SOME UNEXPECTED RESULTS OF THE HETEROPLASTIC
TRANSPLANTATION OF LIMBS

BY ROSS G. HARRISON

Osborn Zoological Laboratory, Yale University

Read before the Academy, Nov. 12, 1923

The embryos of distinct but closely related species of amphibians often
differ from one another in certain qualities, such as pigmentation, size,
time of appearance of organs, etc., which render them useful in the analysis
of the factors of growth and development by means of grafting experiments.
Two of our native salamanders, Ambystoma punctatum Linn. and A.
tigrinum Green, afford advantages of this kind in connection with the
development of the limbs.

In the spotted salamander, A. punctatum, the fore legs begin to show
externally at a comparatively early stage and but little later than the
external gills. By the time the yolk is resorbed, they are fully developed
both in form and in function. On the other hand, in the tiger salamander
(A. tigrinum) the fore limbs at this period are small nodules still, scarcely
visible superficially. It is not until some weeks later that they reach the
degree of development attained by the fore limbs of the punctatum larva
at the close of the embryonic period. With respect to the development
of most of the other organs the embryos of these two species show much
greater concurrence, but there are several characteristic differences that
require brief notice here.

The tigrinum egg is smaller and contains much less yolk, so that this
store of food is exhausted sooner than in the punctatum egg. The tigrinum
larva consequently begins to feed when quite small, although, except for
the difference in the fore limbs, the embryos of the two species are ap-
proximately at the same stage of development at this period. During
the first part of larval life the rate of growth is not very different in the
two species, but after five or six weeks, when they have reached a length
of about 4 cm., the tigrinum larvae begin to forge ahead rapidly and
ultimately attain a much larger size than the punctatum. Sexually mature
larvae (axolotls) that have come under my notice measure from 25 to 32.5
cm. in length. The two longest adult specimens mentioned by Cope\(^1\)
measured 25 and 27.5 cm., respectively. In the strain used in the present
investigation metamorphosis has not been observed to take place in speci-
mens less than 13 cm. in length, and it often, apparently, does not occur
at all, while in A. punctatum it takes place when the larvae are about
5 cm. long. This size may be reached under favorable circumstances in
the latter species in about ten or twelve weeks, at which time tigrinum
larvae of the same age, kept under similar conditions, are about 6 cm. long,
without, however, showing any sign of metamorphosis. Adult punctatum individuals measure about 16 cm. in length.

For the purpose of investigating the factors concerned in the development and growth of the fore limbs, limb buds were interchanged between embryos of the two species. The grafting was done in the usual way at an early period of development (stages 29–31), before the limb buds could be distinguished as such, and before peripheral nerves or blood vessels were present.

The initial effect of the operation was as expected, especially in the one series of experiments. The punctatum limb implanted on a tigrinum embryo grows rapidly, and is soon far beyond the normal tigrinum limb in development. It does not, however, keep pace with the normal undisturbed punctatum limb of the donor, and it does not during this period acquire its normal powers of movement.

In the reciprocal experiment the tigrinum limb on the punctatum embryo remains very small for a short time, while the normal limb on the opposite side grows out rapidly. In this case, however, the effect of the transplantation soon becomes evident in a positive manner, for the grafted limb begins to grow far more rapidly than it would have, had it been left in place. When the punctatum larva begins to feed (stage 46) the normal limb has three digits with the beginning of the fourth, and it functions perfectly. While the grafted tigrinum limb is less advanced than this, it has, nevertheless, lengthened far beyond the normal tigrinum limb, which is still a nodule, and it shows the notch marking the first two digits, a condition reached by the normal punctatum limb in stage 42. Such grafted limbs even show slight movements at the shoulder, although the normal punctatum limb does not reach this functional condition until stage 44, when the hand is much better developed.

The subsequent behavior of the grafted limbs in both series of experiments is notable for the increasing influence of the host on their growth.

The transplanted punctatum leg lags more and more behind the normal fore leg of the donor. For a time it appears atrophic, and its only movement is a slight twitching at the shoulder. This lack of motility is in itself interesting, for sections of individuals preserved during this period show that the nerves of the limb are well-developed. The absence of normal function is, therefore, probably due to conditions within the central nervous system, corresponding to the rudimentary state of the normal tigrinum limb. In several cases the transplanted leg seemed for a time to be undergoing degeneration. At the same time the tigrinum host grew rapidly, and its own fore leg, in the course of about six or seven weeks from the date of operation, overtook and soon exceeded in size the grafted one (figs. 1 and 2), which remained very small, though it later acquired perfect form and function, all traces of degeneration disappearing. Three such
specimens have been kept through this stage. One (fig. 1) was preserved and sectioned, showing a diminutive shoulder girdle as well as limb. Two are still alive. One of the latter metamorphosed when 13 cm. long, at which time the grafted limb was resorbed to a short stump. The other, now 14.7 cm. long is still a larva. The transplanted limb is very small as compared with the normal limb of the host, but it is now larger than the limb of the punctatum donor, which latter is a much smaller animal, 6.6 cm. long. The function of the grafted limb is perfect. Its shape is that of the donor species (punctatum), but the skin markings and coloration are those of A. tigrinum, showing that extensive pigment migration must have occurred.

Fig. 1.—Larva of Amblystoma tigrinum with a punctatum fore limb (gr) on the right side; n, normal limb. Exp. NE.10; specimen preserved 76 days after operation.

Fig. 2.—Larva of A. punctatum, the donor of the limb shown in fig. 1. Regeneration of the lost limb has not occurred. Specimen preserved 76 days after operation.

Fig. 3.—Larva of A. punctatum, showing a gigantic, but otherwise normal, tigrinum limb (gr), grown to this size after having been grafted in the limb bud stage; n, normal limb. Exp. NE.13, specimen preserved 72 days after operation.

Fig. 4.—Normal control larva of A. tigrinum of the same age.

All figures magnified 1.4 diameters.

The tigrinum limb grafted to the punctatum larva grows very rapidly after it once begins, so that shortly after the yolk is resorbed it overtakes the normal limb in degree of development, at the same time having become
more massive. It is at first white and almost without pigment cells, like
the developing tigrinum leg, whereas the punctatum leg is well-pigmented.
The grafted limb soon outstrips the normal one on the opposite side, grow-
ing rapidly until the time of metamorphosis, when in the most extreme case
its linear dimensions are fully double those of the normal (figs. 3 and 4).
These animals are very grotesque in appearance, especially when viewed
from the front. The gigantic limbs, however, like the diminutive ones in
the reciprocal experiment, are well proportioned and function perfectly.
The shoulder girdle and pectoral muscles are likewise enormously developed
for the size of the animal. None of the five specimens of this series sur-
vived metamorphosis.

Sections show that the constituent parts of the limbs in both kinds of
transplantations are proportionately developed. Skeleton, muscles, and
nerves are small in the miniature transplanted punctatum limb and large
in the giant tigrinum limb on the punctatum larva. However, neither the
central nervous system nor the spinal ganglia show hypoplasia in the one
case or hyperplasia in the other. This may seem odd in connection with
Detwiler's discovery that dimunition or increase in the peripheral area
innervated by a given nerve results, respectively, in the hypoplasia or
hyperplasia of the ganglion of that nerve. It should be recalled, however,
that the limb does not immediately show its ultimate growth powers after
either type of transplantation. On the contrary, the early tendencies
are opposite to the later ones in this respect, both kinds of limb buds at
first manifesting their own inborn characters, so that the punctatum limb
on the tigrinum embryo is for some time actually larger than the normal
tigrinum limb, and the grafted tigrinum limb requires about a month to
outtake the normal punctatum limb in size. In this early formative
period, therefore, the tendency would be toward hyperplasia of the spinal
ganglia innervating the limb that ultimately remains smaller, and toward
hypoplasia of those innervating the limb that ultimately becomes much
larger. Apparently the limb does not reach its ultimate disproportionate
relation sufficiently early to produce an effect on the sensory nerve
centers.

In order to account for the above results it is necessary to assume that
two distinct factors, aside from the ordinary nutritional ones, are concerned
in the growth of the limbs. One of these, immanent in the cells of the
transplanted limb and derived from the general constitutional qualities
of the species, may be termed the growth potential (G). This is much
greater in A. tigrinum (t), as shown by the much larger ultimate size of
individuals of this species in comparison with A. punctatum (p). The
other factor may be designated as a regulator (R), since it acts by control-
ling the rate of growth. It exists not in the individual cells of the trans-
planted tissue, but in the circulating medium, and is probably a hormone
derived either from the hypophysis or the thyroid gland. Since A. punctatum metamorphoses very early as compared with A. tigrinum, there is justification for the assumption that this regulating factor is more effective in the former species. The relative effect of the four combinations (two normal and two experimental) of these factors, acting until the time of metamorphosis of the punctatum larvae (ten or twelve weeks after grafting), may be expressed by the inequality

\[ G_tR_p > G_tR_t > G_pR_p > G_pR_t \]

What the ultimate relation of these effects would be if the salamanders were kept until they all reached full size remains unknown, because no individuals of the first combination have survived metamorphosis. In other words, we do not know whether the grafted tigrinum leg would ultimately exceed its normal size, or whether it would simply reach this condition in less time and then remain stationary. We do know, however, that the order of the last two terms will be reversed later, for the actual size of the grafted punctatum limb in the oldest specimen under observation is larger than that of the normal control limb of that species. Here, however, there is a likelihood that nutritional factors are concerned, for the tigrinum individual with the grafted leg is very much larger than the punctatum donor, and presumably a larger supply of nutrient material reaches the grafted leg than the leg of the latter.

The foregoing experiments show with great clearness that various influences are involved in growth. However, it is desirable here to emphasize the importance of the constitutional factors, because they are more likely to be overlooked. The grafted limb is subject to exactly the same nutritional and hormonal influences as the normal limb of the same individual, and yet, by virtue of its specific constitution, its growth is enormously greater in the one and correspondingly less in the reciprocal combination than the growth of the normal control limb on the opposite side of the body. Again when a comparison is made between the grafted limb and the control left on the body of the donor, then the importance, in growth, of the regulatory hormone stands out, for the relative acceleration or retardation, as the case may be, is very marked. This too is a constitutional factor, but in a different sense, for the production of the regulator is probably local and its distribution is effected by the circulating medium, whereas the growth potential is a quality of the tissue cells in general.

Experiments to test the hypothesis regarding the source of the regulating factor have been devised and will be carried out as soon as material is available.

Summary.—The fore limb of Amblystoma punctatum develops during the embryonic period, but that of A. tigrinum relatively much later. The
latter, however, preserving its proportion to the body as a whole, attains ultimately a much greater absolute size.

The forelimb bud of *A. punctatum*, engrafted in normal position on a *tigrinum* embryo, develops at first much more rapidly than the natural limb on the opposite side of the host, but is retarded in comparison with the limb of the donor left in place. In the reciprocal transplantation the *tigrinum* limb bud remains for a time small, but its growth is soon greatly accelerated and the limb later becomes much larger than that of either species in its normal surroundings.

These results may be explained by the assumption that two factors are concerned in the growth process: the growth potential, a property of the cells of the graft; and a regulator, probably an internal secretion of the host, carried to the limb through the circulation.


**SETS OF COMPLETELY INDEPENDENT POSTULATES FOR CYCLIC ORDER**

**BY EDWARD V. HUNTINGTON**

**HARVARD UNIVERSITY**

*Communicated, January 4, 1924.*

The "universe of discourse" in this paper consists of all systems (K,R), where K is a class of elements, A,B,C,..., and R is a triadic relation, denoted by R(ABC), or simply ABC.

Within this universe of discourse, two classes of systems (K,R) are of special importance; first, the *betweenness-systems* characterized by any one of twelve sets of postulates, studied elsewhere; and second, the *cyclic-order-systems* characterized by any one of three sets of postulates studied in the present paper.

The three sets of postulates for cyclic order are:

(1) E, B, C, D, 2,
(2) E, B, C, D, 3,
(3) E, B, C, D, 9;