FURTHER STUDIES UPON THE NERVE SUPPLY AND FUNCTION OF SUPERNUMERARY GRAFTED LIMBS

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Introduction

Evidence has accumulated from former experiments to show that the anterior limb rudiment of *Amblystoma,* when grafted four segments caudal to the normal position (autoplastic grafts), may receive one or more of the normal brachial (third, fourth and fifth) nerves. Typically, the grafted limbs are supplied by the fifth (brachial), sixth and seventh nerves. The coördinated activities which such limbs display have been shown to be due to their connection with the normal brachial reflex mechanism through the fifth nerve, for when this nerve is sectioned, leaving the sixth and seventh intact, coördinated activities cease (Detwiler and Carpenter, 1929).

It has been reported also that, when the anterior limb is left intact and an additional forelimb is grafted just caudal to the intact normal (homoplastic graft), the graft never receives any of the normal brachial nerves (third, fourth and fifth), and its function is defective and uncoördinated (Detwiler, 1920, 1925). The supernumerary limb under such conditions was found to be innervated most frequently by the sixth, seventh and eighth spinal nerves (Fig. 1), a fact which explains the non-coördinated motility, since it has been shown that some connection with the brachial region of the cord is essential for coördinated movements.

When the anterior limb rudiment is grafted the distance of four segments caudal to the normal site (autoplastic graft) but under conditions allowing for regeneration of a limb in the orthotopic position; the brachial nerves may become redistributed to both limbs. Typically the fifth grows caudally to the graft, whereas the third and fourth supply the orthotopic regenerant. The characteristic caudal growth of the fifth nerve to the graft has been interpreted as a growth response to an attractive influence which is apparently stronger in the rapidly growing grafted appendage than in the regenerating one (Detwiler, 1925, p. 486).

In the earlier experiments, as reported above, it was found that supernumerary limbs, when grafted just caudal to the normal intact limb, did not receive any nerves from the normal brachial region of the cord. It was thus concluded that the presence of the normal limb, by reason of its proximity to the growing brachial nerves, and the attrac-

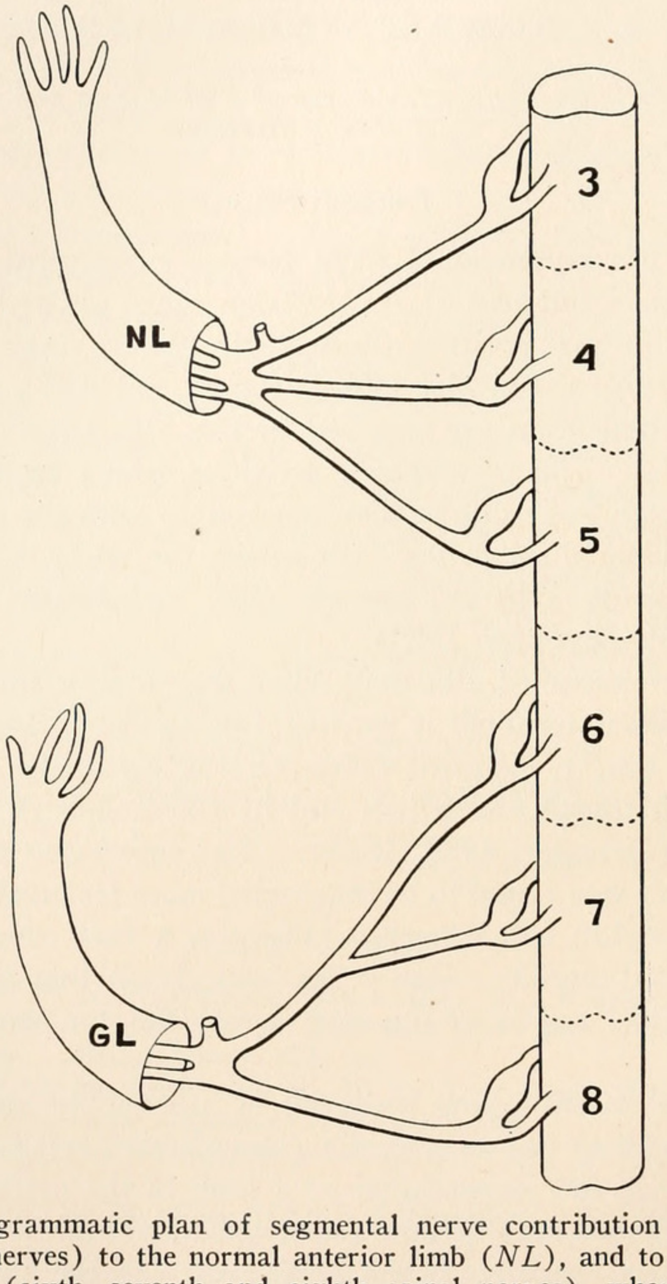


FIG. 1. Diagrammatic plan of segmental nerve contribution (third, fourth and fifth spinal nerves) to the normal anterior limb (*NL*), and to a supernumerary limb (*GL*) (sixth, seventh and eighth spinal nerves), when grafted four segments caudal to the normal limb position.

tive influence which it apparently exerts, is sufficient to prevent these nerves from supplying any additional rudiment implanted in the adjacent field. These conclusions have become subject to modification, however, as a result of recent experiments of the same type, since we have obtained a number of cases with coördinated activities in supernumerary limbs grafted close to the normal, and the coördinated function has been shown again to be due to the connection of the graft with the brachial correlation mechanism through the fifth (brachial) nerve.

Experimental

During the operating season in 1928 we performed fifty experiments in which a supernumerary limb rudiment was grafted, with normal (dorso-dorsal) orientation, caudal to the intact rudiment so that the graft centered ventral to the eighth somite. This brings the center of the graft just four segments caudal to the center of the normal limb disc, and a space approximately the width of one somite separates the two rudiments (Fig. 3). The operations were performed upon embryos in the tail-bud stage (Harrison's stages 28, 29).

The results of the experiments which are given in Table 1, *A,* show

Table I

Showing Results of Grafting a Supernumerary Right Anterior Limb Rudiment Four Segments Caudal to the Normal Position (Figs. 3 and 5)

	Orientation	
	A	B
	Normal (Dorso-dorsal)	Inverted (Dorso-ventral)
No. of Operations	50	60
Positive Cases	48	58
Single Limbs	38	42
Reduplications	10	11
Abortive Limbs	2	16
Limbs with Coördinated Function	7	1

a higher percentage of single limbs than is usually obtained in heterotopic dorso-dorsal grafts. Whereas most of the grafted limbs, whether single or reduplicated, gave evidence of function upon tactile stimulation, there were seven cases in which the grafted appendage functioned in coördination with the normal limbs. In such cases the movements in the graft and in the intact limb of the same side were homologous and synchronous. This phenomenon has been observed and discussed previously (Detwiler, 1925, Weiss, 1924, 1928).

Six of the seven cases exhibiting coördinated activities of the grafted limb were sectioned serially for a study of the nerve supply. The results of this study are given in Table II, *A,* where it can be seen that

TABLE II *

Showing Segmental Nerve Supply to Supernumerary Limbs Grafted Caudal to the Right Intact Anterior Limb (Figs. 2, 3, 4, 5)

Group	Case	Orientation of Graft	Distance Caudal to Normal Limb Site	Segmental Nerve Supply to Normal Limb	Segmental Nerve Supply to Supernumerary Grafted Limb
A	4	Dorso-dorsal	4 Segments	3, 4,	5, 6
	5	" "	4 "	3, 4	5, 6, 7
	7	" "	4 "	3, 4, 5	5, 6, 7, 8
	43	" "	4 "	3, 4	5, 6
	49	" "	3 "	3, 4	5, 6
	50	" "	4 "	3, 4, 5	5, 6, 7
B	12	Dorso-ventral	3½ Segments	3, 4, 5	6
	24	" "	2 "	3, 4, 5	6
	25	" "	4+ "	3, 4, 5	7, 8
	28	" "	3 "	3, 4, 5	5, 6
	39	" "	4 "	3, 4, 5	6, 7, 8, 9
	42	" "	3+ "	3, 4, 5	6

* The normal brachial plexus is made up of the third, fourth and fifth spinal nerves.

in every case the supernumerary grafted limb received contribution from the fifth (brachial) nerve. In four of the six cases the entire fifth nerve grew caudally to the graft, whereas in the other two cases the fifth nerve bifurcated so that both normal and grafted limbs were supplied by this nerve. A graphic reconstruction of the segmental nerve contribution to the normal limb and the grafted limb in cases 7, 49 and 50 are shown in Figs. 6, 7 and 8 respectively.

It is to be noted also from the table that in case 49, the final position of the grafted appendage was only three segments caudal to the normal intact limb.

With these unlooked-for results, we carried out during the operating season of 1929, sixty experiments in which the limb was grafted to the same position, but with inverted (dorso-ventral) orientation (Fig. 5 and Table I, *B*). Since the bulk of the limb tissue is concentrated in the antero-dorsal quadrant of the limb disc (Fig. 3), it becomes apparent that when the graft is inverted, the distance between the outgrowing fifth nerve and the concentrated mass of limb-forming cells is considerably increased as compared with the conditions in dorso-dorsal orientations (*cf.* Figs. 3 and 5).

The question arose as to whether under these circumstances (with inverted discs) the fifth nerve would grow caudally to the graft as frequently as in the experiments with dorso-dorsal orientation.

The results of the experiments are given in Table I, *B*. In 58 positive experiments there were six cases in which the graft was capable of considerable function, but only one case with definite coördinated movements.

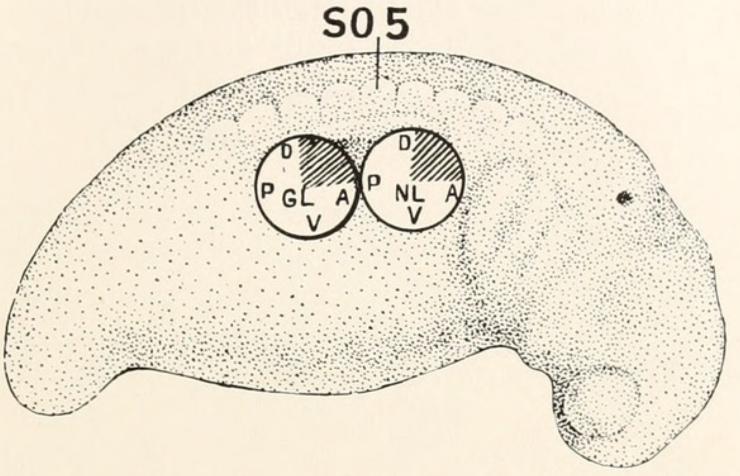


FIG. 2. Drawing of *Amblystoma* embryo, showing supernumerary anterior limb rudiment (*GL*) grafted (with normal orientation) so as to center ventral to the seventh somite. *NL,* normal anterior limb rudiment; *D,* dorsal; *V,* ventral; *P,* posterior; *A,* anterior; *SO*5, fifth somite. The shaded regions indicate the quadrants of the rudiments in which the main mass of the limb-forming cells is concentrated. × 12.

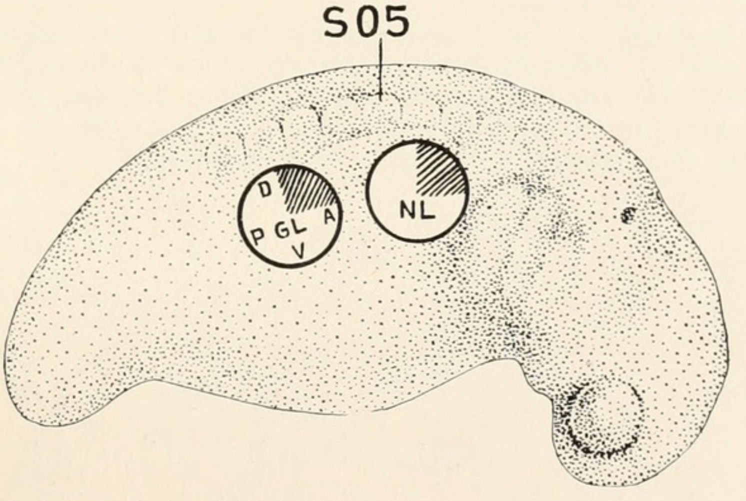


FIG. 3. Drawing of *Amblystoma* embryo showing supernumerary limb (*GL*) grafted (with normal orientation) so as to center ventral to the eighth somite. *NL,* normal anterior limb rudiment; *SO*5, fifth somite. × 12.

The results of a study of the nerve contribution to the six cases selected are given in Table II, *B*. Here it is seen that the one case (No. 28) which definitely displayed coördinated movements, received a branch of the fifth nerve. A graphic reconstruction of this nerve showing its contribution to both limbs is given in Fig. 9. In all of the other cases sectioned, the fifth nerve followed its typical course to the normal intact limb.

The distance between the fully differentiated transplant and the normal limb varied much more in this series than in the dorso-dorsal grafts. This is shown in Table II, *B,* in which it is seen that the final position of the grafted limb varied from two to four segments caudal to the normal. In spite of the fact that in four of the six cases the grafted limb was less than four segments caudal to the normal, only one received contribution from the fifth nerve (case 28).

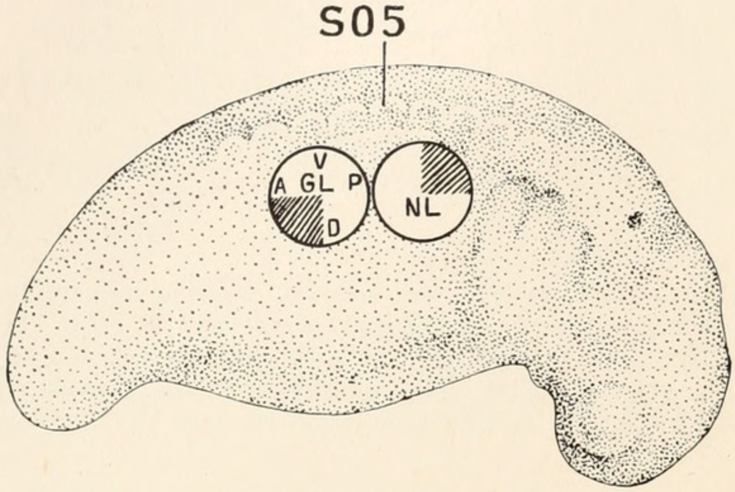


FIG. 4. Drawing of *Amblystoma* embryo showing supernumerary limb rudiment (*GL*) grafted (with inverted orientation) so as to center ventral to the seventh somite. The original antero-dorsal quadrant (shaded quadrant), which contains the bulk of the limb-forming cells, is brought into a postero-ventral position. *SO*5, fifth somite; *NL,* normal limb rudiment. × 12.

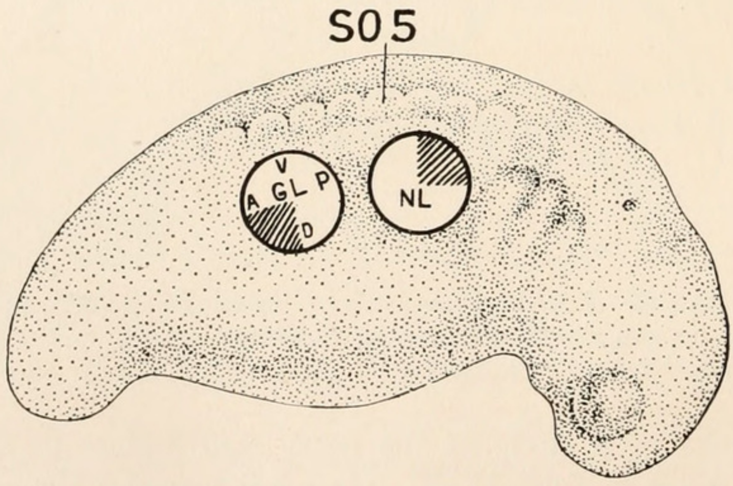
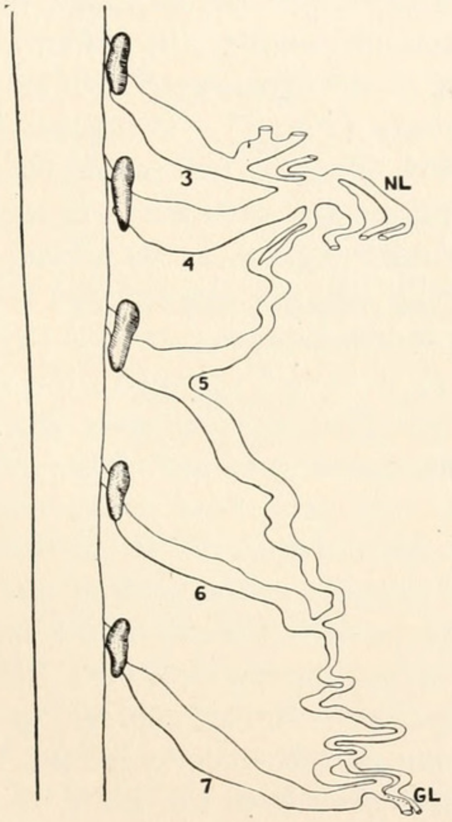


FIG. 5. Drawing of *Amblystoma* embryo showing supernumerary limb rudiment (*GL*) grafted (with inverted orientation) so as to center ventral to the eighth somite. *SO*5, fifth somite; *NL,* normal limb rudiment. × 12.

Although we did not study the nerve supply to any of the heterotopic limbs of the dorso-dorsal series which lacked coördinated function, we feel safe in assuming that such limbs were innervated entirely from the postbrachial region of the cord. In all previous experiments it has been found that whenever grafted limbs exhibit movements which are not coördinated with those of the normal intact limbs, they lack connection with the brachial reflex mechanism (Detwiler, 1920, 1925; Detwiler and Carpenter, 1929).

Discussion

The results of the experiments cited in this paper differ from those described in former communications (Detwiler, 1920, 1925) in showing that, when a supernumerary anterior limb bud is grafted just caudal to the normal intact limb rudiment, the most caudal of the normal brachial nerves (fifth) may (in some cases) be taken over by the graft rather



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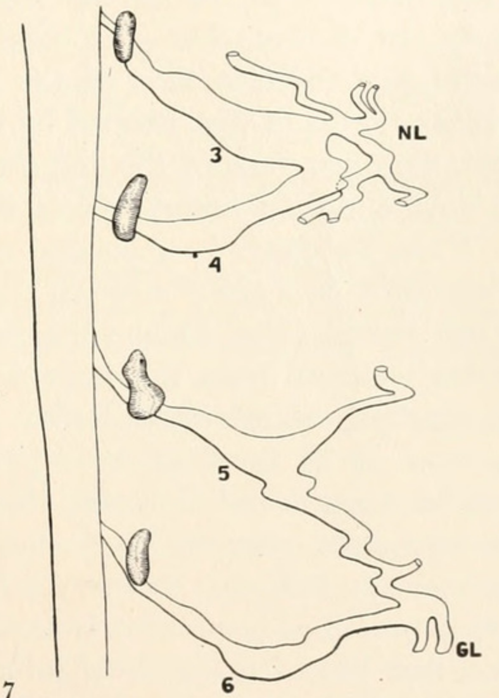

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FIG. 6. Graphic reconstruction of the segmental nerve supply to the normal limb (*NL*) and the grafted limb (*GL*) in case *HS*4–7. A supernumerary anterior limb rudiment was grafted (with dorso-dorsal orientation) so as to center ventral to the eighth somite (Fig. 3). $\times 20$.

FIG. 7. Graphic reconstruction of the segmental nerve supply to the normal limb (*NL*) and the grafted limb (*GL*) in case *HS*4–49. A supernumerary limb was grafted (with dorso-dorsal orientation) so as to center ventral to the seventh somite (Fig. 2). $\times 20$.

than by the normal (Table II). This has occurred almost exclusively in limbs with dorso-dorsal orientation rather than in those in which the limb was inverted. In order to account for this difference, we have tentatively advanced the hypothesis that a stronger attractive influence upon the fifth (brachial) nerve is exerted by the graft when normally oriented, since, under these conditions, the bulk of the limb-forming cells in the grafted disc is closer to the growing fifth nerve than when the disc is inverted (Figs. 2 and 3; *cf.* Figs. 4 and 5).

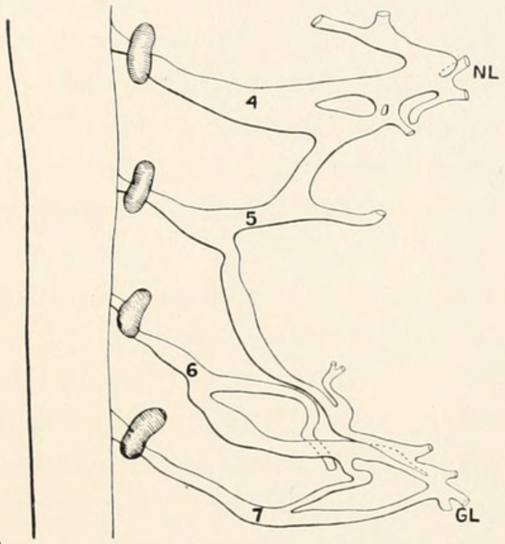
In the normal limb disc which extends from the anterior border of the third somite to the caudal border of the fifth, the cells may be said to be concentrated chiefly in the antero-dorsal quadrant (Fig. 2), even though the postero-dorsal quadrant has been shown by Swett (1923) to form a definite part of the limb. The antero-ventral and the postero-ventral quadrants contribute only very slightly to the limb (Swett, *op. cit.*).

When an additional rudiment is grafted just caudal to the normal so that it centers ventral to the seventh somite, the antero-dorsal quadrant of the graft is about as close to the outgrowing fifth nerve as is this quadrant in the normal appendage (Fig. 2). Consequently, as far as the distance factor is concerned, it seems not unreasonable to assume that the attraction of the graft on the fifth nerve is approximately as great as that exerted by the normal since, in the normal rudiment, the main mass of the limb-forming cells are concentrated beneath the third and the anterior half of the fourth somite.

When the graft is implanted so as to center ventral to the eighth somite there is a space between the two discs approximately the width of one somite (Fig. 3) and the antero-dorsal quadrant of the graft is further removed from the outgrowing fifth nerve than the corresponding quadrant of the intact limb. In spite of this, we found that in 7 cases out of 48 the fifth nerve grew caudally to the grafted limb. It must be remembered, however, that in the early growth of the embryo, the myotomes elongate in a caudo-lateral direction, which tends to mechanically influence the nerves to pursue a similar course, especially in their proximal portions. It seems not unreasonable to assume, therefore, that the fifth nerve, in its caudal growth to the graft, is aided initially by the characteristic caudal elongation of the myotomes. Only a very short growth in this direction would bring the growing nerve under the attractive influence exerted by the antero-dorsal quadrant of the graft. This is then apparently sufficient to insure its further growth to the grafted limb.

That the attraction of the normal limb rudiment, however, is typically greater is evident by the fact that in the majority of the cases, the fifth nerve supplies the orthotopic rather than the heterotopic appendage.

When the graft is inverted, its original antero-dorsal quadrant is brought into a ventro-caudal position and the distance between the concentrated mass of limb-forming cells and the outgrowing fifth nerve is greatly increased as compared with the distance in the dorso-dorsal orientations (*cf.* Figs. 4 and 5 with 2 and 3). It has been noted also



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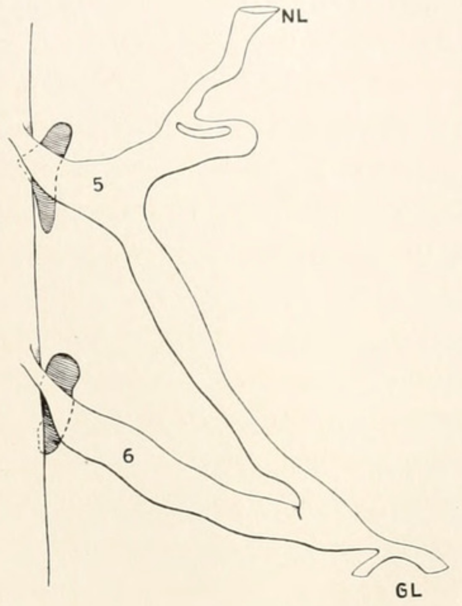

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Fig. 8. Graphic reconstruction showing segmental nerve supply to the normal limb (*NL*) and the grafted limb (*GL*) in case *HS*4–50. Conditions same as described for Fig. 6. × 20.

Fig. 9. Graphic reconstruction showing segmental nerve contribution to the grafted limb (*GL*) in case *HS*4–28. A supernumerary limb was grafted (with inverted orientation) so as to center ventral to the seventh somite (Fig. 4). × 20.

from Table I that in the dorso-ventral series only one case was obtained in which the graft developed coördinated activities and in this case it was supplied by the fifth nerve (case 28, Table II). In many cases in this series, the graft was closer to the normal limb than in the dorso-dorsal series, yet no connection was made with the fifth nerve. It appears, therefore, that the failure of this nerve to supply the graft in the dorso-ventral combinations is associated with a more remotely located center of attraction as compared with the dorso-dorsal grafts.

Although the interpretation which we have pictured is largely hypothetical for the present results, we do have considerable evidence from former experiments (Detwiler, 1920, 1922, 1925) to indicate that the limb rudiment does attract spinal nerves, and it seems not unreasonable to suppose that when the grafted limb is inverted, as in the present experiments, the distance between the outgrowing fifth nerve and the bulk of the limb-forming cells is so greatly increased, that this nerve falls entirely under the influence of the more closely situated attraction center represented by the normal limb.

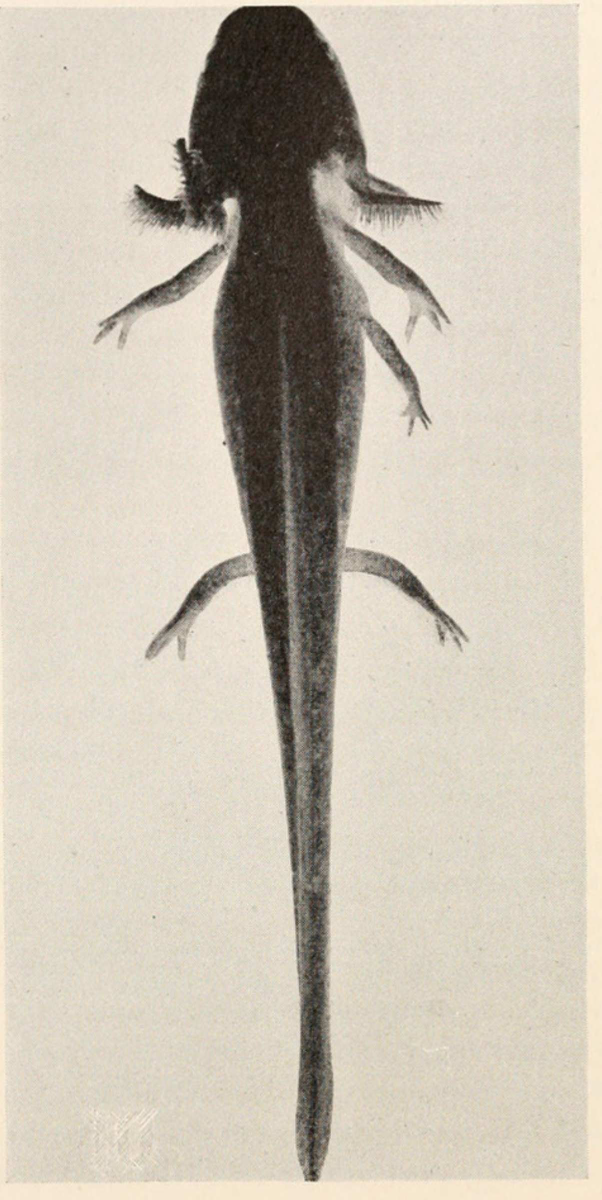

FIG. 10. Photograph of *Amblystoma* larva *HS*4–37 showing supernumerary limb grafted four segments caudal to the intact normal limb. ×2.7.

SUMMARY

1. When a supernumerary anterior limb disc is grafted (with normal orientation) caudal to the intact rudiment, so as to center ventral to the seventh or eighth somites (Figs. 2, 3), the fifth nerve, which normally supplies the orthotopic limb, may grow caudally to the graft. This has been found to occur in seven out of forty-eight cases. The entire nerve may be taken over by the graft (Fig. 7) or it may bifurcate so as to send one branch to the normal and the other to the supernumerary limb (Figs. 8, 9).

When the graft is supplied by the fifth nerve its activities are coördinated with those of the normal limbs. The movements of the muscles in the grafted appendage are synchronous with those in the homologous muscles of the normal limb of the same side.

2. When the supernumerary limb disc is grafted to the same position, but with inverted orientation (dorso-ventral, Figs. 4, 5), there is a greatly reduced number of cases in which the fifth (brachial) nerve supplies the graft. In fact in only one case in fifty-eight was this condition obtained.

3. The greater number of cases with brachial nerve contribution (fifth nerve) to the supernumerary limb in the experiments with normally oriented (dorso-dorsal) grafts, as compared with those which have been inverted (dorso-ventral), is interpreted as due to the fact that a stronger attraction is exerted upon the fifth nerve. The bulk of the cells in the limb disc is concentrated in the antero-dorsal quadrant (Fig. 2). When the graft is inverted, the antero-dorsal quadrant occupies a caudo-ventral position and the distance between the outgrowing fifth nerve and the concentrated mass of limb cells is thus considerably increased (*cf.* Figs. 4, 5 with 2 and 3). Any attractive influence of the graft upon the fifth nerve, therefore, cannot be realized by reason of the distance factor.

4. More evidence has accumulated in the present experiments to bear out former conclusions (Detwiler, 1925, Detwiler and Carpenter, 1929) that when grafted anterior limbs exhibit movements which are coördinated with those of normal intact anterior limbs, they have some connection with the central brachial reflex correlation mechanism.

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