Some effects of de-afferentation on the developing amphibian nervous system

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WITH TWO PLATES

An adult anuran can still walk or swim if the nerves supplying one or even two limbs are de-afferentated (Gray, 1950). However, in a developing amphibian, a limb at motile stages becomes paralysed when deprived of its sensory input. A sequence of degenerative changes then follow in the cord and in peripheral nerves.

Tadpoles of *Bufo marinus* and late embryos of *Eleutherodactylus martinicensis* have been submitted to this experiment; in these tropical forms the subsequent events follow rapidly. Most attention has been paid to *Eleutherodactylus*, on which a quantitative study of the numbers of fibres in nerves to the hind limb during development has recently been published (Hughes, 1965a). This work, together with a study of the behaviour of the normal embryo (Hughes, 1965b) has been used as a basis for the present experimental observations.

MATERIAL AND METHODS

The source of the embryos of *E. martinicensis* and the methods of culturing and observing them remain the same as in previous studies (Hughes, 1962, 1964a & b, 1965a & b). Except at the drier times of the year tadpoles of *Bufo marinus* are everywhere common in standing water in Jamaica. Early stages were collected and kept in the laboratory at about 25°C. They were fed on scraps of meat. Under these conditions, the interval between early limb stages and metamorphosis was about 3 weeks.

*Bufo* tadpoles at swimming stages were anaesthetized in a solution of 1:2000 Sandoz MS 222 in water. They were then transferred to Holtfreter saline containing 0.45 per cent. NaCl, in which chloromycetin at 1:10000 w/v had been dissolved. Under a dissecting binocular, the skin of the dorsal surface of the lumbar region was then medially incised, and the longitudinal strips of muscle over the vertebral column displaced to either side. The neural arches of the lumbar vertebrae were severed and the meningeal envelope of the cord cut open.

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The dorsal roots of the lumbar nerves were then exposed. The aim of a preliminary experiment was to see whether a tadpole could survive after exposure of the cord in this way; no further operative interference was then attempted. The animal was placed in saline containing antibiotic, and allowed to recover; the dorsal musculature closed over the cord.

Next day these tadpoles were found to be swimming normally, and were fixed in Bouin's fluid. In serial sections the cord appeared undamaged, even though in direct contact with extravasated blood; a thin sheet of ectoderm had already grown over the wound. In the next experiment, the central processes of the lumbar nerves were cut on one side only. Not all were severed in each case, but of ten operated tadpoles, eight were found to be paralysed on the operated side after recovery from the anaesthetic. Next day, nine tadpoles were alive, and in four the initial paralysis had persisted. All were then fixed, and subsequently studied in serial sections.

In *Eleutherodactylus* embryos, which are much smaller than *Bufo* tadpoles, the dorsal longitudinal muscles are very thin, and at operation it seems that the cord is covered only by two membranes, the skin and the meninges. The central processes of the dorsal roots are too fine to be individually discerned. It was, however, found possible to expose the cord without injury to them and to retain the action of the hind limb. To sever the central processes of the lumbar nerves it was necessary to run a fine sharp blade along the dorso-lateral surface of the cord. The embryos were immersed in Holtfreter saline containing 0.45 per cent. NaCl during the operation and afterwards; no anaesthetic was used. The reaction of the leg clearly indicated when a dorsal root had been touched by a metal instrument. In all cases the ipsilateral leg was paralysed after the operation.

The operated embryos were fixed in Bouin's fluid from 1 to 8 days after the operation. They were subsequently sectioned serially, and were impregnated by Palmgren's method (Palmgren, 1948), with slight modifications found advisable for this material (Hughes, 1965a).

**Observations**

Seventy embryos of *E. martinicensis* from 7–10 days of development were submitted to lumbar de-afferentation and fixed from 1 to 8 days afterwards. In all but the youngest there was some degree of limb motility at the time of the operation. In normal embryos at 7½ days, the hind limbs show a generalized movement during trunk wriggles, while at 9½ days, the leg first exhibits a strong extension thrust which is independent of trunk movement (Hughes, 1965b). The subsequent history of these embryos is shown in Table 1. The results varied both in the extent to which the action of the limbs was impaired and in the amount of degeneration subsequently detected in histological study. In another group of embryos fixed 8 hr. after operation, it was seen that within the cord and ganglia the damage was confined to the central processes of the lumbar nerves and to the
dorsal funiculi of the cord in that region. This degree of operative interference by itself is enough to cause a complete paralysis of the limbs.

After severance of a central process fibre, the cut end swells into a club-like structure (Plate 1, Figs. B and H). They are seen in both *Bufo* and in *Eleutherodactylus*, in the latter as early as 8 hr. after the operation and up to 5 days afterwards in fibres which then still end blindly. They may be found at any level within the ganglia, in the stump of a central process, or, in *Bufo*, within the dorsal funiculus. For an end-club to form, it is not necessary for a fibre to be cut. Blindly ending terminals in abnormal situations present much the same appearance. If thwarted from reaching their normal destination, central process fibres

**Table 1**

Records concerning seventy embryos of *Eleutherodactylus* in which lumbar central processes were removed

<table>
<thead>
<tr>
<th>Stage at operation (days)</th>
<th>Totals</th>
</tr>
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<tr>
<td>7</td>
<td>7½</td>
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</table>
| Number operated | 3 | 21 | 13 | 7 | 22 | 4 | 70  
| Result next day | | | | | | |
| (1) Dead or moribund | 7 | 7  
| (2) Limbs not yet motile | 3 | 6 | 9  
| (3) Both paralysed | 1 | 3 | 4  
| (4) Both move, little difference | 6 | 7 | 4 | 5 | 4 | 26  
| (5) One moves, other paralysed, or largely so | 2 | 5 | 3 | 14 | 24  
| Further history of Group (5) | | | | | | |
| (5a) Fixed after 24 hr. | 1 | 1 | 1 | 1 | 4  
| (5b) Condition later maintained | 4 | 4 | 8  
| (5c) Later recovery of i/l limb | 2 | 1 | 3  
| (5d) Later loss of motion in c/l limb | 1 | 5 | 6  
| (5e) Moribund 2 or more days later | 3 | 3  

will enter the regenerating margins of the skin ectoderm lateral to the cord (Plate 2, Figs. M & O). Here they may ramify freely among the ectodermal cells at a time when the fibres of normal cutaneous nerves are all outside the epidermis. At the end of each branch is a dilated club. Such appearances exactly recall the abnormal dilated fibres found within the epithelium of the *uterus masculinus* of the male human foetus in the 3rd and 4th month, as described by Boyd & Hughes (1960). Ramon y Cajal (1928, e.g. Fig. 215, p. 543) has described the development of end clubs in severed central process fibres in kittens fixed several days after operation. The only new feature which the present results disclose in that end clubs may be formed within a few hours of the severance of a fibre and before any regeneration has begun. The dilation of nerve terminals in this way can only be interpreted as due to a piling up of axoplasmic material. In the central process of a spinal ganglion, the flow which this phenomenon reveals is
in the same direction as that of the excitatory impulse. These considerations sug-
gest that in developing amphibia, material of some kind is conveyed into the cord
along the incoming sensory fibres. A proximo-distal flow along adult ventral root
axons is well established (Weiss & Hiscoe, 1948; Ochs, Dalrymple & Richards,
1962).

In most *Eleutherodactylus* embryos in which central processes have been cut,
some fibres find their way back into the cord after a day or so. The initial obstacle
to their growth was often the blood clot surrounding the ganglion. They enter
in the normal dorsal position if possible, but otherwise in lateral, or even ventro-
lateral positions. They are only able to enter the cord at sites where the meningeal
envelope is not present. Only where regenerating central process fibres enter the
cord in the normal dorsal position do they give rise to a longitudinal funiculus.
Laterally entering fibres may sweep across the white matter of the cord and
enter the mantle layer in an abnormal manner (Plate 2, Fig. Q). It can sometimes
be seen that dendrites of ventral horn cells have grown towards them.

In many operated embryos of *Eleutherodactylus* the cord became protruberant,
particularly in the younger members of the series, where the central canal opened
widely, and a condition of spina bifida was established (Plate 2, Fig. N). This was
accompanied by a variable degree of oedema and necrosis of the exposed layers
of cells in the alar plate of the cord, still largely neuroblastic at these stages. On
the supposition that the swelling of the cord was partly osmotic, the concentra-
tion of NaCl in the Holtfreter solution was increased to 0.45 per cent. with some

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**EXPLANATION OF PLATES**

All figures are of sections impregnated by Palmgren's method. The horizontal line in each
figure represents 10 μ.

**PLATE 1**

**Fig. A.** From late *Bufo marinus* tadpole in which the central process of DRG 8 was severed
17 hr. previously, showing degenerating nerve fibres in spinal nerve.

**Fig. B.** From late *Eleutherodactylus* embryo in which central processes of lumbar nerve root
were severed 8 hr. previously, showing end clubs on central process fibres which have already
made contact with the lateral surface of the cord (right).

**Fig. C.** Same embryo as in Fig. B, showing degeneration of fibres in nerve to *triceps femoris*.

**Figs. D & E.** From same *Bufo marinus* tadpole as in Fig. A, showing ipsilateral (D), and con-
tralateral (E) ventral horn cells in neighbourhood of DRG 8, with severed central process.
In (D), the cell bodies are shrunken and the ventral root axons swollen in comparison with
(E).

**Figs. F & G.** From 84-day embryo of *Eleutherodactylus* (f, Text-fig. 1) with lumbar central
processes severed on one side 24 hr. previously, showing swollen ipsilateral ventral root axons
(Fig. F, arrows) compared with contralateral (Fig. G, arrow).

**Fig. H.** From 94-day embryo of *Eleutherodactylus* (d, Text-fig. 1) in which central processes
of DRG's 7–9 on one side were severed 24 hr. previously, to show end bulbs on central
process fibres of DRG 8 (arrows).

**Figs. I & J.** Nerve to *triceps femoris* on each side in late embryo of *Eleutherodactylus* in which
the lumbar central processes were severed on one side 8 days previously. The ipsilateral leg
became functional again after 3 days. Ipsilateral nerve to triceps (Fig. I) has fewer (33)
fibres than the contralateral (Fig. J, 45), though in both the largest are about 2.0 μ in diameter.
Plate 2

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improvement. The effect was also tried of covering the exposed cord with a strip of the fin of the animal's own tail. This hastened the regeneration of epithelium over the cord, even though the graft itself became necrotic.

Some degree of exposure of the cord is not incompatible with movement of the leg, provided that sufficient fibres of the lumbar central processes are intact. In such instances, the ventral horn cells and their axons remain largely free of the degenerative changes which occur when all contact with lumbar spinal ganglia is lost. Herniation of the cord, however, even though unaccompanied by necrosis, blocks the regeneration of the dorsal funiculus.

In Text-fig. 1, the state of the lumbar central processes and of the dorsal funiculus in that region is represented in a series of embryos of Eleutherodactylus fixed at varying times after operation, and of different ages. The white matter of the normal Eleutherodactylus embryo at 8–9 days exhibits a well developed ventral funiculus, a much smaller dorsal funiculus, and very few fibres laterally which are as yet stainable with silver. The lateral funiculus in the lumbar region only becomes prominent after 9½ days, when the leg is first able to extend. In none of the operated embryos of the present series was there any interruption of the lateral funiculus. Text-fig. 1 shows in the first place that limb action depends on sensory input from dorsal root fibres and that what descending impulses from the lateral column there may be at these stages do not evoke any movement of the leg.

Relatively young embryos fixed at stages when the hind limb is able to retract but not to extend can still move the leg even where central process fibres are present in only one main lumbar nerve, and with the dorsal funiculus much attenuated and incomplete longitudinally. After 9½ days, however, the results in Text-fig. 1 suggest that for the limb to extend, central process fibres from both the main lumbar nerves (S8 and S9) are necessary, together with the section of dorsal funiculus between them.

Plates 2

FIGS. K & L. Nerve to triceps femoris (lower half of figure) on each side in late embryo of Eleutherodactylus (k, Text-fig. 1), 5 days after lumbar central processes were severed on one side. The ipsilateral leg was still paralysed at fixation. Only fibres less than 1.2 μ in diameter are seen in the ipsilateral nerve (Fig. K), while in the contralateral nerves (Fig. L) there are some fibres twice this diameter. In both figures the sciatic nerve is in upper half of figure.

FIG. M. From a late embryo of Eleutherodactylus (j, Text-fig. 1) in which lumbar central processes were removed on one side 4 days previously. Central process fibres of DRG 9 have entered the regenerating ectoderm, and formed end clubs.

FIG. N. Diagram of transverse section of 8½-day embryo of Eleutherodactylus (c, Text-fig. 1) 1 day after operation. The open cord is flanked by two regenerating ridges of ectoderm, into which on either side fibres from the central process of DRG 9 have penetrated. Each of these ridges is photographed in Figs. O & P.

FIG. O. Here among the ectodermal cells, branches of central process fibres have formed end clubs.

FIG. P. Here central process fibres have grown right through the ectodermal ridge to enter the exposed dorsal surface of the cord.

FIG. Q. From the same embryo as in Figs. K & L, showing central process fibres from DRG entering cord laterally (right). Some dorsal funicular fibres are seen at top of figure.
Text-Fig. 1. Diagrams showing conditions of central processes in lumbar segments S₇ to S₁₀, and of the dorsal funiculus within the cord at these levels, together with the degree of movement seen in the hind limbs before fixation of twelve Eleutherodactylus embryos in which removal of the central processes was attempted on one side 1 to 5 days previously. Thick lines, dashes and dots indicate decreasing densities of fibres numbers in the dorsal funiculus. N, central process normal; F, containing few fibres; O, with none, or very few; h, herniation of cord.

The ages of these embryos at fixation were: (a–c), 8 days; (d–i) 9 to 10 days; (j–l) 12 to 13. Diagrams d, e, g, j and k refer to the embryos analysed quantitatively in Text-fig. 2.
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In the kitten, for the first 10 days after birth, Malcolm (1955) found that the motor response of a limb to the stimulation of afferent fibres is ‘strictly limited to the motor neurones of the segment at which the afferent volley arrives’. The present results are consistent with the view that the withdrawal reflex of the hind limb is similarly monosegmental in *Eleutherodactylus* embryos at 8–9 days.

We are, however, not yet able to say how far fibres extend longitudinally in the dorsal funiculi from their point of entry at the dorsal root. In the adult frog, according to Liu & Chambers (1957), such fibres extend rostrally to the medulla and caudally for five to six segments. So far, in no *Eleutherodactylus* embryos has a single dorsal root alone been severed. This result has been achieved in *Bufo* tadpoles, but here the whole dorsal funiculus is missing in the immediate neighbourhood of the severed dorsal root, while the ends of those fibres which originate from adjacent segments show end clubs similar to those of central process fibres. It may be that in such tadpoles the dorsal funiculus was locally injured at the same time that the dorsal root was severed.

Changes within ventral horn cells and their axons

Eighteen hours after operation in *Bufo marinus*, the lumbar ventral horn cells on the side on which the central processes have been severed are noticeably shrunken in comparison with the contralateral cells (Plate 1, Figs. D & E). At the same time, among their axons traversing the white matter, dilated and irregular fibres may be seen. Under the same circumstances this swelling of ventral root axons is also found in *Eleutherodactylus* embryos 1 day after operation (Plate 1, Figs. F & G). Further distally, in operated tadpoles of *Bufo marinus*, granular debris of nerve fibres can be discerned in the main lumbar spinal nerves (Plate 1, Fig. A), though it cannot be decided whether the fibres which have disintegrated belong to dorsal or to ventral roots, for in *Bufo* the operation provokes a variable amount of degeneration within the dorsal root ganglia themselves.

Unilateral loss of fibres in peripheral nerves can be detected by differential counting of the constituent fibres on each side. Such counts were made in operated *Eleutherodactylus* embryos, where the normal values for the nerves in the hind limb during development are already known (Hughes, 1965a). Through the whole series of embryos, search was made for those in which the operative interference was concentrated on one side only. Five were found in which the contralateral damage was insufficient to affect the movement of the leg. In Text fig. 1 (e, f, g, h, j, k) are diagrams relating to these embryos, one of which was fixed on each of the first 5 days after operation. In all five, the central processes of all contralateral lumbar spinal ganglia reach the cord, though there is some reduction in the number of the constituent fibres in the first three embryos. In Text-fig. 2, the results of quantitative study on the nerve supply of the hind limbs of these embryos are shown, expressed in the form of the ratios of the numbers of cells or fibres on each side. It must be remembered, however, that
the absolute numbers of fibres in muscular nerves at these stages may be as low as ten. Figures for normal embryos of *Eleutherodactylus* are given in Table 2 of Hughes (1965a).

In the study of these five operated embryos, the first point of enquiry was the extent to which the dorsal root ganglia had suffered either directly or indirectly from the effects of the operation. In each embryo the total number of cells in each of the lumbar ganglia S7 to S10 were counted, and also the total number of dorsal root fibres immediately distal to each ganglion. Text-fig. 2A shows that throughout the loss of cells in the lumbar spinal ganglia is less than 20 per cent. For the first 2 days after the operation, the ratio of the dorsal root fibres is again within 20 per cent. of unity. On the motor side, there is no loss of ventral horn

**Text-fig. 2.** Ratios of numbers of fibres or cells on the two sides of the operated embryos (d, e, g, j and k) of Text-fig. 1, in which damage at operation was largely concentrated on one side only. A, ratios for sensory nerves and cells; B, for motor roots and cells.
cells, or of ventral root fibres during the 1st day (Text-fig 2B) though some ventral root axons become swollen.

Peripherally, however, there is a loss of from 35 to 55 per cent. in the nerves supplying individual muscles (Text-fig. 2D). Fibre degeneration in the nerve supplying the triceps femoris has been seen as early as 8 hr. after the operation (Plate 1, Fig. C). Yet cutaneous nerves are unaffected throughout the period of the experiment (Text-fig. 2A). These circumstances, together with the fact that the ipsilateral leg is paralysed, imply that the loss within muscular nerves mainly concerns their motor fibres. In these developing Anura, therefore, it seems that when a ventral horn cell is deprived of contact with incoming dorsal root fibres, it is unable to maintain the terminal part of its axon. Axoplasm which normally flows centripetally through the whole course of the fibre, accumulates in the proximal region (Plate 1, Figs. D & F).

During the 2nd and 3rd days, the ratio of the numbers of fibres in muscular nerves remains at the same level as at 24 hr. after the operation (Text-fig. 2). At the same time, the corresponding ratio falls for the sciatic nerve (Text-fig. 2C), both

![Graph](image)

**TEXT-FIG. 2.** C, for main limb nerves; D, for nerves to limb muscles.
at its entry into the limb and at the point of bifurcation into tibial and peroneal nerves. There is also a fall in the number of ventral horn cells and ventral root fibres (Text-fig. 2B). These observations imply a retrograde atrophy of motor axons which in some instances finally involve the perikaryon itself.

After the 3rd day, a period of regeneration begins, although paralysis of the ipsilateral limb persists. Text-fig 1 shows that in most lumbar segments some central process fibres enter the cord by the 4th day. Centrally the number of ventral horn cells and of ventral root fibres has then risen. These new axons, however, do not as yet reach peripherally to muscular nerves (Text-fig. 2D). In the embryo of this series fixed 5 days after operation, however, the numbers of nerve fibres reaching some muscles has markedly increased (Text-fig. 2D) even though the paralysis of the limb still persists. The new fibres supplying the ilio-psoas, for instance, are very fine and much smaller in diameter than most of those in the corresponding contralateral nerve.

In the normal embryo, the diameter of some nerve fibres which supply the main muscles of the limb markedly increases towards the end of development (Hughes, 1965a), as, for instance, in the nerve to the triceps femoris. In the embryo of the present series fixed 5 days after operation these nerves show a marked difference on the two sides. Large fibres are seen in the contralateral nerve (Plate 2, Fig. L) while in the paralysed ipsilateral limb the triceps is innervated only by small fibres (Plate 2, Fig. K), some of which are newly regenerated. An opposite condition was seen in another embryo where the ipsilateral leg was paralysed only for the first 3 days after operation. Both legs were able to kick on the 4th day, and on the 5th normal swimming was observed. The embryo was fixed 8 days after operation. Although the nerve to the ipsilateral triceps femoris contains fewer fibres than that on the unoperated side, little or no difference in calibre of the largest fibres in both can be detected (Plate 1, Figs. I & J). There is evidence from another quarter, to be presented in a further paper on the influence of the thyroid on the development of the nervous system in Eleutherodactylus, that ability of the leg to extend is correlated with the appearance of relatively large fibres in the nerve to the triceps femoris.

**DISCUSSION**

In adult mammals, evidence for degenerative change in neurones when deprived of synaptic connections has mainly been derived from that seen in the lateral geniculate body of the mid-brain following the interruption of the optic nerve by enucleation of the eye (Minkowski, 1913; Cook, Walker & Barr, 1951; Matthews, Cowan & Powell, 1960; Matthews, 1964). In this way the separate terminations of crossed and uncrossed fibres in the optic nerve has been charted. The changes, however, mainly involve the shrinkage of the perikaryon, a decrease in size of the nucleus and a loss of Nissl substance in the cytoplasm. Only after many months, or even years, after operation is there any loss of cells (Goldby, 1957; Matthews, 1964).
There is some doubt whether any comparable effects are always seen in ventral horn cells of the adult mammalian cord after severance of dorsal roots. Warrington (1898) found that in cats killed 10 to 28 days after operation, basophilic material in these cells aggregated round the nucleus, which moved to an eccentric position. Similar chromatolytic changes were described by Foerster, Gagel & Sheehan (1933) in ventral horn cells of Rhesus and in man following section of the lumbar dorsal roots. Cook, Walker and Barr (1951), however, found no cellular changes in the spinal cord of cats after such operations.

This apparent difference between the neurones of the mid-brain and of the spinal cord might be attributed in the first place to the fact that in the latter, only some of the synaptic connections of ventral horn cells are lost with the destruction of dorsal root fibres. However, Tower (1937) combined this operation in the lumbo-sacral region of puppies with transection of the cord on either side of the de-afferentated roots, thus isolating a segment of the cord from all incoming impulses. These conditions necessarily precluded any comparison with an uninjured contralateral half. Tower's results, however, clearly show that not only can the perikarya of ventral horn cells survive under these conditions, but that many axons must have remained intact. The ventral roots were 'remarkably well preserved in parts', while some muscular activity was seen in the leg, both spontaneously and after mechanical stimulation of the skin area over the isolated cord. The effects of this double operation on the leg muscles have been further studied in cats by Eccles (1944) and by Buller, Eccles & Eccles (1960). From their results it is clear that under these circumstances motor axons and nerve-muscle junctions remain intact, although there is atrophy of the muscles, and among their fibres, loss of the distinction between slow and fast elements.

Recently, in a series of studies which rank as an outstanding contribution by tissue culture methods to neuroembryology, the survival of isolated portions of the embryonic spinal cord has been demonstrated by Crain & Peterson (1963, 1964). Explants of chick, rat and human tissues have been used. While in some experiments the cord alone has been explanted and has exhibited complex electrical responses to stimuli, in most others an attached dorsal root ganglion has been included in the culture. In such, electron microscopy has revealed evidence of synaptic connections. Furthermore, Bornstein & Breitbart (1964) have maintained in vitro explants of the embryonic mouse cord, together with dorsal root ganglia and the adjacent myotomes. Here the presence of functional nerve muscle junctions was evident (Crain, 1964). In such preparations, further evidence could be obtained whether dorsal root fibres have a controlling influence on the development of ventral horn cells and their axons.

Meanwhile, one point concerning the present experiments remains to be made. In these developing Amphibia at the stages with which we have been concerned, the effect of deafferentation on the ventral horn cells is sharply marked from that of amputation of the limb. In both, there is a retrograde degeneration of motor axons, though under different circumstances. Severance of dorsal root fibres
distal to the ganglia as in limb amputation, however, results after a few days in a chromatolytic swelling of the cell body (Hughes, 1962), together with a further proliferation of new ventral horn cells (Hughes, 1964b). Neither of these effects is seen when central processes are severed. It is hoped that further analysis of these various effects will lead to a fresh understanding of the mutual interrelationships during development of dorsal root ganglia, the cord, and the peripheral field of innervation.

**SUMMARY**

1. The hind limbs of late tadpoles of *Bufo marinus* and of *Eleutherodactylus martinicensis* become paralysed after section of the central processes of the dorsal root ganglia.
2. Within a day, the cut ends of the central process fibres show an accumulation of axoplasmic material in end clubs.
3. At the same time, the axons of the related ventral horn cells undergo a retrograde degeneration.
4. It is suggested that in these developing Amphibia, some substance associated with material entering the spinal cord from the dorsal root ganglia is necessary to maintain the integrity of the synaptically related motor cells.

**RÉSUMÉ**

*Quelques effets de la suppression des voies afférentes sur le système nerveux des Amphibiens en cours de développement*

1. Les membres postérieurs de têtards âgés de *Bufo marinus* et d’*Eleutherodactylus martinicensis* deviennent paralysés après section des racines dorsales.
2. En une journée, les extrémités sectionnées des fibres du prolongement central montrent une accumulation de matériel axoplasmique dans les masses terminales.
3. En même temps, les axones des cellules de la corne ventrale associée subissent une dégénérescence rétrograde.
4. On suppose que chez ces Amphibiens en cours de développement, un substance associée à du matériel pénétrant dans la moelle épinière partir des ganglions de la racine dorsale est nécessaire pour maintenir l’intégrité des cellules motrices associées par voie synaptique.

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