The regeneration of double dorsal and double ventral limbs in the axolotl

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SUMMARY
Surgically made double dorsal and double ventral upper arms, comprising double extensor and double flexor muscles respectively, were amputated immediately following operation; both limb types regenerated. In terms of skeletal anatomy, a range of limb types was found. These included four-digit limbs of normal cartilage pattern, and hypomorphic limbs having from one to three digits.

All of the double dorsal and the majority of the double ventral limbs were symmetrical in the dorsal–ventral axis. This was detected by analysing their muscle patterns at carpal and mid-forearm level, and muscle and epidermal characteristics in the metacarpal region are discussed in terms of the significance of healing time and stump pattern on the regenerative ability of surgically created limb regions.

INTRODUCTION
It is becoming increasingly clear that in the amphibian limb, the behaviour of the dorsal–ventral axis does not conform to the same rules that determine pattern along the anterior–posterior axis. The most striking example is the discovery that supernumerary limbs resulting from a variety of experimental situations can show a range of abnormalities in their dorsal–ventral structure; being either symmetrical double dorsal or double ventral, or possessing anatomical discontinuities in this axis. These have been found in supernumeraries formed following 180° ipsilateral blastemal rotations (Maden, 1982, 1983; Maden & Mustafa, 1982a; Tank, 1981; Papageorgiou & Holder, 1983), and following skin transplantation and nerve deviation experiments (Reynolds, Holder & Fernandes, 1983; Maden & Holder, 1984). Recently, it has been shown that mixed-handed limbs, which have discontinuities in the dorsal–ventral axis, and so are similar in structure to the abnormal supernumeraries, can regenerate from surgically made mixed-handed stumps (Holder & Weekes, 1984). In addition, symmetrical double dorsal and double ventral limbs can regenerate following amputation of stumps bearing symmetrical skin grafts (Maden & Mustafa, 1982b).

In contrast, surgically made limbs symmetrical in the anterior–posterior axis never show anatomical discontinuities (Holder, Tank & Bryant, 1980; Krasner &

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Bryant, 1980; Stocum, 1978; Holder & Weekes, 1985). In addition, the upper arm experiments have shown that a difference in regenerative ability between double anterior and double posterior limbs exists at this level. Double posterior upper arms can produce symmetrical outgrowths having more than the normal number of digits; but double anterior upper arms never result in more than a single digit. Both types, however, only attain their maximum regenerative potential if amputation is carried out immediately following grafting; there is a considerable reduction in structure if amputation is delayed even for a few days (Tank & Holder, 1978; Stocum, 1978). It is known that double dorsal and double ventral upper arms have little regenerative ability if they are amputated 30 days after operation (Tank, 1978). Whether double dorsal and double ventral limbs regenerate if amputation is carried out at earlier times has not yet been determined.

The purpose of the present study is twofold. First, to establish whether double dorsal and double ventral limbs show a healing time effect comparable to that seen for double anterior and double posterior upper arms; and second, to discover if a difference in regenerative ability exists between the two types of upper arms, as is characteristic of the anterior–posterior axis. The results show that double dorsal and double ventral upper arms regenerate limbs of appropriate symmetry when amputated immediately, therefore demonstrating a clear healing time effect. Comparison of the structure of these regenerates shows that both limb types have an equivalent regenerative potential.

MATERIALS AND METHODS

Experiments were carried out on the upper forelimbs of larval axolotls (Ambystoma mexicanum), which had been spawned in the colony at King's College. They were kept in tap water in individual plastic containers, throughout this study, and fed on raw heart three times a week. All animals were anaesthetized in MS222 (Sigma) during surgery and amputation of limbs.

Experimental

The axes used in this experiment correspond exactly to those described for the newt (Bryant, 1976). Furthermore it is now clear following extensive discussions between all the investigators in the papers cited below, that they also correspond to those used previously to make symmetrical upper arms in axolotls (Tank, 1978; Tank & Holder, 1978; Holder & Tank, 1979; Holder et al. 1980; see Fox, 1982), despite descriptions to the contrary in these papers. Hence the double dorsal and double ventral upper arms made by Tank (1978) are directly comparable in anatomy to those of the present experiment. Dorsal halves of left upper arms were exchanged with ventral halves of right upper arms, within the same animal (Fig. 1). The proximal–distal and anterior–posterior axes of graft and host remained aligned, and the grafts were sutured in place. Limbs were amputated immediately through the symmetrical region, and bone and soft tissue trimmed to give a flat amputation surface, while leaving at least 2 mm of grafted tissue firmly in place.

Control operations, involving removal and replacement of either dorsal or ventral halves, but no contralateral exchange, were carried out using the same technique. These were similarly amputated through the mid-upper arm immediately after surgery.

The forelimbs of both experimental and control animals were checked initially every few days, and then at weekly intervals, to examine the condition and viability of the graft. Limbs were allowed to regenerate for 60 days, and then amputated.
Analysis of experimental limbs

All limbs were fixed in Bouins solution decalcified in EDTA for at least a week, and then dehydrated, stained with Victoria Blue and cleared in methyl salicylate to reveal the skeletal exchange.

Fig. 1. Diagram showing details of the grafting procedure. At the top is shown the dorsal view of the forelimbs, and below are sections through the upper arms, both before and after operation. The ventral flexor muscles (dotted), and the dorsal extensors (unmarked) surround the centrally located humerus, which was left in situ. A, anterior; P, posterior; D, dorsal; V, ventral.

Table 1. Skeletal structure of regenerates following immediate amputation of symmetrical upper arms

<table>
<thead>
<tr>
<th></th>
<th>Number of digits</th>
<th>Spikes or cartilage elements only</th>
<th>No regeneration</th>
<th>Mean no. of digits*</th>
<th>Mean no. of skeletal elements†</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>4 3 2 1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>DD</td>
<td>14</td>
<td>3 0 1 2</td>
<td>5</td>
<td>2.7</td>
<td>11.2/7.2</td>
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<tr>
<td>VV</td>
<td>16</td>
<td>3† 4 2 1</td>
<td>2</td>
<td>2.6</td>
<td>11.6/8.3</td>
</tr>
</tbody>
</table>

* Only limbs which regenerated digits were used to calculate the mean.
† The first figure is the mean for all limbs which regenerated skeletal elements, and the second includes all operated limbs.
† Two limbs had muscle only on the host ventral side. These were not included in assessment of the mean number of digits or skeletal elements.
Fig. 2. Anatomy of the normal axolotl forelimb. (A) Skeletal structure, dorsal view of a right limb. The dashed lines correspond to the levels of the transverse sections. Scale bar, 2 mm. (B) Proximal metacarpal level; camera-lucida drawing of a haematoxylin- and eosin-stained section. The cap-shaped extensor digitorum breves (edb) muscles lie on the dorsal side of the metacarpals; ventrally are found the thicker flexor muscles (dotted). (C) Transverse section through the proximal carpals, showing the names and arrangement of the muscles. (i) Dorsal extensor muscles. adl, abductor minimi I; edc, extensor digitorum communis. (ii) Ventral flexor muscles. abdm, abductor brevis digitii minimi; ppI, II, III, palmaris profundus muscles; ps, palmaris superficialis; uc, ulno carpalis. (D) Transverse section through the mid-forearm region. (i) Dorsal extensor muscles. eacu, extensor antebrae carpi ulnaris; ear, extensor antebrae radialis; ecr, extensor carpi radialis; edc, extensor digitorum communis. (ii) Ventral flexor muscles. facr, flexor antebrae carpi radialis; fcu, flexor carpi ulnaris; pq, pronator quadratus; ps, palmaris superficialis; uc, ulno carpalis. A, anterior; P, posterior; D, dorsal; V, ventral; m, metacarpals; c, carpals; r, radiale; u, ulnare; i, intermedium; 1, 2, 3, 4, digits, numbered from anterior to posterior. Scale bars, 0.5 mm.

structure (Bryant & Iten, 1974). Camera-lucida drawings were made of all regenerates. The limbs were then processed for wax embedding. Serial transverse sections, 10 μm thick, were cut and stained with haematoxylin and eosin. Muscle patterns were determined for each limb by examining sections at three distinct limb levels; proximal metacarpals, proximal carpals and mid-forearm.
RESULTS

(A) Experimental limbs

These consisted of a total of 14 double dorsal upper arms and 16 double ventral upper arms.
(1) Cartilage patterns

Nine of the double dorsal operations and twelve of the double ventral operations regenerated; the majority produced typical limb structures, having between one and four digits (Table 1). Three of the double dorsal upper arms

Fig. 3. Cartilage patterns of hypomorphic regenerates from double dorsal upper arms. Camera-lucida drawings of Victoria-Blue-stained specimens. Dorsal view of right forelimbs. Scale bars, 2 mm. (A) Single-digit regenerate, which has both radius and ulna, and then tapers distally. (B) Two-digit regenerate, having just a single forearm element. A, anterior; P, posterior.
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produced four-digit regenerates, which had normal cartilage structure (Fig. 2A). Three hypomorphic limbs resulted in this group: one having two digits (Fig. 3B), and two having single digits. Of the latter, one converged distally, having both radius and ulna, four carpal elements, and then tapering to a single digit (Fig. 3A); the other limb had one forearm element and two carpals.

The double ventral upper arm regenerates showed a wider variety of skeletal pattern. Three limbs having four digits resulted. One was missing the ulna, but was otherwise skeletally normal; one had slight branching of two digits, and in the third

Fig. 4. Hypomorphic regenerates from double ventral upper arms; dorsal view of left forelimbs. Scale bars, 2 mm. (A) Two-digit limb. The radius is fused with the humerus, the posterior two digits are at right angles, and their associated carpals are at slightly differing proximal–distal levels. (B) Three-digit regenerate. The humerus did not regenerate distal to the amputation plane. A, anterior; P, posterior.
Fig. 5. Light micrograph of the dorsal epidermis in the metacarpal region of the normal forelimb; taken from the section drawn in Fig. 2B. The abundance of Leydig cells (L) can be clearly seen, amongst the less-frequent epidermal cells (e), which are considerably smaller in size. The basement membrane (bm) is visible, and part of an edb muscle. (B) Light micrograph of the ventral epidermis, from the same transverse section as A. There are few Leydig cells; the majority of cells are of the smaller epidermal type, so the epidermis is comparatively thin in this region. Note also that there appears to be a distinct gap between muscle and epidermis; this is similarly characteristic of the ventral side at the metacarpal level. Scale bars, 80 μm.
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the digits curled ventrally to an exaggerated degree. Four of the double ventral stumps regenerated three-digit limbs, all of which had additional proximal–distal skeletal abnormalities. All lacked a normal elbow, and had missing or misaligned cartilage elements in the hand and forearm regions (Fig. 4B). Two limbs having two digits also resulted (Fig. 4A), and one having a radius, four carpals, and a single digit along its posterior edge. These should be compared to the more regular patterns of the hypomorphic limbs that regenerated from the double dorsal upper arms.

If the mean number of digits for both types of limb are compared, it is found that both are approximately equal; for limbs which produced digits, an average of 2.7 resulted from the double dorsal upper arms, and 2.6 from the double ventral upper arms (Table 1). The mean number of skeletal elements, however, reveals a slight difference in regenerative ability, with the double ventral operations giving a higher figure. This difference is largely due to the greater percentage of double dorsal upper arms that did not regenerate at all, and so contributed nothing to the total.

(2) Muscle patterns

A clear asymmetry of dorsal and ventral muscle patterns exist in the normal axolotl limb. Sections taken through three distinct regions, the proximal metacarpals, proximal carpals and mid-forearm, were used to ascertain the dorsal–ventral axial pattern of the regenerates in this study.

Briefly, the muscle pattern of the normal forelimb is as follows (Fig. 2). At the metacarpal level, the dorsal muscles (extensor digitorum breves), are crescent-shaped, and lie as caps over each of the cartilage elements. Those of digits 2 and 3 reach furthest distally; while for the lateral digits, muscle terminates in the proximal metacarpal level; tendons then extend to the distal ends of the phalanges. Ventrally, five separate flexor muscles form a continuous mass which extends across the underside of the hand; and eventually reaches to the distal ends of the digits (see Maden, 1980, 1982; Maden & Mustafa, 1982a). In addition, at this level, a clear dorsal/ventral difference in the nature of the epidermis is apparent (Fig. 5). Dorsally, more of the epidermal cells are Leydig cells, and due to their larger size the epidermis is thicker on this side (Holder & Weekes, 1984). Also, a distinct gap between muscle and epidermis appears to be a characteristic of the ventral side at this limb level. More proximally, these differences disappear.

At carpal and forearm levels, characteristic muscles are again found, and in specific locations. In addition, in the mid-forearm, dorsal and ventral are easily distinguished by the presence of a distinctive muscle, the pronator quadratus (pq), which lies beneath the radius and ulna on the ventral side: the fibres appear longitudinal in transverse section, and so can be unequivocally identified (see Holder & Weekes, 1984).

All of the double dorsal upper arms that regenerated typical limb structures had double dorsal muscle patterns (six limbs in total: Table 1). The three limbs that regenerated four digits had two complete mirror image copies of the normal
dorsal extensor muscle pattern (Fig. 6). At the metacarpal level, \( \text{edb} \) muscles can be clearly seen, forming caps on both sides of the cartilage elements. Thick dorsal epidermis completely surrounds the limb, with an even distribution of Leydig cells at all axial positions. Sections taken further proximally similarly show the symmetrical pattern of the muscles; and there is no \( pq \) in the forearm.

Fig. 6. \textit{Camera-lucida} drawings of transverse sections from a double dorsal four-digit regenerate, taken at the same proximal–distal levels as in Fig. 4. (A) Section through the metacarpal region. The cap-shaped \( \text{edb} \) muscles are found on both sides of the metacarpals; thick dorsal-type epidermis completely surrounds the limb at this level. (B) Proximal carpals; again a complete set of dorsal extensor muscles lies on either side of the cartilage elements. Abbreviations as for Fig. 2C. (C) Mid-forearm; showing the double dorsal symmetry of the muscles. Abbreviations as for Fig. 2D. Scale bars, 0.5 mm.

Fig. 7. \textit{Camera-lucida} drawings of sections from the double ventral four-digit regenerate. (A) Metacarpal level. The thick sheets of ventral flexor muscle completely enclose the cartilage elements; the surrounding epidermis is ventral in character, containing few Leydig cells. (B) Proximal carpal level; showing the symmetrical arrangement of the two sets of ventral muscle. Abbreviations as for Fig. 2C. Scale bars, 0.5 mm.
Both of the single-digit limbs similarly had symmetrical muscles at all levels. The two-digit limb sectioned poorly, and its muscle pattern could not be identified. For this reason it was re-amputated through the graft region; the second regenerate

Fig. 8. Mid-forearm section from a two-digit regenerate from a double ventral upper arm regenerate; the cartilage pattern is shown in Fig. 3A. Transverse sections taken at more distal levels showed that the muscles were symmetrical; however, due to the reduction in width of the anterior–posterior axis, and the proximal–distal mismatch and abnormal orientation of the digits, the actual nature of the muscles could not be identified at these levels. In this section, the double ventral character of the muscles can be clearly seen. Abbreviations as in Fig. 2D. Scale bar, 0.5 mm.

Fig. 9. Metacarpal section from a double ventral upper arm regenerate, having a single copy of the ventral muscles only. The flexor muscle is located on the outer curved edge; on the opposite side of the metacarpals only diffuse connective tissue is present. Scale bar, 0.5 mm.
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had four digits and was clearly double dorsal in structure. The first regenerate has therefore been classified as symmetrical in Table 1.

Eight of the ten regenerates arising from the double ventral upper arms had double ventral (flexor) symmetry. One was a four-digit limb, which although lacking an ulna was otherwise skeletally normal (Fig. 7). Thick sheets of ventral muscle extended across both limb surfaces in the metacarpal region; only ventral epidermis is present, and the characteristic ventral gap is found on both sides. The symmetry of the muscle patterns was clearly apparent at all levels. However, the majority of these limbs, in addition to a reduction in the anterior–posterior axis, also had proximal–distal skeletal abnormalities, especially in the hand region. The double ventral nature of these limbs could frequently be most easily determined at the mid-forearm level, where the presence of two copies of the pronator quadratus muscle could often be seen (Fig. 8).

The two remaining four-digit regenerates resulting from this operation had an asymmetrical muscle pattern. In the digit region characteristic ventral muscle was present on one side of the cartilage, diffuse connective tissue only being present on the opposite side. One limb was transiently double ventral at forearm level. Thin epidermis surrounded the metacarpals, although the distribution of Leydig cells was biased towards the muscleless region. The digits curled extensively inwards on this side (Fig. 9), giving further confirmation that abnormal dorsal–ventral pattern cannot be interpreted by analysis of skeletal structure alone. In the other, dorsal muscle was found in the carpals, and dorsal epidermis was present in the metacarpal region. These limbs were not included in calculation of the mean number of digits and skeletal elements; they may have regenerated from the host ventral half only, possibly due to reduced viability of the graft.

(B) Control limbs

Five control operations were carried out for both types of symmetrical upper arm. All regenerated four-digit limbs of normal skeletal structure, and having normal dorsal–ventral asymmetry. Following the second amputation, one of the double ventral controls failed to regenerate, but all others again gave normal four-digit limbs.

DISCUSSION

The results presented here show that both double dorsal and double ventral surgically made upper arms, do regenerate if amputated immediately, and produce limbs having the symmetry of the stump. Both have been shown to have the same regenerative ability, and to be capable of producing four-digit proximal–distally complete limbs. Hypomorphic limbs also resulted from each type of operated upper arm; more skeletal abnormalities were found amongst the double ventral regenerates.

This work extends previous findings for limbs symmetrical in the anterior–posterior axis. Double posterior surgically made upper arms can produce double
posterior distally complete regenerates following immediate amputation. Over 50% of the resulting limbs had a higher number of digits than normal; between three and six digits resulted. Double anterior limbs, however, are the least successful of any of the four symmetrical limb types at regenerating: they have never been found to produce more than a single digit (Holder et al. 1980; Holder, 1981). So the profound difference in regenerative ability that characterizes double anterior and double posterior upper arms does not exist for limbs that have been made symmetrical in the dorsal–ventral axis at this level, although some qualitative differences were found. This conclusion is consistent with that drawn from an assessment of the frequency of regeneration of double dorsal and double ventral limbs formed from a variety of different grafting procedures ranging from blastemal rotation (Maden & Mustafa, 1982a; Papageorgiou & Holder, 1983), skin transplantation and nerve deviation (Reynolds et al. 1983; Maden & Holder, 1984), mixed handed limb stumps (Holder & Weekes, 1984), to stumps bearing symmetrical skin (Maden & Mustafa, 1982b). Although the frequency of regeneration of double dorsal and double ventral limbs varies considerably from experiment to experiment, the overall picture clearly indicates a similar potential for regeneration of both of these limb types.

Although double posterior stumps are capable of regenerating proximal–distally complete limbs with three or more digits, they do not always do so: certain experimental conditions are necessary. The most profound influence on their regenerative ability is the healing time allowed between grafting and amputation (Tank & Holder, 1978). The results obtained here show that a similar healing time effect appears to exist for upper forelimbs made symmetrical in the dorsal–ventral axis. Double dorsal and double ventral upper arms allowed a healing time of 30 days and gave markedly less outgrowth (Tank, 1978) than has been shown here to result from immediate amputation. As was discussed above (see Materials and Methods) the symmetrical limbs constructed in both experiments are identical in terms of muscle patterns at the amputation site.

To compare the regenerative ability of limbs resulting from the two series of operations, three particular points need to be assessed. First is the percentage of symmetrical upper arms that initiate a regenerative response, and also those that produce typical limb structures, in terms of possessing complete proximal–distal pattern. Second is the maximum regenerative potential for each group. Third, the average amount of structure can be compared: this is best considered in terms of the mean number of cartilage elements produced, so that those that did not regenerate to the most distal levels can also be included.

The percentage of upper arms that initiated regeneration, giving at least a single cartilage element, as opposed to not regenerating at all, was approximately equal both for our results and those of Tank (1978). However, in all other respects regeneration following immediate amputation proved to be much more successful.

The maximum regenerative potential of the double ventral upper arms amputated 30 days after operation consisted of spike-like structures, having one or two skeletal elements only. None had any distinctive transverse axial organization, or
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typical proximal–distal pattern. Immediate amputation not only resulted in a seven-fold increase in the mean number of skeletal elements, but the majority of limbs that initiated regeneration had typical limb structures, and were here found to be capable of complete regeneration, to give four-digit double ventral limbs.

Similarly, skeletally complete double dorsal limbs resulted following immediate amputation, but not if it had been delayed by 30 days. Two-digit regenerates constituted the maximum regenerates for the latter category, and the mean number of skeletal elements per operated limb was more than doubled by allowing no healing time. None of the 30-day regenerates were sectioned, however, so whether or not they possessed double dorsal symmetry is not known.

A similar result was found in the newt; only slight regeneration occurs if 30 days healing time is allowed (Bryant & Baca, 1978). So clearly a similar healing time effect exists for surgically made upper arms that are symmetrical in the dorsal–ventral axis as for those made symmetrical in the anterior–posterior axis.

The significance of the healing time effect is not clear. Long healing times do not give a reduction in the regenerative ability of double anterior and double posterior lower arms (Krasner & Bryant, 1980). Also, limbs symmetrical in the dorsal–ventral axis result from a variety of grafting experiments: these have been described above. The restriction in regenerative potential appears to be specific to upper arms allowed a long healing time, rather than a reflection of the underlying patterning mechanisms. Further support for this is that despite the increasing evidence that pattern specification in the two transverse axes is different, healing time has now been shown to affect both axes in the same way. Whether there is any difference between surgically made symmetrical limbs and those that have arisen by other means is currently being investigated.

Two recent models for pattern specification in the amphibian limb must be evaluated in the light of the results presented here. Meinhardt (1983) has proposed that two distinct boundaries exist in the normal limb, one having anterior-to-posterior orientation and one dorsal-to-ventral orientation. These are then responsible for organizing pattern in the transverse axes, intersection of the boundaries being a necessary prerequisite for limb outgrowth. Thus double dorsal and double ventral stumps would only be expected to regenerate if a dorsal–ventral boundary remained after the operation, and on the posterior side of the limb. No such boundary regions were evident in any of the symmetrical limbs described in this study, or have ever been noted elsewhere. However, this may be because they are not apparent at the level of muscle patterns, and that a more sensitive assay is needed for their detection.

That double dorsal and double ventral upper arms have a similar regenerative potential supports the proposal that dorsal and ventral are equivalent cell states. However, surgically made symmetrical limbs cannot be an unequivocal test of Meinhardt’s proposal. Depending on whether or not the patterning boundary coincides with the anatomical dorsal–ventral boundary, neither limb of a reciprocal pair, or one type only would be expected to regenerate. In the present
experiment both limbs regenerated in five pairs, giving a total of between four and eight digits per pair, and four of these pairs produced at least three forearm elements. In one case only the double dorsal upper arm resulted in a limb, giving a four-digit symmetrical outgrowth; in three pairs only double ventral regenerates were found, which had either two or three digits. The remaining cases either failed totally to regenerate, or produced only a small number of cartilage elements. It could be argued, however, that slight variations in cutting could lead to the presence of some oppositely coded cells in both upper arms of a reciprocal pair, which then may be sufficient to establish the necessary boundary.

The polar co-ordinate model (Bryant, French & Bryant, 1981) also cannot satisfactorily explain the results presented here. Patterning and distal outgrowth are proposed to occur by a process of short arc intercalation, due to local interactions between normally non-adjacent cells (Bryant, 1978; Bryant et al. 1981). Specific influences on cell contact patterns must therefore lead to particular changes in the type and degree of structure regenerated.

Two such influences are relevant here. Firstly, the healing time effect for surgically made upper arms has previously been explained on this basis. If the presence of an unhealed wound in the centre of the amputation plane prevents cell contact across the line of symmetry, then cells with like positional values would not be brought into contact, and so intercalation proceeds until the most distal levels are reached. However, it must be questioned whether the healing time effect is a specific response of the patterning mechanisms, or a consequence of the trauma of surgery at this arm level. Double dorsal and double ventral supernumeraries result from a variety of grafting experiments in which no unhealed wound is present across the line of symmetry (see Maden, 1982, 1983; Tank, 1981; Maden & Holder, 1984). Also no healing time effect has been found for surgically made double anterior and double posterior lower arms (Krasner & Bryant, 1980).

Secondly, the transition in blastemal shape is predicted to alter cell contact patterns (Holder & Reynolds, 1983, 1984; Holder, 1984). This changes from being rounded in the upper arm to being elliptical at lower limb levels, with the short axis oriented dorsal–ventrally. Expansion of the anterior–posterior axis following amputation of double posterior limbs in the lower arm is then explained as a consequence of dorsal and ventral cells being more likely to be brought into contact than cells from the anterior and posterior extremities. But limbs that are symmetrical in the dorsal–ventral axis must then be expected to terminate abruptly as soon as levels distal to the elbow are reached, which is clearly not the case. So although a difference in behaviour between upper and lower arms is expected in terms of short arc intercalation, again all of the experimental findings cannot be accounted for.

We interpret our results as supporting the view that pattern in the two transverse axes is specified by different mechanisms. Posterior dominance and continuity appear to underlie the patterning mechanism in the anterior–posterior axis: this is compatible either with a gradient model, or local cell interactions leading to directional intercalation (Muneoka & Bryant, 1984) in this axis only. The
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equivalence of dorsal and ventral and maintenance of this component of positional information throughout morphogenesis are indicated by the behaviour of the dorsal–ventral axis during experimental manipulation. Relative contribution of cells with dorsal and ventral codings then determines the pattern of the resulting regenerate, and so double dorsal and double ventral limbs would both be expected to regenerate from symmetrical upper arms, and show the same regenerative ability.

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