

retarded from 1 to 3 weeks. In adults the degeneration of the optic nerve accompanies that of the retina and is accomplished during the first month. During the second month the growth of the new optic nerve from the bulb accompanies resolution of the retina. The nerve frequently approaches normal in size and passes to the optic chiasma usually along a tortuous course. In only 2 cases did the optic nerve fail to leave the bulb by the end of the second month. In these cases the nerve travelled a short distance between the retina and the choroid. In larvae a complete degeneration of the optic nerve took place during the first week although retinal change was slight compared to adult grafts. By the end of the third week it was completely regenerated in all but 4 cases (3 were rotated about  $180^\circ$ , one was normally oriented). The optic nerve was usually tortuous and less than normal in size.

Regeneration in a young adult transplanted eye was so rapid that return of vision was proven as early as the thirty-eighth day. Usually this was not determined in adults until after the second month. In larvae the earliest tests for vision which gave positive results were made on the forty-eighth day (a transplant). This host was still in a larval stage. Vision was proven in all types of grafts and in many specimens by methods already published in detail.<sup>1</sup> In them the microscopic studies showed complete optic nerves. Ocular movements were present and when the hosts were in the adult stage corneal and pupillary reflexes were demonstrated.

## 5786

Experiments on the Development of the Hind Limb of *Amblystoma*.

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Experiments with the hind limb of *Amblystoma punctatum* were undertaken to compare its developmental behavior with that of the fore limb, as described by Harrison.<sup>1</sup>

In normal development the hind limb does not appear as a definite bud until approximately  $2\frac{1}{2}$  weeks later than the fore limb, so that it lies latent in the host for a much longer period after transplantation than does the fore limb bud. Furthermore, the 2 halves of the

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<sup>1</sup> *J. Exp. Zool.*, 1921, **32**, 4.

pelvic girdle are intimately fused in the median line, while the corresponding halves of the pectoral girdle are separate.

A preliminary experiment was done to determine the location of the hind limb rudiment at the stages used for operation. During the tail bud stage it was found that it lies ventral to myotomes 16, 17 and 18. The posterior rounded part of the body wall is its approximate caudal limit.

The following experiments were performed to test the equipotentiality of the hind limb. The limb rudiment was removed and the wound allowed to heal without covering. In this case a complete limb and girdle developed from the surrounding tissue. The same result followed when only a part of the rudiment was removed and the wound left uncovered. When the limb rudiment was extirpated and the area blocked with indifferent ectoderm, no limb developed, and the pelvic girdle was absent. When all, or a sufficiently large part of the hind limb rudiment was transplanted to an abnormal location, such as the flank, a complete limb and girdle developed at the site of implantation.

Hence, the hind limb of *Amblystoma* constitutes a harmonic equipotential system, just as Harrison found to be the case with the fore limb.<sup>2</sup> The pelvic girdle, however, differs from the pectoral girdle in that it also constitutes a harmonic equipotential system, while the girdle of the fore limb has been shown by Detwiler to be a mosaic system.<sup>3</sup>

To ascertain the symmetry relations of the hind limb of *Amblystoma*, the 8 experiments made by Harrison upon the fore limb were made.<sup>4</sup> That is, limb rudiments were transplanted both in normal and in abnormal locations, thus constituting orthotopic and heterotopic grafts respectively. In each, grafts were made both on the same side and on the opposite side of body to origin; the antero-posterior axis of the graft was oriented both normally and with 180° rotation. The medio-lateral axis was not varied.

The results obtained by the heterotopic transplantations show that when the antero-posterior axis of the limb disc is reversed in grafting, the resulting limb is always disharmonic, *i. e.*, it is of opposite laterality to the side on which it is placed; while it always develops harmonically (with the same laterality as the side on which it is placed) when the antero-posterior axis is directed normally.

The results obtained with the orthotopic transplantations show

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<sup>2</sup> *J. Exp. Zool.*, 1918, **25**, 424.

<sup>3</sup> *J. Exp. Zool.*, 1918, **25**, 523.

<sup>4</sup> *J. Exp. Zool.*, 1921, **32**, 7.

that the limb rudiment always develops into a harmonic limb whether its antero-posterior axis is reversed or not in grafting.

Reduplications took place in a minor number of cases in both orthotopic and heterotopic experiments. In the heterotopic operations they occurred only after 2 of the 4 orientations, namely, after transplantation to the same side of the body as that of origin, with the antero-posterior axis reversed, and after transplantation to the side of the body opposite its origin with the antero-posterior axis not reversed. That is, reduplications took place only in cases where the dorso-ventral axis had been inverted. In the orthotopic series reduplications occurred in most cases only after reversal of the antero-posterior axis of the graft. In both heterotopic and orthotopic grafts the primary member of the reduplicated limbs had the same laterality as a single limb would have had in the same location. With a few heterotopic cases, however, in which the graft was very large, 2 limbs were formed in serial alignment.

It is seen that the heterotopic grafts follow the same rules as apply to the fore limb according to Harrison's experiments.<sup>5</sup> The orthotopic grafts, however, follow different rules in that the reversal of the antero-posterior axis of the rudiment does not prevent the harmonic development of the limb, just as occurs when this axis is not reversed. The result with orthotopic grafts shows that the regulatory influence of the normal position of implantation predominates over the axial polarization which exists in the limb rudiment at the time of transplantation. It is possible that this regulatory influence may be due to the length of time during which the hind limb rudiment lies latent in the host. As an alternative explanation it is also possible that the intimate connection between the two halves of the hind limb girdle may exert an influence in producing harmonic limbs.

## 5787

### Innervation and Function of Grafted Hind Limbs in *Amblystoma Punctatum*.

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Experiments were carried out to study the effects of a heterotopic limb upon the growth of the spinal nerves in the vicinity of the lum-

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<sup>5</sup> Roux. *Arch. f. Entw. Mech.*, 1925, **106**, 497.