

Nerve Pattern in Regenerated Urodele Limbs.

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The factors determining the course, grouping, distribution, and size of peripheral nerves in development are still very much in the dark. One way of investigating these factors is direct experimentation on the embryo (Harrison,¹ Braus²). Another way is to study the development of nerves in regenerating limbs of adult urodele Amphibians. Limb regeneration in these animals is known to be a sort of second development (P. Weiss), differing from the first development in the mass of tissue involved, the time relations, the conditions of muscle differentiation and of nerve supply. A comparison of nerve patterns established during regeneration with those formed during embryogenesis can be expected to yield some clue as to which factors may, and which may not be significant. If merely the nerves supplying a limb are severed, nerve regeneration into the limb takes place from the proximal stumps, the outgrowing fibers mainly following the old degenerated peripheral pathways which have persisted as such. If the whole limb is amputated, differentiation of a new limb occurs out of an undifferentiated blastema in which no old pathways are present to guide the outgrowing nerve fibers. The question arises as to what sort of pattern will be assumed by the nerves in the regenerating limb.

In metamorphosed individuals of the newt, *Eurycea bislineata* (Green), of varying age and size, the left fore limb and the right hind limb were amputated at their bases. The limb nerves were cut at about the level of the girdle plexus. Eight animals were kept for from 176 to 202 days, and were then preserved for dissection. Regeneration had taken place in all of the hind limbs and in 5 of the fore limbs. The regenerates varied from one-half to three-quarters the length of controls on the opposite side. They were slenderer in proportion but otherwise well formed. Some of the animals died before observations were made on the function of the limbs, but in all those observed there was normal coordination of movements. In one case the extension of the elbow was somewhat impaired.

All 4 limbs were dissected, the distribution of nerves in the regenerated limb being compared with that of its mate on the opposite

¹ Harrison, R. G., *J. Exp. Zool.*, 1907, **4**, 239.

² Braus, H., *Anatom. Anzeiger*, 1905, **26**, 433.

side. It was assumed that there would normally be less variation between the members of a pair of limbs than between the homologous limbs of different individuals. Attention was directed chiefly toward the plexuses, the nerves of the upper arm, and the sciatic and its ramifications as far as the knee. The results were as follows:

The main muscle masses of the arm and thigh are essentially normally arranged in the regenerating limbs, though minor aberrations of subdivision or attachment are found, and in some cases there are notable deficiencies in relative volume. In general the nerves keep a normal spatial relation to these muscle masses and to the skeleton. For instance, the superior (radial) and inferior (ulnar) long brachial nerves always enter the arm next to each other between the heads of the anconaeus, and then reach the flexor surface of the forearm by their usual opposite spirals around the humerus, the radial constantly lying between the 2 more lateral heads of the anconaeus, and the ulnar running deep to the anconaeus coracoideus and the coraco-brachialis longus. In the thigh the sciatic and most of its branches always run in the space between the femur and the flexor and extensor masses, covered superficially by the m.ilio-fibularis. The points where the minor branches leave the main trunks are variable, but scarcely more so than in the normal limbs of different individuals, and each muscle group seems always to be innervated by the usual nerve. There is some plexus formation between, and subdivision of the nerves along their course in the free limb, especially in the thigh. Such plexus formation is also present in the control limbs, but there is significantly more of it in the regenerates. In direct comparison of the two sides, there is a range of cases from very close symmetry of the two limbs, to very irregular plexus formation in the regenerated limb. Even in the latter cases, however, the main course of the nerve trunks is the same on both sides.

One striking point is the close correlation between the volume of the muscles in the regenerated limb and the diameter of the nerves innervating them. The nerves proximal to the level of amputation, however, show no reduction. The extreme of this condition is found in those cases where the fore limb had entirely failed to regenerate. Here the trunks of the 3rd and 4th spinal nerves of the two sides correspond exactly in diameter and position, but on the operated side stop short at the level of the plexus, giving off only a few branches to the girdle muscles.

It would seem that there are factors present in the regenerating limb which lead to the growth and grouping of entering nerve fibers

along essentially typical major pathways, and to their distribution to the muscles according to typical spatial relations.

7329

Reaction of Anterior Pituitaries of Immature and Mature Female Rats to Injection of Pregnancy Urine Extracts.

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It has been previously reported¹ that injections of an oestrin-free placenta extract, containing the A. P. L. factor, into immature female rats induced a marked increase in the weight of the ovaries and a moderate increase in the weight of the pituitaries. Histologically, the pituitaries of the injected rats showed a slight to moderate decrease in the level of the eosinophiles; a considerable number showed evidence of granular loss. The most evident changes were found in the basophiles, which showed marked evidence of granular depletion.

Injection of pregnancy urine extracts into immature female rats gives similar results. Thirty rats, 21 to 25 days old, were given daily injections of pregnancy urine extract† (25 to 75 R. U. daily for 6 to 15 days). Twenty-eight littermate sisters served as controls. The ovaries of the injected rats weighed from 50 to 80 mg., while those of the controls weighed from 15 to 25 mg. The pituitaries of the injected animals were usually, but not invariably, increased in weight. Complete serial sections of all pituitaries were cut, at 2 micra, in the horizontal plane. One section from each ribbon of 15 was mounted and stained. Cell counts were made on 5 sections, equidistant apart from each set of serial sections (110,039 cells were counted on the 208 sections studied). The anterior lobes of the injected animals were definitely altered from those of the

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¹ Wolfe, J. M., Phelps, Doris, and Cleveland, Rucker, *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 1092.

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