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Experimentos sobre la transplatación de los miembros en
Amblystoma.

La formación de plexos nerviosos y la función de los miembros.

El miembro anterior de Amblystoma transplataado en una parte del cuerpo situada en uno de los segmentos comprendidos entre el primero y el séptimo de estos, a partir de la posición normal del órgano, presenta un decrecimiento normal en su capacidad para funcionar a medida que se transplanta mas lejos de su posición normal. El cambio de posición del miembro en un cierto número de segmentos no implica un cambio correspondiente en la posición de la inervación segmentaria de su plexo, existiendo por consiguiente, una marcada tendencia en los miembros transplataados a recibir inervación del nivel normal del miembro, en la médula espinal. Los miembros implantados tan posteriormente que es imposible que reciban inervación desde el nivel correspondiente de la médula obtienen la mayor parte de su inervación de los segmentos situados delante de ellos, en vez de recibirla de los segmentos correspondientes a la nueva posición de dichos miembros. El decrecimiento gradual de la función en los miembros transplataados en un sitio cada vez mas lejano del punto en que aparecen normalmente parece estar directamente relacionado con la inervación segmentaria, siendo mas perfecta la función cuando la inervación se deriva del nivel normal del miembro en la médula. Esta pérdida gradual de función se atribuye a conexiones centrales defectuosas mas bien que a una disminución en la inervación efectiva periférica. Los resultados de los experimentos indican que el miembro transplataado ejerce una influencia que guía a los nervios segmentarios que contribuyen a su inervación. La reacción hacia esta influencia parece ser mayor en los nervios procedentes del nivel normal del miembro en la médula, puesto que estos nervios se alargan mas para ponerse en conexión con el miembro que los procedentes de los segmentos posteriores al nivel del miembro.

EXPERIMENTS ON THE TRANSPLANTATION OF LIMBS IN AMBLYSTOMA

THE FORMATION OF NERVE PLEXUSES AND THE FUNCTION OF THE LIMBS

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TWENTY-TWO FIGURES

INTRODUCTION

The transplantation of limbs constitutes an experimental method which has been applied to the solution of a number of fundamental questions concerning the development of the peripheral nervous system. Without doubt the most important applications of this method were made in connection with the question of the genesis of the nerve fiber in the work of Braus ('04 and '05), Banchi ('06), Gemelli ('06), and Harrison ('07).

Aside from their bearing upon the specific problem of the genesis of the nerve fiber, the transplantation experiments with the exception of those of Banchi showed in general that if a limb bud is transplanted to an abnormal (heterotopic) position it will acquire a system of nerves which are connected with that part of the central nervous system of the host corresponding to the position occupied by the implanted limb rudiment.

Although Braus ('05) claimed that aneurogenic limb buds (those taken from nerveless larvae) did not acquire nervous connection with the host, Harrison's experiments showed that aneurogenic as well as euneurogenic limb buds (those taken from normal larvae) became supplied with peripheral nerves. That such nerves were partially functional was shown by slight voluntary movements of the limbs as well as by movements in response to electrical stimulation.

Apparently in all these cases the function of the limb was greatly restricted, and in no case cited was there any adaptive or coördinated movements. Harrison ('07, p. 256), in describing his first experiment, says in regard to the function of an aneurogenic limb, "No attempts were made to stimulate electrically, but spontaneous movements, though slight, were unmistakable."

Both Braus and Harrison observed that, regardless of the segmental nerve contribution, the architecture of the intrinsic nerve distribution is exactly the same as that in a normal limb. This is not an unusual phenomenon, for we know that in both normal and transplanted limbs, nerves reach the limb when it is still in the blastema stage. The union of the nerve with the differentiating limb system is made very early, so that in either case the final plan of nerve distribution is patterned according to skeletal-muscular differentiation and growth, probably in accordance with Naussbaum's law: that the course of the nerve within the muscle is an index of the direction in which the muscle has grown.

The experiments to which reference has thus far been made were carried out on the anuran embryo, *Bombinator* being used by Braus, *Bufo vulgaris* by Banchi and Gemelli, and *Rana sylvatica* and *Bufo lentiginosus* by Harrison. In the majority of cases the limb buds were transplanted at a stage when the peripheral nerves were in part or completely developed. Accordingly, when the wound in the host was made for the reception of the transplant, the terminal branches of the nerves of that region were severed and the implanted limb rudiment was placed in close apposition to the cut ends. Although these nerves so disturbed were originally intended to innervate other muscles, it was found that they would readily grow into the implanted embryonic rudiment and innervate the differentiating limb muscles as do the nerves in the normal situation. This fact would indicate that there is no specificity of a given motor neurone for any particular muscle fiber, so that the ultimate distribution and connection of the nerve fiber cannot be an intrinsic factor of the neurone.

This leaves open the question of whether or not the limb rudiment, either in the normal or the heterotopic position, exerts any directive influence upon the segmental contribution of the spinal nerves or upon the final path taken by them in the innervation of the limb. The possibility of the limb's exerting any such influence on the developing peripheral nerves could hardly be adequately tested by transplanting limb buds to larvae in which the peripheral nerve paths have already been formed, such as in the experiments hitherto described. As we have seen, limb buds in these cases are placed in the direct pathway of a number of already formed spinal nerves, the peripheral ends of which are severed in preparing the wound, and it is expected that these nerves should continue their growth into the rudiment so placed.

Evidence as to whether or not the limb rudiment does exert any influence on the segmental contribution and on the path of the spinal nerves entering into the formation of the nerve plexus ought to be obtained by transplanting limb rudiments in embryos at a period before the spinal nerves have begun to develop. Then, any possible influence on the part of the end organ can exert itself on the nerve fibers at a time when initial outgrowth takes place. In the Urodele, *Amblystoma punctatum*, the anterior limb rudiment can readily be transplanted at such a period. In the present paper, which contains a description of a series of both autoplasmic and homoplasmic transplantations on this form, certain findings are set forth which strongly suggest such an influence on the part of the limb.

Another very important question on which the experimental results contained in this paper throw some light deals with the part played by functional activity of the limb on the differentiation of neuroblasts.

The general problem of the effect of functional activity of an end organ on the differentiation of neuroblasts is one which has been tested but little by the experimental method, and the results of different investigations as they stand to-day are not entirely compatible.

The method of attacking this problem up to the present time has consisted in extirpating the end organ at a period either before or shortly after the peripheral nerves have begun to develop and of observing the effect of its absence on that part of the central nervous system ordinarily supplying it with nerve components.

Braus ('06) extirpated the forelimb buds of *Bombinator* at a period prior to the outgrowth of the brachial plexus, with a view of determining the effect of the absence of the limb on the ventral horn region of the spinal cord corresponding to the limb level. It was found, from a study of larvae preserved ten days after the operation, that the brachial nerves had grown out to the limbless area and that they were as well formed as those on the uninjured side. No reduction in size or number of the ventral horn cells could be detected. Observations, however, on operated larvae which were kept alive until just before metamorphosis showed that not only was the brachial plexus on the limbless side diminished in size when compared with its counterpart, but that, in addition, there was a distinct reduction in the size of the ventral horn area ordinarily supplying the limb. As a corollary to the general developmental theory of Roux ('85), Braus concluded that the development of the 'central nervous system,' is readily divisible into two periods: the first, in which growth and differentiation are independent of functional activity, and, the second, in which further differentiation and growth continue only when under the influence of functional activity.

Miss Shorey ('09), who performed a series of extirpation experiments on the limb buds of the chick and the amphibian embryos, came to the rather sweeping conclusion from her findings that no neuroblasts are self-differentiating and that all are alike dependent for differentiation on stimulation from end organs or from the products of the activities of end organs.

Although the experiments of Harrison ('10) left no doubt that the initial differentiation of the nerve fiber is a factor predetermined within the neuroblasts, they did not attempt to give any definite information on the part played by functional activity on the later differentiation of neuroblasts.

It was suggested by Doctor Harrison that the best way of testing the influence of functional activity on the differentiation of neuroblasts would consist not solely of removing the end organ and noting the effects of its absence, but rather of transplanting the end organ so that it might function in a new environment. In this way not only could the effects of removal be noted, but still better, the effect of continued function of the transplanted end organ on that part of the central nervous system from which its innervation is derived.

Accordingly, experiments were begun with this problem in mind, and while positive evidence has been attained from these experiments to show that the functional activity of the transplanted limb will initiate a hyperplasia of the sensory neurones contributing innervation to the limb, the results of this phase of the experiments are taken up in a separate publication (Detwiler, '20). The present paper will consider questions mainly concerned in the formation of nerve plexuses and the function of the limbs.

I wish to express here my thanks to Doctor Harrison for his suggestions and criticisms.

ANATOMICAL

Even though a transplanted limb may be well innervated by spinal nerves of the host, it is obvious that the degree of function of the limb is conditioned by still other factors. Structural deficiencies of the shoulder-girdle or deficiencies in the shoulder musculature would greatly restrict its function, even though the limb were copiously supplied with nerves and the structures within it were perfectly developed.

Although the developmental intimacy of the shoulder-girdle and limb led Wiedersheim ('92) to conclude that the shoulder-girdle can develop only when under the formative influence of the free extremity, the lack of interdependence of these two systems has been shown experimentally (Braus, '09, and Detwiler, '18). It has also been shown (Braus, *op. cit.*, Harrison, '18, and Detwiler, *op. cit.*) that in the transplantation of a typical

limb bud, only a portion of the girdle rudiment is included. From his experiments on *Bombinator*, Braus ('09) claimed that from this fraction of the rudiment a complete girdle of reduced size is developed, and he concluded that the shoulder-girdle rudiment, like that of the limb, constitutes an equipotential restitution system. Experiments hitherto reported (Detwiler, op. cit.) have shown that this rudiment in *Amblystoma punctatum* constitutes a mosaic and is incapable of qualitative restitution. When transplanted, only such components of the girdle develop as are represented in the corresponding portions of the implanted rudiment. Such girdles, however, although qualitatively incomplete, may become topographically complete by a process of hyperplasia which compensates for qualitative deficiencies. This is well illustrated in cases where in the absence of the suprascapular rudiment the dorsal zone of the girdle becomes practically completed by hyperplastic development of the intact portion. The above is not always the case, however, and inasmuch as there is considerable variability in the degree of development of the shoulder-girdle in the heterotopic position, restricted function of the limb may be a result of marked deficiencies in the girdle.

Intimately associated with the development of the shoulder-girdle is the development of the shoulder muscles. These, however, may develop and differentiate independently of one another so that with a well-developed girdle there may be muscular deficiencies.

The lack of interdependence of muscle and skeletal differentiation has previously been shown. Braus ('06 a) showed this to be true in the development of the pectoral fin of the *Elasmo-branch* embryo. Personal observations on the development of the shoulder-girdle of *Amblystoma* have shown that in the absence of skeletal differentiation, shoulder muscles may develop. It is obvious, therefore, that restricted function may result from muscular deficiencies in addition to those of the girdle.

Even though skeletal and muscular differentiation may be complete, restricted function of the limb might be due to the failure of certain of the individual muscles to receive innervation, so that this offers a third factor conditioning the degree of function.

With the above possibilities in mind it became necessary, for proper interpretation of the results, to make a study of the normal anatomy and the normal conditions of innervation. The brief description which is herewith presented is based upon a study of a series of cross-sections (10μ) of a specimen preserved seventy days after the closure of the medullary folds. This has been augmented by a dissection of the shoulder region of an adult specimen.

A. Shoulder-girdle

A description of the cartilaginous girdle as found in a larva twenty days after the closure of the neural folds has previously been given (Detwiler, '18, p. 501, and fig. 23). The conditions found in a larva of seventy days do not show fundamental alterations in general topography. The suprascapula has extended its growth somewhat more dorsal and has lengthened out in an anteroposterior direction so as to form a flat plate of cartilage. The procoracoid has expanded in an anteroventral direction and the coracoid has undergone a ventral expansion so as to overlap its counterpart in the midventral line. The scapula has undergone partial ossification and that portion of the procoracoid which enters into the formation of the glenoid cavity, although still cartilaginous, is about to undergo ossification. This is suggested by the greatly enlarged cartilage lacunae in that region. The adult shoulder-girdle is illustrated in figure 1. The entire scapula, together with those portions of the procoracoid and the coracoid which enter into the formation of the glenoid fossa, have become ossified. The greater part of the procoracoid and coracoid and also the entire suprascapula remain cartilaginous throughout life.

B. Shoulder muscles

So far as could be ascertained, no description of the shoulder muscle of *Amblystoma* appears in the literature. The musculature, however, so far as has been studied, closely resembles that of *Salamandra*, a European tailed Amphibian described

by Fürbringer ('73). In referring to the musculature, Fürbringer's nomenclature will be employed.

The shoulder muscles may be separated into two groups, viz., the trunk muscles or those of myotomic derivation and the so-called limb muscles or those of somatopleural origin. The former group contains the following: *m. cucullaris* (*trapezius*: Mivart), *m. serratus magnus* (*serratus magnus anticus*), *m. levator scapulae*, and the *m. pectori-scapularis internus*. None of these muscles derive their innervation from nerves entering into the formation of the brachial plexus.

Fig. 1 Outline drawing of the normal shoulder girdle of an adult specimen of *Amblystoma*. Stippled area represents bony portion. $\times 3\frac{1}{2}$. *s.sc.*, suprascapula; *sc.*, scapula; *p.cor.*, procoracoid; *cor.*, coracoid; *g.c.*, glenoid cavity.

The second group contains the following: *m. supracoracoideus*, *m. procoraco-humeralis*, *m. dorsalis scapulae* (*deltoid*: Mivart; *infraspinatus*, *deltoideus* and *teres minor*: Humphrey), *m. pectoralis* (*pectoralis major* and *minor*), *m. subcoraco-scapularis* (*subscapular*: Mivart), *m. latissimus dorsi*, *m. coraco-brachialis brevis*, *m. coraco-brachialis longus*, *m. anconaeus scapularis medialis*. This last muscle is undoubtedly the homologue of the long or scapular head of the *triceps brachii* of man. All of the muscles in the second group receive their innervation from branches of the brachial plexus.

Since the rudiments of the muscles in the first group never become implanted along with the limb rudiment in a typical transplantation, it will not be necessary to consider the normal anatomy of these muscles. However, the rudiments of the muscles of the second group are involved in the transplantation, and for this reason a brief description of these muscles as they occur in their normal situation becomes necessary.

1. *M. supracoracoideus*. This is a broad, flat, fan-shaped muscle which takes its origin from the entire external surface of the coracoid with the exception of the medial and posterior margins. Its fibers converge to be inserted on the proximal part of the processus lateralis humeri.

Innervation: n. supracoracoideus (fig. 2, n. sp. cor.), a branch of the third spinal nerve.

2. *M. procoraco-humeralis*. This is a somewhat longer and narrower muscle than the m. supracoracoideus. Its fibers take origin from the external surface of the procoracoid with the exception of the distal anteromedial and anterolateral margins. The fibers converge proximally and become inserted on the processus lateralis humeri between the insertion of the m. supracoracoideus and the m. dorsalis scapulae.

Innervation: through a branch of the third spinal nerve (fig. 2, n. p. cor.).

3. *M. dorsalis scapulae*. This is a somewhat thicker muscle than the m. supracoracoideus and the m. procoraco-humeralis and takes origin from the entire external surface of the cartilaginous suprascapula with the exception of the dorsal, anterior, and posterior margins, the latter two of which serve for respective insertions of the m. levator scapulae and a portion of the m. serratus magnus. The muscle passes down over the external surface of the scapula, crosses over the tendon of the m. latis-simus dorsi, and inserts by means of a flat tendon on the processus lateralis humeri, medial to the insertion of the m. procoraco-humeralis.

Innervation: by several short branches from the third spinal nerve (fig. 2, n. ds. c).

4. *M. pectoralis*. This constitutes a triangular-shaped muscle taking origin from the aponeurosis between it and the *m. obliquus externus abdominis*, the *linea alba*, and from a cartilaginous sternum. Its fibers converge into a short tendon which inserts on the distal part of the *processus lateralis humeri*.

Innervation: *n. pectoralis*, a branch of the common trunk formed by the union of the fourth and fifth spinal nerves (fig. 2, *n. pc.*).

5. *M. subcoraco-scapularis*. This is a short, thick muscle which takes origin from the medial side of the contiguous portions of the scapula and the *procoracoid*. The fibers pass out across the inner aspect of the shoulder joint and insert on the medial surface of the proximal part of the humerus.

Innervation: *n. subscapularis*, a branch of the third spinal nerve (fig. 2, *n. sb. sc.*).

6. *M. latissimus dorsi*. This is a broad triangular muscle which arises from an aponeurosis between it and the longitudinal dorsal trunk muscles. The fibers converge into a long tendon, a part of which unites with the tendon of origin of the *m. anconaeus scapularis medialis*; the remainder passes across the lateral aspect of the shoulder capsule and inserts on the proximal dorso-lateral surface of the humerus close to the *processus lateralis humeri*.

Innervation: *n. latissimus dorsi*, a branch of the fourth spinal nerve (fig. 2, *n. lt. dor.*).

7. *M. coraco-brachialis brevis*. This is a short, rather thick muscle which takes origin from the posterior margin and from the outer surface of the posterior part of the *coracoid*, internal to the *m. supracoracoideus*. The muscle passes to the medio-ventral surface of the humerus and inserts on its proximal two-fifths and under cover of the fibers of the *m. brachialis inferior* (*m. biceps brachii*).

Innervation: by the *n. coraco-brachialis*, a branch of the fourth and fifth spinal nerves (fig. 2, *n. cor. br.*).

8. *M. coraco-brachialis longus*. This is a long, rather thick muscle which takes origin from the internal posterior margin of the *coracoid* close to the *glenoid cavity*. It passes out along the

ventral surface of the humerus, ventral to the m. anconaeus scapularis medialis and medial to the m. brachialis inferior. It inserts on the ventral surface of the distal two-fifths of the humerus.

Innervation: by the n. coraco-brachialis (fig. 2, *n. cor. br.*).

9. *M. anconaeus scapularis medialis*. This muscle takes origin by means of a long, flat tendon from the glenoid margin of the scapula and from the capsule of the shoulder joint. A portion unites with the tendon of the m. latissimus dorsi; the remainder passes out on the dorsomedial surface of the humerus, between the m. coraco-brachialis and the m. anconaeus humeralis lateralis with which it finally unites. It inserts in common with the m. anconaeus humeralis lateralis and the m. anconaeus humeralis medialis on the olecranon process of the ulna.

Innervation: through the n. brachialis longus superior.

According to Fürbringer some of the deep posterior fibers of the m. supracoracoideus in *Salamandra* leave this muscle, join the fibers of the m. brachialis inferior and insert with it on the proximal part of the radius and ulna. This muscle he calls the m. coraco-radialis proprius. A similar structure in *Amblystoma* could not be identified either in the larval or in the adult individual.

C. The brachial plexus

In the so-called tail-bud stage, the period of development in which the experiments reported in this paper were carried out, the position and extent of the anterior limb rudiment, as has already been shown by Harrison ('15), constitutes a slightly thickened region of somatopleural mesoderm lying just ventral to the pronephros and extending from the anterior border of the third somite to the posterior border of the fifth. The normal anterior limb is supplied by a plexus composed of the third, fourth, and fifth spinal nerves (fig. 2). The outgrowing nerves above enumerated, effecting connection with the limb rudiment at a period when it occupies its maximum extent (anterior border of the third somite to the posterior border of the fifth) become converged into a plexus as a result of concentration of the rudi-

ment into the definitive limb bud which centers ventral to the fourth myotome. Such convergence having taken place, a typical normal plexus as illustrated in figure 2 would be produced.

It has long since been pointed out, especially by Fürbringer ('79), that the nerve plexus from which a limb is supplied might, in two cases, have a different segmental origin and yet the intrinsic nerves arising from the plexus might be distributed in the same manner in each.

From his own transplantation experiments, Harrison ('07) showed that there are two main factors involved in the development of the innervation of a limb: first, the position and extent

Fig. 2 Graphic reconstruction of the normal left brachial plexus of case AS4₂₈ (fig. 13), preserved sixty-eight days after the operation. $\times 20$. *n.sp.cor.*, nerve to musc. supracoracoideus; *n.p.cor.*, nerve to musc. procoracohumeralis; *n.d.sc.*, nerve to musc. dorsalis scapulae; *n.pc.*, nerve to musc. pectoralis; *n.sb.sc.*, nerve to musc. subcoraco scapularis; *n.cor.br.*, nerve to musc. coraco-brachialis; *n.lt.dor.*, nerve to musc. latissimus dorsi.

of the extremity at the time of origin which determines the source of the nerve supply, and, secondly, the mode of segregation and growth of the individual structures of the limb which governs the intrinsic distribution of its nerves.

As the result of a study of the normal plexus formation, in addition to the observations of Harrison, the evidence at hand tends to show that the position and extent of the limb rudiment serves as an index of the number of spinal nerves supplying the extremity, the number corresponding with that of the segments occupied by the limb rudiment at the period when initial connection is made. This, then, would not only readily account

for differences in the number of spinal segments which contribute nerves to the limbs in different species, but it would also account for slight variations in the segmental contribution to the limb plexus in a given species—a condition which, as has been referred to above, has long since been observed.

If the extent of the limb rudiment were the only factor to account for this latter phenomenon, then the limb rudiment, if displaced the distance of several segments, should so effect the nerve contribution that the new plexus would come from segments corresponding to the position occupied by the displaced limb rudiment. The results of the experiments reported in this paper indicate that, in addition to the position and extent of the limb rudiment, still other factors govern the innervation of limbs when developed from rudiments lying beyond the confines of the orthotopic position.

Fig. 3 Camera-lucida drawing of an embryo of *Amblystoma* in the tail-bud stage. The circle situated ventral to the pronephros (*pn*) indicates the position of the limb rudiment. $\times 15$.

EXPERIMENTAL

The experiments were carried out upon embryos in the tail-bud stage (fig. 3). The extent of the limb rudiment at this period has been described above. The technique employed in embryonic limb transplantation is so well known through the papers of Harrison, Braus, and others that no special description is here required. All of the experiments were made upon the anterior limb rudiment. The majority consisted of transplanting the right anterior limb rudiment to an abnormal (heterotopic) position on the same side of the body from which it was

taken, and at distances ranging from one to seven segments posterior to the normal situation. These experiments constitute the autoplasic transplantations.

In other experiments the normal anterior limb was left intact and additional right anterior limb rudiments from other embryos were implanted respectively the distance of three, four, and five segments posterior to the normal intact limb rudiment of the host. These latter experiments constitute the homoplastic transplantations.

The larvae were preserved in sublimate-acetic fixing fluid at intervals from thirty to eighty days after the operation, the majority being under observation for about sixty days. A study of the animals was made from transverse sections cut 10μ in thickness and stained with Ehrlich's haematoxylin and erythrosin.

A. Autoplasic transplantations

Series AS1. In this series of experiments the limb rudiment was excised and reimplanted the distance of one body segment posterior to its normal position so that the rudiment, instead of centering ventral to the fourth somite as it does in the normal condition, now centers ventral to the fifth somite (fig. 4). The results of this series of experiments are presented in table 1, series AS1. By examination of this table it is seen that 95 per cent of limb rudiments so placed developed into normal limbs, there being only one case in twenty positive experiments in which the rudiment developed into a reduplicated appendage.

The criteria used in establishing the category 'normal limbs' were based purely upon superficial conditions, the limb being classed as 'normal' only when a brachium, antibrachium, and a manus with the proper number of digits were developed.

It is also seen (table 1) that 95 per cent of the limbs so transplanted functioned perfectly, the movements being adaptive and well coördinated with those of the opposite intact limb.

The criteria employed in establishing the category 'perfect function' were: 1) movements of the arm on the shoulder, viz., flexion, extension, abduction, adduction, rotation, and circum-

duction; 2) flexion and extension of the forearm; 3) flexion and extension of the carpus, and, 4) movements of the digits. When the transplanted limb showed inability to carry out all of these movements such as occur in the normal intact limb, it was placed in the category, 'function impaired' (table 1).

The functional behavior of the transplanted limbs in all series was closely observed and recorded, these observations being

TABLE 1

A. Showing the results of transplanting the right anterior limb rudiment a given number of segments (AS1, AS2, AS3, etc.) posterior to its normal position

B. Showing the results of transplanting an additional anterior limb rudiment to an embryo in which the normal limb rudiments have been left intact. HS3, HS4 and HS5 indicate the successive number of segments the additional limb rudiment was implanted posterior to the normal right anterior limb of the host

SERIES	OPERATIONS	POSITIVE EXPERIMENTS	NORMAL LIMBS		REDUPLICATIONS		FUNCTIONAL LIMBS		NO FUNCTION		FUNCTION IMPAIRED		FUNCTION PERFECT	
			Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent	Cases	Per cent
A														
AS1	25	20	19	95.0	1	5.0	20	100.0	0	0	1	5.0	19	95.0
AS2	21	18	17	94.5	1	5.5	17	100.0	0	0	3	16.7	15	83.3
AS3	24	17	13	76.5	4	23.5	15	88.2	2	11.8	4	23.5	11	64.7
AS4	34	29	18	67.0	11	33.0	23	79.3	6	20.7	11	37.9	12	41.4
AS5	35	30	11	36.7	19	63.3	20	66.7	10	33.3	16	53.3	4	13.4
AS6	35	29	22	75.9	7	24.1	15	51.7	14	48.3	15	51.7	0	0
B														
HS3	10	9	2	22.2	7	77.8	9	100.0	0	0	6	66.7	3	33.3
HS4	30	21	8	38.1	13	61.9	11	52.4	10	47.6	11	52.4	0	0
HS5	30	25	11	44.0	14	56.0	13	52.0	12	48.0	13	52.0	0	0

frequently made from a period when normal limb reflexes first began to appear until preservation of the animal. The method employed in studying the limb reflexes consisted in the temporary transfer of the animal from the individual aquarium (battery jar) in which it was reared, to a small receptacle, such as a watch-glass. In this way the animal could be brought under direct observation either through the use of a binocular microscope or

a dissecting microscope. The general behavior of the limbs in response to externally applied stimuli could thus be carefully studied. The most successful method of studying the behavior of the limbs in response to applied tactile stimulation consisted in inhibiting total swimming movements which so characteristically dominate the action system at this period, by placing the animal in very shallow water. By so doing, swimming movements could be performed only with considerable difficulty, resulting thereby in an increased effort on the part of the animal to progress solely by means of its limbs.

The first appearance of reflexes in the transplanted limbs of series ASI did not appear until about two days after the first appearance of those in the normal intact limb, which were first noticed about fourteen days after the operation. This slight delay is probably an index of the period required for reorganization and readjustment of the limb rudiment subsequent to its reimplantation.

The time after the operation when the normal limb reflexes first appear cannot adequately be stated in terms of days, owing to the fact that the various factors which determine the rate of growth were not entirely controlled. In general, however, it can be stated that normal limb reflexes begin at a period when the third digit makes its first appearance. At this time the yolk is almost, if not entirely, absorbed and the larva begins to feed. A description of the normal development of the external features of the limb up to this period has been given by Harrison ('18, page 417).

Of the series ASI, case 12, preserved sixty-four days after the operation, was sectioned and studied. The transplanted limb is innervated by a plexus composed of the normal segmental nerve components (table 2) and the intrinsic nerve distribution within the limb is practically identical with that in the normal limb. No shoulder muscles of the group which typically develop in the heterotopic position (table 3) were lacking and all received peripheral innervation. The shoulder-girdle was somewhat atypical. The coracoid was rather short, the procoracoid and scapula were abnormally thick and the more dorsal portion of

the girdle was continuous with the original suprascapula which had developed in situ from the unremoved suprascapula rudiment when the limb was transplanted.

It has previously been shown that the operation employed in extirpating the limb leaves in the embryo portions of the rudi-

TABLE 2

Showing the segmental nerve contribution of the right anterior limb when implanted successively a number of segments (1 to 7) posterior to its normal position

SERIES	CASES	POSITION OF LIMB. NUMBER OF SEGMENTS POSTERIOR TO NORMAL	SEGMENTAL NERVE CONTRIBUTION								
			3	4	5	6	7	8	9	10	11
Normal.....	1	0	3	4	5						
AS1.....	12	1	3	4	5						
AS2.....	5	2	3	4	5						
	12	2	3	4	5						
AS3.....	9	3		4	5	6					
	18	3		4	5	6					
AS4.....	12	4		4	5	6	7	8			
	27	4			6	7	7				
	26	4			5	6	7				
	30	4			5	6	7				
AS5.....	25	5			5	6	7	8	9		
	27	5				6	7	8	9		
	23	5					7	8	9		
	30	5				6	7	8	9		
	26	5					7	8	9		
AS6.....	29	6						8	9	10	
	5	6							9	10	
AS7.....	5	7								10	11

ments of the suprascapula, the coracoid and occasionally the procoracoid (Harrison, '18, and Detwiler, '18), so that the shoulder-girdle which develops in the heterotopic position does so from the more central portion of the rudiment which is included with that of the limb in a typical bud transplantation.

Series AS2. In this series of experiments the limb rudiment was excised and reimplanted the distance of two segments posterior to the normal position, so that the rudiment which originally extended from the anterior border of the third myotome to the posterior border of the fifth, now occupies a region from the anterior border of the fifth myotome to the posterior border of the seventh; and this rudiment, subsequent to its convergence into the definitive limb bud, centers ventral to the sixth myotome instead of the fourth, as in the normal situation (fig. 5). The denuded area underneath the third and fourth somites was covered by ectoderm taken from another individual.

In eighteen positive experiments of this series there occurred only one in which a reduplicated appendage developed. The remainder gave rise to normal limbs (table 1). The results of the study of the behavior of these limbs showed that, although all were capable of movements, 16 per cent showed restricted movements. The remainder functioned normally (table 1 and fig. 9).

Two individual cases of this series, viz., AS2₅ and AS2₁₂ (preserved, respectively, fifty-six and fifty-two days after the operation, were sectioned and studied. The heterotopic limbs in both cases were found to receive the normal segmental nerve contribution, the plexus in each case being derived from the third, fourth, and fifth nerves (table 2). Such being the case, it is highly probable that all of the series AS1 were likewise innervated by the third, fourth, and fifth nerves, such as was found in case AS1₁₂ (table 2).

An examination of the shoulder musculature in cases AS2₅ and AS2₁₂ showed that here, too, all of the muscles which typically differentiate in the heterotopic position were developed (table 3), and that all were supplied with peripheral nerves. The structures within the limbs showed essentially a normal arrangement and the nerves a normal intrinsic distribution. The shoulder-girdles showed no defects other than a short suprascapula and a poorly developed procoracoid in case AS2₁₂ (table 4).

Whether all of the heterotopic limbs of this series received the normal segmental nerve contribution cannot be definitely stated.

If so, the 16 per cent which exhibited impaired function is most likely a result of structural deficiencies in the girdle and shoulder muscles or is due to defective peripheral innervation.

Fig. 4 Outline drawing of case AS1₁₂. Right anterior limb excised and reimplanted the distance of one segment posterior to the normal position. Animal preserved sixty-four days after the operation. $\times 5$.

Fig. 5 Outline drawing of individual AS2₁₂. Right anterior limb transplanted the distance of two segments posterior to the normal position. Animal preserved fifty-two days after the operation. $\times 5$.

The period at which the limb reflexes began to appear in the heterotopic limbs of this series was no more delayed when compared with the normal intact limb than in the series AS1—the reflexes first appearing in the normal limb about fourteen days

TABLE 3
Enumerating the shoulder muscles which developed with the transplanted limb, and the presence or absence of their respective nerve supply¹

INDIVIDUAL CASES	MUSCLES									
	M. procoraco- numeralis	M. subcoraco- scapularis	M. supra- coracoideus	M. dorsalis scapulae	M. latissimus dorsi	M. anconaeus scapularis medialis	M. coraco- brachialis brevis	M. coraco- brachialis longus	M. pectoralis	
AS1 ₁₂	Present +	Present	Present +	Present	Present +	Present	Present	Present	Present	+
AS2 ₁₂	Present +	Present	Present +	Present	Present +	Present	Present	Present	Present	+
AS3 ₉	Present +	Present	Present +	Present	Present +	Present	Present	Present	Present	+
AS3 ₁₈	Present +	Present	Present +	Present	Present +	Present	Present	Present	Present	+
AS4 ₂₆	Present +	Present	Present +	Present	Present +	Present	Present	Present	Present	+
AS4 ₂₇	Present -	(Small)	Present -	(Abnormal)	Present -	Present	(Short)	Present	(Very short)	+
AS5 ₂₃	Present +	(Small)	Present +	(Abnormal)	Present +	Present	(Short)	Present	(Very short)	+
AS5 ₂₅	Present +	(Small)	Present +	(Very small)	Present +	Present	(Short)	Present	(Very short)	+
AS5 ₂₆	Present +	(Small)	Present -	(Very small)	Present -	Present	(Short)	Present	(Very short)	+
AS5 ₂₇	(Short) -	(Small)	Present -	(Very small)	Present +	Present	(Short)	Present	(Very short)	-
AS6 ₂₉	(Short) +	(Small)	Present +	(Very small)	Present +	Present	(Short)	Present	(Very short)	-

¹ + indicates that the muscle is innervated; - indicates that the muscle lacks innervation.

after the operation, those in the heterotopic limbs appearing about two days later. This would indicate that the period of delay in this series has no correlation with the length of time required for the nerves to grow back to the heterotopic limb. As was the case in series AS1, the delay probably represents the time required for reorganization of the rudiment subsequent to its reimplantation.

Series AS3. Reimplanting the limb rudiment the distance of three segments posterior to the original position results in

TABLE 4

Showing the development of the different components of the shoulder-girdle when implanted successively a number of segments (1 to 6) posterior to the normal position

CASES	THE SHOULDER-GIRDLE			
	Suprascapula	Scapula	Procoracoid	Coracoid
AS1 ₁₂	Normal	Thick	Abbreviated	Short
AS2 ₁₂	Short	Normal	Very short	Normal
AS3.....	Short	Normal	Short	Short
AS3 ₁₈	Very short	Normal	Very small	Normal
AS4 ₂₆	Short and thick	Normal	Small	Normal
AS4 ₂₇	Short and thick	Normal	Small	Very short
AS5 ₂₃	Short	Normal	Well developed	Short
AS5 ₂₆	Short	Normal	Short and thick	Normal
AS5 ₂₇	Short	Normal	Absent	Normal
AS6 ₅	Short	Normal	Very small	Short
AS6 ₂₉	Short and thick	Normal	Very small	Well developed

a decreased number of cases with normal limbs, there being only thirteen such cases in seventeen positive experiments (table 1). A study of the functional behavior of limbs so transplanted shows also a decreased percentage of cases in which coördinated movements of the limbs could be perfectly carried out. Although 88 per cent of the limbs were capable of movements, only 64 per cent functioned perfectly, 23 per cent exhibiting impaired function, and 11 per cent being entirely incapable of movements (table 1 and fig. 7).

An examination of serial sections of cases 9 and 18 (table 2), preserved, respectively, sixty-four and sixty-six days after the operation, showed that in both cases the innervation was derived from plexuses formed by the fourth, fifth, and sixth spinal nerves (table 2). Although the limbs of this series were displaced posteriorly the distance of three segments, the segmental nerves contributing to the plexus were displaced by only one segment, so that the bulk of the innervation was derived from segments situated anterior to those corresponding to the position occupied by the transplanted limb.

All of the shoulder muscles in case AS3₉ were found to be present (table 3), the coraco-brachialis muscle, however, being very short. All of the shoulder muscles were supplied with nerves. The shoulder-girdle was well formed except for a short suprascapula and a curtailed development of the coracoid (table 4), the latter being a condition responsible for the brevity of the m. coraco-brachialis.

In case AS3₁₈, muscular differentiation was also qualitatively complete, although the m. dorsalis scapulae and the m. procoracohumeralis were quite short, due to an imperfect development of those parts of the girdle giving origin to these muscles, viz., the suprascapula and procoracoid. The remaining parts of the shoulder-girdle were typically developed (table 4).

The m. latissimus dorsi received no nerve supply, resulting in an inability to the animal to draw the arm dorsoposteriorly. Defective innervation also of the m. triceps brachii resulted in partial extensor paralysis of the forearm. Other than these defects, peripheral innervation was complete.

In general, initial reflexes in the transplanted limbs of this series did not appear quite as early, when compared with the normal, as did those in the series AS1 and AS2, the period extending from two to ten days after the first appearance of reflexes in the normal limb, although the number of cases in which there was only slight delay exceeded those in which the delay was greater.

Series AS4. Transplanting the limb the distance of four segments posterior to its original position, so that the limb

rudiment centers ventral to the eighth somite, results in still greater decrease in the number of cases with normal limbs, and a corresponding increase in the number of cases in which reduplication of the rudiment occurred (table 1 a, series AS4). Seventy-nine per cent of limbs so transplanted became functional, although only 41 per cent exhibited perfect movements, the function of the remaining 38 per cent being impaired in one way or another (table 1 a, series AS4 and fig. 8).

The transplanted rudiments of this series, although placed ventral to the seventh, eighth, and ninth somites, became innervated by plexuses derived mainly from segments situated anterior to those corresponding to the position occupied by the limb rudiment, as was found to be the case in the series already described. The segmental nerve supply, although preëminently coming from the fourth, fifth, and sixth segments, exhibits some variation, as is shown in table 2. In case AS4₁₂ (fig. 14) the rudiment developed into a reduplicated appendage, the anterior disharmonic member of which became fully differentiated and functional, while the posterior harmonic member developed into a structurally deficient and atrophic appendage. As a consequence of reduplication, the anterior member, being nearer the original limb level than the posterior, was found to receive practically all of the nerve supply, which was contributed by the fourth, fifth, sixth, and seventh segments of the cord (table 2, case AS4₁₂).

The anterior (disharmonic) member in this case did not begin to develop until eighteen days after the operation. At this period it began as a small knob-like growth on the radial side of the base of the harmonic limb, which up to this period had developed normally. The reduplicating bud developed with marked rapidity, and eleven days later it had differentiated into a normal appendage and had become functional. From this time on it rapidly improved in growth and function, while the posterior harmonic limb underwent gradual atrophy. The relative size of the two limbs at sixty-four days after the operation is shown in figure 14.

Reduplication of the limb rudiment was not accompanied by a reduplication of the shoulder-girdle, there being only one girdle, which, however, was reversed and in which there occurred two glenoid cavities, one for each member of the reduplication.

Braus ('09) claimed from his experiments on Bombinator that when reduplicating limbs arose from a single transplant, there occurred a corresponding reduplication of the shoulder-girdle—observations on which he partly based his conclusions that the shoulder-girdle, like the limb, constitutes an equipotential system. From the case (AS4₁₂) under consideration as well as from cases previously reported (Detwiler, '18), it can be said that reduplication of the limb in *Amblystoma* is not accompanied by reduplication of the shoulder-girdle.

The shoulder muscles to the anterior disharmonic limb in case AS4₁₂ were normally developed and were well supplied with nerves, while the shoulder muscles to the harmonic limb showed marked deficiencies and no muscular differentiation whatsoever had taken place in the arm. No nerves could be traced to the limb.

Case AS4₂₆. In this specimen (fig. 13), preserved sixty-eight days after the operation, the transplanted limb was innervated by a plexus of nerves coming from the fifth, sixth, and seventh segments of the cord (table 2 and fig. 10). Limb reflexes in this case began about six days later than did those in the normal intact limb of the left side, although perfect coördinated function was not attained until fifteen days later. The shoulder musculature was typically developed (table 3) except for a rather short m. procoraco-humeralis, the result of an imperfect development of the coracoid.

The shoulder muscles were well supplied with peripheral nerves, as were also the muscles of the limb itself. Figure 2 represents a graphic reconstruction of the left normal brachial plexus of case AS4₂₆. Figure 10 is a reconstruction of the plexus supplying innervation to the transplanted limb which was centered ventral to the eighth myotome. The tendency for transplanted limbs to be innervated by nerves coming from segments of the cord situated anterior to the limb itself is well illustrated in this figure.

Fig. 6 A graph showing the relative percentage of functional limbs developed from transplanting the anterior limb rudiment a successive number of body segments (S1, S2, S3, etc.) posterior to the normal position (curve A); and from transplanting an additional anterior limb rudiment successively three, four, and five segments (S3, S4, S5) posterior to the normal anterior intact limb of the host (curve B).

Fig. 7 Graph showing the relative percentage of transplanted limbs developed with total loss of function. Conditions as in figure 6.

Fig. 8 Graph showing the relative percentage of transplanted limbs developed with impaired function. Conditions as in figure 6.

Fig. 9 Graph showing the relative percentages of transplanted limbs with perfect function. Condition as in figure 6.

The shoulder-girdle was quite typically developed for the heterotopic position, its only defect being a slightly reduced procoracoid and a short, thick suprascapula (table 4).

Reflexes in the majority of the transplanted limbs of this series began at different periods ranging from fifteen to twenty-six days after the operation, the majority of the limbs beginning to function about the eighteenth or nineteenth day; in other words, about four or five days after the first appearance of reflexes in the normal limb.

Series AS5. When the limb buds were transplanted the distance of five segments posterior to the normal position so that they centered ventral to the ninth somite, 63 per cent developed reduplicated appendages, there being only eleven cases in thirty positive experiments which developed into normal limbs (table 1 a, series AS5). The limbs of this series showed a still greater decrease in function than did those of the series AS4. Although there were twenty cases in thirty which could be classed in the category of functional limbs, only four cases functioned perfectly (table 1 a). Of this series of experiments five cases were sectioned and studied. The segmental nerve contribution to a number of cases of this series is shown in table 2. Although some variability in the segmental contribution is noticeable, it is also seen that, as in the series previously described, the bulk of the innervation comes from segments of the cord situated anterior to the position of the limb itself. The limb, although centering ventral to the ninth segment and extending over into the region of the eighth and tenth segments, receives no innervation from the latter segments of the cord.

Of the individuals sectioned, case AS5₂₅ (figs. 16 and 17) was the only one which exhibited perfect function. It is also found by an examination of table 2 that this was the only case of those sectioned which received innervation from the fifth segment, which, as has hitherto been shown, belongs to the normal limb level. Figure 11 represents a graphic reconstruction of the nerve plexus supplying the transplanted limb. Although the limb receives innervation from five segments, the bulk of the nerve fibers come from the fifth, sixth, and seventh segments of the cord.

Fig. 10 Graphic reconstruction of the right brachial plexus of case AS₄₂₆, showing the segmental nerve supply to the right anterior limb transplanted the distance of four segments posterior to the normal position. Animal preserved sixty-eight days after the operation. $\times 20$.

Fig. 11 Graphic reconstruction showing the segmental nerve supply to the right anterior limb of case AS₅₂₅, the limb being transplanted the distance of five segments posterior to the normal position. Animal preserved sixty-four days after the operation. $\times 20$.

Fig. 12 Graphic reconstruction of the segmental nerve supply to the right anterior limb of case AS₅₂₇, the limb transplanted the distance of five segments posterior to its normal position. Animal preserved sixty-five days after the operation. $\times 20$.

A study of the shoulder region of this individual showed that the shoulder muscles were well developed (table 3). Peripheral innervation to these muscles was also found to be complete. The shoulder-girdle was almost typical. The procoracoid, however, was poorly developed. The procoracoid is frequently considerably reduced in size or entirely lacking in the transplanted girdle. Experiments hitherto reported (Harrison, '18, and Detwiler, '18) have shown that, when the limb rudiment is transplanted, the procoracoid rudiment may be entirely excluded so that its entire development takes place in the old situation.

Since the girdle lacks the power of qualitative restitution (Detwiler, '18), variations, therefore, in its size and completeness are the results of a greater or less amount of the respective rudiments having been included in the transplant, added to, perhaps, certain mechanical factors governing its shape as it develops in different heterotopic positions.

In the remaining cases sectioned shoulder-muscle differentiation was on the whole as complete as in case AS5₂₅, although the peripheral innervation was less perfect, a fact that would partly account for the restricted function which these cases exhibited (table 3).

Case AS5₂₇ (fig. 15), though exhibiting fair movements of the forearm, was very defective in shoulder movements. Although four segmental nerves contributed to the innervation of the transplanted limb (fig. 12), the shoulder muscles were poorly innervated, even though all had undergone complete differentiation, the following muscles being without nerve supply: m. procoraco-humeralis, m. supracoracoideus, m. dorsalis scapulae, m. coraco-brachialis longus, and the m. pectoralis (table 3).

The shoulder-girdle also exhibited some imperfections, the suprascapula being short and the procoracoid being entirely wanting (table 4). In such a case, incomplete or restricted function of the shoulder is clearly a result of defective peripheral innervation to its muscles.

Case AS5₂₆ showed a distinctly poor nerve supply to both shoulder and limb, both of which had undergone complete

muscular differentiation, in addition to a typically developed shoulder-girdle.

In general, however, the defective function of limbs in this position cannot be regarded as a result of factors already considered, but it is conditioned by other factors, which will be considered later. The completeness of function is in no way correlated with the time in days after the operation when reflexes first begin to appear. For example, in case AS5₂₅ limb reflexes began twenty-two days after the operation, while in some other cases with impaired function reflexes began as early as seventeen days after the operation.

Series AS6. Limbs transplanted six segments posterior to the normal position so that they centered ventral to the tenth somite were so far removed from the normal situation that there were no cases which functioned perfectly. Although 51 per cent were capable of some movement, in all cases the movements were greatly restricted (table 1 and fig. 8). Case AS6₂₉ of this series (fig. 20) was the only one in which were observed movements coordinated with those of the opposite intact limb, yet these movements were very restricted, especially movements of the arm on the shoulder. The segmental nerve contribution to the limb is shown in table 2.

In case AS6₂₉ all of the shoulder muscles were differentiated and all but three received peripheral innervation from a plexus composed of the eighth, ninth, and tenth segments of the cord. An examination of table 2 shows that peripheral innervation in this case was as complete as in limbs of the series AS5. The shoulder-girdle also was quite well developed and showed no more defects than in those not so distantly removed from the normal situation.

It has hitherto been observed (table 1) that there occurred a general increase in the number of reduplications as the limbs became transplanted farther and farther away from the normal situation. This, however, was not the case with series AS6, in which only 24 per cent developed reduplication, which is in marked contrast with the 63 per cent which reduplicated in series AS5. The small number of reduplicated appendages of the

series AS6 is a very striking and unlooked-for phenomenon, and since all of the factors underlying reduplications are not as yet understood, the decrease in this series is not explicable. In a great many cases the anterior or disharmonic member of the reduplication became more functional than the posterior or harmonic member, which probably is accounted for by the fact that it was closer to the source of the nerve supply and received the bulk of innervation. The fact that the disharmonic members of the reduplicated appendages frequently functioned more perfectly than the harmonic members would indicate that reduplication in itself can, in no wise, be adaptive. The non-adaptiveness is illustrated by such a case as AS4₂₆, in which the posterior member of the reduplication underwent almost complete atrophy, while the anterior member functioned quite perfectly, even though disharmonic (fig. 14). These general observations would well agree with those of Harrison ('17), viz., that when innervation and vascularization are sufficient, a functional condition may arise which is independent of the harmony of the combination.

Although in the autoplasic experiments there occurred a gradual increase in the number of reduplications as the limbs became transplanted farther and farther away from the normal situation, the reverse was found to be the case in the homoplasic operations; i.e., those in which an additional limb was transplanted a given number of segments posterior to the normal intact limb (table 1b). All of these reduplicated appendages functioned very imperfectly.

With the present knowledge of the factors underlying reduplications, the results obtained from these experiments are not entirely explicable. It would, therefore, be premature to attempt to discuss the results other than to state as has above been pointed out: that there is no evidence to indicate that reduplication is in any way adaptive.

A few experiments were made in which the limbs were transplanted the distance of seven segments posterior to the normal position. Inasmuch as very imperfect movement was observed in limbs of the series AS6, it appeared highly probable that

limbs still farther removed from the normal region should exhibit little if any movements. Such was found to be true, since in twelve experiments so performed there occurred only one (case AS7₅) in which any motion was observed and this consisted of a slight flexion of the carpus. This individual was sectioned and studied. The limb was found to be innervated from the tenth and eleventh segments of the cord (table 2). The shoulder-muscle innervation was quite defective. The number of cases of this series being small, they have not been included in the table.

Résumé of autoplasic experiments. Viewing the autoplasic experiments as a whole, we find that as the limbs become transplanted more and more remote from the normal situation there occurs a corresponding decrease in their ability to function until a position is reached (series AS6) in which all limb movements are very imperfect, there being no cases with perfect adaptive function. This gradual decrease, in so far as movements of the shoulder are concerned, is unquestionably determined by a gradual decrease in the peripheral innervation; yet, the decrease in the function of the forearm, wrist, and hand is not based upon defective limb innervation. Further, the gradual loss of coördination as the limbs are farther and farther removed from the normal site must be conditioned by factors other than its peripheral innervation. These factors, which are thought to be most important in conditioning the function of the limbs in the various positions, will be more fully discussed later on.

B. Homoplastic transplantations with normal limb intact

The homoplastic experiments consisted in transplanting an additional anterior limb rudiment a given number of segments posterior to the normal intact limb, the respective series being designated as HS3, HS4, and HS5.

The results of these experiments when compared with those of the autoplasic experiments furnish additional evidence which explains more fully the factors underlying the function of transplanted limbs.

As has already been pointed out, the normal anterior limb rudiment occupies a region extending from the anterior border

of the third somite to the posterior border of the fifth, centering ventral to the fourth somite. In the series HS3 the additional rudiment centered ventral to the seventh somite and its anterior border was contiguous with the posterior border of the normal intact limb rudiment. In these experiments the two limb buds always began independent growth; but in seven cases out of nine positive experiments, the posterior rudiment sooner or later reduplicated so that its anterior or disharmonic member fused with the normal limb to form a fused double limb. The posterior (harmonic) member which remained as a free limb functioned in all cases, but there was only one case, however, in which it functioned perfectly. Both of the two cases in which a single limb developed exhibited normal function. The results of this series of experiments are seen in table 1 b.

Series HS4. In this series of experiments, the additional anterior limb centered ventral to the eighth somite, so that the anterior margin of the transplanted limb bud was separated from the intact bud by the distance of one segment. In twenty-one positive experiments only eight developed normal limbs, the remainder forming reduplicated appendages. All of the reduplications functioned very imperfectly, as did also seven of the single limbs. In no case did function consist of more than slight flexion and extension of the forearm; yet in others motion was entirely restricted to twitchings of the hand.

The complete absence of cases with perfect function is in striking contrast with the 41 per cent which exhibited perfect function of the autoplasic series AS4, in which case the anterior limbs were transplanted to the same relative position (compare table 1 a, series AS4, with table 1 b, series HS4).

Series HS5. In the series HS5 the transplanted limb centered ventral to the ninth segment. The results of these experiments are seen in table 1 b. In twenty-five positive experiments eleven developed normal limbs, eight of which showed very imperfect function. Of the fourteen cases which reduplicated, only five exhibited any function. This consisted mostly of slight twitchings in the hand and in several cases slight movements of the forearm. The same was also true of the normal limbs.

By comparing series HS4 with series HS5 (table 1b), it is noticed that limbs of the latter series exhibited no better function than did those of the former series. The superior function of the limbs in the series HS3 to that in the aforementioned series is explicable on the grounds of the close proximity of the transplanted limb with the normal limb, which resulted in the additional limb's receiving some nerve supply from the normal plexus.

No cases of the series HS4 were sectioned. However, those of series HS5 which were sectioned showed that the main nerve supply came from segments corresponding to the position occupied by the transplanted limb; e.g., in case HS5₁₂ (fig. 22), which centered ventral to the eighth and ninth segments, the innervation was derived from these same segments of the cord. This observation is in accord with those made by Braus ('04, '05) and Harrison ('07) on anuran embryos, viz., that when the normal limbs are left intact the transplanted limb becomes innervated from that region of the cord corresponding to the position of the implanted limb rudiment.

No experiments were made to test the function of additional limbs transplanted the distance of six segments posterior to the normal intact limb. From the results obtained in the series HS4 and HS5 it appeared improbable that they should function to as great an extent as did those of the series above mentioned. Viewing these experiments as a whole, therefore, we see that the limbs functioned much more imperfectly than did the autoplasmic limbs transplanted to the same relative positions, and also that their innervation was derived from segments of the cord corresponding to the position of the implanted limb rudiment rather than from segments situated anterior to the position occupied by the limb. The significance of these points will be considered more fully in the discussion.

Regeneration

Although the position and extent of the limb mesoderm can be quite accurately located by means of certain landmarks, it

is not always possible to remove the entire rudiment in a typical limb extirpation. Owing to its equipotentiality, certain unrecovered cells then may be sufficient in number to regenerate the appendage after the bulk of the rudiment has been removed. This regeneration has been shown to be greater in wounds which have not been covered (Harrison, '15). In the series AS1, in which the limb rudiment was removed the distance of only one segment posterior to the normal position, the wounds were not covered, the denuded area being so small that it could not easily be covered. From the anterior border of these uncovered wounds there occurred twelve cases in twenty-five which showed signs of regeneration. These consisted of small nodules of cells which began to project from the body about four days after the operation. These regenerating nodules, however, were very close to the transplanted rudiment, being separated only by the distance of one segment. They gradually decreased in size, however, until the eighth day after the operation they entirely disappeared and in no case did regeneration occur.

In the series AS2, where the limb rudiment was removed the distance of two segments, only five of the wounds were uncovered. Here ten cases in twenty-one began to regenerate the limb from small nodules of cells at the anterior border of the wound, as in the series AS1. These nodules of regenerating cells also began to appear on the surface of the body about four days after the operation. Some of these nodules began to increase in size, so that on the fifth and sixth days regeneration appeared very probable; however, they soon began to decrease in size, and by the eleventh day all signs of regeneration had entirely disappeared. Although the initial regenerating nodules in this series were about as large as those in the series AS1, their total disappearance did not occur until three days after total disappearance of those in the series AS1.

In the series AS3, in which the limbs were removed by the distance of three segments, the wounds were cleaned and covered. In twenty-three experiments there occurred ten cases in which regenerating nodules appeared. Some of these subsided about the fifth and sixth days, while others had developed so that by

the tenth or eleventh day they were almost as large as the transplanted rudiment. They then began to undergo reduction in size, but did not finally disappear until eighteen to twenty days after the operation.

All regenerating nodules of cells which appeared in the series AS4 and AS5 completely regenerated into a normal appendage at the original site.

It is readily seen, therefore, from these observations that there is an inhibitory influence of the transplanted limb upon the unremoved portion of the rudiment which attempts to regenerate, so that the more remote the transplanted limb bud becomes, the longer the time required for total disappearance of the regenerating nodule of cells. Since no sections of these cell nodules were made and since it could not be ascertained from external observations, it was impossible to say whether the regenerating cells were actually drawn over into the transplanted limb bud or whether, lacking the necessary stimulus for growth, they underwent atrophy and were resorbed. However, no matter what influence the limb bud exerts on these regenerating nodules, it is no longer manifested when the transplanted bud is removed the distance of four segments, for in the series AS4 and AS5 when regenerating nodules of cells appeared they always completely regenerated an appendage.

DISCUSSION

When the anterior limb of *Amblystoma punctatum* is excised and reimplanted to the same embryo at distances ranging from one to seven segments posterior to the normal position (autoplastic transplantation), corresponding decrease in the function of such limbs occurs as they are implanted more and more remote from the normal situation. A position is finally reached (six segments posterior to the normal) in which all limbs exhibit imperfect function, there being no cases with perfect adaptive movements (table 1a and fig. 9). This gradual decrease in the function of the limbs seems to be directly correlated with the segmental nerve supply (table 2), the function of the limbs

being more perfect when innervated from the limb level of the cord.

It has hitherto been stated that in the tail-bud stage, the period of development in which the experiments were carried out, the anterior limb rudiment constitutes a region of somatopleural mesoderm extending from the anterior border of the third somite to the posterior border of the fifth. The normal anterior limb is supplied by a plexus composed of the third, fourth, and fifth spinal nerves (fig. 2). These nerves, effecting connection with the limb rudiment at a period when it occupies its maximum extent (anterior border of third somite to posterior border of fifth), become converged into a plexus as a result of concentration of the rudiment into the definitive limb bud which centers under the fourth myotome. Such convergence having taken place, a typical normal plexus as illustrated in figure 2 would be produced. In the normal position, the number of segments occupied by the limb rudiment apparently serves as an index of the number of segments which will contribute nerves to the appendage.

The results of homoplastic experiments carried out by Braus ('05) and Harrison ('07), as have already been pointed out, show that transplanted limbs also received innervation from segments corresponding to the extent and position of the rudiment. In the majority of their experiments, all of which were carried out on anuran embryos, the nerve paths were in part or totally laid down at the period when the rudiment was transplanted. In preparing the wound for the reception of the transplant, the peripheral ends of the nerves were severed and the rudiment placed in the direct pathway of the cut ends, which were found to continue their growth into the rudiment so placed. Any influence which the limb might have on the segmental nerve contribution and the direction of the nerve paths could not be adequately tested by these experiments; since, in order to do so, the rudiment would have to be transplanted at a period before initial outgrowth of the nerves begins. The results of the autoplastic limb experiments in *Amblystoma* show that other factors, in addition to the position and extent of the limb rudiment,

govern the innervation of the limbs, when transplanted at a period before initial outgrowth of the nerves begins.

Were this not so, then the limb rudiment, if displaced the distance of several segments should so effect the nerve contribution that the plexus ought to come from segments corresponding to the position occupied by the displaced limb rudiment. That is, by shifting the position of the limb bud the distance of two segments so that it lies ventral to the fifth, sixth, and seventh somites, there should be a corresponding change in the segmental nerve contribution so that the limb plexus should be derived from the fifth, sixth, and seventh nerves. However, in an examination of table 2 the strong tendency for the limb to receive innervation from the limb level is quite evident. In fact, it requires a shifting of the limb rudiment the distance of three segments (series AS3, table 2) before a change in the segmental nerve contribution can be at all effected. The results of these experiments strongly suggest that the transplanted limb bud exerts a guiding influence on the segmental nerve supply and determines the paths taken by the spinal nerves effecting its innervation. The results also tend to show that the positive reaction toward this influence is greater in the nerves developing from the normal limb level of the cord. Especially is this true of the fourth and fifth nerves, both of which were found to elongate in a posterolateral direction a greater distance to effect innervation than did the third nerve or those developing from segments of the cord posterior to the limb level. The remarkable posterior elongation of the nerves to innervate transplanted limbs is well illustrated in figures 10, 11, and 12. Figure 11 shows the segmental contribution to the plexus supplying the limb which was transplanted so as to center ventral to the eighth, ninth, and tenth somites. We find that this limb receives a considerable amount of its nerve supply from the fifth segment of the cord. The question arises: why should these nerves situated such a great distance anterior to the limb effect limb innervation? It is found that the nerves of the limb level do not grow out to the limbless area as they do upon simple extirpation of the limb, but almost immediately after their exit from the

vertebral foramina they begin to elongate posteriorly and in the general direction of the transplanted limb. It could easily be assumed that these nerves, not finding a sufficient number of muscles to accommodate the sum total of their fibers as they grow posteriorly, would so continue to grow until the limb muscles were reached and total connections were made. However, in the absence of any directing influence of the transplanted limb on these nerves, there is no apparent reason to expect that they should enter the limb. Further, why should not the outgrowing nerves corresponding to the segments occupied by the transplanted limb effect its sole innervation? For example, if a limb is transplanted so that its position corresponds to the eighth, ninth, and tenth segments, the outgrowing nerves corresponding to these same segments are closer to the limb rudiment than are those coming from segments of the cord situated anterior to the limb, yet those nerves situated anterior to the position of the displaced rudiment are the ones which contribute the main bulk of the nerve supply. Only by recognizing the presence of a guiding influence and a positive reaction of the nerves toward it, can the nerve supply to transplanted limbs be adequately explained at present. By the use of such an hypothesis we can explain why the nerves of the normal limb level, especially the fourth and fifth, will grow a longer distance posteriorly to effect limb innervation than those from more posterior segments. Such an explanation can only be made by assuming that the positivity toward this influence is greater at this level of the cord. The facts as shown in table 2 would seem to agree with such an assumption, for this table shows that the farther away from the normal level the limbs are transplanted, the less the tendency to be innervated from segments situated anterior to the position of the limb rudiment and the greater the tendency to be innervated by segments corresponding to the position occupied by the limb. The presence of such an influence as seems to be exerted by the autoplasmic limbs may be an expression of a mechanism whereby the anterior limbs, when set back a number of segments, can function coordinately with the opposite intact limb, for we have already seen that limbs transplanted poste-

riorly cannot function to any degree unless they receive innervation from the limb level or from segments of the cord just posterior to the limb level.

It thus seems likely that the influence bringing about the posterior elongation of the nerves from the limb level, in addition to certain probable conditions existing within the central nervous system (which will be discussed later on), provide a mechanism whereby the transplanted limbs may, within limits, perform functionally adaptive movements. One cannot help but be impressed with the fact that the peculiar posterior elongation of the nerves to the transplanted limb, as is shown in figures 10 and 11, is not the result of mere accident or the result of a non-directive agent. Especially is this true in those cases where the nerves, after having grown out posterolaterally to the body wall, will turn their course in a ventral direction and wind their way through a mass of muscle tissue to meet a limb more ventrally situated.

Although further experiments are necessary to prove or disprove definitely that the transplanted limb does so exert an influence on the outgrowing nerves as to determine the segmental nerve supply and the paths taken by them in the innervation of the limb, this hypothesis appears at the present time to be the one which most nearly fits the facts.

A brief explanation of the factors conditioning the function of the transplanted limbs has already been published (Detwiler, '19), but it will be necessary to discuss this point again.

It has previously been shown (table 2 and fig. 9) that as the transplanted limbs receive less and less innervation from the normal limb level of the cord, there occurs a corresponding decrease in the ability of the limbs to exhibit movements coördinated with those of the opposite intact limb, so that a position is finally reached (six segments posterior to the normal) in which the limbs receive no segmental contribution anterior to the eighth segment of the cord and show nothing more than greatly impaired movements in response to tactile stimulation. This gradual decrease in the function of the limbs as they become implanted more and more remote from the normal limb region, depends

upon a number of factors outside the structure of the limb itself. Limbs transplanted only a short distance from the normal situation have been found, in general, to grow slightly larger than those removed farther away; however, the degree of structural deficiency within the limb itself is not strictly proportional to the distance from its normal position. The limb being an equipotential system, it is to be expected that complete structural differentiation, exclusive of vascularization and innervation, should not be effected by location.

All limbs classed in the category 'normal limbs,' which was based on purely topographic completeness, were also found to be for the most part structurally complete. Hence, it is seen that the gradual loss of function in limbs more remotely removed is in no wise the result of structural deficiencies. The gradual decrease, therefore, in the function of limbs as they become implanted more and more remote from the normal limb region suggests the following possible factors conditioning the degree of function: *a)* structural deficiencies in the shoulder-girdle; *b)* deficiencies in the shoulder musculature; *c)* the failure of certain shoulder muscles to receive innervation, and, *d)* the absence of proper central neurone connections.

In a typical limb-bud transplantation the major portion of the shoulder-girdle rudiment is included. Since it is difficult to excise and transplant always exactly the same region of mesoderm and since the girdle rudiment in *Amblystoma* constitutes a mosaic (Detwiler, '18), there is considerable variation in the degree of its development in the heterotopic position. Yet its development, in general, is no less complete in cases where the limb has been removed a considerable distance from the normal situation than in those where it has been removed only a short distance. An examination of table 4 shows that the heterotopic girdles exhibit no specific deficiencies which can be correlated with the distance they have been transplanted from the normal situation. Hence, the gradual loss of function which the limbs exhibit as they are farther and farther removed cannot be the result of corresponding shoulder-girdle deficiencies. It is found, however, that girdles developing close to the normal

situation are likely to be somewhat larger than those developing more posteriorly, a condition very likely due to the fact that in the former position there is more room for development. With the development of larger girdles, of course, there would be a corresponding development of larger muscles which would permit of slightly greater shoulder movements than in the more posterior positions where the shoulder-girdle is not quite so large; but the difference in the degree of function of the limbs could not be a result of this factor alone, for girdles developing the distance of four segments posterior to the normal position are no larger than those developing six segments posterior to it, yet the difference in the degree of function of the limbs in these two positions is considerable, as is shown in table 1 a.

Another factor, which must be considered when dealing with function, is the differentiation of the shoulder muscles, since movements of the limbs cannot be perfectly performed unless shoulder-muscle differentiation is complete. A study of the cross-section anatomy of the shoulder region of the transplanted limbs shows that shoulder-muscle differentiation is no less complete in cases where the limbs are removed considerable distances than in those where they are only slightly removed from the normal situation. An examination of table 3 shows that no cases were found in which there was complete absence of any of the shoulder muscles that typically develop in the heterotopic position. So, in limbs transplanted considerable distances away from the normal position and which exhibit practically no function, a skeletomuscular mechanism exists adequate for all movements of the arm on the shoulder; hence, it is necessary to look elsewhere for the factors governing function of the limbs.

This brings up the third factor, that of peripheral efferent innervation to both limb and shoulder muscles, deficiencies in which would readily account for restricted movements. The result of this study shows that the peripheral innervation to shoulder and limb is somewhat less complete quantitatively when transplanted to the more posterior positions than in cases where the transplanted limb receives segmental nerves from all or only a portion of the normal limb level of the cord, and to a

certain degree somewhat less developed qualitatively as is seen by an examination of table 3. This would naturally account for considerable deficiencies of movement, yet by an examination of individual cases it is found that the degree of motion is not directly correlated with the number of muscles innervated, nor with the number of segments contributing nerve fibers. For example, case AS4₂₇ with only four of the nine muscles innervated showed a considerable degree of shoulder movement. In case AS5₂₇ with an equal number of muscles innervated (table 3), there was practically no shoulder movement. The same was true in case AS5₂₆.

Further evidence which shows that the completeness of shoulder movement is not entirely dependent upon the number of muscles innervated is afforded by an examination of homoplastic limbs transplanted the distance of four and five segments posterior to the normal intact limb of the host. For example, in case HS5₁₂ (fig. 22) all of the shoulder muscles received nerve fibers, yet practically no function was observed.

That the degree of function of the limbs cannot be a result of the number of segments supplying nerves to the brachial plexus is also shown by the fact that the function of the limbs is more complete in some cases where only three segmental nerves contribute to the plexus than in others where four nerves are involved, even though the number of shoulder muscles innervated in both cases is the same. Of course, the number of segmental nerves contributing to the brachial plexus is not as important as the actual number of nerve fibers entering the shoulder muscles and the limb. However, it is practically impossible to count the number of nerve fibers in a given segmental nerve, since in larvae of the age studied the fibers are non-myelinated and cannot be counted with any degree of accuracy. Although the segmental nerves contributing to the innervation of the transplanted limb were larger than their counterparts which do not supply a corresponding end organ, the increase in size was found to be the result of a hyperplasia of the peripheral afferent neurones, there being no positive evidence that the functional activity of the transplanted limb had brought about any definite hyperplasia of the somatic motor neurones.

The increase in the number of sensory neurones was determined by counting the number of cell bodies in the spinal ganglia. Attempts to count the anterior horn cells were not successful, inasmuch as the anterior horn areas are not well differentiated at the age in which the larvae were studied and no accurate differentiation could be made between the anterior horn cells and the non-nervous cells (spongioblasts). The question dealing specifically with the effect of continued function of the limb upon the development of peripheral neurones, both afferent and efferent, will be discussed more fully in a later publication. However, from a complete survey of the peripheral innervation, both from the standpoint of the number of muscles innervated and the amount of nerve contribution, it is highly improbable that the defective peripheral innervation can account for the great degree of imperfect movements that are exhibited by limbs in the series AS5 and AS6.

The remaining factor, therefore, viz., defective connections within the central nervous system, appears to be the only one which will adequately account for the marked deficiency of function in limbs transplanted so far posteriorly as to be beyond the point where they will receive peripheral innervation from all or only a portion of the normal limb level of the cord.

Although in normal larvae of this age the most obvious motor responses to various types of peripheral stimulation consists of total swimming reactions, under certain controlled conditions motor responses may be almost entirely limited to coördinated movements of the limb. Such responses may be carried out perfectly by the transplanted limbs when their peripheral innervation is derived from the normal limb level of the cord, but the ability to exhibit movements coördinated with those of the opposite intact limb decreases markedly when their peripheral innervation is derived from segments well beyond the normal limb level (series AS6).

As has been shown by Herrick ('14), we have here a central nervous architecture by means of which peripheral sensory stimuli pass through more or less localized ascending sensory tracts from the cord to the medulla (tractus spinobulbaris); to the mid-

brain (tractus spinotectalis), and to the thalamus (tractus spinothalamicus). These stimuli may become finally discharged into the somatic motor centers of the spinal cord by means of descending tracts such as the tractus thalamobulbaris, tractus tectobulbaris, the fasciculus longitudinalis medialis, and the tractus bulbospinalis. According to Herrick (op. cit.), the cell bodies of the tractus bulbospinalis lie in the general motor tegmentum of the medulla and their axones are directed ventrally into the ventral funiculi of the same and opposite sides. It is highly probable that a certain number of these fibers normally develop only as far as the third, fourth, and fifth segments of the cord for specific discharge into the normal appendicular somatic motor centers. The fact that transplanted limbs, which receive peripheral innervation from these levels, do exhibit movements coördinated with the opposite intact limb strongly suggests such a condition. The behavior of limbs innervated mainly from the sixth, seventh, and eighth segments of the cord (series AS5) suggests that these descending neurones, which normally end in the limb level, may be induced to continue their growth an additional segment or two in order to meet the functional demands imposed upon them by the transplanted limb. Their incapacity for further functional regulation is suggested by the loss of coördinated function and the greatly impaired movements such as are exhibited by limbs of the series AS6 (table 1 a)—probably none of which receive peripheral innervation from segments of the cord anterior to the eighth (table 2).

The increase in the number of cases with total loss of function as the limbs are implanted more and more posteriorly (table 1 a and fig. 7) would also suggest that, in addition to an inadequate supply of descending neurones, there probably occurs a corresponding increase deficiency in the connections of purely intraspinal correlation neurones.

Additional homoplastic limbs transplanted respectively three, four, and five segments posterior to the normal intact limb of the host (table 1 b), never attain the completeness of function attained by autoplastic limbs transplanted to the same relative positions. Such limbs may be well supplied with peripheral

nerves derived from segments of the cord posterior to the normal limb level, so that the restricted function which they do exhibit cannot be the result of a lack of peripheral innervation. It appears, therefore, highly probable that the greatly impaired movements which these additional limbs exhibit are conditioned by a lack of supply of central efferent neurones. As has been stated above, there are probably a certain number of these descending neurones belonging to the tractus bulbospinalis which develop only as far as the normal limb level, where they discharge into the normal appendicular somatic centers. Assuming this to be true, the greatly restricted movements of the additional limb would readily meet an explanation on this basis; for, these descending neurones being taken up by the peripheral neurones of the normal limb, the movements of the additional limbs must very likely be effected through more or less imperfectly connected intraspinal, intersegmental correlation neurones of the levels from which peripheral innervation is derived.

SUMMARY

1. When the anterior limb of *Amblystoma* is excised and re-implanted to the same embryo at distances ranging from one to seven segments posterior to the normal position (autoplastic transplantations), there occurs a corresponding decrease in the function of such limbs as they are implanted more and more remote from the normal situation (table 1 a).

2. Shifting the position of the limb a given number of body segments does not effect a corresponding shifting of the segmental nerve contribution to the plexus. There is a marked tendency for the transplanted limb to receive innervation from the normal limb level of the cord (table 2 and figures 10 and 11).

3. Limbs removed so far posterior to the normal situation as to receive no innervation from the normal limb level of the cord received the main bulk of their nerve supply from segments situated anterior to the transplanted limb rather than from segments corresponding to the position occupied by the limb (table 2).

4. The gradual decrease in the function of the limbs as they become implanted more and more remote from the normal limb region seems to be directly correlated with the segmental nerve supply, the function of the limbs being more perfect when innervated from the limb level of the cord.

5. Transplanted limbs receiving less and less innervation from the normal limb level of the cord show a corresponding decrease in their ability to exhibit movements coördinated with those of the intact limb of the opposite side.

6. The gradual loss of function of limbs, as they become transplanted farther and farther away from the normal situation, is attributed to increased defective connections within the central nervous system rather than to a corresponding decrease in effective peripheral innervation and structural deficiencies of the limb and the shoulder-girdle.

7. In general, as the distance between the normal position and the transplanted limb is increased, a corresponding increase occurs in the time required for initial limb reflexes to appear. Limbs in which the appearance of initial reflexes is considerably delayed are less apt to attain perfect function.

8. The number of segments occupied by the transplanted limb rudiment does not determine the number of segmental nerves contributing innervation to the limb.

9. The results of the experiments suggest that the transplanted limb rudiment exerts a guiding influence on the segmental nerve contribution and determines the path taken by the nerves effecting innervation of the limb. The positive reaction toward this influence appears to be greater in the nerves coming from the normal limb level of the cord.

10. Regardless of the segmental nerve contribution, the architecture of the nerve distribution within the transplanted limb is exactly the same as that in the normal limb. These results corroborate those of Braus ('04, '05) and Harrison ('07).

11. Gradually increasing the distance between the normal position and the transplanted limb brings about a gradual increase in the number of reduplicated appendages.

12. The non-adaptiveness of reduplication is shown by the fact that a functional condition frequently arises which is independent of the harmony of the combination. The results accord with those of Harrison.

13. Additional anterior limb rudiments transplanted a given number of segments posterior to the normal intact limb of the host (homoplastic transplantations) never attain the completeness of function attained by autoplastic limbs transplanted to the same relative position (tables 1 a and 1 b). The increased deficiency in function when compared with the autoplastic limbs is attributed to greater defective connections within the central nervous system rather than to increased defects in the peripheral innervation.

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PLATES

EXPLANATION OF PLATES

All of the figures in plates 1 and 2 are photographs of living specimens. The animals were anaesthetized by chloretone (1:3000), and photographed under water.

PLATE 1

EXPLANATION OF FIGURES

13 Ventral view of case AS4₂₆, photographed fifty-two days after the operation, showing the right anterior limb implanted the distance of four segments posterior to the normal position. $\times 2$.

14 Dorsal view of case AS4₁₂, showing reduplication of the anterior limb which was transplanted the distance of four segments posterior to the normal position. The anterior (disharmonic) member of the reduplication has become functional. The posterior member failed to function and has undergone considerable atrophy.

15 Dorsal view of case AS5₂₇. Right anterior limb transplanted the distance of five segments posterior to the normal position. Animal preserved sixty-five days after the operation. $\times 2\frac{1}{4}$.

16 Dorsal view of case AS5₂₅. Right anterior limb transplanted the distance of five segments posterior to the normal position. Animal preserved sixty-three days after the operation. $\times 2\frac{1}{2}$.

17 Ventral view of case AS5₂₅.

18 Ventral view of case AS5₂₇.

PLATE 2

EXPLANATION OF FIGURES

- 19 Ventral view of case AS3₉₀. Right anterior limb transplanted the distance of three segments posterior to the normal position. Animal preserved sixty-six days after the operation. $\times 4$.
- 20 Ventral view of case AS6₃₉. Right anterior limb transplanted the distance of six segments posterior to the normal position. Animal preserved sixty-nine days after the operation. $\times 4$.
- 21 Dorsal view of case HS4₇. Additional anterior limb transplanted the distance of four segments posterior to the normal intact limb of the host. Animal preserved seventy-five days after the operation. $\times 3$.
- 22 Dorsal view of case HS5₁₂. Additional anterior limb transplanted the distance of five segments posterior to the normal intact limb of the host. Animal preserved seventy days after the operation. $\times 4$.