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.[5] 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.

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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-

[5] *Roux. Arch. f. Entw. Mech.*, 1925, **106**, 497.

bosacral plexus and to analyze the muscular movements of the limbs.

The hind-limb rudiment of *Amblystoma punctatum* was excised at stages varying from 39 to 45 and implanted from one to 5 segments anterior to its normal position. Two types of operations were performed, an autoplastic series in which the right hind-limb rudiment was excised and reimplanted to the flank, and a homoplastic series in which either a right or a left hind-limb bud was transplanted to corresponding positions anterior to the normal limb.

The limb rudiments transplanted at stages 42 to 45 were given various orientations. It was found that both the antero-posterior axis and the dorsoventral axis are irreversibly fixed at stage 42.

When the wound made by the removal of the limb bud was covered with indifferent ectoderm from the flank region, regeneration occurred in only 9 out of 51 cases. When the wound was not cleaned or covered regeneration took place in 59 out of 96 cases.

The hind-limb is normally innervated by the ventral rami of the 15th, 16th, and 17th spinal nerves. Coordinate and homologous movements were observed in many grafted limbs which had been implanted from one to 3 segments anterior to their normal position. The type of function was similar to that reported by Weiss,[1] Detwiler,[2] and Detwiler and Carpenter.[3] The homologous muscle groups in the heterotopic limb, irrespective of limb orientation, flexed and extended synchronously with those of the ipselateral normal limb, and alternate with those of the contralateral limb. The grafted limbs in such cases were found to be innervated by one or more branches from the normal lumbosacral plexus.

No definite coordinated movements could be observed in grafted limbs which were supplied entirely by nerves anterior to the 15th. Several grafts innervated by the 15th spinal nerve, together with nerves anterior to it, failed to function synchronously. In these cases the limbs were located in the dorsal portion of the flank in the epaxial musculature and received only the enlarged dorsal ramus of the 15th spinal nerve. This ramus normally has no connection with limb musculature. Another type of function observed was a sharp flexion of the ankle, foot, and digits of the graft, synchronous with the movements of the jaw muscles during feeding. The normal limbs and the animal as a whole were stationary during these movements. These heterotopic limbs were found generally to be

[1] Weiss, Paul, *Arch. fa. Mikros. Anat. u. Entw.-Mech.*, 1924, **102**, 635.

[2] Detwiler, S. R., *J. Comp. Neur.*, 1925, **38**, 461.

[3] Detwiler, S. R., and Carpenter, R. L., *J. Comp. Neur.*, 1929, **47**, 427.

innervated by nerves anterior to the lumbosacral plexus, but in 3 cases they received, in addition, a dorsal ramus from the 15th spinal nerve.

Whether functional or not, the grafted limbs received extensive innervations from the spinal nerves. In order to reach the limbs the nerves traversed abnormal pathways through the body musculature. When limbs were grafted high on the side of the body they became innervated by enlarged dorsal rami which grew laterally or dorsolaterally to reach them. Ventrally situated grafts were supplied by ventral rami which followed their normal paths until they reached the level of the limb and then turned abruptly laterally to enter them. Since the spinal nerves were already laid down at the time the operations were performed, it is probable that the ends of the nerves were cut, and that they regenerated into the graft as it differentiated.

The 2 chief nerves entering the thigh of a normal hind-limb are the cruralis and ischiadicus. By means of graphic reconstructions of the plexuses to grafted limbs both these nerves could be identified in their usual positions. This was true even for limbs whose antero-posterior axes had been reversed. At the base of the grafts, as in normal limbs, the ischiadicus communicated with the cruralis.

Cell counts of the spinal ganglia showed that there is extensive sensory hypoplasia following the removal of the hind-limb, and extensive hyperplasia in the ganglia supplying a supernumerary limb.

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Experiments on the Determination and Development of the Pronephros of Amblystoma Punctatum.

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This investigation deals with two problems; first the time at which determination of the pronephros occurs and second, the question of the transplantibility of this organ and its relation to the various problems of development and function.

Prospective pronephric mesoderm from embryos of early stages was transplanted to the flank region of animals in the early tail bud stages. When this method is used, the capacity to form pronephric tubules is determined at the stage of widely open neural folds. (stage 15). The tubular pattern of 2 nephrostomal canals and a