PERIPHERAL NERVE AND REGENERATION IN CRUSTACEA. II

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In the previous paper of this title (Needham, 1945) denervation of a thoracic limb of the isopod *Asellus aquaticus* at the time of amputation was shown to result in a marked retardation of regeneration, as compared with the partner limb amputated, without denervation, at the same time. In the present work it was hoped to throw more light on this neurotropic effect by experiments involving denervation at different times relative to amputation. Schotté & Butler (1944) find that the peripheral nerve is necessary for the early stages but not for the later stages of regeneration of amphibian limbs. In one series of the present experiments denervation was performed 5 days after amputation, and in a second series after normal regeneration for one instar, that is, at the first appearance of an externally visible regenerate, an average of 10 days after amputation. Such a regenerate is already partly functional, so that the possibility must be envisaged that denervation might lead to some disuse-atrophy in addition to its possible effect on regenerative growth; in a third series, therefore, the normally growing limb was denervated (without amputation), in order to obtain some measure of the extent of this disuse-atrophy. The results of this third series may include an effect of denervation on normal (i.e. non-regenerative) growth also. In a fourth series the limb was denervated 5 days before amputation, the object being to ascertain whether tissue denervated some time previously to amputation and undergoing the consequent atrophic changes is capable of developing a regeneration blastema at the surface of amputation, and of regenerating at the normal rate as soon as the regenerating nerve reaches that surface (cf. Needham, 1945, p. 146).

Table 1. Asellus aquaticus. *Inhibitory effect of denervation, at various stages relative to amputation, on regeneration of a thoracic limb; the partner limb, as control, regenerating under normal conditions, for one instar*

Series no.	Nature of the experiment
1	Control. Wound, not involving peripheral nerve, applied to limb base.
2	Denervation simultaneous with amputation.
3	Denervation 5 days *after* amputation.
4	Denervation 10 days (one instar) after amputation.
5	Denervation of normally growing limb, without amputation.
6	Denervation 5 days *before* amputation.

Series no.	No. of individuals	Mean abdomen width	Mean control limb length	Mean experimental limb length	Mean difference between control and experimental limb length	Probable error of mean difference	Figures appropriate to common mean abdomen width of 100 units		
							Control limb length	Experimental limb length	Difference between control and experimental limb length
1	18	136	155·7	145·7	9·95	3·93	114·5	107·1	7·31
2	18	140	170·0	112·7	57·26	6·36	123·0	80·5	40·90
3	18	124	157·8	112·6	45·15	3·66	127·1	90·7	36·41
4	23	96	194·6	170·8	23·81	2·33	201·9	177·2	24·70
5	25	116	265·9	237·9	28·33	3·29	229·0	204·9	24·40
6	18	119	132·6	113·0	20·21	3·25	111·4	95·0	16·98

All dimensions in mm. × 56.

The four series will be referred to as series 3, 4, 5 and 6 and the two of the previous paper as series 1 and 2 (Table 1).

METHOD

All essentials of procedure were as in the previous paper, and the same tests were applied for the significance of the mean difference in length between experimental limb and control limb of the other side of the body. Data for about twenty individuals were obtained in each series. The limbs concerned were measured after the first ecdysis following the final operation, that is, following denervation in series 3, 4 and 5, and amputation in series 6.

RESULTS

The relevant data, together with those for series 1 and 2, are summarized in Table 1. The mean body size (measured by abdomen width) varied somewhat in the different series, and it is useful, for purposes of comparison, to convert all measurements to those appropriate to a common abdomen width of 100 units, assuming an approximately linear relation between abdomen width and limb length, at corresponding stages of regeneration or of normal growth.

Denervation is seen to have a clearly and uniformly significant retarding effect on regeneration at all stages. The magnitude of the effect, however, decreases progressively with increasing interval between amputation and denervation (series 2, 3 and 4), in spite of the fact that complete reinnervation of the growing limb must occupy an increasingly long time. The decrease is probably logarithmic rather than linear, so that the effect on a one-instar regenerate (series 4) is already no greater than on a normally growing limb (series 5). The decrease probably does no more than parallel the decrease in the rate of normal regeneration itself.

It is not possible to estimate the effect of denervation on normal, non-regenerative growth, though some such effect seems *a priori* probable, as in the Amphibia (Hamburger, 1928). Much of the effect of denervation in both series 4 and 5 was undoubtedly due to disuse-atrophy. There was frequently an evident shrinkage of the denervated limb so that it became significantly shorter than the control limb, even before the next ecdysis, that is, before there could be any growth of the control limb. The available data for pre-ecdysal limb lengths indicate a mean figure of about 14 units for the control-experimental difference in limb length, in both series, that is, between one-half and two-thirds of the total, post-ecdysal difference. The increase in the difference at ecdysis is also approximately the same in both series, and certainly not greater in 4 than in 5. By contrast, the increase in limb length at ecdysis was at least twice as great in 4 as in 5 (57 : 27 units for the control and 47 : 16 units for the experimental limb); if, as suggested above, retardation of growth is roughly proportional to the growth rate itself, then it must form a small fraction of the total observed control-experimental difference in series 4 and 5.

In series 6 there was a clearly significant retardation of regeneration, but of much smaller magnitude than in series 2–5. Tissue denervated 5 days previously to amputation appears to be capable of developing a regeneration blastema, which probably commences normal regeneration as soon as the nerve, already in process of regeneration, reaches the surface of amputation. The data are in accordance [illegible]h this assumption. The effect in this series was gr[illegible]er and more significant than in series 1: that is to say, destruction of other tissue, at the time of amputation, affects regeneration less than does the absence of nerve for the short period of 2 or 3 days.

The visible results of denervation of a limb are of incidental interest. The limb is not usually cast off: autotomy is a reflex act and is abolished by denervation. Otherwise series 4, 5 and 6 would be impracticable. The limb is paralysed, usually with the proximal segments in extension. The muscle proteins become visibly opaque, and there is a varying degree of atrophy accompanied by shrinkage and by crumpling of the exoskeleton. Rarely, the most distal segments degenerate completely and subsequently regenerate; when this occurred in series 4 and 5 the figures for these segments, for both control and experimental limb, were omitted from the estimate of total limb length. Denervated limbs are less easily withdrawn from the exuvia at ecdysis than are normal limbs. It is probable that the disappearance of the opacity and shrinkage of the tissues is synchronous with reinnervation and the recovery of motor function.

It seems probable that in early stages of regeneration, also, there is no marked degeneration following denervation, and that regeneration is resumed in the regeneration bud as soon as it is reinnervated. Otherwise the control-experimental difference in series 3 could scarcely be less than in series 2 (for in series 3 the control limb regenerated for 5 days before denervation), or the experimental limb length relatively so much greater (90·7 : 80·5 units).

DISCUSSION

The main conclusions to be drawn from the experiments are that the extent of inhibition of regeneration by denervation depends on (1) the time required for the regenerating nerve to reach the regenerating tissue, and (2) the normal rate of regeneration at the time of denervation. Probably little degeneration follows denervation, and regeneration is resumed where it was interrupted.

It cannot be decided from the present data if denervation of a limb in the later stages of regeneration or during normal growth has any retarding effect on growth as distinct from the effect of disuse-atrophy. Evidence for this might be sought in one of the larger species of Crustacea, by section of other components of the peripheral nerve leaving the motor component intact, if, as in Amphibia, it is the sensory nerve (Singer, 1943), or the autonomic supply to the limb (Schotté, 1926) which is responsible for the tropic effect on growth. Whatever the nature of the effect of denervation in these stages, however, there is no doubt that the peripheral nerve is essential for the normal condition and behaviour of the limb, and there is probably no st[illegible] during which the peripheral nerve is not of vi[illegible]mportance.

The close similarity between the mean length of the experimental limb in series 2, 3 and 6 is striking but probably only a fortuitous consequence of the differences in mean body size in the three series. The figures appropriate to a common mean abdomen width differ considerably (Table 1). On the other hand, the mean length of the control limb for the four series with first instar regenerates (1, 2, 3 and 6) shows much less variation when this difference in mean abdomen width is allowed for. Normal regeneration is thus fairly constant in amount in an instar (cf. Przibram, 1909, p. 114). The mean control limb length for series 2 and 3 is, nevertheless, somewhat greater than for series 1 and 6, in which the experimental limb was less inhibited, thus supporting the unconfirmed suggestion of the previous paper (1945, p. 145) that the regeneration of the control limb is accelerated in proportion to the degree of inhibition of the experimental limb. Statistical tests of this have not been applied to the present data.

The results of series 6 suggest that in Crustacea the co-operation between a transient local 'wound factor' and an 'emanent' factor, probably due to the nerve supply, is not so important for regeneration as in Amphibia (Needham, 1941, p. 81). In this series there could scarcely be any normal nerve tissue at the surface of amputation within 2 days of the time of amputation. Reinnervation would seem to initiate regeneration whatever the conditions of amputation and denervation. Experiments have, in general, failed to reveal any significant stimulus to regeneration by a wound factor in this crustacean.

SUMMARY

1. The peripheral nerve is probably essential for all stages of regeneration of thoracic limbs in the crustacean *Asellus aquaticus*. The inhibitory effect of denervation decreases, however, with the rate of regeneration itself.

2. When a limb reaches the functional stage denervation leads to some degree of disuse-atrophy, which masks the possible neurotropic effect on late stages of regeneration and on normal growth.

3. A limb denervated previously to amputation is capable of forming a regeneration blastema at the surface of amputation, and normal regeneration probably begins as soon as the regenerating nerve reaches that surface.

4. There is probably no general degeneration following denervation at any stage of regeneration or of normal growth, and growth is probably always resumed with reinnervation.

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