The timetable of innervation and its control in the chick wing bud

By GAVIN J. SWANSON1 AND JULIAN LEWIS1

From the Department of Anatomy, King’s College, London

SUMMARY

How is the timing of nerve outgrowth controlled during development? This question has been examined by grafting early limb buds between chick embryos of different ages, before innervation, and assessing the morphological pattern of nerves at later stages. Grafted limbs continued to develop according to their own timetable and were invaded by nerves from the host. Irrespective of the age of the host, the development of the pattern of innervation followed the time course appropriate to the age of the grafted limb. Young nerves in an old limb showed accelerated development; old nerves in a young limb showed retarded development. The process of innervation is apparently governed not by the intrinsic developmental timetable of the neurons, but rather by the rate of construction of pathways for them in the peripheral tissue, and by the times at which their specific targets, such as muscles, differentiate.

INTRODUCTION

Peripheral nerves grow out along predictable paths at predictable times in development. How then are the paths and the times of outgrowth controlled? It has been known for a long time that the routes which nerves follow in a vertebrate limb are largely determined by the structure of the limb itself, rather than by the character of the nerves. In amphibians (Braus, 1905; Harrison, 1907; Hamburger, 1929; Piatt, 1956) and chicks (Hamburger, 1939; Narayanan, 1964) limb buds transplanted to various sites on the body or inverted show a subsequent pattern of innervation which resembles that of a normal limb, no matter what the source of the innervation. Thus, for example, if the wing bud of a chick embryo is cut off at an early stage, before it has become innervated, and is grafted onto the rudiment of the jaw, cranial nerves grow into it along very nearly the same routes that normal wing nerves follow (Fig. 1). Similar results are obtained when the limbs are left in situ but are supplied with innervation from pieces of spinal cord that have been transplanted to brachial or lumbar sites from elsewhere (Straznicky, 1963). There are some discrepancies (Piatt, 1956, 1957), and portions of the normal pattern of innervation are often missing; but to a good first approximation, foreign nerves enticed into a developing limb

1 Authors’ address: Department of Anatomy, King’s College London, Strand, London WC2R 2LS, U.K.
Fig. 1. (a) The right wing bud of a chick embryo is cut off at 3½ days of incubation, before nerves have grown into it, and is grafted onto the first branchial arch. Four days later, it has developed into a wing attached to the retromandibular region of the head, as shown. The undisturbed left wing is visible on the opposite side of the body. (b) When the limbs are silver stained as whole mounts, it can be seen that cranial nerves (facial and/or trigeminal) have grown into the grafted right wing (labelled g), forming a pattern that is closely similar to that seen in the control left wing (labelled c), that is innervated in the normal way by brachial nerves. The two limbs are shown here from the dorsal aspect, with dark-field illumination.

are restricted to the standard set of routes that limb nerves take under normal circumstances. The limb may thus be said in effect to provide ‘public highways’ for nerve outgrowth. Very little is known as to which components of the limb serve to define these highways, and how; though it has been shown that the nerve branches leading to individual muscles depend for their formation on the presence of muscle cells (Lewis, Chevallier, Kieny & Wolpert, 1981).

Still less is known as to the factors that control the time of outgrowth of the nerves along their predictable routes. Do the axons grow out at a rate determined by their own intrinsic properties, along highways that are preformed in the limb? Or is the rate of axon outgrowth controlled by changes in the non-neural tissues of the limb, and limited by the rate at which highways are created or opened up? These questions have been investigated in the present paper by grafting embryo chick wing buds, before they have become innervated, onto younger or older hosts, in place of the normal host wing buds. The maturity of the resulting pattern of nerves a few days later can be assessed by silver staining. The results show that the invading axons produce patterns appropriate to the age of the limb tissue, irrespective of the age of the nerve cells themselves.
MATERIALS AND METHODS

Chick eggs (White Leghorn × Sykes Tinted, from Needle Farm, Elstree, Herts.) were incubated at 38 ± 1 °C and windowed at 2–3 days. The embryos were staged according to Hamburger & Hamilton (1951), the windows resealed with adhesive tape, and the eggs returned to the incubator until they reached the appropriate stage for the operations. They were then taken in pairs for grafting, the younger member of each pair being at about stage 18.

The vitelline and amniotic membranes were carefully torn to expose the embryos. Whole, right wing buds were cut off and exchanged between the two embryos of the pair. Fine (25 μm) platinum wire pins were used to attach the graft to the stump, in the normal orientation (Fig. 2). The undisturbed, left wing buds of the two embryos served as controls for the grafted limbs. A few drops of Hepes-buffered Hanks Balanced Salt Solution with penicillin (50–100 i.u. ml⁻¹) and streptomycin (50–100 μg.ml⁻¹) were added to prevent infection before the eggs were resealed and returned to the incubator. The embryos were fixed after a further 3–3½ days when they had reached stage 29–31.

The fixed embryos were silver stained as whole mounts, dehydrated, and cleared in methyl salicylate, as described previously (Lewis, 1978; Lewis et al. 1981). Drawings were made using a camera lucida attached to a Zeiss stereo-
Fig. 3. Camera-lucida drawings of the developing pattern of innervation in a series of normal wings, from stage 26 to stage 35. The ventral pattern only is shown: the development of the dorsal wing innervation follows a similar time course. The drawings were made from specimens silver stained as described in 'Materials and Methods'. Mixed nerves and motor nerve branches are shown in solid black; cutaneous nerves are shown by dashed lines. Note that at stage 26 the nerves are much less tightly fasciculated than at later stages, and gaps can be seen between the individual fascicles that compose each major nerve trunk. In the pictures for stages 27/28 and 29, the letter D marks that point where the nerve branches off to the dorsal side of the wing. The spinal roots and nerves to the shoulder muscles are shown only up to stage 29. Scale bar = 1 mm in each case.
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(d) Stage 30/31

(e) Stage 32

(f) Stage 35
Fig. 4. Schematic diagram of the pattern of innervation, as seen at stage 32, identifying the nerve branches to individual muscles. (a) Dorsal nerves; (b) ventral nerves. The number accompanying the name of each muscle gives the stage at which the nerve branch to that muscle first becomes apparent in more than 50% of normal limbs examined. (At least 6 limbs were scored for each stage, and at least 12 for stages 28–32.) The slight discrepancies between the timetable indicated here and that reported by Lewis et al. (1981) may be due partly to differences between the Wyandotte x Rhode Island Red chicks used in the previous experiments and the White Leghorn x Sykes Tinted chicks used here, and partly to differences in the external criteria used for staging, which is a rather subjective procedure, and accurate only to within about ±1 stage.

microscope. In a few cases where the staining was poor and the issue was critical, we examined the nerves also at higher magnification using Zeiss Nomarski optics under an ordinary compound microscope. The musculature was inspected by viewing specimens between crossed polaroid filters. The lengths of grafted and control wings were measured from the mid-point of the elbow to the tip of the hand; since our limbs were not stained for cartilage, it was difficult to find any better fiducial points than these.
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Table 1. Summary of experimental limbs analysed

<table>
<thead>
<tr>
<th></th>
<th>Total number</th>
<th>Haemorrhagic necrosis</th>
<th>Deformed skeleton</th>
<th>Grossly defective innervation</th>
<th>Well formed and well innervated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young bud grafted onto old host</td>
<td>76</td>
<td>14</td>
<td>19</td>
<td>13</td>
<td>30</td>
</tr>
<tr>
<td>Old bud grafted onto young host</td>
<td>74</td>
<td>55</td>
<td>8</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Grafts between embryos of the same stage</td>
<td>14</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

A total of 196 embryos bearing grafted wings survived. Of these, 32 were at unsuitable stages at fixation, or were poorly stained, leaving 164 for analysis, as tabulated above.

Muscle nomenclature is according to Sullivan (1962). We abbreviate the names of muscles as follows:

Dorsal series: Tric, triceps; EMR, extensor metacarpi radialis; Sup, supinator; Anc, anconaeus; EDC, extensor digitorum communis; EMU, extensor metacarpi ulnaris; EML, extensor metacarpi longus; EIL, extensor indicis longus; EIB, extensor indicis brevis; Adi, adductor indicis; EMB, extensor metacarpi brevis; IOD, interosseus dorsalis; UMD, ulnometacarpalis dorsalis.

Ventral series: Bic, biceps; Brach, brachialis; PS, pronator superficialis; PP, pronator profundus; EECU, entepicondyloulnaris; FCU, flexor carpi ulnaris; FDP, flexor digitorum profundus; UMV, ulnometacarpalis ventralis; FDS, flexor digitorum superficialis; AbI, abductor indicis; FI, flexor indicis; AbM, abductor medius; IOP, interosseus palmaris; FDQ, flexor digiti quarti.

RESULTS

To study the factors controlling the maturation of the wing nerve pattern, we fixed our embryos during a period of rapid morphological change, in which nerve patterns at different stages of development can be easily distinguished. Before discussing the experimental findings, we briefly describe the normal course of events during this period and just before and after it.

The normal time course of innervation

Figure 3 shows the pattern of innervation in a series of normal chick wings, from stage 26 to stage 35, as seen in silver-stained whole mounts. The nerve patterns in limbs at stages earlier than this have been well described from sectioned specimens by Tello (1917), Roncali (1970) and Bennett, Davey & Uebel (1980). Axons begin to grow into the bud at about stage 24. At first, two main
Table 2. *The lengths of grafted wings, compared with host and donor controls*

<table>
<thead>
<tr>
<th></th>
<th>Young donor, old host</th>
<th>Donor and host same (intermediate) age</th>
<th>Old donor, young host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host control wing length</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Grafted wing length</td>
<td>79 ± 3</td>
<td>89 ± 3</td>
<td>112 ± 4</td>
</tr>
<tr>
<td>Donor control wing length</td>
<td>81 ± 3</td>
<td>100</td>
<td>124 ± 5</td>
</tr>
</tbody>
</table>

Wing lengths (from elbow to tip) are expressed as percentages of the host control wing length. Absolute lengths were in the neighbourhood of 2 mm. The figures are means (± standard errors) of measurements on six representative pairs of old/young exchanges, and on five pairs of same-stage exchanges (that is, \( n = 6 \) for the first column, \( n = 10 \) for the second column, and \( n = 6 \) for the third column). Note that the wings grafted at the later stages are slightly stunted in their growth by comparison with the donor control wings.

Fascicles can be seen, one dorsal (n. brachialis longus superior) and one ventral (n. brachialis longus inferior). At stage 26, the leading fibres of these two nerve trunks are just passing the region of the future elbow. At stage 27, further subdivisions have developed at and just distal to the elbow, giving rise to the main mixed nerves that travel down the forearm towards the hand – the median and interosseous nerves on the ventral side, and n. radialis profundus on the dorsal. At stage 29, the median and interosseous nerves have begun to arch round towards one another at the wrist; at stage 30, they have met to form an arch or plexus from which various side branches diverge to innervate the hand. In general, the main mixed nerve trunks develop first, and the side branches to individual muscles become visible a little later, nearly all between about stage 27 and about stage 31 (Fig. 4 and Lewis et al. 1981). This is the period in which the dorsal and ventral wing muscle masses begin to differentiate and to split into individual wing muscles (Shellswell & Wolpert, 1977). After stage 31, the various nerve branches, both muscular and cutaneous, grow and develop wider ramifications, but there is little change in the basic plan. Practically all the features that are invariant from embryo to embryo and the same on the two sides of the body are evident already at stage 30 or 31 – the wider ramifications that each nerve branch develops subsequently are randomly variable in their details. The development of the ventral wrist plexus and of the nerve branches to individual muscles, both dorsal and ventral, provided the criteria by which we assessed the maturity of the pattern of innervation in our experimental wings.

*Grafted wings*

Right wing buds were exchanged (Fig. 2) between embryos of two different stages, mostly stage 19 ± 1 and stage 22 ± 1, corresponding to an age difference
of about 18 h. Grafts between embryos of the same stage were also exchanged, mostly at stage 19, to control for any effects due to the operation itself. The embryos were fixed 3–3½ days later, when the young ones were roughly at stage 29 and the old ones roughly at stage 31. A total of 164 embryos were analysed, after silver staining (Table 1). We were limited in our choice of stages at the time of operation by the fact that wing buds transplanted after stage 22 do not develop normally; they usually show regions of haemorrhagic necrosis, and their development is almost always stunted and abnormal, even when the host is at the same stage as the donor. Thus a large proportion of our old wing buds grafted onto young hosts developed abnormally, leaving only 11 out of 74 that had well-formed skeletons and no regions of haemorrhagic necrosis. By contrast, the skeletal development of the young buds grafted onto old hosts was practically normal in the majority of cases (43 out of 76), as was the development of buds of intermediate age grafted onto hosts of the same age (10 cases out of 14). Of the grafted wing buds with an apparently normal skeleton, the majority (47 out
Fig. 6. Camera-lucida drawings of the ventral nerves of the wings whose photographs are shown in Fig. 5. Note that the young wing (a) grafted onto the old embryo resembles the control wing (d) of the young embryo in its pattern of innervation, while the old wing (c) grafted onto the young embryo resembles the control wing (b) of the old embryo. Scale bar = 1 mm.
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of 64) were plentifully innervated. The account that follows is based on these well-formed and well-innervated wings – 30 young ones grafted onto older hosts, 7 old ones grafted onto younger hosts, and 10 of intermediate age grafted onto hosts of the same age.

As described previously (Summerbell & Lewis, 1975), the grafted wing tissues developed according to their own autonomous timetable, apart from a slight (~ 10%) reduction in size, presumably due to trauma. The wings that formed from old buds grafted onto young hosts were longer than the contralateral host control wings and more advanced in their skeletal development, closely resembling the contralateral control wings of the donor embryos. Correspondingly, the wings grafted from young donors onto old hosts were retarded relative to the host controls and resembled instead the donor controls (see Table 2). Thus the grafted wings confronted the invading nerves with a pattern of tissues whose degree of maturity was truly incongruent with that of the centres from which the nerves arose.

Young wing buds grafted onto older embryos

A typical case is illustrated in Figs. 5 and 6(a)–(d), showing the result of grafting a stage-17 wing bud onto a stage-22 host, and fixing when the host was at stage 30. In the control (left) limb of the host, the wrist nerve plexus has already formed (Fig. 5b) and the nerve branches to the muscles EMR, Anc, EMU and EDC are present. But in the right wing (Fig. 5a), grafted from the younger donor, the pattern is retarded and resembles that of a stage-28 wing. The ventral nerves have not yet met one another at the wrist, and, of the muscle nerve branches, only that of EMR is visible. The nerves in the grafted limb are smaller than in the host control limb, in both diameter and length. The pattern is closely similar to that in the control (left) wing of the young donor (Fig. 5d), which was fixed at the same time as the old host.

To make a systematic objective assessment of the results, we compared the innervation of each grafted wing with two controls – the left wing of the young donor embryo from which the graft had been taken, and the left wing of the old host which had supplied the nerves. Since we wished to decide which of these two controls the graft most closely resembled, the features of interest were those in respect of which the two controls differed. We had a total of 20 young grafted wings for which both the host and the donor controls were available. In 15 of these 20 cases, the ventral nerve trunks had met to form the wrist nerve plexus in the host control wing, but not in the donor control wing. In all these cases, the grafted limb resembled the donor control in this respect. In the remaining 5 out of 20 cases, the ventral nerve trunks had met to form a relatively mature nerve plexus in the host control wing, and had only just contacted one another in the donor control wing; and in these cases also the young grafted wing resembled the young donor control wing. Besides examining the wrist plexus, we looked in every wing for each of 10 muscle nerve branches which lay distal to
the level of the host-graft junction (EMR, EMU, EDC, Anc and UMD on the
dorsal side of the wing, and PS, PP, FDP, EECU and FCU on the ventral side).
After excluding those cases where a particular muscle nerve branch was present
in both the donor control and the host control, or absent in both, and those
cases where the staining was not adequate to permit a judgment, we were left
with a total of 29 instances in which a muscle nerve branch could be seen in the host
control wing and not in the donor control wing. In all but one of these instances
the muscle nerve branch in question was absent in the grafted limb. Thus with
the exception of one muscle nerve branch in one specimen, the nerve pattern of
the grafted wing resembled that of the control wing of the donor in its degree of
maturity, by all our criteria. This suggests that it is the age of the wing itself,
rather than that of the nerves, that determines the timetable of development of
the nerve pattern.

An objection can be raised to this interpretation of the results, however. The
young grafted wing has suffered surgical trauma and may be somewhat mis-
aligned on the host limb stump, and it could be that this is the reason why it
lacks nerve branches that are present in the host control wing, independently
of whether or not they are present in the young donor control wing. Our observa-
tions allow us to judge how far we are likely to have been misled in this way.
Of the muscle nerve branches scored, there were 115 that were present in both
host and donor control wings. If our other results are to be interpreted in terms
of surgical damage, we should expect that these branches too should generally
be missing in the grafted limbs. In fact they were present in 88 (77 %) of the
instances, and absent only in 27 (23 %). Thus deficiencies resulting from surgical
disruption occur, but they occur relatively rarely, and it is unlikely that they
are the main explanation of the apparent retardation of nerve development
in the young wings grafted onto old hosts.

Old wings grafted onto young hosts

The old wings grafted onto young hosts provide strong direct evidence in
favour of the same conclusion. In these specimens the development of the nerves
in the grafted wing appeared to be accelerated by comparison with the control
wing of the young host (Figs. 5 and 6b, c and d). Thus, in six out of seven cases,
the ventral nerves in the old grafted wing had met to form the wrist nerve plexus,
whereas they had not yet done so in the host control wing. Further analysis
involves comparisons with the donor control wing as well as with the host
control wing. We had altogether five complete sets of well-stained wings, each
comprising an old grafted wing, an old donor control wing and a young host
control wing. In these, we found a total of 16 muscle nerve branches that pro-
vided a useful criterion of maturity, in that they were present in the old donor
control wing but not in the young host control wing. In eight of these instances,
the muscle nerve branch was present in the grafted wing; in the other eight it
was absent. The instances where the branch was present strongly support our
main conclusion that the maturity of the nerve pattern is determined by the maturity of the limb itself. But how are the absences to be explained? Can they be interpreted as deficiencies due to surgical disruption? As before, we have an independent estimate of the frequency of such deficiencies. In this series of grafts, there were 21 muscle nerve branches that were present in both host and donor control wings. In four (19%) of these instances, the muscle nerve branch was missing in the grafted wing. If the age of the grafted wing determines whether or not a given nerve branch will be present, then making due allowance for deficiencies due simply to surgical disruption, we should expect to find, out of our 16 critical instances, only three in which the nerve branch in question was absent in the grafted limb, rather than eight. This would be a statistically significant discrepancy if each muscle nerve branch was a statistically independent case; but it is not. In fact, four out of the eight missing nerve branches were from a single grafted wing which was found to have been pinned onto the host stump at an unnatural angle, rotated through 90° about its proximodistal axis. It is thus entirely plausible that the cases where a nerve branch was missing from the grafted wing though present in the donor control wing represent simply the consequences of surgical disruption, rather than any tendency of the maturity of the nerve pattern to be limited by the age of the host. On the other hand, the eight cases where a muscle nerve branch that had not yet developed in the young host control wing was visible in the older grafted wing show unequivocally that young nerves can be induced to develop precociously in an old limb.

Control grafts

In addition to the grafts between embryos of different stages, we did a series of control grafts between pairs of embryos at the same stage (roughly stage 19), or differing only slightly. These provided a further estimate of the proportion of muscle nerve branches missing simply as a consequence of the trauma of the grafting operation.

Ten grafted wings were analysed together with their host and donor controls. After excluding those instances in which the host, the donor or the graft was not well enough stained to allow a judgment as to whether or not a particular muscle nerve branch was present, we were left with a total of 73 muscle nerve branches in the grafted wings whose state of development could be compared with both host and donor controls. As expected from the results already described, there was no case in which a muscle nerve branch was present in a grafted wing but absent in the corresponding donor control wing. Out of 50 instances in which a muscle nerve branch was present in the donor control wing, we found the corresponding branch to be present in the grafted wing on 38 occasions, and absent on 12 occasions. Thus, 24% of the expected muscle nerve branches were missing, simply as a consequence of the trauma of grafting. This agrees well with the estimates of the effects of trauma in the grafts between embryos of different ages, and helps to support our previous arguments.
DISCUSSION

The developing pattern of nerves in the chick is largely determined by the limb tissues, rather than by the nerves themselves. The present results show that this is true not only with respect to geometry, but also with respect to timing. Young axons growing into an old wing bud behave precociously; old axons growing into a young bud are retarded. Highways for nerve outgrowth are apparently created in the limb as it matures, and the development of the nerves indicates the time at which these highways are first formed, or first opened up. If one of the highways is blocked artificially, for example by insertion of a small mica barrier into the early limb bud, the axons are blocked in their outgrowth, but generally are not deviated into regions where no highways exist (Lewis, unpublished). What then is the nature of the highways?

Electron microscope studies (Al Ghaith & Lewis, 1982) have shown that the growth cones of the pioneer axons of a nerve in the chick wing are in contact, over almost all their surfaces, with surfaces of other cells. Though the filopodia of the pioneer growth cones were not examined, this observation suggests that the route and timing of initial axon outgrowth are chiefly influenced by the surface properties of the mesenchymal cells of the limb. Subsequent axons follow the pioneers, which thereby determine the course of the mature nerve.

Two contrasting views of the influence exerted on axons by the tissue that they invade are relevant here. In the central nervous system, Singer, Nordlander & Egar (1979), Silver & Sidman (1980), Krayanek & Goldberg (1981) and others have described small channels that form between the neuroepithelial cells in advance of the growing axons, and have suggested that the direction and time of axon outgrowth are governed by the opening up of these channels. This idea does not seem applicable to the development of peripheral nerves, which advance through a connective tissue with extracellular spaces extending in all directions, rather than through a tightly packed epithelium (though we cannot exclude the possibility of some weak orienting effect due to a tendency for channels to be aligned more often in one direction than another). Another viewpoint, however, is provided by studies in tissue culture. Here, growth cones appear to be sensitive to the stickiness of the substratum, which affects their behaviour in several ways (Letourneau, 1975): given a choice, growth cones advance along the routes where the substratum is stickiest, at a speed which depends on the stickiness of the substratum; also, on very sticky substrata they show an increased tendency to branch. By analogy, one might suggest that in the limb bud the mesenchymal cells create the highways for axon outgrowth by altering their surfaces so as to make themselves sticky for growth cones. (Similarly, it has been proposed that the surface properties of the connective tissue cells in the limb bud may direct the grouping of myoblasts to form separate muscles (Chevallier, Kieny & Mauger, 1977; Wolpert, 1978; Jacob & Christ, 1980).) There is little to be said for or against this suggestion so far as
the main mixed nerve trunks are concerned. It is known, for example, that cells in the chick leg bud secrete fibronectin as the limb develops (Dessau, von der Mark, von der Mark & Fischer, 1980; Melnick et al. 1981; Tomasek, Mazurkiewicz & Newman, 1982) but the distribution of fibronectin does not bear any obvious relation to the paths of nerve outgrowth. The same is true of the distribution of collagen (see for, example, Shellswell, Bailey, Duance & Restall, 1980). Another suggestion, based on studies on the frog (Hamburger, 1929), is that blood vessels serve as guides for nerve outgrowth; but the observations of Piatt (1942) argue against this, as do our own observations on the chick (Al Ghaith & Lewis, 1982, and unpublished). Sections of developing chick wings show that, although nerves sometimes run closely parallel with blood vessels, they also often go separate ways.

One can, nevertheless, make some more specific statements about the nerve branches that go to individual muscles. These branches form only if myoblasts are present in the limb (Lewis et al. 1981). They originate in most cases as short tufts of axons that turn aside from pre-existing mixed nerve trunks where they pass close (within a few tens of µm) to the developing muscle rudiments. The axons that turn aside presumably arrive at the branch point by following pioneer fibres in the mixed nerve trunk. The muscle nerve branches first become visible just after myotubes have begun to form by fusion of the myoblasts; and, as our present results show, the time of their first appearance is controlled by the timing of changes in the limb tissues. Taken together, these observations strongly suggest that the branch routes that axons take towards developing muscles are defined by a short-range influence of the myoblasts or myotubes on the growth cones of the motor axons, an influence that could very well be exerted through a change in surface adhesivity of the developing muscle cells. Studies of human cells in vitro have indeed shown that the surface antigens of myoblasts change as they fuse to form myotubes (Walsh & Ritter, 1981). On the other hand, several recent papers (Dribin & Barrett, 1980; Henderson, Huchet & Changeux, 1981; Pollack, Muhlach & Liebig, 1981) have reported that axon outgrowth from neural tube explants in culture is stimulated, and perhaps guided, by diffusible factors released by muscle cells or other cells of developing limb buds. Of course, contact interactions and interactions depending on diffusible products are not mutually exclusive, and may shade into one another in the case of materials such as fibronectin, which can be released into the extracellular medium and also can cling to cells as a surface coat.

The molecular nature of the highways for axon outgrowth and the cellular mechanisms of guidance remain problematical. The findings reported here help to indicate where and when one must look in the developing limb for clues to solve the problem. Until the crude features of the guidance mechanism – that is, those that operate indiscriminately on nerve fibres from different sources – have been elucidated, it seems unlikely that we shall be able to explain satisfactorily the more subtle and specific forms of guidance that cause axons from
different regions of the CNS to follow particular branches of the public highway system preferentially, and to innervate specific targets in the periphery (Lance-Jones & Landmesser, 1980; Summerbell & Stirling, 1981; Landmesser, 1980; Hollyday, 1980).

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REFERENCES


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