THE CENTRAL NERVOUS SYSTEM AND REGENERATION IN CRUSTACEA

By A. E. NEEDHAM

Department of Zoology and Comparative Anatomy,
University Museum, Oxford

(Received 15 March 1952)

INTRODUCTION

It has been shown (Needham, 1945, 1946, 1950) that the rate of regeneration of appendages in Asellus aquaticus (L.) is dependent upon innervation, as in amphibia (Schotté & Butler, 1941, 1944; Singer, 1943) and in other groups of animals (Korschelt, 1927). The object of the present study was to obtain further information on the origin and nature of this neural factor, and in particular to ascertain whether it emanates from the segmental ganglion or from higher centres of the c.n.s. A trophic effect of the vertebrate nervous system on non-regenerating structures (Wyburn-Mason, 1950), which appears to be related to the effect of the system on wound-healing and regeneration, originates locally, since atrophy of organs in the vertebrates does not immediately follow spinal transection, but is correlated with the subsequent atrophy of the local nerve-centres (Wyburn-Mason, 1950, p. 192). There is evidence (Singer, 1943) that in amphibia the regeneration-effect likewise is thus localized.

To test this it is necessary to isolate the local centre from the rest of the c.n.s. The crustacea are ideal for this purpose since their cytons are virtually restricted to the segmental ganglia, and individual body-segments are easily isolated by transection of the intersegmental connectives. In the present experiments the regeneration of the right seventh thoracic limb (th. 7) in A. aquaticus, following degrees of such isolation of its segmental ganglion, has been compared with normal regeneration and again with regeneration following partial ablation of the segmental ganglion, without transection of the connectives. As an additional control, the rate of regeneration of the right sixth and eighth limbs was measured simultaneously. In this animal the exoskeleton is sufficiently transparent to permit operation through it.

METHODS

The general technique of operation and measurement has been described (Needham, 1945, 1946b). In the control series, of animals regenerating normally, the right limbs of thoracic segments 6, 7, 8 (subsequently designated th. 6, 7, 8) were removed at the autotomy-plane and allowed to regenerate under standard laboratory conditions. In a first experimental series the intersegmental nerve-connectives between th. 6 and 7 were severed between the edges of two fine steel needles, the ends of
which had been bent at right angles, tempered and sharpened. The tips were passed transversely to the nerve-cords from either side, one above, the other through the exoskeleton and below the cords. The lower needle was slightly curved at the tip to ensure retention of the cords during the lateral shearing action. On severance of the cords their cut ends sprang apart, and complete sensory and motor independence resulted between limbs anterior and posterior to the lesion. The right th. 6, 7, 8 were removed immediately prior to the operation. Mortality was high in this series and therefore a further series was subjected to incomplete transection, by merely crushing the connectives between the needle-blades. By this method varying degrees of functional isolation were obtained between the limbs on either side of the lesion, and persisted with little change during the period of regeneration studied. In these two series it was anticipated that the relative effects of transection on the regeneration of th. 6 and 7 would measure the relative importance for regeneration of any influences passing respectively forwards and backwards in the c.n.s. The effect on th. 8, relative to that on th. 7, was expected to show how far such influences are restricted to the immediate vicinity of the lesion. This was further tested by comparison with a fourth series in which the intersegmental cords were crushed between th. 7 and 8, as well as between th. 6 and 7. The main object of this last series, however, was the complete neural isolation of th. 7. Comparison with the preceding series also permitted an estimate of the general effect of increasing severity of operation (p. 155).

Partial ablation of the right segmental ganglion of th. 7, with the tip of a needle, is a simple operation. Under bright illumination the ganglion is easily visible, and it is held stationary between the ventral nerve cord and the peripheral nerve. The amount of damage to other tissues, and of haemorrhage, in this series was probably less than in the preceding experimental series. The right th. 6, 7, 8 were removed as before. In a further small group of individuals regeneration-rate was measured, for th. 6 and 7 only, in two successive acts of regeneration, following damage to the right 7th segmental ganglion.

The six series will be numbered and considered in the following order:
1. Normally regenerating (controls).
2. Intersegmental connectives crushed, th. 6 and 7.
3. Intersegmental connectives crushed, th. 6 and 7, th. 7 and 8.
4. Intersegmental connectives completely severed, th. 6 and 7.
5. Right segmental ganglion partially destroyed, th. 7.
6. Two successive regenerations following damage to right th. 7 ganglion.

The regenerating limbs were measured at eclosion and the mean eclosion-length (e.l.) computed for each of the three limbs, in each series. From this the specific daily mean increment in each (s.d.m.i.) was computed (Needham, 1949a) by dividing the mean e.l. by the product of the mean body-size and mean eclosion-time (i.e. the mean interval between operation and eclosion). This measure of specific regeneration-rate which involves relatively little labour was considered adequate for estimating general effects of the experimental treatments, and is justified by the precision with which it reveals general trends (Fig. 1).
Differential effects on the three limbs were estimated by a more precise method, the comparison of the observed values of e.l. 7 and e.l. 8 with those to be expected, in normal regeneration, from the observed values of e.l. 6. This comparison was made for each individual animal in each series, and the mean difference, expected/observed, for limbs 7, 8 was the property tested for significance. Variables common to the three limbs are eliminated by this method, which measures purely differential effects.

All measurements are quoted in the units mm. × 60. Abdomen-width was used as the measure of body-size.

RESULTS

The regressions of e.l. 6 and e.l. 8* on e.l. 7 in normal regeneration were:

\[
\text{e.l. 6} = (164.52 \pm 1.159) + (0.898 \pm 0.029) \text{ (e.l. 7 - 181.202)}, \quad (1)
\]

\[
\text{e.l. 8} = (190.04 \pm 0.504) + (1.015 \pm 0.012) \text{ (e.l. 7 - 181.202)}, \quad (2)
\]

* Initially it was considered that these regressions would be the most useful.
from which it may be calculated that:

\[ e.l. 7 = 1.113 \times (e.l. 6) - 1.567, \quad (3) \]

\[ e.l. 8 = 1.130 \times (e.l. 6) + 4.153. \quad (4) \]

It is clear that the independent term of equation 3 is not significantly different from zero, whereas that of equation 4 may be just significant, implying either that th. 8 begins its regeneration earlier than the other two or that its rate, relative to theirs,

Table 1. Summary of Results

(All units mm. x 60)

<table>
<thead>
<tr>
<th>Series Number (see p. 152)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6 First regeneration</th>
<th>6 Second regeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of records</td>
<td>41</td>
<td>36</td>
<td>17</td>
<td>13</td>
<td>30</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Mean abdomen-width</td>
<td>127.94</td>
<td>149.98</td>
<td>187.47</td>
<td>188.69</td>
<td>170.90</td>
<td>137.70</td>
<td>142.75</td>
</tr>
<tr>
<td>Mean eclosion-time (days)</td>
<td>10.90</td>
<td>13.50</td>
<td>12.91</td>
<td>16.40</td>
<td>13.95</td>
<td>13.50</td>
<td>14.88</td>
</tr>
<tr>
<td>Mean eclosion-length</td>
<td>164.52</td>
<td>170.56</td>
<td>145.57</td>
<td>135.86</td>
<td>201.17</td>
<td>160.88</td>
<td>166.70</td>
</tr>
<tr>
<td>(e.l.) of:</td>
<td>181.20</td>
<td>187.71</td>
<td>151.22</td>
<td>136.12</td>
<td>101.13</td>
<td>162.78</td>
<td>178.95</td>
</tr>
<tr>
<td>th. 6</td>
<td>190.04</td>
<td>188.31</td>
<td>149.25</td>
<td>133.15</td>
<td>219.94</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>th. 7</td>
<td>190.04</td>
<td>188.31</td>
<td>149.25</td>
<td>133.15</td>
<td>219.94</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>th. 8</td>
<td>190.04</td>
<td>188.31</td>
<td>149.25</td>
<td>133.15</td>
<td>219.94</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mean deficit of e.l. 7, e.l. 8,</td>
<td>—</td>
<td>12.08</td>
<td>8.89</td>
<td>13.35</td>
<td>8.35</td>
<td>14.38</td>
<td>9.83</td>
</tr>
<tr>
<td>below values expected from observed value of e.l. 6:</td>
<td>—</td>
<td>12.08</td>
<td>8.89</td>
<td>13.35</td>
<td>8.35</td>
<td>14.38</td>
<td>9.83</td>
</tr>
<tr>
<td>e.l. 7</td>
<td>0.1180</td>
<td>0.0847</td>
<td>0.0571</td>
<td>0.0437</td>
<td>0.0815</td>
<td>0.0867</td>
<td>0.0764</td>
</tr>
<tr>
<td>e.l. 8</td>
<td>0.1299</td>
<td>0.0932</td>
<td>0.0593</td>
<td>0.0438</td>
<td>0.0774</td>
<td>0.0877</td>
<td>0.0820</td>
</tr>
<tr>
<td>Specific daily mean increase (s.d.m.i.) in regeneration of:</td>
<td>0.1360</td>
<td>0.0936</td>
<td>0.0585</td>
<td>0.0429</td>
<td>0.0801</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>th. 6</td>
<td>—</td>
<td>0.0313</td>
<td>0.0609</td>
<td>0.0743</td>
<td>0.0365</td>
<td>0.0313</td>
<td>0.0416</td>
</tr>
<tr>
<td>th. 7</td>
<td>—</td>
<td>0.0207</td>
<td>0.0706</td>
<td>0.0561</td>
<td>0.0252</td>
<td>0.0422</td>
<td>0.0479</td>
</tr>
<tr>
<td>th. 8</td>
<td>—</td>
<td>0.0244</td>
<td>0.0775</td>
<td>0.0041</td>
<td>0.0469</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

is higher at the outset than later. The slope of the regression of e.l. 8 on e.l. 7 is not significantly different from 1.0, whereas that of e.l. 6 on e.l. 7 is significantly less than 1.0; after the initial irregularity, therefore, the rate of regeneration of th. 8 is strictly proportional to that of th. 7, whereas th. 6 regenerates more slowly, although it begins at the same time as th. 7. As a consequence the eclosion-lengths of the three limbs (Table 1) come to bear approximately the same proportions to their definitive lengths, which likewise show a smaller difference between 8 and 7 than between 7 and 6. The regressions of the definitive lengths of th. 6 and 8 on th. 7 are:

\[ th. 6 = (246.938 \pm 1.014) + (0.849 \pm 0.010) (\text{th. 7} - 290.144), \quad (5) \]

\[ th. 8 = (308.521 \pm 1.181) + (1.077 \pm 0.012) (\text{th. 7} - 290.144); \quad (6) \]

giving

\[ \text{th. 7} = 1.180 \times (\text{th. 6}) - 0.873, \quad (7) \]

\[ \text{th. 8} = 1.271 \times (\text{th. 6}) - 4.699. \quad (8) \]

That of th. 7 on th. 6 is not significantly different from that in normal regeneration,
and the difference in th. 8/th. 6 is to be expected from the fact that th. 8 is inhibited during early ontogenesis (Needham, 1937, p. 295). It is noteworthy that the regression of th. 8 on th. 7, in normal growth, nevertheless passes through the origin, so that the rapid growth of th. 8 in the post-inhibitory period (Needham, loc. cit.) very soon must bring its proportionate length to the definitive value, which is subsequently maintained throughout growth. The regression of th. 6 on th. 7 also passes through the origin, and a simple proportion is maintained throughout growth. Apart from the irregularity due to the peculiar ontogenesis of th. 8, therefore, it seems probable that the relative regeneration-rates of limbs are very similar to their relative growth-rates in ontogeny. However, while regeneration-rate, like normal growth-rate, is progressively less in the order th. 8 > 7 > 6, the gradient is less steep, implying a relative advantage to the more anterior limbs, in regeneration. This is probably an indication of the differential effect which more strongly characterizes general inhibitory conditions in regeneration (see below).

In all experimental series (p. 152) the specific mean regeneration-rate (s.d.m.i., Table 1) was below normal in all three limbs. This general retardation has not been tested statistically in all series, since it was not the main object of the experiments, but it was an invariable feature, and its magnitude was correlated with the magnitude of the operation. In series 3, 4 it was so great as to obviate any need for quantitative tests. The absolute magnitude of this retardation increased in the order 6 < 7 < 8 but the relative magnitude also increased in that order. Such a differential effect may be an exaggeration of that seen in normal regeneration as compared with normal growth (see above).

In series 2 (connectives crushed, 6–7 intersegment) the general inhibition was considerable. The relative inhibition of th. 7 was a little greater than that of th. 6, as measured by the mean (expected-observed) difference in e.l. (Table 1), and that of th. 8 was proportionately greater when allowance was made for two exceptionally inhibited limbs (which were included in the computation for Table 1). It seems reasonable to conclude, therefore, that transection of the connectives between two segmental ganglia did not specifically retard the regeneration of the limb on either side of the lesion (th. 6 and 7); there was merely a general inhibitory effect, acting differentially on the three limbs in the order of their normal regeneration-rates.

In series 3 (connectives crushed 6–7 and 7–8 intersegments) the general retardation of regeneration was nearly twice as great as in series 2 and was therefore proportional to the extent of the injury at operation. Further the gradient in relative inhibition along the series of limbs was correspondingly steeper, so that the absolute rate of regeneration of th. 8 was actually lower than that of th. 7 (Table 1). The values of e.l. 8 fell short of those to be expected from the observed values of e.l. 6 by a mean figure of 17·5 ± 5·82 units, which may be regarded as significant.

In series 4 (complete transection of connectives, 6–7 intersegment) the general retardation was even greater and the gradient in differential inhibition proportionately steeper, so that the absolute regeneration-rate of th. 8 was lower than that of both th. 7 and th. 6. E.l. 8 fell short of the value to be expected from the observed value of e.l. 6 by an average of 24·53 ± 7·48 units, which again is significant.
Although the amount of tissue destroyed in the operation for this series was less than in that of the previous series, haemorrhage was greater, and the interruption of the c.n.s. was complete and permanent.

Comparing the results of the three transection-series it seems that there is no good evidence of a specific effect on the regeneration of any of the three limbs but only of a general inhibitory effect on all, which therefore was probably systemically mediated. It was a function of the magnitude of operational injury and a differential function of the normal regeneration-rate of the limb.

The results of series 5, 6 (partial ablation of 7th right segmental ganglion) contrasted sharply with those of the preceding series, showing, in addition to the general inhibition, a specific inhibition of th. 7. The general inhibition, as measured by the reduction in mean specific regeneration-rate of th. 6 and 8, was not significantly greater than in series 2, and the relative inhibition of th. 8, also, was virtually the same as in that series. By contrast the deficit of e.l. 7 below expectation, virtually zero in series 2, was highly significant in series 5 (31.25 ± 4.16). The magnitude of the specific inhibition was greater, and its standard error less, than the greatest inhibition produced by complete transection. The effect was virtually restricted (Fig. 1) to th. 7, the specific regeneration-rate of which was absolutely less than that of th. 6 (Table 1).

For the eight individuals of series 6 the mean specific regeneration-rate of th. 6, in the first act of regeneration, was virtually the same as in the larger series (5). That of th. 7 was considerably reduced by the operation, though less than in the larger series, largely owing to variation in the degree of injury to the ganglion. In the second act of regeneration the mean specific regeneration-rate of th. 6 was lower than in the first act, no doubt due to the normal effect of age and of repeated regeneration (Needham, 1949), but the decline for th. 7 was relatively less, and an improvement in innervation is implied. This could be due entirely to regeneration of cut fibres and partially damaged cytons and does not necessarily imply replacement of destroyed cells. The recovery was such that the residual inhibition of the limb was virtually that characteristic of general inhibition (Fig. 1).

DISCUSSION

The main conclusions to be drawn from the results are that the nervous control of regeneration-rate in this crustacean emanates from the neurons of the local segmental ganglion. Section of the intersegmental connectives has virtually no specific effect on the regeneration of those limbs supplied from the ganglia immediately fore and aft of this lesion, so that the higher centres and other parts of the c.n.s. are probably not involved.

It is scarcely possible in this small crustacean to ascertain which functional component of the segmental nerve-supply is most active in the promotion of regeneration. This problem is incompletely solved for the vertebrates. Singer (1943) finds that the dorsal root-fibres are most important in the regeneration of amphibian limbs, as in neuro-trophic activity (Wyburn-Mason, 1950), though the motor supply also has some effect (Singer, 1945) as again in the neuro-trophic effect on normal
The central nervous system and regeneration in crustacea

The view of Wyburn-Mason (1950, p. 59) that the trophic effect in vertebrates is due to unmyelinated autonomic efferent fibres running in both dorsal and ventral roots, would simplify the picture and might assimilate the earlier conclusion of Schotte (1926) that the sympathetic system promotes regeneration of amphibian limbs. However, it cannot be reconciled at present with the evidence that the trophic fibres of the dorsal root are antidromic afferent fibres, with their cell-bodies in the dorsal root-ganglion (Fulton, 1943, p. 27).

A possible, if minor, role in regeneration must be envisaged for the cutaneous nerve-net of crustacea (Nusbaum & Schreiber, 1897; Holmgren, 1898). At present there is no direct evidence of this but it is interesting that epidermal components of the insect limb may regenerate in the absence of the segmental ganglion (Suster, 1933), which is essential only for the mesodermal structures.

The differential operation of the general inhibition observed in these experiments recalls the differential inhibition observed in the regeneration and ontogenesis of various animals by Child (1941) and others. There, also, inhibition was proportional in magnitude to the normal activity. In Asellus the most probable basis would seem to be a differential distribution of the response of local tissues to the level of some essential, distributed by the blood. If damage to the c.n.s. plays any part, it is through systemic mechanisms, for example through a disturbance of cardiovascular control. The results seem to rule out any direct control by the c.n.s. of this general component of the inhibition, and this is important since in other groups there is much evidence (Child, loc. cit.) that a graded organization of the nervous system may determine and control morphogenetic gradients. In the present instance the nervous system is not necessarily ruled out as the initial determinant, in ontogeny, of the morphogenetic gradient, but it is not continuously necessary for its subsequent expression.

When the values of s.d.m.i. for th. 7 and 8 are plotted (Fig. 1) against those for th. 6, in the six series, a straight line graph is obtained in each case. These lines do not pass through the origin but indicate that with increasing general inhibition, first th. 8 and then th. 7 would be completely suppressed. In other words the limbs require a progressively higher threshold of systemic factor, in the order $6 < 7 < 8$, for the initiation of regeneration. To increments above this threshold, however, the proportionate responses of the three limbs are in the same order as the thresholds, and the proportionate responses are constant for all increments above the threshold. It is not yet clear if this is true for differential inhibition in general. Child and his school, working mainly with soft-bodied animals, have been more restricted to qualitative and simple geometrical representation of the phenomenon. However, complete suppression of the higher levels of a gradient, under conditions which permit some growth of lower levels, is a familiar result (Child, loc. cit.) and implies some such differential threshold, though not necessarily the linear proportionality between the responses of different parts of the gradient to supraliminal conditions.

In passing it may be noted that the present gradient has its high point posteriorly (cf. Needham, 1943) in the body.
On the graph of s.d.m.1 7/s.d.m.1 6 the points for series 5 and series 6 (first act) fall below the line fitting all other points, confirming very strikingly the conclusion (p. 156) that the effect of partial ganglionectomy on th. 7 was the only specific effect produced in the experiments.

The extent of powers of regeneration in the c.n.s. of crustacea merits further investigation. After damage to the segmental ganglion there was some improvement of regenerative power (p. 156), and after crushing the intersegmental connectives there was a progressive, though often incomplete, restoration of sensory and motor co-ordination across the lesion. Both of these recoveries could have been due entirely to regeneration of cell-processes, and as yet there is no good histological evidence that cell-bodies are replaced, as they are in lower invertebrates and in the urodeles.

SUMMARY

1. The neural influence on the rate of regeneration of a limb in Asellus emanates from the segmental ganglion. It is independent of other centres of the c.n.s.
2. Operations on the animal cause a non-specific retardation of all limbs regenerating immediately afterwards. The immediate cause is probably haemorrhage, since the effect is both systemic and proportional to the severity of injury.
3. This general retardation acts differentially on a series of limbs, in proportion to their normal regeneration-rates. It has been possible to obtain a quantitive measure of this 'differential inhibition'.

I am indebted to Professor A. C. Hardy, F.R.S., for the facilities I have enjoyed while carrying out this work and to Dr A. C. Willis for references to the subcutaneous nervous system in Crustacea.

REFERENCES

The central nervous system and regeneration in crustacea


