Transection of the Spinal Cord in Developing *Xenopus Laevis*

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*WITH ONE PLATE*

**INTRODUCTION**

The literature on regeneration in the central nervous system of vertebrates has been reviewed exhaustively by Windle (1955, 1956). Adult fish and urodeles re-establish physiological and anatomical continuity of the spinal cord after it has been completely transected while adult anurans (Piatt & Piatt, 1958) and mammals on the whole do not. In all groups of vertebrates regeneration is more successful in the period of early embryonic development, and becomes less so as development proceeds. Experiments designed to investigate the factors responsible for this change demand an animal in which the difference in the regenerative capacity of embryonic and adult form is marked, and all stages of development are easily accessible for operative procedures. These criteria are satisfied by Anura. For this reason regeneration in the anuran central nervous system merits further investigation. After spinal cord transection in urodele larvae, Piatt (1955) found that the Mauthner axons did not regenerate although other axons around them did. This suggests that the regeneration of the axons was controlled by factors which were independent of the local changes occurring consequent to the trauma. The fundamental nature of this hypothesis justifies more experiments to test it. Larvae of *Xenopus laevis* are especially suitable for such experiments as in the ventral white columns of their spinal cord are large descending axons of primary motor neurones as well as the Mauthner axons. The large axons of the primary motor neurones are almost as big as the Mauthner axons (Hughes, 1959). Also, Piatt (see Windle, 1955) has pointed out that observations on the behaviour of the Mauthner axons after spinal cord transection in Anura are lacking.

Hooker (1915, 1925) showed that physiological and anatomical regeneration followed transection of the spinal cord in frog larvae at all stages of development. Lorente de Nó (1921) found that regeneration occurred after cutting partly across the spinal cord of frog larvae 20–35 mm. long. Recently Piatt & Piatt (1958) showed that regeneration did not take place after transection of the spinal
cord in adult *Rana pipiens*. It was decided to test the capacity for regeneration in *Xenopus* larvae at a late stage of development so that it would be unlikely to occur. A preliminary experiment showed that the latest stage at which larvae would survive after cord transection was stage 56. Metamorphosis progresses rapidly after this stage and it appeared that when the operation was performed on older larvae they died because their respiratory requirements could not be satisfied by their skin and regressing gills.

**METHODS**

Amplexus and ovulation were induced in adult *Xenopus* with chorionic gonadotrophin. The larvae were reared in tapwater in large plastic bowls which were kept in a cupboard maintained at a temperature of 20° C. and controlled by a thermostat. They were fed with a suspension of nettle powder in water. The methods used to rear and stage the animals were those recommended by Nieuwkoop & Faber (1956).

The operation was performed on animals at stage 56. They were anaesthetized with a 1:5,000 solution of MS 222 (Sandoz). A dorsal midline incision was made through the skin and carried down between the axial musculature of the two sides as far as the neural arches. Then the spinal cord and dorsal part of the notochord were divided between the fifth and sixth neural arch. The scar on the notochord was used as evidence that transection of the spinal cord was complete. Postoperatively it was found essential to aerate the water of the operated animals to keep them alive. They were not able to swim to the surface for air and were entirely dependent on cutaneous and branchial respiration. Aeration of the water of the controls was unnecessary. They were able to swim to the surface for air and supplemented their branchial respiration with pulmonary respiration. Weisz (1945) questioned the respiratory function ascribed to the lungs of *Xenopus* larvae by Bles (1905) and Dreyer (1914).

The observations presented here were made on 33 larvae which survived the operation. In such a group of larvae the rate at which development proceeds varies from individual to individual; so some complete metamorphosis before others. It is likely that the rate of regeneration varies with the rate of development and so, in order to obtain a progressive sequence regarding stage of development, the most immature larva present was always taken when one was to be fixed. Details of the length of time the larvae were allowed to survive following the operation and the stage of development they had reached are given in Table 1.

Thirty unoperated larvae at stage 56 were kept under the same conditions as the operated ones to act as controls. The length of time which elapsed from the beginning of the experiment until they were fixed and the stage of development they had reached are indicated on Table 1. Only 12 controls were prepared for sectioning.
Observations were made on posture, spontaneous movements, and response to stimuli of the normal and operated animals. Touching with a blunt coloured glass rod was the stimulus used. Cinematograph films were taken and the
wavelengths and frequencies of the movements passing along the tail were estimated on them.

The trunk spinal cord, together with the structures immediately surrounding it, was fixed for 24 hours in an aqueous solution of 5 per cent. formalin and $\frac{1}{2}$ per cent. acetic acid, decalcified in acidified 70 per cent. ethanol, and embedded in paraffin wax. Sections were prepared at a thickness of 10 $\mu\text{m}$ and were either stained with cresyl violet or silvered by Bodian's protargol method. The plane of section and stain used for each specimen are given in Table 1. Measurements were made on the sections with a micrometer eyepiece.

**OBSERVATIONS**

**Movements**

**Normal animals**

The normal larvae at stage 56 exhibited movements associated with locomotion, posture, and respiration. Locomotion was effected by the passage caudally of waves in the lateral plane. They had a wavelength of 25 mm., an amplitude of 6 mm., and a frequency of 4 per second. Each wave involved movement of the head, trunk, and tail of the animal. During spontaneous swimming two or three complete waves were followed by a glide through the water before they were repeated. A gentle touch with a glass rod anywhere on the surface of a larva resulted in co-ordinated swimming of the animal as a whole. The movement did not begin at the site of stimulation and move caudalwards. The latest stage at which co-ordinated tail movements during swimming were seen was stage 63. A vigorous response accompanied by flexion of the oral tentacles was evoked by a knock on the side of the bowl containing the animals. They darted through the water in what appeared to be an avoiding reflex.

Larvae at stage 56 held the hind limbs away from the ventral fin at an angle of about 10° when at rest. During swimming they were held immobile in contact with the ventral fin. Stimulation of one hind limb resulted in adduction and extension of both of them. At stage 59 co-ordinated swimming movements of the hind limbs during locomotion began and the response to stimulation of one hind limb changed to flexion of the stimulated limb. At rest they were held in the adult position with the thigh and foot in a plane vertical to the trunk and the leg parallel to the trunk.

When feeding, the larvae adopted a position with the head down and the long axis of the trunk almost vertical—a curious posture which excited the attention of Beddard (1894) and Bles (1905). It was maintained by the posterior third of the tail executing a continuous series of waves passing caudalwards. They had a wavelength of 5 mm., an amplitude of 1.5 mm., and a frequency of 8 per second. The latest stage at which the movement was present was stage 63. This movement was less powerful than that concerned with locomotion as Weisz (1945) observed. Swimming did not begin by a gradual increase in strength of the postural movement: when swimming began the postural movement ceased.
The respiratory movement performed by the larvae produced the flow of water through the pharynx essential for branchial respiration and filter feeding. It consisted of opening of the mouth and lowering of the pharyngeal floor, followed by closing of the mouth and raising of the pharyngeal floor. It was repeated about 40 times a minute. The last stage at which it was seen was stage 61.

**Operated animals**

The movements performed by the larvae after transection of the spinal cord divided the postoperative period into two phases. Until the fourteenth day after the operation none of the animals were able to transmit swimming movements past the site of transection, and the caudal region of the animals never performed swimming movements when stimulated. Light touching of the skin with a glass rod posterior to the site of transection resulted in abduction of the hind limbs. Stronger stimulation resulted in a single flick of the tail. No movement of the animals cranial to the site of transection could be attributed directly to these stimuli. Persistent swimming movements confined to the region of the animals cranial to the site of transection resulted from lightly touching the skin of the head. These were not sufficient to enable locomotion. A knock on the side of the bowl containing the animals produced rapid flexion of the oral tentacles and angulation of the larvae about the site of transection.

During the earlier postoperative phase the larvae rested on the bottom of the container. Whenever they were examined the rapid postural movement of the caudal third of the tail was present in one or two of them. It seemed to stop and start spontaneously. When absent it could be started by lightly pinching the ventral fin with fine forceps. This caused the tail to lash and then to begin the postural movement.

The movements of the pharynx associated with branchial respiration and filter feeding were unaffected by the operation.

On the fifteenth postoperative day 11 of the 16 remaining animals were able to transmit swimming movements past the site of transection. Two operated animals were fixed, specimen number 18 which did not transmit swimming movements past the site of transection, and specimen 19 which did.

On the sixteenth postoperative day all the remaining larvae resumed the characteristic posture in the water and performed co-ordinated swimming movements involving the whole animal. Purposive swimming movements of the hind limbs appeared when the larvae reached stage 59. The response to knocking the side of the bowl containing the larvae never returned to the region of the animals caudal to the site of transection.

**White Matter**

**Normal animals**

The white matter of the spinal cord in the normal control larvae was found to be disposed in ventral, lateral, and dorsal columns as in other vertebrates.
The ventral white column of each side contained the Mauthner axon which was 10 \( \mu \) in diameter and axons from the large primary motor neurones which were 6 \( \mu \) in diameter. A detailed account of the development and relations of the latter has been given by Hughes (1959). The dorsal white columns contained the ascending and descending branches of the fibres that enter the spinal cord from the dorsal root ganglia. It was not possible to decide upon the nature of other fibres in the white columns at the stages of development covered by this material. There was no evidence of mitosis occurring in the white matter of the sections of normal larvae.

**Operated animals**

In specimens 1 and 2, fixed 24 hours after transection, retraction buds were present at the ends of the cut fibres. The hiatus between the two cut ends of spinal cord was occupied by escaped blood-cells and necrotic tissue. On the third postoperative day (specimen 3) branches were found on fine fibres still in continuity with their cell-body. The branches arose on the region of the fibres adjacent to the cut and terminated in cones of growth. Fine fibres passing completely across the site of transection were seen first in specimen 6, which was killed on the seventh day after the operation (Plate, fig. A). It was not possible to determine their origin as they were lost among the arborizing fibres at the edges of the cut. After the seventh day the only appreciable change in the fibres crossing the site of transection was an increase in number (Plate, fig. B). No difference was found between the sections prepared from specimen 18, which did not conduct swimming movements past the site of transection on the fifteenth postoperative day, and those from specimen 19, which did. The diameter of the regenerated region of the cord was always smaller than that of the intact region.

The Mauthner axons were identified in all the material silvered by Bodian's protargol method. The part of the axon remaining in continuity with its cell-body invariably ended between half to one segment rostral to the site of transection. In specimen 1, fixed 24 hours after the operation, the axon tips were not swollen and were surrounded by a clear unstained region 50 \( \mu \) in diameter (Plate, fig. C). By 5 days after the operation the end of the axon had doubled its diameter over a length of 30 \( \mu \) (specimen 4; Plate, fig. D). A club-like ending, 20 \( \times \) 25 \( \mu \), was found in specimen 10 fixed 10 days after the operation, and in all the older operated specimens (Plate, fig. E). None of the material showed any evidence of the formation of new collaterals by branching or attempts to regenerate by the axon tip.

The large axons of the primary motor neurones presented changes similar to those of the Mauthner axons, differing in degree only. The part of the axons in continuity with its cell-body ended in the region of the spinal cord between the ends of the Mauthner axons and the ends of the fine fibres at the cut surface. In contrast to the Mauthner axons the formation of retraction balls on these large axons was not delayed. They were present in specimen 1, fixed 24 hours after the
operation. No evidence of the formation of new collaterals or the regeneration of the axon tips was found.

Mitotic figures were present in the white matter only in specimen 5 which was fixed 5 days after the operation. The axis of division bore no constant relation to the long axis of the axons.

Grey matter

Normal animals

In the normal material at stage 56 the cells of the primary motor system were established (Plate, fig. F) and those of the ventral horn still differentiating. To avoid confusion of the changes associated with this differentiation with those due to transection of the spinal cord, observations on perikarya were confined to primary motor neurones. The perikarya of the primary motor neurones occupied a characteristic position along the ventral boundary of the grey matter. At stage 57 about 1 out of 3 of these cells had lost their Nissl granules. The proportion increased to about 2 out of 3 at stage 59. All of them were devoid of Nissl substance at stage 63.

Ciliated ependymal cells lined the central canal of the spinal cord and mitotic figures were found among them in all the normal material stained with cresyl violet.

Operated animals

In specimen 2, fixed 24 hours after transection, the cytoplasm of the perikarya situated within half a segment of the cut, both cranially and caudally, was completely bare of Nissl substance and contained many vacuoles at its periphery. These cells had stained lightly with cresyl violet in specimen 5 fixed 5 days post-operatively. In some the nucleus had disintegrated and in others small cells resembling microglia were in the necrotic debris. The cranial limit of this change was one segment from the site of transection. The perikarya situated in the second segment cranial to the cut showed a less intense chromatolytic reaction: Nissl substance was absent only from the perinuclear region of the perikarya (Plate, fig. G). Caudally chromatolysis occurred only in perikarya adjacent to the cut. Cells in this condition were present at the same position relative to the transection in all the material stained with cresyl violet which had been fixed up to 13 days after the operation. No comment is made on chromatolysis at stages later than 56, as it was also present in the controls. However, cells in the phase of recovery after chromatolysis were found in specimens 23 and 26 (Plate, fig. H) fixed 17 and 21 days after the operation, indicating that chromatolysis following axon section did not necessarily continue without recovery into the chromatolytic changes of metamorphosis.

The continuity of the central canal, ependyma, and grey matter was restored in only 4 of the 33 operated animals. These were specimens 11, 18, 23, and 24.
No increase in the number of mitoses occurring in the ependymal layer was apparent at any time. There was never any indication of neuroglial scar formation.

**DISCUSSION**

The motor activity displayed by the operated larvae during the first 14 days after spinal cord transection indicates that the central nervous system on each side of the cut was functionally independent. This independence was most evident in the limitation of swimming movements to the region of the larvae cranial to the transection. This result, and the observation on normal *Xenopus* that swimming was always performed by the larva as a single unit, is consistent with the conclusion of Hughes (1959) that there is a centre for the co-ordination of swimming movements in the central nervous system at the level of the otocyst. The observation on normal larvae that when swimming begins the postural movement stops suggests that they are produced by two separate central systems. This possibility is confirmed by the behaviour of the operated animals during the early postoperative period. In these circumstances the two types of movement were separated by the lesion of the spinal cord. Swimming movements were effected only by the region of the larvae cranial to the lesion. The isolated caudal part of the spinal cord was sufficient for the performance of the postural movement but not of the swimming movement.

The distribution of chromatolytic primary motor neurones for two segments cranial and half a segment caudal to the lesion is the one expected from the course of their axons. Hughes (1959) observed that the axons of these motor neurones develop by growing caudalwards along the neural tube before leaving it in the ventral roots.

The first operated specimen in which fibres crossing the site of transection were found was fixed 7 days after the operation. This was 8 days before co-ordination of function across the lesion was established. The only change apparent in the sectioned material which had been fixed during this period of delay was an increase in the number of fibres bridging the site of transection. The observations made by Piatt (1955) on regeneration of the spinal cord show that a similar delay occurs in the salamander.

The rate at which regenerating axons within the central nervous system of frog larvae increase in length is not known. Speidel (1933) found that growth cones of regenerating peripheral nerves in frog tadpoles travel about 0.2 mm. in 24 hours. If the rate of growth in the spinal cord is of the same order the regenerating axons in the present experiment would have grown 1 to 2 mm. into the opposite stump during the 8 days delay preceding recovery of motor co-ordination. The isolated part of the spinal cord caudal to the lesion was at least 30 mm. in length. If motor co-ordination across the site of transection required the growth of regenerating fibres along a considerable length of the spinal cord a growth rate of the order of 4 mm. a day must be postulated. This is 20 times greater than the
rate found by Speidel and therefore can be dismissed as improbable. It follows that although the isolated spinal cord could not mediate co-ordinated swimming movements, one which had only a short region under the direct control of descending motor pathways could. This raises the problem: had the normal pathway for swimming been re-established?

The other conditions which may limit functional co-ordination across the cut region of the cord are the number of fibres crossing the gap, the pattern of synaptic connexions, and possibly myelination of the regenerated fibres to give sufficiently rapid conduction rates. It is certain that the number of fibres crossing the gap increased during the delay preceding the establishment of motor co-ordination, but the significance of this cannot be assessed in the absence of information about the other two factors. More experiments are required before this problem can be resolved.

Two types of large, heavily myelinated axons were cut in the spinal cord of the *Xenopus* larvae; the Mauthner axons confined to the central nervous system, and the axons of primary motor neurones distributed to the axial musculature. Neither of these types showed any sign of regeneration. The presence of regenerating fibres around them indicates that their environment was favourable to regeneration and suggests that the limiting factor is associated with the neurones themselves. The features that the two types of axon have in common are: they are situated in the ventral white column of the spinal cord, they have axons of large diameter with thick myelin sheaths, they are fully differentiated and functional when cut, and they disappear during metamorphosis. Further investigations are required to unravel the roles which these factors might be playing to prevent regeneration of these axons.

The changes displayed by the fibres in the white matter after transection have been described in detail by Cajal (1928) for mammals and by Lorente de Nó (1921) for frog larvae. In *Xenopus* larvae the regenerative capacity of the axons of small diameter contrasts with that of the axons of large diameter. Coupled with the ability of the former to regenerate is their ability to branch. It may be that regeneration of an axon within the central nervous system is initiated by the formation of a new branch. Inability to branch may preclude regeneration of the larger axons and be a consequence of differentiation or myelination.

After the operation the motor behaviour of the larvae returned to normal with one notable loss. Their response to knocking on the side of their bowl was restricted to the region cranial to the site of transection. It is possible that the Mauthner axons are the motor pathway concerned in this response. This suggestion is compatible with the claim of Berkowitz (1956), Retzlaff (1957), and Wilson (1959) that the Mauthner fibres are the pathway concerned in the rapid flexion of the tail produced as a startle reflex of fish. It is apparent that intact Mauthner axons are not essential for the performance of the undulatory swimming movements of the trunk and tail.
SUMMARY

1. The spinal cord of *X. laevis* larvae at stage 56 of development was completely transected between the fifth and sixth neural arch. Observations were made on 33 larvae which survived the operation and a suitable group of normal control animals. They were fixed at intervals up to 6 months after the operation. Sections were prepared and either stained with cresyl violet or silvered by Bodian’s protargol method.

2. Normal larvae exhibited movements associated with locomotion, posture, and respiration. A knock on the side of their bowl evoked an avoiding reaction in which they darted through the water.

3. During the first 15 days after the operation the locomotory movement was performed only by the region of the experimental animals cranial to the lesion and the postural movement was performed only by the region caudal to the lesion. On the sixteenth postoperative day functional co-ordination across the lesion had returned in all the experimental animals. The response to a knock on the side of their container never returned to the region of these animals caudal to the site of transection.

4. The first axons to regenerate across the lesion were found on the seventh postoperative day—8 days before the return of functional co-ordination. The only change at the site of transection after this was an increase in the number of axons. The Mauthner axons and the large axons of primary motor neurones did not regenerate.

5. Perikarya of primary motor neurones showing chromatolytic changes were found for two segments cranial and half a segment caudal to the site of transection in the operated animals.

6. It was concluded that the movements associated with locomotion are mediated by a different central system to that mediating the postural movements of these animals.

7. It is suggested that the Mauthner axons of these animals are the central motor pathway for the avoiding response stimulated by a knock on the side of their container.

8. The evidence is consistent with the hypothesis that regeneration of axons after transection of the spinal cord is not limited by local changes consequent to the lesion.

RÉSUMÉ

*La section transversale de la moelle épinière chez l'embryon de Xenopus laevis.*

1. La moelle épinière de larves de Xénopé au stade 56 a été complètement sectionnée entre le 5e et le 6e arc neural. Les observations ont été faites sur 33 larves ayant survécu à l'opération et sur un nombre convenable de témoins normaux. Les individus ont été fixés à divers intervalles (jusqu'à 6 mois) après
l'opération. Les coupes histologiques ont été soit colorées au violet de crésyle, soit imprégnées à l'argent selon la méthode de Bodian au protargol.

2. Les larves normales présentaient des mouvements associés à la locomotion, au maintien de la position sur place, et à la respiration. Un coup donné sur le bord du récipient provoquait une réaction de fuite, au cours de laquelle elles s'élançaient à travers l'eau.

3. Pendant les 15 premiers jours suivant l'opération, les mouvements de locomotion ont été accomplis seulement par la partie du corps antérieure à la lésion, et les mouvements liés à la station sur place seulement par la partie du corps postérieure (caudale) à la lésion. Le 16e jour après l'opération, la coordination fonctionnelle était rétablie à travers la lésion chez tous les animaux opérés. La réaction au choc sur le bord du récipient ne s'est jamais étendue à la partie du corps de ces animaux caudale par rapport à la section transversale.

4. On a observé les premiers axones régénérant à travers la lésion le 7e jour après l'opération, soit 8 jours avant le rétablissement de la coordination fonctionnelle. Après ceci, la seule modification intervenue à l'emplacement de la section a été une augmentation du nombre d'axones. Les axones de Mauthner et les grands axones des neurones moteurs primaires n'ont pas régénéré.

5. Chez les animaux opérés, des modifications chromatolytiques des péri-caryons ont été observées dans les neurones moteurs primaires des deux segments antérieurs et de la moitié du segment postérieur au niveau de la section.

6. On a conclu que les mouvements associés à la locomotion sont assurés par l'intermédiaire d'un système central différent de celui qui assure les mouvements de position sur place.

7. On suggère que les axones de Mauthner constituent la voie motrice centrale de la réaction de fuite provoquée par un choc sur le bord du récipient.

8. Les faits s'accordent avec l'hypothèse selon laquelle la régénération des axones, après section transversale de la moelle épinière, n'est pas limitée par des modifications locales consécutives à la blessure.

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REFERENCES


EXPLANATION OF PLATE

**Fig. A.** Horizontal section of specimen 6 which was fixed 7 days after transection of the spinal cord; fine axons (indicated by arrow) are seen passing across the site of the lesion. Bodian protargol stain. $\times$120.

**Fig. B.** Horizontal section of specimen 31 which was fixed 112 days after transection of the spinal cord; many fine axons are seen passing across the site of the lesion. Bodian protargol stain. $\times$120.

**Figs. C, D, and E** illustrate the changes which occurred at the cut end of the Mauthner axon (indicated by arrows).

**Fig. C.** Sagittal section of specimen 1 which was fixed 1 day after transection of the spinal cord; the cut end of the Mauthner axon is seen with an unstained region around it. Bodian protargol stain. $\times$200.

**Fig. D.** Horizontal section of specimen 4 which was fixed 5 days after transection of the spinal cord; the dilated cut end of the Mauthner axon is seen. Bodian protargol stain. $\times$200.

**Fig. E.** Horizontal section of specimen 10 which was fixed 10 days after transection of the spinal cord; the dilated cut end of the Mauthner axon is seen. Bodian protargol stain. $\times$200.

**Figs. F, G, and H** illustrate the changes which occurred in the perikarya of the large primary motor neurones after their axons had been cut.

**Fig. F.** Horizontal section of normal tadpole—specimen C2; the normal appearance of Nissl granules is shown. Cresyl violet stain. $\times$800.

**Fig. G.** Sagittal section of specimen 2 which was fixed 1 day after transection of the spinal cord; the appearance of the perikaryon is shown during chromatolysis. Cresyl violet stain. $\times$800.

**Fig. H.** Horizontal section of specimen 26 which was fixed 21 days after transection of the spinal cord; the appearance of the perikaryon is shown during recovery after chromatolysis. Cresyl violet stain. $\times$800.

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