.

5 *Larvæ of Amblystoma opacum. Nos. 2000-2031*

A mass of eggs with the adult coiled over them was collected on October 1, 1906. The hatched salamanders were put into individual dishes about December 15 to avoid cannibalism. Four kinds of operations were made on December 15 with eight individuals for each kind. The essential precautions to avoid error as described on pp. 514 to 518 were taken. All individuals were killed in Gilson's fluid on January 8, twenty-four days after the operation. The four kinds of operation were:

- a* Right fore-leg removed at its base.
- b* All four legs removed.
- c* Tail alone removed.
- d* Tail plus all legs removed.

The data are given in Tables I and II. The lengths are in millimeters and the tail length equals the distance from the level of the hind legs to the tip of the tail.

Two kinds of comparisons may be made from the data:

a The length of the regenerated tail when it alone is removed may be compared with the same when there is an additional injury involving the removal of the four legs. The specific amount regenerated¹⁴ gives the best basis for a comparison. Seven individuals in each group are available and are arranged in order of their specific amounts in Table 3. Notwithstanding an overlapping in individual cases it is evident that on the whole the faster regeneration of the tail takes place when there is an additional injury involving all four legs.

b The regenerated right foreleg when it alone is removed may be compared with the same when all four legs or the tail plus all four legs are removed. Unfortunately it is not possible on account of the various bends in the legs to make an accurate measurement. A general side-by-side comparison of the right forelegs in the sets with the three different degrees of injury did not reveal any differences of sufficient degree to be readily

¹⁴ The specific amount is the regenerated length divided by the removed length. The specific rate is the rate per unit of removed length per day.

CHART I

	ECTODERM	NOTOCHORD	NEURAL TUBE	MUSCULAR TISSUE REGION	FIN REGION
3 days	Three irregular layers of cubical cells with mitotic figures.	Lacking.	Extending halfway to the tip of the regenerating tail. Relatively large.	Specialized cells lacking. Muscle region occupied by cells observed in the fin regions.	Filled with closely packed round or cubical cells with large nuclei. No tissue of fibrous appearance.
5 days	Three layers more regular. The outer one relatively thinner, of somewhat flattened cells.	A solid rod of round cells.	Same as three days.	Muscle fibers absent; a very few small sarcoblasts on and very near the level of injury.	Not a solid mass of cells as before. Some cells with fibrous outgrowth. Cells with large nuclei.
7 days	Three layers distinct, cells of the outermost flat, those of the innermost columnar.	Larger, somewhat reticular. Wall very thin, composed of two or three layers of connective tissue in which nucleated cells are still persistent.	Not different in structure but relatively much longer.	A layer of sarcoblasts under the ectoderm in the muscle region.	Fins filled with loose fibrous tissue. Nuclei few except between the muscle regions.
10 days	The same as at seven days.	Core an open reticulum with nucleated cells in a layer lining the wall. Wall thicker and without nucleated cells. 3-5 layers of connective tissue, with nuclei, drawn very closely around the wall.	No evident change. Relatively smaller. — Dorsal aorta definable.	Many of the sarcoblasts differentiated into small muscle fibers that take an eosin stain, and are grouped like the fibers of the old muscles. Many sarcoblasts still present among the developing fibers.	Very fibrous tissue throughout. Nuclei few except in region of the notochord. Connective tissue between the muscle regions forming a vertical partition.
12 days	Middle layer less evident. The inner and outer layers quite regular.	Core unchanged. The wall thicker. 3-5 layers of connective tissue still bearing nuclei are drawn very closely around the outside of the wall and seem in process of being added in concentric layers to the wall.	No change in neural tube. — Dorsal aorta with a thicker wall.	Muscle fibers larger; new groups of fibers appearing.	The same changes noted on the tenth day continue.
14 days	The same as at twelve days.	Changes observed for 12 days are continued.	No change in neural tube. — Dorsal aorta extending relatively nearer the tip of the new tail.	Changes observed upon the twelfth day are continued.	No evident change in structure since the twelfth day.

EXPLANATION OF CHART

The changes in state of differentiation of ectoderm, notochord, neural tube, muscle tissue, and connective tissue are written in vertical columns and correspond horizontally with the number of days after operation named in the column to the left.

TABLE 1

Amblystoma opacum. Nos. 2000-2031

CHARACTER OF OPERATION	CATALOGUE NUMBER	ORIGINAL MEASUREMENTS IN MMS., DECEMBER 15, '06		
		Total body length	Total tail length	Length of re- moved tail
Right fore leg.....	2002	33.1	16.2	
	2006	35.1	16.5	
	2010	31.2	13.8	
	2015	30.0	13.2	
	2021	37.2	18.1	
	2024	33.0	15.3	
	2025	35.0	15.7	
	2027	37.2	17.6	
All legs.....	2000	35.0	16.7	
	2001	35.2	16.3	
	2005	37.4	17.7	
	2011	33.7	15.4	
	2019	31.2	13.5	
	2022	29.2	13.7	
	2023	33.0	15.2	
	2029	37.0	17.5	
Tail.....	2003	31.8	15.2	12.3
	2007	35.8	17.0	12.2
	2012	35.3	17.1	13.7
	2013	32.7	14.7	12.2
	2017	38.0	19.0	15.6
	2018	35.0	16.8	13.7
	2020	28.9	13.0	9.7
	2030	34.1	15.3	13.3
Tail plus all legs.....	2004	34.2	16.0	14.4
	2008	31.8	15.2	12.4
	2009	34.7	15.9	12.9
	2014	35.6	17.3	12.8
	2016	28.2	12.0	9.2
	2026	38.1	18.0	
	2028	35.6	16.5	12.9
	2031	32.0	15.1	13.0

TABLE 2

Amblystoma opacum. Nos. 2000-2031

CHARACTER OF OPERATION	CATALOGUE NUMBER	FINAL MEASUREMENTS. PRESERVED ANIMALS, JANUARY 8, 1907				
		Total body length	Total tail length	Length of regenerat- ing tail	Specific amount	Specific rate
Right fore leg. {	2002	34.4	16.3			
	2006	35.8	16.8			
	2010	32.9	14.5			
	2015	30.7	13.7			
	2021	33.0	14.4			
	2024	30.5	13.9			
	2025	31.5	13.7			
	2027	33.5	15.5			
All legs..... {	2000	36.2	16.9			
	2001	35.6	16.8			
	2005	36.0	16.7			
	2011	34.6	16.0			
	2019	31.1	13.6			
	2022	29.1	13.2			
	2023	32.6	14.7			
	2029	33.8	14.8			
Tail..... {	2003					
	2007	29.2	10.3	4.5	0.37	0.015
	2012	28.9	10.2	5.2	0.38	0.016
	2013	27.4	9.8	4.9	0.40	0.017
	2017	29.0	10.1	4.5	0.22	0.009
	2018	28.5	10.0	4.9	0.36	0.015
	2020	25.0	9.6	4.9	0.51	0.021
	2030	24.4	7.9	4.6	0.35	0.015
Tail plus all legs..... {	2004	29.3	9.3	5.0	0.35	0.015
	2008	28.1	10.3	5.9	0.48	0.020
	2009	28.8	10.2	5.8	0.45	0.019
	2014	28.0	10.5	5.7	0.45	0.019
	2016	25.0	9.3	5.2	0.57	0.024
	2026	28.8	9.9	5.0		
	2028	29.7	11.1	4.9	0.38	0.016
	2031	26.5	9.1	5.3	0.41	0.017

TABLE 3

Amblystoma opacum. Nos. 2000-2031. Individuals arranged in order of specific amounts of tail regeneration, twenty-four days after the operation

TAIL ALONE REMOVED	TAIL PLUS ALL LEGS REMOVED
0.22	
0.35	0.35
0.36	
0.37	
0.38	0.38
0.40	
	0.41
	0.45
	0.45
	0.48
0.51	
	0.57

noticeable. The numbers of compared individuals in the three sets are eight, eight, and seven respectively beginning with the lowest injury.

The following four experiments on *Amblystoma* (Nos. 6, 7, 8 and 9) were planned only partly with regard to the question of the effect of degree of injury on the rate of regeneration. They give some data on the present problem, though in most cases the comparisons are merely between a very few individuals.

6 *Tail of the Larva of Amblystoma opacum*. Nos. 967-990

The rate of regeneration of the tail when it alone was removed was compared with the rate when one or both of the fore-legs were removed at the same time.

From a mass of eggs belonging to a single salamander collected on October 1, 1905, the young were hatched on October 4.

Twelve of the thirty larvæ are available for present purposes. Four groups of three individuals each were operated upon November 8 and 9 and all the individuals were killed seventeen days

after the operation. The necessary precautions employed are given in the general outline of the sources of error (pp. 514 to to 518). In each group there is an individual with the tail alone cut off, one with the tail plus the right fore-leg and one with the tail plus both fore-legs removed. The lengths of the regenerated tails and other measurements are given in Table 4. With the exception of one individual (No. 969) it is seen that the per cent

TABLE 4

Amblystoma opacum

T = Tail alone removed

T + r. F. L. = Tail plus right fore-leg removed

T + b. F. L. = Tail plus both fore-legs removed

	CATALOGUE NUMBER	PARTS REMOVED	TOTAL ORIGINAL BODY LENGTH IN MMS.	LENGTH OF REMOVED PART OF TAIL IN MMS.	LENGTH OF REGEN- ERATED TAIL IN MMS.	PER CENT REGEN- ERATED
Group one....	967	T	25.2	6.2	2.6	42
	968	T + r. F. L.	22.4	?	3.1	50+
	969	T + b. F. L.	23.9	5.9	2.0	34
Group two....	974	T	20.1	6.0	3.7	62
	975	T + r. F. L.	20.4	5.8	3.8	66
	976	T + b. F. L.	20.0	5.1	3.7	73
Group three....	981	T	22.3	6.6	3.0	45
	982	T + r. F. L.	21.9	6.0	3.1	52
	983	T + b. F. L.	20.9	5.2	3.6	69
Group four....	988	T	22.3	6.6	2.2	33
	989	T + r. F. L.	22.0	6.6	2.4	36
	990	T + b. F. L.	21.7	5.5	2.4	44

of the removed length of the tail regenerated is least in the individuals with the tail alone removed and increases with increase in additional injury.

The special objection to the results of this experiment applies equally well to the following observations on *Amblystoma*. These observations were made as parts of a larger experiment and in all only a few individuals are available for comparison. The

our groups in the present case should be considered separately since no special attempt was made to keep the conditions alike throughout all four.

7 *The Right Fore-leg of the Larva of Amblystoma opacum*
Nos. 1026-1034

The size and stage of differentiation of the regenerating right fore-leg in individuals with no additional injury were compared with the regenerating right fore-leg in cases in which the other three legs and the tail were removed at the same time. Nine individuals were used. These came from a set of eggs collected on October 1, 1905, and hatched on October 4. The operations were made on February 7. The animals were killed in 85 per cent alcohol on March 17, thirty-eight days after the operation. Only three individuals are valid for the comparison. In individual No. 1033 with the tail and all four legs removed each regenerating fore-leg is larger and its toes are longer and more developed than in the right fore-legs of individuals Nos. 1027 and 1030, in which the right fore-legs alone were removed. The character of the regenerating organ makes it impossible to give exact measurements. However there is no doubt that each of the two individuals with the lesser degree of injury has a smaller and less differentiated right fore-leg than the one with an additional injury involving the other three legs and the tail.

8 *Larvæ of Amblystoma opacum. Nos. 1035-1046*

As before, all the larvæ are from a single egg mass. They were collected October 1, 1905, and hatched October 10. The operations came on February 8 except Nos. 1043 and 1044, which were made on February 9. All were killed on March 17, thirty-six or thirty-seven days after the operation. Three separate comparisons on the effect of degree of injury are possible.

a The size and stage of development of the right fore-leg was compared in the cases with different degrees of injury. In individuals Nos. 1040 and 1044 the right fore-leg alone was removed. In Nos. 1035, 1042 and 1046 the right fore-leg plus

the other three legs and the tail were removed. No differences in size of the regenerated right fore-leg were apparent. The toes however showed differences in degree of development.

In Nos. 1035 and 1042 the fourth toe was well developed.

In Nos. 1044¹⁵ and 1046 the fourth toe was beginning to develop.

In No. 1040 there was no fourth toe.

Evidently the fourth toe on the whole appears earlier in the individuals with a greater injury than in those with a lesser injury. In this group there is no apparent difference in size of the regenerating organs but an evident difference in the stage of differentiation.

b The size and stage of development of the right hind-leg were compared in cases with different degrees of injury. In Nos. 1037, 1039 and 1043 the right hind-leg alone was removed.

In Nos. 1035, 1042 and 1046 the right hind-leg plus the three other legs and the tail were removed. No difference in the size or stage of development of the regenerated right hind-legs could be made out.

In this group as far as could be seen additional injury neither retarded nor accelerated the rate of regeneration.

c The length of the regenerated tail in individuals in which the tail alone was removed was compared with that of individuals in which there was an additional removal of all four legs. The lengths of the regenerated tail in the three individuals with the lesser injury were 2.6, 2.9, and 2.9 mm. respectively. In those with the greater injury, 2.4, 2.5 and 2.6 mm. This is an advantage in favor of the lesser injury.

Salamanders Nos. 1035-1046 thus show a more rapid differentiation of the right fore-legs and a less rapid growth of the tail in the cases with a greater injury.

9 *Larvæ of Amblystoma opacum.* Nos. 1047-1057

All these salamanders came from one egg mass collected on October 1, 1905, and hatched on October 10. The operations were made on February 8 and the animals were killed on May

¹⁵ The regenerating foot is double.

17, thirty-seven days after the operation. Three sets of comparisons similar to those of Experiment 8 may be made.

a The regeneration of the right fore-leg when it alone was removed was compared with the regeneration of the same organ: (1) when the other three legs were removed at the same time and (2) when the other three legs plus the tail were removed. Two individuals are valid for comparison in each case.

The two groups with the greater injury were alike in size of the legs and stage of development of the toes. When compared with the one individual in which the right fore-leg alone was removed all four individuals with additional organs removed have a larger right foreleg but there is no difference in the stage of development of the toes. Difference when present is here in favor of the greater injury, though this comparison by itself would have but little weight.

b The regenerated right hind-leg (No. 1051) and the regenerated left hind-leg (No. 1057), one leg alone being removed in each case, were compared with the regenerated right or left hind-legs in cases with an additional injury involving

1 All four legs (Nos. 1048 and 1054).

2 Tail plus all four legs (Nos. 1049 and 1055).

In stage of development of the toes these six individuals can be arranged as follows beginning with the one having the greatest development:

1049, 1048, 1055 = 1054 = 1051, 1057. Individuals with an additional injury are evidently ahead of those with no additional injury. On the whole also the ones with the greater additional injury have an advantage over those with a lesser additional injury.

c One individual with the tail alone removed had regenerated 2.8 mm. of its tail, while of the two individuals with an additional removal of the four legs, one had regenerated an equal amount (2.8 mm.) and the other only 2.3 mm. While insufficient for a definite conclusion the data favor the lesser degree of injury.

Taking the five sets of experiments on salamander larvae together the results on the whole evidently favor the individuals with additional injury.

10 *The Tail in Tadpoles of the Green Frog, Rana clamitans*
Nos. 911-962

The length of the regenerated tail in individuals with that organ alone removed was compared with the length in others with an additional removal involving one or both hind-legs. Twenty-three of the fifty-six tadpoles give data that are valid for the present comparison. The operations were made on October 20 and 21, 1905; the first measurements of length of the regenerating tail on November 13 and 14, twenty-four days after the operation; the second measurements, January 27, ninety-nine or one hundred days after the operation and the third and final measurements from specimens killed on March 18, one hundred and forty-nine or one hundred and fifty days after the operation. The last were preserved in 85 per cent alcohol and the apparent decrease in the lengths as given is due to shrinkage caused by the alcohol.

The data showing the total lengths, the stage of development of the hind-legs, the tail lengths, the removed tail lengths, the amounts regenerated and the specific amounts of regeneration or the amounts per unit of removed length are given for each of the four measurements in Tables 5 and 6.

The tails were removed by a transverse cut near their middle and the hind-legs by cuts through the thighs. The catalogue numbers are given for comparison with the same individuals as used in other experiments.

The individual variations are so great that nothing more than a general determination of the effect of degree of injury to the individual can be made from the present data alone. The difference shown is on the whole without any doubt in favor of the tadpoles with an additional injury as opposed to those without such injury. The data are most complete at twenty-four days after the operation. If the individuals in each group are compared separately with regard to absolute amounts of regeneration it is found that in a majority of cases the length of the regenerating tail is greatest when the tail plus the left hind-leg is removed the tail plus both hind-legs coming next and the tail alone removed

TABLE 5

Tadpoles of Rana clamitans. Nos. 911-962

T = Tail removed

T + l. h. l = Tail plus left hind leg removed

T + b. h. l.'s = Tail plus both hind legs removed

Lengths are in mms.

Measurements at time of operation,
October 20-21, 1905.Measurements, November 13-14, 24 days
after operation

CHARACTER OF OPERATION	CATALOGUE NUMBER	TOTAL LENGTH	HIND LEGS ANGLE AT KNEE JOINT	TOTAL TAIL LENGTH	LENGTH OF PORTION OF TAIL REMOVED	TOTAL LENGTH	HIND LEGS		TOTAL TAIL LENGTH	LENGTH OF REGENERAT- ING PORTION OF TAIL	SPECIFIC AMOUNT REGENERATED
							Right	Left			
			degree				degree	degree			
T + b. h. l's.	911	63.5	100	39.4	21.0	52.2		reg. knob	29.7*	11.0*	0.52
T + l. h. l.	912	64.8	obtuse	41.4	23.3	55.4	100		34.8	12.8	0.55
T.....	913	59.5	obtuse	38.2	22.2	51.0	135	135	30.8	12.0	0.54
T + b. h. l's.	918	60.6	100	37.5	19.3	54.1			33.3	11.6	0.60
T.....	920	58.8	130	36.0	21.7	50.8	90	90	29.1	10.4	0.48
T + b. h. l's.	925	70.9	85	47.0	21.5	60.0			37.5	9.5	0.44
T + l. h. l.	926	72.8	80	48.2	23.0	61.1	80		37.9	12.8	0.56
T.....	927	65.6	120	43.0	22.5	52.2	130	130	30.3	9.4	0.42
T + b. h. l's.	932	72.2	95	48.0	23.0	58.6			37.0	9.9	0.43
T + l. h. l.	933	69.8	90	45.7	22.5	57.5	90		35.6	10.8	0.48
T.....	934	75.5	80	50.6	22.0	62.4	60	60	39.4	10.4	0.47
T + b. h. l's.	939	66.3	95	43.6	20.0	56.6			35.7	11.3	0.56½
T + l. h. l.	940	69.0	110	47.3	21.4	58.0	100	reg. knob	37.2	10.1	0.47
T.....	941	69.9	110	46.4	19.5	58.8	125	125	38.8	9.0	0.46
T + b. h. l's.	946	65.3	100	45.0	22.5	53.9			31.9	9.3	0.41
T + l. h. l.	947	73.0	110	50.3	23.2	58.2	110	small knob	38.0	9.0	0.39
T.....	948	70.3	80	48.6	24.0	55.5	90	90	34.7	9.3	0.39
T + b. h. l's.	953	65.6	95	42.1	19.7	52.1			30.4	7.3	0.37
T + l. h. l.	954	62.7	150	39.9	17.7	51.0	135	small knob	30.2	8.6	0.49
T.....	955	71.2	85	47.5	25.7	51.6	90	90	31.1	7.6	0.30
T + b. h. l's.	960	62.1	150	41.8	18.0	48.9	reg. knob		30.3	6.9	0.38
T + l. h. l.	961	64.0	150	43.0	22.2	51.0	135		30.6	8.5	0.38
T.....	962	58.9	150	39.5	19.0	46.7	135	135	28.6	8.1	0.43

TABLE 6

*Tadpoles of Rana clamitans. Nos. 911-962**Measurements January 27,
99-100 days after the operation**Individuals killed, March 18,
149-150 days after the operation*

CHARACTER OF OPERATION	CATALOGUE NUMBER	TOTAL LENGTH	HIND LEGS		TOTAL TAIL LENGTH	TOTAL REGENERATED PORTION OF TAIL	SPECIFIC AMOUNT OF REGENERATION	TOTAL LENGTH	HIND LEGS		TOTAL TAIL LENGTH	LENGTH OF REGENER- ATED PORTION OF TAIL	SPECIFIC AMOUNT OF REGENERATION
			Right	Left					Right	Left			
T + b. h. Ps...	911	55.3	degs.	reg. bud	33.6	13.9	0.66	51.5	degs.	degs.	31.4	14.7	0.70
T + l. h. l...	912	57.9	90		38.5	15.8	0.68						
T.....	913	55.3	135	135	35.5	15.6	0.70						
T + b. h. Ps.	918	57.0			37.0	17.0	0.88						
T.....	920	55.9	90	100	35.3	17.5	0.81	50.2	110	110	30.3	16.0	0.74
T + b. h. Ps.	925	60.0			39.0	12.2	0.57	50.4			31.7	10.7	0.50
T.....	927	53.7	135	135	34.1	13.5	0.60	45.5	100	100	26.9	9.6	0.43
T + l. h. l...	933	58.2	95		38.2	12.7	0.57						
T + b. h. Ps.	939	58.6			37.2	13.0	0.65	54.9			33.7	12.5	0.62½
T + l. h. l...	940	57.2	120	small reg. bud	38.0	13.0	0.61						
T.....	941	57.4	120	120	38.8	9.1	0.47	49.5	110	110	32.4	8.8	0.45
T + b. h. Ps.	946	53.5			33.2	11.4	0.51						
T + l. h. l...	947	59.0	90	small reg. bud	38.6	10.3	0.44						
T.....	948	56.4	80	80	36.4	11.9	0.50	46.9	90	90	29.0	9.6	0.40
T + b. h. Ps.	953	51.2			32.0	10.7	0.54						

coming last. The same is true when the specific amounts of regeneration are compared. The comparison of individuals within a group is more accurate than an inter-group comparison because the control of the factors involved in general treatment of the animals is better. The dishes within a group were always kept together and operations were made at the same time. However, if all the individuals of the experiment are taken without regard to group boundaries the same general result is obtained. The animals with an additional injury of one hind-leg show the greatest average amount of regeneration of the tail, those with both hind-legs removed nearly as great an average amount, while those with the tail alone removed show the least tail regeneration. The average specific amounts are 0.487, 0.464 and 0.436 respectively.

The later measurements suffer considerably from the large number of deaths, the series with one hind-leg removed suffering more than the others. Ninety-nine or one hundred days after the operation the average specific amounts are 0.623 for the greatest injury, 0.572 for the medium injury and 0.616 for the tail alone removed. One hundred and forty-nine or 150 days after the operation all those with the medium injury had died. The ones with an additional injury of both hind-legs show an average specific amount of regeneration of the tail equal to 0.605 as opposed to 0.505 for those with the tail alone removed. The apparent shrinkage in the last measurements is not real but due to preservation in alcohol, the other measurements having been made from the living animals.

The specific amounts of regeneration are given in Table 7. The individuals are arranged according to magnitude of specific amount.

Summary of Experiments on Animals Without a Molting Habit

A general survey of the foregoing experiments on animals without a molting habit may be profitable. All my experiments that give any data on the problem are included. Taken as a whole the data are favorable to the view that within moderate

TABLE 7

Tadpoles of Rana clamitans. Nos. 911-962. Specific Amounts of Tail Regeneration

TWENTY-FOUR DAYS AFTER OPERATION			NINETY-NINE TO ONE HUNDRED DAYS AFTER OPERATION			ONE HUNDRED AND FORTY-NINE TO ONE HUNDRED AND FIFTY-DAYS AFTER THE OPERATION		
Tail + both hind legs	Tail + left hind leg	Tail alone	Tail + both hind legs	Tail + left hind leg	Tail alone	Tail + both hind legs	Tail + left hind leg	Tail alone
0.37		0.30	0.51	0.44	0.47	0.50		0.40
0.38	0.39	0.39	0.54	0.56	0.50	0.625		0.43
0.41	0.47	0.42	0.57	0.61	0.60	0.70		0.45
0.43	0.47	0.43	0.65	0.68	0.70			0.74
0.44	0.48	0.46	0.66		0.81			
0.52	0.49	0.47	0.81					
0.565	0.55	0.48						
0.60	0.56	0.54						
Av ..0.464	0.487	0.436	0.623	0.572	0.616	0.608		0.505

degrees of injury an additional injury to an individual increases rather than decreases the rate of regeneration of a part. These data are based on experiments with several widely separated groups of animals. They support the general proposition that when an organ involving only a moderate disturbance of an animal's activity is removed, its rate of regeneration is less than it is in the case the individual is injured to a moderate degree in other parts at the same time. In general terms the rate increases up to an optimum degree of injury to the individual; it then remains stationary, and with still greater injury it decreases.

EXPERIMENTS ON ANIMALS WITH A MOLTING HABIT

Animals with a hard cast that has to be shed at intervals have an obvious disadvantage for the present purpose as compared with non-molting animals. It has been shown that the relation of the time of the operation to the time of the molt has a very great influence upon the amount of regeneration taking place in a given time. Likewise there is a variation in the rate of regen-

eration with the length of the molting period. A study of the problem has revealed a very complicated interrelation of age molting time, degree of injury, rate of growth and rate of regeneration. On the whole the specific amount of regeneration per molting period as a unit is more constant than the amount per day, other factors being alike. For this reason in the majority of the experiments the molting period is used as the unit.¹⁶

Notwithstanding their molting habit Crustacea have some very distinct advantages in a study of the problem in hand which partly offsets the disadvantages. The presence of a definite breaking joint in the chelæ makes it possible to be sure of the constancy of the level of regeneration. Furthermore Crustacea can be readily preserved after the completion of an experiment without danger of shrinkage and measurements can be more accurately made on them than on living animals.

1 *The Fiddler-crab, Gelasimus pugilator*¹⁷

In the male fiddler-crab one of the two chelæ is much larger than the other. In the female both are small and of equal size. The following plan of experiment was followed:

A Experiments on Males

- Set a* Large chela alone removed. Twenty individuals were kept for sixty-two days.
- Set b* Small chela alone removed. Ten individuals were kept for sixty-two days.
- Set c* Both chelæ removed. Ten individuals were kept for sixty-two days and eighteen others for forty-two days.

B Experiments on Females

- Set a* One chela was removed in six individuals.
- Set b* Both chelæ were removed in three individuals.

¹⁶ See paper on Successive Regeneration. This journal, vol. vii, no. 3.

¹⁷ Journal of Exp. Zool., vol. ii, no. 1, p. 81.

It was found that the crabs with both chelæ removed molted sooner than those with only one chela removed. The regenerating buds were in general larger in the specimens with two removed chelæ than in those with one removed. This was true of both molted and non-molted individuals.

12 *The Chelæ of Alpheus dentipes*¹⁸

In *Alpheus dentipes* the two chelæ have different functions. One is larger than the other and different in structure. The larger is called the "snapping" chela, the smaller the "cutting" chela. In nature either one may be on the right side. When the cutting chela alone is removed a new cutting chela grows out in its place. When the snapping chela is removed the uninjured cutting chela is transformed into a new snapping chela while in place of the removed snapping chela a new cutting chela develops. Finally, when both chelæ are simultaneously removed a new snapping chela grows out in place of the old snapping and a new cutting in place of the old cutting chela. The gradation in degree of injury to the individual as a whole beginning with the lowest injury is,

- 1 Cutting chela alone removed.
- 2 Snapping chela alone removed.
- 3 Both chelæ removed.

In each case a cutting chela is developed, in the first two cases from the single cut surface and in the last case from one of the two cut surfaces.

The rate of molting increases with increase in degree of injury. The average molting period for the set with the lowest injury was 29.6 days, for the next 28.7 days and for the set with the greatest injury 22.9 days.

Notwithstanding the shorter absolute time allowed to the series with the greater injuries the lengths of regenerating cutting chelæ¹⁹ were greater in these than in the series with the lesser

¹⁸ Journal of Exp. Zool., vol. ii, no. 1, p. 85.

¹⁹ "Cutting chelæ" refers to resultant organs.

injuries. Thus at the end of the second molting period the series with the least injury had regenerated 74.3 per cent of the cutting chela length, the second series 75.1 per cent and the series with the greatest injury 87.0 per cent. The difference between the last and first two is striking especially in view of the difference in molting times.

13 *The Chelæ of the Crayfish, Cambarus propinquus*²⁰

The chelæ are approximately equal in size. A comparison of the rate of regeneration of a chela in individuals with it alone removed was made with its rate in individuals in which both chelæ and the last two pairs of walking legs were removed. Seventy-seven mature crayfish ranging in cephalo-thoracic lengths from 10 to 20 mm. were used. Thirty-six of these had the lesser and forty-one the greater injury. The data are given in the Journal of Experimental Zoölogy, vol. ii, no. 3, pp. 350 ff.

It was found that the crayfish with the greater injury molted more rapidly than the ones with the lesser injury. The amount of regeneration at the end of a molt was approximately equal in the two series, but because of the acceleration in the molting time the actual rate was greater in the series with the greater injury. Thus among the males in the series with the lesser injury (fourteen available individuals) the specific rate of regeneration²¹ of the right removed chela was 0.0049 ± 0.0003 against 0.0080 ± 0.0005 for the same chela in individuals with the additional injury (thirteen cases).

Among females the corresponding figures are 0.0030 ± 0.0001 (fourteen cases) for the lesser injury and 0.0083 ± 0.0007 (twenty cases) for the greater injury.

In each case the difference is very strikingly in favor of the greater injury notwithstanding the considerable variations among individuals.

²⁰ Journal of Exp. Zool., vol. ii, no. 3, p. 347.

²¹ The specific rate of regeneration is the rate in mms. per unit of thoracic length per day.

14 *The Chelæ of the Shrimp, Palæmonetes vulgaris (Stimp)*
*Nos. 1806-1861*²²

The object of the experiment was the comparison of the rate of regeneration of a single chela when it alone is removed with the rate of the two chelæ when both are removed.

Fifty-six shrimps were collected at Wood's Hole on September 2, 1906. Eighteen of these were "control" specimens without an operation, twenty had both chelæ removed and eighteen had one chela removed, the right in nine and the left in the other nine. The operations were made on September 2 and all animals were killed on September 10 giving a regeneration interval of eight days.

Only sixteen out of the thirty-seven available operated shrimps molted during the eight days of the experiment. Eight of these had the greater and eight the lesser injury. All thirty-seven individuals are however available for the comparison of rates of regeneration because in *Palæmonetes vulgaris* the new chela grows out directly and is not curled up in a chitinous sac as in the crabs. It is therefore unnecessary to wait for the molt. As a matter of fact the data show that the molted individuals are not necessarily the ones with the greatest length of regenerating chelæ.

The data are given in Tables 8, 9, 10 and 11. Table 8 gives the data for the shrimps in which the left chela alone was removed. Table 9 gives the data for those in which the right chela alone was removed. Table 10 gives the corresponding data for the ones in which both chelæ were removed. In Table 11 the specific amounts of regeneration are compared. They are arranged in order of magnitude beginning with the lowest, except the left chela in shrimps with both chelæ removed and regenerating. In these last the left chelæ are put opposite the right ones of the same individuals. Since the period of regeneration is eight days in every case the specific rates are comparable in the same way.

In individuals with both chelæ removed (nineteen cases) the average specific amount of regeneration of the left chela is 0.282

²² I am indebted to the acting director of Wood's Holl Marine Biological Laboratory, Professor Frank R. Lillie, for the use of a room during August and September, 1906.

TABLE 8

Palamonetes vulgaris. Nos. 1806-1861. Left chela alone removed. Animals killed eight days after the operation

CATALOGUE NUMBER	FINAL CEPHALO- THORACIC LENGTH	FINAL RIGHT CHELA LENGTH	LEFT CHELA		SPECIFIC AMOUNT OF REGENERATION
			Original length	Regenerated length	
1810	9.3	2.85	2.88	0.62	0.22
1816	9.3	2.80	2.80	0.77	0.27
1822	11.6	3.89	3.68	0.62	0.17
1828	12.1	4.04	4.00	0.69	0.17
1834	8.4	2.70	2.64	1.10	0.42
1840	12.2	4.43	4.52	0.79	0.17
1846	11.1	3.81	3.81	0.82	0.22
1852	10.5	3.41	3.41	0.83	0.24
1858	9.1	2.65	2.66	0.73	0.27
Average	0.239

TABLE 9

Palamonetes vulgaris. Nos. 1806-1861. Right chela alone removed. Animals killed eight days after the operation

CATALOGUE NUMBER	FINAL CEPHALO- THORACIC LENGTH	FINAL LEFT CHELA LENGTH	RIGHT CHELA		SPECIFIC AMOUNT OF REGENERATION
			Original length	Regenerated length	
1807	br	2.96	2.93	0.82	0.28
1813	6.1	2.00	1.88	0.60	0.32
1819	11.1	4.07	4.02	0.55	0.14
1825	10.0	3.11	3.04	0.70	0.23
1831	8.0	2.51	2.52	0.52	0.20
1837	9.7	2.95	2.95	0.79	0.27
1843	11.1	3.77	3.59	0.48	0.13
1849	9.1	2.74	2.62	0.52	0.20
1855	9.7	2.75	2.81	0.85	0.30
Average230

TABLE 10

Palamonetes vulgaris. Nos. 1806-1861. Both chelæ removed. Animals killed eight days after the operation

CATALOGUE NUMBER	FINAL CEPHALO- THORACIC LENGTH	LEFT CHELA			RIGHT CHELA		
		Original length	Regenerated length	Specific amount	Original length	Regener- ated length	Specific amount
1806	8.3	2.48	0.79	0.32	2.48	0.80	0.32
1809	8.9	2.62	0.59*	0.23	2.62	0.59*	0.23
1812	6.5	1.93	0.76	0.39	1.93	0.76?	0.39?
1815	br	2.74	0.76	0.28	2.69	0.80	0.30
1818	11.8	3.92	0.94	0.24	3.92	0.90	0.23
1821	10.0	3.40	1.41	0.41	3.40	1.37	0.40
1824	10.4	3.12	0.81	0.26	3.14	0.89	0.28
1827	11.9	3.55	0.62	0.18	3.55	0.56	0.16
1830	9.8	3.06	0.65	0.21	3.13	0.68	0.22
1833	9.2	2.89	0.52	0.18	2.90	0.52	0.18
1836	9.1	2.69	0.59	0.22	2.69	0.59	0.22
1839	12.7	3.96	0.77	0.19	3.96	0.77	0.19
1842	10.0	3.11	1.48	0.48	3.11	1.48	0.48
1845	12.1	4.24	0.84	0.20	4.24	0.93	0.22
1851	11.6	3.35	0.83	0.25	3.35	0.89	0.27
1854	8.4	2.56	0.74	0.29	2.56	0.74	0.29
1857	9.0	2.44	0.62	0.25	2.52	0.66	0.26
1860	7.9	2.37	1.26	0.53	2.37	1.36	0.57
1861	11.7	3.48	0.85	0.24	3.48	0.81	0.23
Average	0.282			0.286

and of the right, 0.286. When the right chela alone is removed (nine cases) the average is 0.239. When the left chela alone is removed (nine cases) the average is 0.230.

The advantage is very evidently in favor of the shrimps with the greater injury. Though the individual data overlap, the difference in the average is great enough to be certainly significant.

15 *Crabs*. Nos. 594-601 *Sp?* and 602-614, *Porcellana platycheles*

In connection with experiments carried on at the Naples Zoölogical Station in 1902-1903 on the question of the reversal of asymmetry data were obtained on the effect of degree of injury

TABLE 11

Palaeomonetes vulgaris. Nos 1806-1861. Specific amounts of regeneration of the chelæ arranged in order of magnitude

BOTH CHELÆ REMOVED		LEFT CHELA REMOVED	RIGHT CHELA REMOVED
Left	Right	Left	Right
0.18	0.16	0.17	0.13†
0.18	0.18†	0.17	0.14†
0.19	0.19	0.17†	0.20
0.22	0.22	0.22	0.20†
0.20	0.22	0.22†	
0.21	0.22†		
0.23	0.23		0.23
0.24	0.23		
0.24	0.23†	0.24	
0.25	0.26	0.27	
0.25	0.27	0.27	0.27
0.26	0.28		0.28†
0.29	0.29		
0.28	0.30		0.30
0.32	0.32†		0.32†
0.39	0.39†		
0.41	0.40†	0.42†	
0.48	0.48†		
0.53	0.57†		
Av..0.282	0.286	0.239	0.230

† = cases that moulted after the operation.

The individuals used were crabs of an undetermined species and others of *Porcellana platycheles*. They all showed asymmetry in the chelæ. The operations consisted of the removal (a) of the shorter chela alone, (b) the larger chela alone, and (c) both chelæ. The operations were made on January 16, 1903, and the animals were killed on March 8, fifty-one days after the operation. Several individuals did not molt. Others molted once and these are used in the following data. Table 12 gives the data for the crab of undetermined species and Table 13 for *Porcellana platycheles*.

TABLE 12
Chelæ of crabs, Nos. 594-601

PARTS REMOVED	CATA- LOGUE NO.	ORIGINAL CHELA LENGTHS				FINAL CHELA LENGTHS				SPECIFIC LENGTHS REGENERATED	
		Right		Left		Right		Left		Right	Left
		Length	Width	Length	Width	Length	Width	Length	Width		
Right.....	594	4.9	3.3	4.9	2.5	3.4	1.8	4.7	2.4	0.69	
Right.....	598	3.5	1.4+	3.5	2.3	3.0	1.4	3.1	2.0	0.86	
Left.....	601	4.5	2.3	?	?	4.4	2.2	3.4	1.6		?
Both.....	596	3.6	2.5	4.1	2.1	3.0	1.4	2.9	1.3	0.83	0.71
Both.....	597	3.0	1.3+	2.5	1.9	2.2	1.0	2.2	1.0	0.73	0.88
Both.....	599	2.9	1.3	2.5	1.9	2.2	1.0	2.4	1.0	0.76	0.96

TABLE 13
Chelæ of Porcellana platycheles, Nos. 604-613

PARTS REMOVED	CATALOGUE NO.	ORIGINAL LENGTHS		FINAL LENGTHS		SPECIFIC AMOUNTS OF REGENERATION	
		Right	Left	Right	Left	Right	Left
Left.....	604	4.4	3.9	5.0	3.7?		0.95?
Left.....	608	3.0	2.7	2.6	?		?
Left.....	610	2.9	?	3.2	2.6		?
Right.....	612	?	1.7	1.4	1.6		0.94
Both.....	605	3.0	2.7	2.8	2.7	0.80	0.64
Both.....	613	1.6	1.9	1.7	1.5	1.06	0.79

A comparison of the specific amounts of regeneration at the end of fifty-one days shows on the whole an advantage in favor of the individuals with the greater injury. In the undetermined species the specific amounts of regeneration of the shorter chela are 0.83, 0.88 and 0.96 respectively for the three individuals with both chelæ removed and 0.86 for the one individual with the shorter chela alone removed. In the case of the larger chela the specific amounts when both chelæ were removed are 0.71, 0.73 and 0.76 in the three individuals against 0.69 in the one individual with the longer chela alone removed.

In *Porcellana platycheles* the data are fragmentary, only three individuals being available. The specific amount of regeneration of the smaller chela when the larger is removed at the same time is 0.80 and 1.06 in the two individuals present against 0.94 in the one individual with the small chela alone removed. These data show no difference between the average of the two sets but no general conclusion can be drawn because of the small number of cases.

The data for both species as far as they show any difference with difference in degree of injury favor the increase in rate with increase in degree of injury.

16 The Chelæ of the Crayfish, Cambarus bartoni, Nos. 2218-2267

A comparison was made of the rate of regeneration of a single chela when it alone is removed with the rate of each of the two chelæ when both are removed. Fifty young crayfish from a single mother were used in the experiment. As soon as the young left the mother they were put into individual dishes and the treatment for all was as nearly alike as possible. The food consisted of *Tubifex*, a supply of the living worms being kept in each dish.

The mother crayfish with eggs was captured on December 14, 1906. The young were put into separate dishes on February 11, 1907. The data fall into five groups: (a) First regeneration in younger individuals.²³ (b) First regeneration in older individuals.²⁴ (c) Second regeneration. (d) Third regeneration. (e) Fourth regeneration,

First Regeneration in Younger Individuals

The crayfish used were in the fourth molt at the beginning and in the sixth at the end of the experiment. Their lengths at the beginning were 6.0-6.5 mm. and at the end 7.7-8.3 mm. The operations were made two days after the fourth molt and the lengths of the regenerated chelæ at the end of the sixth molt were compared. The data are given in Tables 14 and 15.

²³ Used for third regenerations in the data on successive regenerations.

²⁴ Used for first regenerations in the data on successive regenerations.

TABLE 14

Cambarus bartoni. Nos. 2218-2267. Regeneration of a single removed chela. Younger crayfish. Molts 4-6. The right chela was removed in all except No. 2242

CATALOGUE NUMBER	MOLTS	INTERVAL IN DAYS	CEPHALO-THORACIC LENGTHS			AVERAGE CEPHALO- THORACIC LENGTH Molts 4-5	LENGTH OF REGEN- ERATING CHELA	SPECIFIC AMOUNT OF REGEN- ERATION	SPECIFIC RATE OF REGEN- ERATION
			Molt 4	Molt 5	Molt 6				
2218	4-5-6	23	6.2	7.0		6.6	2.9	0.44	0.019
2219	4-5-6	26	6.2		8.2	6.6	3.5	0.53	0.020
2223	4-5-6	23	6.0		8.0	6.5	3.5	0.54	0.023
2227	4-5-6	20		7.3		6.8	3.6	0.53	0.0265
2238	4-5-6	23	6.3	7.4	8.1	6.8	3.6	0.53	0.023
2239	4-5-6	31	6.5	7.0	7.7	6.7	3.2	0.48	0.015
2248	4-5-6	24	6.4	7.0		6.7	3.1	0.46	0.015
2252	4-5-6	28	6.4	7.2	8.2	6.8	3.5	0.51	0.018
2259	4-5-6	19	6.1	7.1	8.1	6.6	3.0	0.45	0.024
2262	4-5-6	19		7.0	8.3	6.5	2.9	0.45	0.024
2242	4-5-6	20	6.4	7.4		6.9	3.1	0.45	0.0225
Average.	23.3	6.28	7.16	8.09	6.68	3.26	0.488	0.0210

The molt numbers indicate the number of molts since hatching. There is a chance for error because the crayfish eat their casts. The specific amount is the amount regenerated per unit of cephalo-thoracic length. The specific rate is the specific amount per day. The "chela lengths" are the lengths of the propodites.

TABLE 15

Cambarus bartoni. Nos. 2218-2267. Regeneration of the chela when both are removed at one time. Younger crayfish. Molts 4-6

CATA- LOGUE NUMBER	MOLTS	INTER- VAL IN DAYS	CEPHALO-THORACIC LENGTHS			AVER- AGE CEPHA- LO-THO- RACIC LENGTH	LENGTH OF REGEN- ERATING CHELAE			SPECIFIC AMOUNT OF REGEN- ERATION	SPECIFIC RATE OF REGEN- ERATION
			Molt 4	Molt 5	Molt 6		Left	Right	Average		
2237	4-5-6	27	6.6	7.6	8.6	7.1	4.2	4.2	4.2	0.59	0.022
2245	4-5-6	26	6.6	7.2	8.3	6.9	4.0	4.0	4.0	0.58	0.022
2253	4-5-6	20	6.2	7.1	8.3	6.7	3.1	3.0	3.05	0.46	0.023
Average	24.3	6.47	7.3	8.4	6.9	3.77	3.73	3.75	0.543	0.0223

The average length of a single regenerated chela is 3.26 mm. for the eleven cases available as compared with a regenerated length of 3.77 mm. for the right and 3.73 mm. for the left chela in the three cases in which both chelæ were removed. The specific amounts of regeneration, in this case the amounts per unit of cephalo-thoracic length, are 0.49 for the single chela when it alone is removed and 0.54 for each of the two chelæ in individuals with both chelæ removed. The specific rates, i. e., the specific amounts per day are correspondingly 0.021 for the single chela and 0.0223 for each of the two chelæ. A regenerating chela in individuals in which additional regeneration is going on thus has an evident advantage over one in which no additional regeneration is present.

First Regeneration in Older Individuals

The crayfish used were in the eighth to tenth molt at the time of the operation and regeneration continued through one molt. The operations were made two days after the molt. The cephalo-thoracic lengths range from 10.5 to 13.6 mm. The data are given in Tables 16 and 17.

TABLE 16

Cambarus bartoni. Nos. 2218-2267. Regeneration of a single removed chela. Older crayfish. Molts 8-9 and 9-10

CATA- LOGUE NUM- BER	MOLTS	INTERVALS IN DAYS	CEPHALO-THORACIC LENGTHS			LENGTH OF REGEN- ERATING CHELA	SPECIFIC AMOUNT OF REGENER- ATION	SPECIFIC RATE OF REGENER- ATION
			Molt 8	Molt 9	Molt 10			
2220	9-10	43		11.9	13.0	6.0	0.50	0.012
2221	9-10	27		11.7	12.1	5.4	0.46	0.017
2241	8-9	27	10.5	11.3		5.3	0.50	0.019
2246	8-9	30	10.5	11.7		5.7	0.54	0.018
2257*	8-9	21*	12.0*	12.9*		2.7*	0.225*	0.011*
2267	8-9	20	?	11.3		4.4	0.42	0.021
Average	29.4	10.5	11.58	12.55	5.36	0.484	0.017

*Not included in the averages because of extreme values in all measurements of length.

TABLE 17

Cambarus bartoni. Nos. 2218-2267. Regeneration of the chelæ when both are removed at one time. Older crayfish. Molts 8-9, 9-10, and 10-11

CATA- LOGUE NUM- BER	MOLTS	INTER- VAL IN DAYS	CEPHALO-THORACIC LENGTHS				LENGTH OF REGENER- ATING CHELAE			SPECIFIC AMOUNT OF RE- GENERA- TION	SPECIFIC RATE OF REGEN- ERATION
			Molt 8	Molt 9	Molt 10	Molt 11	Left	Right	Aver- age		
2222	8-9	27	10.9	11.9			5.4	5.5	5.45	0.50	0.019
2228	8-9	22	br	11.3			5.1	5.0	5.05	0.48	0.022
2233	10-11	30			12.1	13.6	6.8	6.6	6.7	0.55	0.018
2240	8-9	20	10.5	11.6			4.1	4.5	4.3	0.41	0.0205
2258	9-10	25		11.9	13.0		5.9	6.0	5.95	0.50	0.020
Average	24.8	10.7	11.67	12.55	13.6	5.46	5.52	5.49	0.488	0.020

The average lengths of the regenerated chelæ when a single chela is removed is 5.36 mm. for the five available cases as compared with a regenerated length of 5.46 mm. for the right chela and 5.52 mm. for the left chela in individuals in which both chelæ are removed. The average specific amount of a regenerating chela is 0.484 when it alone is removed and 0.488 when the other chela is removed at the same time. The average specific rate of a regenerating chela likewise is 0.017 when it alone is removed and 0.020 when the other chela is removed at the same time.

The advantage on the whole is evidently in favor of the regenerating chelæ in individuals with the greater injury.

Second Regeneration

The data for second regenerations are given in Tables 18 and 19.

The average length of a regenerating chela in the nine cases in which it alone is removed is 4.5 mm. and in the three cases in which both chelæ are removed the averages are 5.1 mm. for the left chela and 5.1 mm. (?) for the right.

The average specific amount for the lesser injury to the individual is 0.521 and for the greater injury 0.563.

TABLE 18

Cambarus bartoni. Second regenerations. Single chela removed. Two molts

CATA- LOGUE NUM- BER	MOLTS	TIME IN- TERVAL IN DAYS	CEPHALO-THORACIC LENGTHS			AVERAGE CEPHALO- THORACIC LENGTHS Molts 6-7	LENGTH OF REGEN- ERATED CHELA	SPECIFIC AMOUNT OF REGEN- ERATION	SPECIFIC RATE OF REGENER- ATION
			Molt 6	Molt 7	Molt 8				
2218	6-7-8	24	br	9.3	10.1	8.8±	4.6	0.52	0.022
2219	6-7-8	32	8.2	9.2	9.9	8.7	4.2	0.48	0.015
2227	6-7-8	30	br	br	10.6	8.7±	5.1	0.59	0.020
2238	6-7-8	45	8.1	9.5	10.9	8.8	5.3	0.60	0.013
2239	6-7-8	50	7.7	8.2	8.7	7.95	3.7	0.47	0.009
2248	6-7-8	42	br	8.9	9.1	8.4±	3.7	0.44	0.010
2252	6-7-8	36	8.2	br	9.9	8.7±	4.6	0.53	0.015
2259	6-7-8	30	8.1	9.3	10.0	8.7	4.6	0.53	0.018
2262	6-7-8	29	8.3	9.3	10.4	8.8	4.6	0.52	0.018
2242	6-7-8	25	br	9.2	br	8.7±	4.6	0.53	0.021
Average	34.3	8.1	9.11	9.96	8.62	4.5	0.521	0.0161

TABLE 19

Cambarus bartoni. Second regenerations. Both chelæ removed. Two molts

CATALOGUE NUMBER	MOLTS	TIME INTERVAL IN DAYS	CEPHALO-THORACIC LENGTHS			AVERAGE CEPHALO- THORACIC LENGTH Molts 6-7	LENGTH OF REGENER- ATED CHELAE			SPECIFIC AMOUNT OF REGENERATION	SPECIFIC RATE OF REGENERATION
			Molt 6	Molt 7	Molt 8		Left	Right	Average		
2237	6-7-8	45	8.6	10.0	11.2	9.3	5.3	5.3	5.3	0.57	0.013
2245	6-7-8	38	8.3	9.3	10.5	8.8	5.4	*	5.4?	0.61	0.016
2253	6-7-8	25	8.3	9.7	10.4	9.0	4.6	4.6	4.6	0.51	0.020
Average	36	8.43	9.67	10.7	9.03	5.1	5.1?	5.1	0.563	0.0163

*Chela deformed.

The average specific rate for the lesser injury is 0.0161 and for the greater injury 0.0163, a practical equality.

In the case of individuals with the greater injury a regenerating chela has the advantage in absolute amount of regeneration and in specific amount. In specific rate there is, however, a practical equality. In the present instance the specific amount makes a better basis for comparison not only for the general reasons given on pp. 534 and 535 but also because of the much greater uniformity of its values within a single group.

Third Regeneration

The data for third regenerations are given in Tables 20 and 21 for cases extending over one molt only and in Tables 22 and 23 for cases extending over two molts.

In the one-molt cases the absolute amounts of regeneration are on the average 4.4 mm. in the case of removal of a single chela and 5.37 for each of the chelæ in case both are removed. The specific amounts are correspondingly 0.434 for the lesser injury and 0.50 for the greater injury and the specific rates 0.017 for

TABLE 20

Cambarus bartoni. Third regeneration. Single chela removed. One molt

CATALOGUE NUMBER	MOLTS	TIME INTER- VAL IN DAYS	CEPHALO-THORACIC LENGTHS		LENGTH OF REGENER- ATED CHELA	SPECIFIC AMOUNT OF REGENERA- TION	SPECIFIC RATE OF REGENERA- TION
			Molt 8	Molt 9			
2218	8-9	21	10.1	11.4	4.1	0.41	0.020
2219	8-9	23	9.9	11.2	2.7	0.27	0.012
2223	8-9	23	10.5	11.3	4.6	0.44	0.019
2227	8-9	31	10.6	12.1	5.4	0.51	0.016
2238	8-9	25	10.9	12.1	5.0	0.46	0.018
2239	8-9	20	8.7	9.4	2.8	0.32	0.016
2252	8-9	24	9.9	br	4.2	0.42	0.0175
2259	8-9	31	10.0	br	5.3	0.53	0.017
2242	8-9	33	br	11.6	5.4	0.55	0.017
Average....	25.7	10.07	11.3	4.4	0.434	0.017

TABLE 21

Cambarus bartoni. Third regeneration. Both chela removed. One molt

CATA- LOGUE NUM- BER	MOLTS	TIME IN- TERVALS IN DAYS	CEPHALO-THORACIC LENGTH		LENGTHS OF REGENERATED CHELAE			SPECIFIC AMOUNT OF REGEN- ERATION	SPECIFIC RATE OF REGEN- ERATION
			Molt 8	Molt 9	Left	Right	Average		
2237	8-9	22	11.2	12.0	5.4	5.4	5.4	0.48	0.022
2245	8-9	26	10.5	11.8	5.3	*	5.3?	0.50	0.019
2253	8-9	30	10.4	11.5	5.4	5.4	5.4	0.52	0.017
Average.	26	10.7	11.77	5.37	5.37?	5.37	0.50	0.0193

*Chela deformed.

TABLE 22

Cambarus bartoni. Third regeneration. Single chela removed. Two molts

CATALOGUE NUMBER	MOLTS	TIME IN- TERVALS IN DAYS	CEPHALO-THORACIC LENGTH			AVERAGE CEPHALO- THORACIC LENGTHS Molts 8-9	LENGTH OF REGEN- ERATED CHELA	SPECIFIC AMOUNT OF REGEN- ERATION	SPECIFIC RATE OF REGEN- ERATION
			Molt 8	Molt 9	Molt 10				
2218	8-9-10	41	10.1	11.4	12.6	10.75	6.0	0.56	0.014
2219	8-9-10	38	9.9	11.2	12.3	10.55	5.4	0.51	0.013
2223	8-9-10	54	10.5	11.3	12.6	10.9	6.4	0.59	0.011
2227	8-9-10	65	10.6	12.1	12.9	10.35	6.7	0.59	0.009
2248	8-9-10	35	9.1	10.2	11.0	9.65	4.9	0.51	0.015
2252	8-9-10	48	9.9	br	12.3	10.65	6.2	0.58	0.012
2259	8-9-10	68	10.0	br	12.0	10.5	6.3	0.60	0.009
2262	8-9-10	36	10.4	11.5	12.7	10.95	6.0	0.55	0.015
2242	8-9-10	71	br	11.6	13.0	10.5	6.7	0.64	0.009
Average		50.7	10.06	11.33	12.38	10.7	6.07	0.57	0.0119

the lesser injury and 0.0193 for the greater injury. The advantage is throughout in favor of the chela that has a regenerating mate.

In the two-molt cases the absolute amount of regeneration is on the average 6.07 mm. when a single chela is removed and 6.85 mm. for the left and 6.6 mm. for the right chela when both are removed. The specific amount is 0.57 for the single chela

TABLE 23

Cambarus bartoni. Third regeneration. Both chelæ removed. Two molts

CATALOGUE NUMBER	MOLTS	TIME INTER-VAL IN DAYS	CEPHALO-THORACIC LENGTH			AVERAGE CEPHALO-THORACIC LENGTH	LENGTH OF REGENERATED CHELAE		SPECIFIC AMOUNT OF REGENERATION		SPECIFIC RATE OF REGENERATION	
			Molt 8	Molt 9	Molt 10		Left	Right	Left	Right	Left	Right
			Molts 8-9	Left	Right	Left	Right	Left	Right			
2245	8-9-10	47	10.5	11.8	12.8	11.15	7.0	6.4	0.63	0.57	0.013	0.012
2253	8-9-10	52	10.4	11.5	12.4	10.95	6.7	6.8	0.61	0.62	0.012	0.012
Average.....		49.5	10.45	11.65	12.6	11.05	6.85	6.6	0.62	0.595	0.0125	0.012

and 0.62 and 0.595 for the left and right chelæ respectively in double removals. Likewise, the specific rate is 0.0119 for the single chela and 0.0125 and 0.0120 for the left and right chela respectively in double removals.

For the third regenerations again the advantage is in favor of the crayfish with the greater injury.

Fourth Regeneration

Only four crayfish had a fourth regeneration. The regeneration in all extended through a single molt only. In three cases with a single chela removed the average absolute amount was 6.5 mm., the specific amount 0.52 and the specific rate 0.019. In the one case with both chelæ removed the average of the two chelæ gave an absolute amount of 5.5 mm., a specific amount of 0.46 and a specific rate of 0.021. The first two values are greater in the crayfish with the lesser injury, the last one is greater in the animal with the greater injury. The number of individuals is too small to make the result of much value.

Taking the present experiment as a whole it is very evident that in young crayfish the advantage is distinctly in favor of the chela with a regenerating mate, as opposed to a chela removed and regenerating alone.

17 *Cambarus propinquus*. Nos. 2309-2378

The present experiment and the following one (No. 18) were planned for a study of other factors than that of degree of injury. However, since they give some data on the problem in hand they are included.

The individuals in Experiment 17 come from a single mother collected with a mass of eggs in April, 1907. All the living young, seventy in number, were taken from the mother on May 12 and put into seventy separate dishes. All but seven of the crayfish had molted twice since hatching. The others molted their second time on May 13. The experiment was planned in part for a study of the effect of the time of the operation with respect to a molt upon the time of succeeding molts. Thirteen individuals are available for the purposes of the present paper. All these were operated upon two days after the third molt. In seven one chela was removed and in six both chelæ were removed. In all except one individual in each set the molt came on May 21 and the operation on May 23. In the exceptional ones the molt came on May 20 in No. 2343 and on May 22 in No. 2358. The crayfish were killed in 85 per cent alcohol on June 20, twenty-eight days after the operation. The data are given in Tables 24, 25, 26 and 27. Tables 24 and 25 include the individuals in which three molts occurred during the regenerating period and Tables 26 and 27 those in which four molts occurred.

The young crayfish were so nearly equal in size that separate measurements of cephalo-thoracic length and removed chela lengths were not taken. It is, therefore, not possible as in the case of the data of the preceding experiment to give the specific amount and specific rate of regeneration. The absolute amounts and the rates per day are given in the tables. Of special value is the fact that the molting periods show only slight differences in length so that two compared groups are nearly equal in absolute time as well as in number of molts.

The data show an advantage in favor of the crayfish with two regenerating chelæ both in absolute amount and in rate per day. For the three-molt cases the removed single chelæ have an abso-

TABLE 24

Cambarus propinquus. Nos. 2309-2378. One chela, the right, removed. Regeneration for three molts after the operation

CATALOGUE NUMBER	DAYS OF REGENERATION	LENGTH OF REGENERATED RIGHT CHELA IN MMS.	
2311	28	2.96	Rate per day = .110 mm.
2319	28	3.33	
2325	28	2.74	
2339	28	3.26	
Average.	28.0	3.07	

TABLE 25

Cambarus propinquus. Nos. 2309-2378. Both chelæ removed. Regeneration for three molts after the operation

CATALOGUE NUMBER	DAYS OF REGENERATION	LENGTH OF REGENERATED LEFT CHELA IN MMS.	LENGTH OF REGENERATED RIGHT CHELA IN MMS.	
2320	28	3.33	3.33	Rate per day: Right chela = .114 mm. Left chela = .114 mm.
2341	29	3.55	3.55	
2355	28	2.81	2.81	
Average.....	28.3	3.23	3.23	

TABLE 26

Cambarus propinquus. Nos. 2309-2378. One chela, the right, Regeneration for four molts after the operation

CATALOGUE NUMBER	DAYS OF REGENERATION	LENGTH OF REGENERATED RIGHT CHELA	
2354	27	3.77	Rate per day = .135 mm.
2358	28	3.33	
2364	28	4.14	
Average.....	27.7	3.75	

TABLE 27

Cambarus propinquus. Nos 2309-2378. Both chelæ removed. Regeneration for four molts after the operation

CATALOGUE NUMBER	DAYS OF REGENERATION	LENGTH OF REGENERATED LEFT CHELA	LENGTH OF REGENERATED RIGHT CHELA	
2317	28	3.92	4.00	Rate per day:
2327	28	3.88	3.88	Right chela = .140 mm.
2343	28	3.92	3.85	Left chela = .140 mm.
Average.....	28.0	3.91	3.91	

lute amount of regeneration of 3.07 mm. and a rate per day of 0.110 mm. while the individuals with both chelæ removed have an absolute amount of regeneration of 3.23 mm. and a rate per day of 0.114 mm. in each of the two chelæ. For the four-molt cases the absolute amount for the single chelæ is 3.75 mm. against 3.91 mm. for each of the chelæ in the double removal and the rates per day are respectively 0.135 mm. and 0.140 mm.

18 *Cambarus propinquus*. Nos. 2391-2483

Ninety-three young of the one hundred and four attached to a single female were used in the experiment which was concerned primarily with other factors than the one discussed in the present paper. These young had molted twice since hatching and were put into individual dishes on May 22, 1907.

Those individuals which were operated upon one day after the third molt give data for the effect of the degree of injury. Eight individuals with one chela removed and nine with both chelæ removed survived until June 20, when all were killed. A comparison is thus obtained between the rate of regeneration of a chela when it alone is removed and its rate when the opposite chela is removed at the same time.

The data are given in Tables 28 and 29. No measurements were made of cephalo-thoracic lengths or removed chela lengths and it is therefore not possible to make a comparison in terms of specific amounts and specific rates of regeneration.

TABLE 28

Cambarus propinquus. Nos. 2391-2483. One chela removed one day after the third molt

CATALOGUE NUMBER	DAYS OF REGENERATION	LENGTH OF REGENERATED RIGHT CHELA	
2402	18	1.67	Rate of regeneration per day = .115 mm.
2406	19	2.90	
2409	18	1.64	
2412	16	1.70	
2415	18	1.61	
2421	18	2.37	
2435	17	2.13	
2477	17	2.20	
Average.....	17.6	2.03	

TABLE 29

Cambarus propinquus. Nos. 2391-2483. Both chelæ removed one day after the third molt

CATALOGUE NUMBER	DAYS OF REGENERATION	LENGTH OF REGENERATED LEFT CHELA	LENGTH OF REGENERATED RIGHT CHELA	
2400	19	1.48	1.55	Rate of regeneration per day: Left chela = .115 mm. Right chela = .113 mm.
2410	18	1.82	1.82	
2424	17	1.44	1.44	
2426	19	1.82	1.52	
2428	18	2.43	2.43	
2437	19	3.10	3.10	
2451	17	2.35	2.28	
2459	18	2.25	2.12	
2470	18	2.13	2.16	
Average.....	18.1	2.09	2.05	

The average regenerated lengths are slightly greater in each of the two regenerated chelæ of a double removal than in the one chela of a single removal. The amounts are 2.09 mm. and 2.05 mm. for the double removal and 2.03 for the single removal. The rate per day is the same for one of the two chelæ of a double removal as for the single chela of a single removal. The other

chela of the double removal is slightly smaller on the average. The rates are 0.115 mm. and 0.113 mm. per day for the double and 0.115 mm. for the single removal.

The individual variations in the present experiment are considerable. The differences between the two degrees of injury are so slight that they are probably not significant. No change in rate of regeneration with degree of injury is indicated in the individuals of the present experiment.

DISCUSSION

The various factors controlling growth and regeneration are so closely interrelated that no final analysis of any one can be expected until there is at least some understanding of all or nearly all of them. This is nowhere more evident than in the case of the rate of regeneration.

Nevertheless the data collected in the preceding experiments point conclusively to the general fact that the rate of regeneration of a part does not necessarily diminish with additional injury to the individual. On the contrary in the majority of the cases the rate of regeneration of a part is evidently higher when other parts of the animal have been removed at the same time than when the part alone has been removed.

It is evident that the amount of injury involved in the removal of the part whose rate of regeneration is being compared as well as the amount of the additional injury must determine the character of the result. The conclusive data in several experiments as well as the general mass of evidence obtained from all of them prove without question that within moderate degrees of injury a part regenerates more rapidly rather than less rapidly when it has regenerating company than when it regenerates by itself. The few exceptional cases showing a decrease in rate are capable of explanation on the grounds either of an incomplete control of subsidiary factors or of the presence of too high a degree of injury. The strength of the statement is made still stronger if possible by the consideration that additional injury to an individual involves a whole train of consequences likely to lead to disturb-

ance of vitality, to infection, and to other means of lessening the rate of regeneration. The accidents of the experiments in other words are all in the direction of an apparent lesser rate for the higher injury. These facts have already been sufficiently discussed under the heading of sources of error (pp. 514-518).

The above considerations seem to be a sufficient answer to the recent criticisms by Emmel, Scott, and Stockard. Stockard states (1) that the individual variation is sufficient to cause an overlapping of the rates in the two compared groups. (2) that in the case of animals with the molting habit this habit constitutes a serious source of error. (3) That even neglecting these difficulties the influence of degree of injury is small in amount as compared with such evident factors as the level of the cut.

The difficulties connected with the molting habit have already been mentioned on pp. 534-535, above and a paper dealing with several of the molting factors is in preparation. The other criticisms have been considered in discussing the sources of error. In a general way these criticisms are directed against the view that increase in injury brings about a decided increase in rate of regeneration. No special arguments against them are needed to support the statement that our evidence at present favors the view that increase in degree of injury to the individual within moderate degrees *increases rather than decreases* the rate of regeneration of a part.

The comparison that has been made between the effect of level of the cut in an organ and the degree of injury to the individual needs further attention. As stated above (p. 515), the level of the cut is not of much value for the determination of the effect of degree of injury to the individual because it involves changes in local conditions. Nor can the increase in rate of regeneration with a lowering of level be compared with the other increase. If the rate of regeneration of a chela for instance is unchanged by the simultaneous removal of the other one there is still twice as much regeneration being accomplished by the individual after a double removal as after a single one, just as in the case of a cut at a deeper level of the tadpole's tail proportionately more new tail is formed than at a higher level. The increase in rate dis-

cussed in the present paper involves more than a proportional increase.

The following general conclusion results naturally from the data as determined at present. The removal of a part of an animal involving a slight or moderate degree of injury is followed by a rate of regeneration that is less than it is in case the removal is accompanied by an additional removal of slight or moderate amount in other parts of the animal. If the degree of injury in either case is considerable, the additional injury results in a decrease in rate of regeneration of the part. For every part capable of regeneration, the statement can therefore be made that its rate of regeneration increases with increase in additional injury up to an optimum degree beyond which further injury causes a decrease in rate.

In a purely descriptive way it may be said that the increase in rate reveals the presence of a kind of inertia in the body of the animal comparable to ordinary physical inertia. The time necessary for the repair of an injury is not least in the case of the lowest injury but a certain degree of injury is necessary before the regenerative powers of the organism can work at their best. In the same way also in the case of the functional operculum of a Serpulid worm a considerable degree of injury is necessary to start the process of reversal of the opercula. The decrease in rate that follows at higher degrees of injury is undoubtedly due to another factor, the active disturbance of the ordinary mechanism of the body.

SUMMARY

1 An attempt was made to determine the pure effect of the degree of injury to the individual upon the rate of regeneration of a removed part.

2 The principal method consisted of the comparison of the rates of regeneration of an organ removed at a constant level when other parts of the animal were or were not removed at the same time.

3 A special study was made of the various other factors influ-

encing the rate of regeneration in order that a more careful determination might be made of the effect of the degree of injury.

4 The data are divided into two groups including on the one hand the animals without a molting habit and on the other those with a molting habit. This was found advisable because the factor involved in molting cannot at present be fully controlled in most cases.

5 A summary is made of former experiments in the first group. These were made on the opercula of the Serpulid worms, *Hydroides dianthus* and *Apomatus ampullifera*, the arms of the brittle-star, *Ophioglypha lacertosa* and the oral arms of the Scyphomedusan, *Cassiopea xamachana*. They show that a removed organ regenerates less rapidly when it alone is removed than it does when other parts are removed at the same time.

6 The new experiments in the first group include five on the larvæ of the salamander, *Amblystoma opacum* and one on the tadpoles of the green frog, *Rana clamitans*. The organs whose rate of regeneration was studied are the tail and the fore- and hind-legs. The data confirm the former results by showing that additional injury to an individual is favorable to the regeneration of these organs.

7 In the group with a molting habit, a summary of former experiments on the chelæ of *Gelasimus pugilator* and *Alpheus dentipes* and on the chelæ and walking legs of the adult *Cambarus propinquus* shows that the regeneration of a chela is less rapid when it alone is removed than it is when the other chela or the other chela and four walking legs are removed at the same time.

8 The new experiments yield data on the chelæ of the shrimp, *Palæmonetes vulgaris*; of two species of crabs, *Porcellana platycheles* and one of undetermined species, and of young individuals of the crayfish, *Cambarus bartoni* and *Cambarus propinquus*. They confirm the results of the former experiments.

9 The experiments as a whole show further that when the additional injury is excessive there is a decrease in the rate of regeneration of the organ under consideration.

10 The following general statement regarding the effect of the degree of injury to the individual upon the rate of regenera-

tion of an organ may be made: The rate of regeneration of an organ increases with increase in additional injury to the individual up to an optimum degree beyond which further injury leads to a decrease in rate. The position of the optimum is different in the different organs of an individual and in corresponding organs of different species. The amount of the optimum degree of additional injury is also changed with change in the level of removal in the organ under observation.

BIBLIOGRAPHY

- DURBIN, M. L. '09—An analysis of the Rate of Regeneration throughout the Regenerative Process. *Journal of Experimental Zoölogy*. Vol. vii, no. 3.
- ELLIS, M. M. '07—The Influence of the Amount of Injury upon the Rate and Amount of Regeneration in *Mancasellus macrourus* (Garman). *Biological Bulletin*, vol. xiii, no. 3, August, 1907.
- '08—Some Notes on the Factors Controlling the Rate of Regeneration in Tadpoles of *Rana clamata* Daudin. *Biological Bulletin*, vol. xiv, no. 5, April, 1908.
- '09—A Consideration of the Relation Between the Amount Removed and the Amount Regenerated in Tadpoles of *Rana clamitans* Latrille. *Journal of Experimental Zoölogy*. Vol. vii, no. 3.
- EMMEL, V. E. '04—The Regeneration of Lost Parts in the Lobster. *Thirty-fifth Annual Report of the Commissioners of Inland Fisheries of Rhode Island*, pp. 80-117.
- MORGAN, T. H. '06—The Physiology of Regeneration. *Journal of Experimental Zoölogy*, vol. iii, no. 4.
- SCOTT, G. G. '07—Further Notes on the Regeneration of the Fins of *Fundulus heteroclitus*. *Biological Bulletin*, vol. xii, no. 6. May, 1907.
- STOCKARD, C. R. '08—Studies of Tissue Growth 1. An Experimental Study of the Rate of Regeneration in *Cassiopea xamachana* (Bigelow). *Carnegie Institution of Washington. Publication No. 103, Tortugas Laboratory Reports*, vol. ii.
- ZELENY, C. '03—A Study of the Rate of Regeneration of the Arms in the Brittlestar, *Ophioglypha lacertosa*. *Biological Bulletin*, vol. vi, no. 1.
- '05-1—Compensatory Regulation. *Journal of Experimental Zoölogy*, vol. ii, no. 1.

- ZELNY, C. '05-2—The Relation of the Degree of Injury to the Rate of Regeneration. *Journal of Experimental Zoölogy*, vol. ii, no. 3.
- '07—The Effect of Degree of Injury, Successive Injury and Functional Activity upon Regeneration in the Scyphomedusan, *Cassiopea xamachana*. *Journal of Experimental Zoölogy*, vol. v, no. 2.
- '08—Some Internal Factors Concerned with the Regeneration of the Chelæ of the Gulf-weed Crab (*Portunus sayi*). Carnegie Institution of Washington. Publication no. 103. Tortugas Laboratory Reports vol. ii.
- '09—Successive Regenerations. *Journal of Experimental Zoölogy*. Vol. vii, no. 3.

APPENDIX

After the manuscript of this paper was sent to press the author received an important paper by Stockard¹ dealing in part with the question of the relation of the degree of injury to the rate of regeneration. Stockard has performed experiments on the arms of the Scyphomedusan, *Cassiopea xamachana* and the arms of the brittle-stars *Ophiocoma riisei* and *Ophiocoma echinata*. He concludes that "the medusa, *Cassiopea*, regenerates each oral arm at a rate which is independent of the degree of injury when replacing either one, two, four or six of its arms. If, however, eight arms are amputated, each arm is regenerated at a rate which, after taking account of the probable error, is significantly greater than the regeneration rates in medusæ injured to any less extent.

"The brittle star, *Ophiocoma riisei*, regenerates either one, two, three, four or all five arms at rates which are not significantly different. In other words, there is no relation between the rate of the individual arms and the degree of injury in this species.

"The rate of regeneration for individual arms in *Ophiocoma echinata*, another species of Ophiuran, is fastest when only a single arm is regenerating and successively slower when two, three, four and five arms are being replaced. The rate of regeneration is slower the greater the extent of injury."

In the first case, that of *Cassiopea*, Stockard finds that the animals decrease in size during the course of the experiments, the ones with a greater number of removed arms decreasing faster than those with a smaller number. He used the original disk diameters for the determination of the specific rates of regeneration. If the final disk diameters are taken the specific rate of regeneration of each arm on the whole increases with increase in number of removed arms. The question as to the use of the original or the final disk diameters depends upon the interpretation of

¹ Studies on Tissue Growth. II. *Journal of Experimental Zoölogy*, vol. vi, pp. 433-469.

the cause of the decrease in size. If it is due to factors only secondarily connected with the removal, such as ability to get food, freedom in movement, etc., it seems to me that it is more valid to use the final rather than the original diameter as a basis. The decrease in size of the animals of necessity means a decrease in the rate of regeneration quite apart from the matter of degree of injury. The new arms are being regenerated in a proportion that is proper for the decreased disk and not for the original one. The smaller animal is certainly not regenerating arms of the original size. If the animals were fed an amount just sufficient to retain their original disk diameters it is probable that the specific regeneration would be greater in those with the greater number of removed arms.

The greatest degree of injury, however, in Stockard's experiments shows the greatest rate for the individual arms under either interpretation. In my *Cassiopea* materials the optimum came at a lower degree. The difference is evidently due to some factor in the conduct of the experiments, probably better living conditions in Stockard's animals.

In the brittle-star *Ophiocoma riisei* the same criticism may be offered of the use of the original rather than the final disk diameter though here it makes only a slight difference in the interpretation of the results. The data show very little if any difference in rate with a different number of removed arms.

In the brittle-star *Ophiocoma echinata* the data furnished by Stockard evidently show a decrease in rate of the individual arms with increase in the removed number. No criticism of the interpretation of the data occurs to the writer.

In comparing these brittle-star experiments with mine on *Ophioglypha lacertosa* it should be borne in mind that apart from specific differences there were also differences in the living conditions in the two cases. The level of the cut in the arms was higher up in Stockard's animals, the latter also had a considerable food supply and a much greater chance of active movement.

Stockard's three experiments thus give one case with increase, a second with no change and a third with a decrease in rate of regeneration as a result of increase in degree of injury. All three such instances are given in the data of my paper though the majority belong to the first group. His data therefore do not modify the general conclusion that on the whole the optimum degree of injury in an individual is not necessarily the lowest degree. In the majority of cases studied so far the optimum degree of injury is above the lowest degree.

The problem now is therefore not whether *all* animals under *all* conditions regenerate parts more rapidly or less rapidly with increase in number of removed parts. It seems to me to be the determination of the conditions affecting the location of the optimum and the relation of these conditions to the general problem of growth.