

LOCAL FACTORS AND REGENERATION IN CRUSTACEA

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The role of normal innervation in the regeneration of crustacean appendages has been illustrated in previous papers (Needham, 1945, 1946); it may, perhaps, be termed the 'central' factor in regeneration. In the present paper 'local' factors, such as properties of the tissues near the site of amputation, have been studied. The results of earlier work, chiefly on Amphibia, Planaria and Oligochaeta (Korschelt, 1927, pp. 639-40), have suggested that the rate of regeneration varies with (1) the extent of tissue damage at the site of amputation and (2) the amount of tissue removed. These two factors have been studied in the present experiments on the regeneration of the 7th thoracic limb of *Asellus aquaticus* (L.).

METHOD

The general procedure was as in previous experiments (Needham, 1945, 1946). The effect of damaged tissue at the site of amputation was assessed by comparing the length of a normally regenerating limb at its first eclosion (its 'eclosion length', e.l.) with that of the partner limb regenerating in the presence of damaged tissue. This was arranged (i) by applying macerated tissue, chiefly muscle from the amputated limb itself ('ipseplasm'), to the stump immediately after amputation (series 7), and (ii) by light maceration of the stump *in situ*, through the autotomy membrane, immediately after amputation (series 8). The adhesion of the macerated tissue in series 7 was ensured by allowing it to dry after application, before returning the animal to water.

The distal aspects of the intersegmental joints were selected as suitable points for amputation in order to measure the relationship between regeneration rate and the fraction of the limb removed. It was hoped to compare, in a series of individuals, the regeneration of a distal fraction of n segments with one of $n+1$ segments in the partner limb, where $n=2, 3, 4$ and 5 in four different series. However, the frequency of autotomy subsequent to amputation (p. 223) prevented the collection of sufficient data for this direct comparison, and the results analysed below (pp. 221-4) were obtained by pooling all data for each value of n and comparing mean regeneration rates. The pooled data for $n=2, 3, 4, 5$ and 6 will be referred to as series 9, 10, 11, 12 and 13 respectively. On account of the shortness of the dactyl it was considered improbable that data for $n=1$ would be reliable. Measurements were taken, as before, at eclosion.

Series 9-13 were designed to measure the average regeneration rate for regeneration initiated in the different segments of the limb. In addition, regeneration from a point of amputation immediately distal to the intersegment was compared with regeneration from a point in the distal half of the segment, in order to detect

variations in 'regeneration-initiating potency' within the segment. This experiment was restricted to the carpus (series 14). As in series 9-13 insufficient complete pairs of results were obtained, and the analysis was applied to all 'basal' and all 'distal' regenerates.

RESULTS

Macerated ipseplasm (p. 220) applied to the stump of an amputated limb exercised an insignificant effect on regeneration rate (Table 1, series 7). Maceration of the stump itself caused (Table 1) a marked retardation of regeneration ($t=3.18$, $P<0.01$). Preliminary experiments involving the actual implantation of macerated tissue into the stump also gave evidence of retardation of regeneration. Macerated ipseplasm would appear to provide no stimulus to regeneration in this limb; on the contrary, maceration of tissues immediately proximal to the autotomy membrane caused a marked loss in regenerative power. These tissues would seem to possess a specifically high regenerative power, a conclusion in agreement with the observations of Przibram (1909, p. 116) that amputation at a point more proximal than the autotomy plane leads to a marked loss in power. This effect is so marked that statistical tests of significance seem unnecessary.

Table 1. *Asellus aquaticus*. Effect of macerated ipseplasm on rate of regeneration

All measurements in mm. $\times 56$ in all tables.

Series 7. Macerated ipseplasm applied to stump after amputation.

Series 8. Tissues of stump macerated immediately after amputation.

Series no.	No. of individuals	Mean abdomen width (mm. $\times 56$)	Mean experimental limb length	Mean control limb length	Mean difference between control and experimental limb length
7	39	110.1	139.4	142.4	3.05
8	38	126.9	113.4	138.7	25.32

The indication of high regenerative potency in these tissues gives added significance to experiments on regeneration from points distal to the autotomy plane (series 9-13). The mean e.l. (p. 220) of the regenerated portion of the limb decreased progressively with the distal movement of the point of amputation. The decrease between $n=6$ and $n=5$ was much greater than elsewhere along the limb, and regeneration is undoubtedly much more rapid from the autotomy plane than from any other point proximally or distally to it.

Although the e.l. of the whole fraction regenerated decreased progressively with the distal movement of the point of amputation, the mean e.l. per segment regenerated increased progressively in the same order, except that, even on this criterion, regeneration potency is greatest at the autotomy plane ($n=6$, series 13, Table 2). The mean e.l. per segment was calculated by dividing the total e.l. of the fraction regenerated by $n - \frac{1}{2}$, using the approximation that segments ischium (*I*), merus (*M*), carpus (*C*), and propus (*P*) are equal in length and the dactyl (*D*) approximately a half of this.

The mean e.l. of individual segments showed the same progressive increase from $n=5$ to $n=2$ (Table 2). In order to obtain a reliable estimate of relative regeneration rates the body size and time interval between amputation and eclosion ('eclosion time', e.t.) should be taken into account. The effect of variations in these two factors was eliminated by the approximate method of dividing the mean e.l. of any dimension by the product of the mean body size and the mean e.t., in days. This quotient may

Table 2. *Asellus aquaticus*. Regeneration of n distal segments of thoracic limb, where $n=2, 3, 4, 5$ and 6 respectively in series 9, 10, 11, 12 and 13

No. of segments amputated (n) ...	6	5	4	3	2
Series no. ...	13	12	11	10	9
No. of records ...	99	37	31	99	45
Frequency of autotomy (%) following amputation ...	—	68	21	14	2
Mean e.t. (in days) ...	16.0	15.2	16.7	17.5	17.5
Mean abdomen width (mm. \times 56) ...	118.4	126.1	117.6	128.1	124.1
Mean e.l. of limb segments:					
Basipreischium	*32.08	56.28	51.44	61.69	54.50
Ischium	23.69	*14.51	40.25	51.94	45.19
Merus	21.97	16.12	*17.46	44.83	41.20
Carpus	22.22	13.97	15.76	*22.95	41.61
Propus	21.76	12.71	14.28	20.12	*23.04
Dactyl	10.15	6.84	7.96	9.57	9.61
Mean e.l. of all segments regenerated	131.7	63.83	55.46	52.64	32.64
Mean e.l. per segment regenerated	23.94	14.18	15.84	21.06	21.76
S.d.m.i. of regenerated segments:					
Basipreischium	0.0169	—	—	—	—
Ischium	0.0125	0.0076	—	—	—
Merus	0.0116	0.0084	0.0089	—	—
Carpus	0.0117	0.0077	0.0080	0.0102	—
Propus	0.0114	0.0066	0.0073	0.0090	0.0106
Dactyl	0.0054	0.0036	0.0041	0.0043	0.0044
S.d.m.i. using the basipreischium as a measure of body size:					
Ischium	—	0.0170	—	—	—
Merus	—	0.0189	0.0203	—	—
Carpus	—	0.0164	0.0184	0.0212	—
Propus	—	0.0149	0.0166	0.0186	0.0242
Dactyl	—	0.0080	0.0093	0.0089	0.0101
M.s.d.i. of propus, using basipreischium as a measure of body size	—	0.0174	0.0178	0.0203	0.0248

The asterisk marks the point of amputation.

be termed the specific daily mean increment (s.d.m.i.) in contradistinction to the mean specific daily increment (m.s.d.i.) obtained by averaging the specific daily increment (s.d.i.) for each individual record of a particular dimension. This latter more accurate measure of regeneration rate was used for specific statistical tests of significance, but the former less laborious method was applied in general. The trends observed in the table of e.l. were preserved in that of the s.d.i. (Table 2); indeed, irregularities in the general trends were reduced, and there can be little doubt of the reality of these trends. The larger differences in s.d.i. were shown to

be statistically significant: the difference in m.s.d.i. of the propus between the series $n=2$ and series $n=3, 4$ and 5 respectively gave values of $t=2.92, 4.13$ and 4.02 for which the probability is less than 0.01 . Both abdomen width and length of the persisting basipreischium were used as a measure of body size.

The results of series 9-13 show another feature of interest. When regeneration rates of individual segments of a fractional regenerate are compared with those of corresponding segments of the whole regenerate ($n=6$) it is seen that the difference is most marked for the most distal segments of the fraction and decreases proximally, suggesting a centre of relatively high regeneration rate at the proximal end of the regenerate. This was observed also in the regenerating whole limb (Needham, 1943, p. 57 and Text-fig. 7). It is clear that the relatively higher rate of regeneration of a particular segment (e.g. propus) or of the average rate per segment when n is small must be due, to some extent, to the progressive elimination of the lower end of this proximo-distal gradient in regeneration rate, as the site of amputation is moved distally along the limb. There would, however, appear to be a genuine progressive increase in 'regeneration-initiating potency' between series $n=5$ and $n=2$, as shown by comparing the values, in the four series, of e.l. or of s.d.i., for the most proximal segment of the fractions regenerated (assuming that I, M, C and P are normally approximately equal in length (p. 221). Similar comparisons for succeeding segments, in order distalwards, show the same trend (Table 2). Moreover, the data for the whole regenerate (series $n=6$) indicate that a relatively steep proximo-distal gradient in segmental regeneration rates may itself be an expression of low potency.

The dactyl probably causes a slight irregularity in the proximo-distal gradient. A more interesting anomaly is seen in the basal segments, I and M , of the fraction $n=5$ (Table 2). In this series the centre of highest regeneration rate would appear to be located in the segment M rather than in I , the basal segment of the fraction. The mean difference between I and M is statistically significant ($t=3.44$) if two individuals, which between them contribute half of the total variance, are discarded. (Note. In one of these two individuals the I was abnormally long on account of a persisting proximal fraction of the segment and in the other, by contrast, that segment was completely absent. The complete absence of a segment is a rare abnormality of regeneration of these appendages, and this example may indicate an inherent poverty of 'regeneration-realization' potency in the segment I . Realization potency is to be distinguished from regeneration-initiating power which also, as judged by regeneration rate per segment, is lowest in this segment (pp. 221-3).)

The frequency of autotomy following amputation decreased progressively with the distal movement of the point of amputation (Table 2).

Within a particular segment tissues near the base would appear to possess a higher regeneration-initiating potency than more distal tissues. The difference between the mean e.l. of segments $P+D$ for basal and distal carpal groups (p. 220) was not statistically significant, but, owing to variable regression of tissue following amputation, the site of regeneration in each group showed some variation. In the

'basal' group regression frequently affected the merus. The data for each group were therefore divided into three subgroups according to the site of initiation of regeneration; this was determined in the 'distal' group by the ratio C/M (i.e. the ratio of the length of persisting fraction of the carpus to that of the intact merus—a measure of the fractional length of the carpus), and in the basal group by the ratio M/I , a measure of the extent to which the merus was affected by tissue regression after amputation. The subgroup with the highest ratio of M/I , virtually unity (Table 3), contained the examples of 'true' basally initiated regeneration in the carpus. The m.s.d.i. of segments $P+D$ for this subgroup differed significantly ($t=4.03$) from that for the subgroup representing regeneration from the middle ($C/M=0.51$) of the carpus. Regeneration-initiating potency would appear to increase again towards the distal end of the segment ($C/M=0.65, 0.79$).

Table 3. *Asellus aquaticus*. Comparison of regeneration rates for regenerates initiated at different points in the segment

Basal group			Distal group		
No. of records	Ratio M/I	M.s.d.i. of segments $P+D$	No. of records	Ratio C/M	M.s.d.i. of segments $P+D$
14	0.80	0.026	9	0.52	0.015
14	0.95	0.026	9	0.67	0.025
15	1.01	0.031	9	0.81	0.023

The main results of series 9–14 may be summarized as follows: (1) Regeneration-initiating power is maximal immediately proximal to the autotomy plane. (2) Subsidiary centres of high potency are located immediately distal to each intersegment. (3) As measured by the total length regenerated in unit time, the potency of these subsidiary centres decreases progressively in the proximo-distal direction, whereas, measured by the length per segment regenerated, the potency decreases equally regularly in the opposite direction (excepting regeneration from the autotomy plane, which, on any criterion, is more rapid than regeneration initiated at any other point of the limb).

DISCUSSION

There would seem to be little evidence that, in these limbs, regeneration is accelerated by a substance of the nature of a 'wound hormone', liberated from damaged tissues at the site of amputation. The autotomy mechanism no doubt normally ensures a minimum of damaged tissue at autotomy, and, moreover, this is separated from the regenerating tissue by the autotomy membrane, and it might be suggested that a sensitivity to possible wound stimulants has in consequence no adaptive value in these limbs. Nevertheless, regeneration from a point of amputation distal to the autotomy plane is not uncommon in nature, and is usually followed by evident and sometimes considerable destruction of tissue, yet regeneration is invariably slower than regeneration from the autotomy plane. Moreover, regeneration is slower from the middle of the carpus (p. 223) than from the base, where the amount of damaged tissue, as measured by tissue regression following amputation, is probably less.

The evidence for a regeneration-stimulating wound hormone, well established for plants (Haberlandt, 1922), is not strong for animals. Apart from the conclusions of Bier (1917), working on human regeneration, there have been few indications (Needham, 1941, p. 81) of such a factor. The familiar phenomenon of supernumerary regeneration or 'superregeneration' (Korschelt, 1927, pp. 413-72) is commonly believed to be due to such a factor, though, from the evidence, a mechanical morphogenetic cause appears at least as possible as a chemical one, and, moreover, the eventually excessive regeneration does not necessarily imply accelerated regeneration.

Far from stimulating regeneration in these limbs, maceration of the tissues immediately proximal to the autotomy membrane appears to cause marked retardation. The equally marked retardation which results from elimination of these tissues by amputation proximally to the autotomy plane (p. 221) strongly supports the suggestion that they possess an inherent maximum in regeneration-initiating potency.

It seems probable that relative maxima of regeneration-initiating potency may be located at those points where the incidence of regeneration initiation is relatively high. This is clearly true of the maximum near the autotomy plane, and it seems probable that regression of tissue, subsequent to amputation at points distal to this plane, may lead to a relatively high incidence of initiation from the intersegmental regions, where the narrowing of the lumen of the limb facilitates the arrest of regression. Again, if the regeneration rate per segment regenerated (pp. 221-3) may be taken as a measure of regeneration-initiating potency, this is also related to the normal incidence of regeneration at the different intersegments (i.e. inversely related to the incidence of autotomy following amputation at the various intersegments (p. 223).

The total regeneration rate of a fractional regenerate, as opposed to its segmental regeneration rate, varies with the amount of the limb removed (p. 221), supporting the classical view (p. 220), though, as shown above (p. 221), the relation is by no means linear.

Although the variations in segmental regeneration rates have been stressed in this paper, their approximate equality is perhaps remarkable. However many segments require replacement each acquires approximately the same length by the time of eclosion, suggesting that once the rudiment of each segment has been determined its further growth is ensured, at a rate which is inherently the same in all segmental rudiments, apart from such environmental accidents as its relative position in the limb, and the regeneration-initiating potency of the tissues at the site of origin of regeneration.

The proximo-distal gradient in regeneration rate along a fractional or complete regenerate (p. 223) is probably related to the familiar basifugal mode of regeneration (Korschelt, 1927, pp. 328-30). It is undoubtedly most marked in relatively small regenerates, due to early eclosion or to retarded regeneration.

SUMMARY

1. No evidence has been obtained of a wound hormone which stimulates regeneration of the thoracic limb of *Asellus aquaticus*.
2. On the contrary, damage to the tissues immediately proximal to the autotomy plane significantly retards regeneration, which depends considerably on intrinsic regeneration-initiating potencies in the intact tissues at the site of amputation.
3. This potency is greatest in tissues immediately proximal to the autotomy membrane, while subsidiary regions of relatively high potency are located immediately distal to each intersegment.
4. The absolute regeneration rate of amputated fractions of this limb increases with the extent of the fraction, according to classical views.
5. The increase is not linear, so that the regeneration rate per segment regenerated, though highest for a complete (6-segment) regenerate, originating at the autotomy plane, is lowest for a 5-segment regenerate, and increases progressively for 4, 3 and 2-segment regenerates.
6. The incidence of autotomy following amputation increases progressively with the disto-proximal movement of the point of amputation.
7. These features probably have adaptive significance.
8. Within a regenerating limb or fraction there is a proximo-distal gradient in regeneration rate, most marked in early stages of regeneration.

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