Pattern formation and growth in the regenerating limbs of urodelean amphibians

By NIGEL HOLDER

From the Anatomy Department, King's College London

SUMMARY

The results of numerous types of grafting experiments involving the amputation of symmetrical limbs are described. These experiments were designed to test the tenets of the polar coordinate model. The analysis of the results of these grafts coupled with a quantitative analysis of blastemal shape strongly indicates that pattern regulation during amphibian limb regeneration can be understood in terms of the model.

INTRODUCTION

The amphibian limb undergoes a specific series of morphologically defined events following amputation at any level of the proximal to distal axis (Iten & Bryant, 1973; Tank, Carlson & Connelly, 1976; Stocum, 1979). Following wound healing the mesodermal cells close to the wound surface undergo a process of dedifferentiation when cells with clear phenotypic characteristics, such as muscle and cartilage cells, become visually homogeneous and form a founding cell population which divides at a consistently high rate to produce a blastema. The blastema then increases in size and cell number until the new parts of the limb begin to reform in a proximal to distal sequence. Once the missing parts have all been replaced they grow to the correct degree and thus result in a complete limb which is the size and form of the part that was initially removed.

Growth is occurring at all stages in this regeneration process, but only part of the process is concerned with pattern formation. The object of this paper is to discuss which part of the growth process is linked to the spatial patterning mechanisms and how this link might be characterized. The term growth is used here in the general sense of increase in size with reference to the new tissues being formed during regeneration. There are three ways in which a tissue can increase in size: the blastemal cell population may increase in number by cell division or cell recruitment, the cells may increase in size (cell hypertrophy) and they can produce extracellular material which swells the tissue mass.

The initial stages of limb regeneration involve both cell division and cell recruitment and some growth is accounted for by swelling of the tissues as a

1 Author's address: Anatomy Department, King's College London, Strand, London WC2R 2LS, U.K.
result of general oedema (Chalkley, 1954). By far the greatest component of growth in the first stages of blastemal outgrowth is an increase in cell number. Cell division occurs at a consistently high level once the blastemal population has been established (Maden, 1976; Smith & Crawley, 1977; Stocum, 1980a). This population of cells is derived from the stump tissues during the dedifferentiation process as a result of cell division and cell recruitment (Chalkley, 1954). The relationship between cell recruitment and cell division in the formation of the blastemal cell population is important to understand in terms of the relationship between pattern formation and growth. It is especially important because amphibian limb regeneration is thought of as an epimorphic process where cell division is crucial for the regeneration of the limb pattern; and it is equally important if we are to establish the relationship between the production of new positional values and cell division.

Pattern formation and growth: the polar coordinate model

The main part of this paper is given over to discussion of the evidence which supports the view that cell division and generation of new positional values are intimately linked. The experiments discussed clearly establish that growth of the regenerating limb can be dramatically affected as a result of surgical manipulations which alter the normal spatial relationship of cells giving rise to the blastemal population. The experiments described were performed in the attempt to test the tenets of a formal model which theoretically links the process of cell division to the generation of new positional values.

The polar coordinate model (French, Bryant & Bryant, 1976; Bryant, French & Bryant, 1981) postulates that the positional values existing in the limb are arranged in a circular and an angular sequence (Fig. 1a). When cells are confronted which have normally non-adjacent positional values, intercalation of the normally intervening values occurs as a result of cell division. With regard to the circular sequence of values this intercalation occurs via the shortest route of the sequence. It is this observation, incorporated into the model as the shortest route intercalation rule, which is of central importance to our understanding of the cellular interactions leading to regeneration of the pattern and to the relationship between pattern formation and growth. The actual number of intercalatory events, and thus the number of new cells with new positional values, will depend upon which cells (i.e. which positional values) contact during the regeneration process. This point is best illustrated by examining how the polar coordinate model accounts for normal limb regeneration.

Blastemal accumulation produces a mound of cells on the distal tip of the amputated limb. This conical mound is narrower at its tip than at its base and therefore, as outgrowth proceeds, cells from different circumferential positions will contact at the distal tip. As cells with normally non-adjacent positional values contact in a localized manner around the circumference intercalation occurs and new cells are produced (Fig. 1b). This process occurs all around the
Fig. 1. (a) The polar coordinate model can be formally represented as a two-dimensional array of positional values. One dimension is angular and is represented by twelve numbers arranged around the circumference, the other is radial and is represented by letters A (proximal) through E (distal). (b) Short-arc intercalation following amputation of a normal limb. The dashed lines represent local cell contacts resulting in intercalation of new circumferential positional values which also have the next most distal radial positional value. Local contacts around the circle produce a complete new, more distal circular sequence. This process continues until the distal end of the pattern is reached. (c) Short-arc intercalation in a double anterior symmetrical upper arm stump from a long-healing-time group. Dashed lines represent cell contacts leading to intercalation of cells with more distal circumferential positional values. Solid straight lines represent cell contacts which will not stimulate such cell divisions. Due to loss of midline circumferential values and non-productive cell contacts, regeneration ceases at radial level C. (d) Short-arc intercalation in a double posterior symmetrical upper arm stump from a short-healing-time group. No cell contacts occur across the central solid line which represents the wound incurred by the initial surgery. This, coupled with the proposed greater number of circumferential positional values in the posterior limb region, allow more distal regeneration to occur than in Fig. 1c.
circumference and results in the build up of a complete new circumferential set of positional values which constitute the next most distal circular set (Bryant et al. 1981; French, 1981). As outgrowth continues, more and more distal sets of circumferential positional values are produced, until the most distal set is formed and the complete pattern is restored. This process of intercalation occurring in localized regions of the circumference during blastema formation emphasizes the importance of the circumference in distal outgrowth. This emphasis is made clearer still if abnormal arrangements of positional values are created at the amputation plane, and the resulting regenerated limb patterns are analysed.

Regeneration of symmetrical limbs

One simple way of altering this circular sequence is to surgically create symmetrical limbs which have two sets of positional values symmetrical about one of the transverse limb axes (Fig. 1c–d). Such symmetrical stumps yield remarkably different patterns of limbs depending on the level of the proximal to distal axis at which they are created, and the local conditions at the amputation site which affect cell contact between cells from each of the symmetrical regions. These conditions include the shape of the amputation plane, that is whether the
amputation plane is round or oval, and the effect of the wound created by the surgery on the ability of cells from either side of the wound to contact. The amount of regeneration achieved by symmetrical limb stumps is also affected by the number of positional values present at the amputation plane in each specific construction.

These last two features, the ability of cells to contact and the number of values present, are demonstrated by examining the effect that graft healing time prior to amputation has on the degree of distal outgrowth achieved by symmetrical upper arms. A clear inverse relationship exists between the degree of distal outgrowth and the time for which grafts creating symmetrical upper arms are allowed to heal before amputation (Stocum, 1978; Tank & Holder, 1978). Figure 2 shows the amount of regeneration occurring from double anterior and double posterior upper forelimbs amputated at periods from 5 to 60 days after the operation. Several points can be gleaned from these results. Double posterior limb stumps regenerate more structure than double anterior limb stumps. If it is assumed that the posterior side of the normal limb contains more than half of the circumferential set of positional values and the anterior side bears the remainder, then the creation of symmetrical limb stumps will accentuate this difference because two sets of these values are present (see Fig. 1c–d). In addition, the fall off in the distal extent of regeneration of symmetrical limbs with healing time may be explained in terms of cell contacts leading to intercalation of new positional values. We argue that in the long-healing-time groups the complete closure of the wound created by the surgery enables cells from either side of it to contact during initial blastema formation (Holder, Tank & Bryant, 1980; Bryant, Holder & Tank, 1981). In this case cells with the same or normally adjacent positional values will contact across the line of symmetry. Such cell contacts will not stimulate intercalation of new values and no cell divisions will occur. Thus the number of new cells and the number of circumferential positional values available at the next most distal level will be reduced. This reduction in positional values will occur at the line of symmetry (Fig. 1c; Holder et al. 1980; Bryant et al. 1981). Midline loss of positional values will continue as distal outgrowth occurs until intercalation, and regeneration, ceases. In the double anterior limbs, because there are assumed to be fewer positional values to start with, midline loss of positional values will lead to the cessation of regeneration faster than in the double posterior limbs which are therefore able to achieve a greater degree of distal regeneration.

Midline loss of positional values and the premature cessation of intercalation in those limbs predicts the formation of tapering, distally incomplete symmetrical regenerates. Should the cessation of intercalation be particularly rapid, virtually no regeneration will occur. These predicted tapering spike-like limbs and regenerates which consist of only nodules of cartilage (non-regenerates) are seen in many cases (Tank & Holder, 1978; Holder et al. 1980) and examples are shown in Fig. 3.
In contrast to these long-healing-time groups, short-healing-time symmetrical limbs regenerate far more structure. This is particularly evident in double posterior limbs (see Figs. 1d, 2); this is again presumed to reflect the unequal distribution of circumferential positional values. Immediate amputation of such limbs leads to the regeneration of double posterior symmetrical structures which vary in the extent of formation of the a-p axis distally and thus show a variation in their number of digits (Holder et al. 1980). Double anterior limbs amputated immediately predominantly regenerate tapered distally incomplete limbs. The proposed explanation for the greater regeneration potential of limbs in short-healing-time groups involves the presence of a wound existing between the symmetrical halves at the amputation plane, which results from the operation. If cells are unable to contact across this central reservation then cells with the same positional value will not contact. Therefore, these non-productive cell contacts which are assumed to be the primary cause of the early cessation of regeneration in long-healing-time groups, are prevented. The short-healing-time symmetrical limb stumps will thus be capable of more distal outgrowth (Figs. 1d, 3). It should be made clear, however, that local cell contacts will still cause loss of midline positional values, as is the case in the long-healing-time groups, and the outgrowths, whether double anterior or double posterior, should show a gradual midline loss of structure as distal outgrowth occurs. Analysis of the skeletal structure shows this to be the case. This midline loss will lead to more rapid cessation of regeneration in double anterior limbs than double posterior limbs but will allow greater degrees of distal outgrowth of both when compared with long healing time groups.

In addition to the various symmetrical outgrowths regenerated by these symmetrical upper arm stumps they also occasionally produce asymmetrical half limb structures. These occurred in both double anterior and double posterior constructions; the double anteriors producing half anterior limbs and the double posteriors half posterior limbs (Fig. 3) (Holder et al. 1980). One conclusion from the explanation for distal outgrowth of short-healing-time limbs is that each half of the surgically created stump distally transforms independently. Thus, if one side is prevented from growing, the other will regenerate regardless. In all of the cases where half limbs developed, they appeared to do so from the

Fig. 3. Victoria-blue-stained whole-mount preparations of forelimbs of the axolotl. (a) Normal regenerated right limb. (b) Spike-like outgrowth regenerated from a double posterior upper arm construction amputated 10 days after grafting. (c) Non-regenerate formed from a double posterior upper arm construction amputated 15 days after grafting. The leader points to the small, regenerated nodule of cartilage. (d) Three-digit symmetrical double posterior regenerate formed after amputation of a double posterior upper arm construction five days after grafting. The dotted line denotes the line of symmetry. Note the distally converging pattern. (e) A posterior half-limb formed following amputation of a double posterior upper arm construction immediately after grafting. d, digits; c, carpals; r, radius; u, ulna; h, humerus. The Roman numerals denote the digital number with i being anterior and iv posterior. The arrows denote the position of amputation. The scale bars equal 1 mm.
Fig. 4. Analysis of the relationship of graft healing time to degree of regeneration. (a) Regenerated limbs of either non-regenerates or spikes (dashed line), or distally complete limbs with digits (solid line) produced following amputation of double-posterior symmetrical upper arms formed following amputation at various times after grafting. Note the sudden fall in appearance of distally complete limbs between 0 and 15 days. The double anterior upper arms stumps all yielded non-regenerates or spikes and are not shown. All data taken from Tank & Holder (1978) and Holder et al. (1980) (see text). (b) The relationship between the appearance of half-limbs (expressed as a percentage of the total number of cases analysed in both 4a, b). The top graph is half posterior limbs formed following amputation of symmetrical double posterior upper arm stumps; the bottom graph is half-anterior limbs formed following amputation of symmetrical double-anterior upper arm stumps.

host side of the symmetrical stump. It seemed therefore that the graft was the half which did not develop. This may be due to poor innervation of the grafted piece because it is well established that a local nerve trophic requirement is needed for cell division and therefore distal outgrowth to occur. Although the appearance of half limbs is consistent with the notion that each half of a symmetrical stump can regenerate independently, the polar coordinate model would require circumferential intercalation to occur on the medial edge of these half limbs during distal outgrowth. The reason why such intercalation does not occur in these cases is at present the subject of further study.

The gradual decline in regenerative ability of both double posterior and double anterior symmetrical stumps with the increase in time before amputation seems paradoxical if healing of a wound is indirectly responsible. This is because the graft and host tissues appear histologically to be completely healed by 10–15 days after the graft was performed (see histological analysis in Tank & Holder, 1978), yet the decline in structure of the regenerates occurs up to 30 days (Fig. 2). This paradox is resolved, at least in part, if the formation of half limbs is taken into account. In the light of the argument that one side of the
symmetrical stump can regenerate independently if the other is unable to regenerate, it seems clear that the formation of half limbs should not bear the same clear relationship with graft healing time as regenerates forming from both sides. For this reason the regenerates produced from all graft healing time groups were re-examined in a different way (data from Tank & Holder, 1978, and Holder et al. 1980). Regenerates were classified into three groups: those where distal outgrowth had produced a clearly symmetrical set of digits (Fig. 3), half limbs which comprised only one forearm element and either 1 or 2 digits (Fig. 3), and distally incomplete regenerates which in fact were either spikes or non-regenerates (Fig. 3). The number of half limbs was then plotted separately in order to examine the relationship of their formation to graft healing time and the other two categories were expressed as percentages of the total of these two classes at each healing time. The results of this analysis are presented in Fig. 4, and several points emerge. The double posterior groups show a steep fall in the number of distally more complete symmetrical limbs between 0 and 15 days, which is the time during which healing between graft and host tissue is completed. This is mirrored by a steep rise in the number of spikes and non-regenerates which are formed (Fig. 4a). The half posterior limbs show a wider distribution which shows no distinct relationship to graft healing time (Fig. 4b). The double anterior groups all yield distally incomplete limbs and never produce symmetrical limbs with digits. The half anterior limbs, which do produce a complete anterior digit, are only seen during the first 20 days of healing, and do show a vague relationship with the time of graft healing. Taken together, these data strongly suggest that half limbs do not follow the same principles as symmetrical outgrowths. This supports the notion that it is the degree of interaction between the cells derived from either side of the symmetrical stump which governs the degree of outgrowth that is achieved. Furthermore, the steep reduction in the number of distally complete symmetrical ‘double-posterior’ outgrowth occurs in close association with the histological time scale of healing.

The symmetrical upper arm experiments demonstrate the importance of the number and arrangement of circumferential positional values and the necessity of local cell interactions to the process of regeneration. The actual cell interactions that result in intercalation will be governed by the preferential healing modes of cells at or near the amputation plane. This point is best demonstrated by experiments where symmetrical limbs are amputated in the forearm region. In these cases, double posterior upper arm stumps were created and amputated immediately to ensure complete distal outgrowth in as many cases as possible (Holder et al. 1980). Following initial amputation through the symmetrical upper arm double posterior symmetrical regenerates which had between 3 and 6 digits were formed. Upon a second amputation through the more distal regenerated forearm region the majority of these limbs underwent a striking distal expansion of their pattern. Thus the limbs with 3 to 6 digits expanded to produce limbs with 5–7 digits (Fig. 8a, b). These remained symmetrical and in
Fig. 5. Camera-lucida drawings of serial transverse sections of (a) a forearm (radius and ulna region) MB blastema and (b) an upper arm (humerus region) MB from the contralateral limb of the same animal. The sections are taken at every 100 μm and run from the blastema tip towards the stump top to bottom, left to right. The lines drawn at right angles on the blastema sections at the 800 μm level represent the measurements taken from which the ratios expressed in figure 6 were calculated. The ratio $r = (x/y)$ where $x$ is the shorter of the two axes, $d$, dorsal; $v$, ventral; $p$, posterior and $a$, anterior. $cx = \text{the number of cells contacted by the line drawn on the } x \text{ axis}$, $cy = \text{the number of cells contacted by the line drawn on the } y \text{ axis.}$
Fig. 6. The ratio of axis length represents a measure of blastemal shape (see text and Figure 5). The ordinate represents the ratio $r$, the abscissa the level of the section measured with 0 as the distal tip of the blastema. The open squares are individual measurements of $r$ taken from upper arm blastemas, the solid squares are individual measurements of $r$ taken from forearm blastemas. The dotted lines represent 95% confidence limits (upper arm blastemas upper limit 0.95, lower limit 0.42; forearm blastemas upper limit 0.59, lower limit 0.02). The two populations show distributions that are statistically significant by the $t$-test at the 1% level.

Some cases where 7 digits were formed a digit 1 appeared in the centre of the digital array. Thus, in these cases the anterior positional values necessary to form a digit 1 (the most anterior digit) had been intercalated as a result of simply amputating these symmetrical limbs at a more distal level. The only position where such an intercalatory event could occur is in the midline of a double
Pattern formation and growth during regeneration

posterior symmetrical limb stump (Fig. 8). However, the previous arguments concerning normal regeneration and regeneration of symmetrical upper arms have been based on local cell contacts and intercalation, and now it seems that cells from the furthest points of the circle are interacting between the dorsal and ventral sides of the symmetrical forearm stump. The reason for this became apparent when the shape of the upper arm and forearm limb amputation planes were compared.

**Blastemal shape and healing modes**

The shape of the forearm and upper arm blastemas was examined in the following way. Right limbs of four larval axolotls (ranging from 68–90 mm) were amputated through the mid-upper arm (humerus) region, while the contra-lateral left limbs were amputated through the mid-forearm (radius and ulna) region. The limbs were then allowed to regenerate to the stage of Medium Bud (MB: Tank et al. 1976) before being removed at the shoulder, fixed in Bouin’s fluid and processed for wax sectioning. Serial transverse 10 μm sections were cut at right angles to the long axis of the limb and stained with haematoxylin and eosin. Camera-lucida drawings were made of every tenth section (every 100 μm) and the axial orientation of these blastemas was determined by the muscle patterns in the stump regions of each limb (see Fig. 7a, b). The lengths of two transverse axes were measured on the drawings (dorsal to ventral and anterior to posterior) and the ratios of these distances calculated (see Fig. 6). (The ratio was calculated by dividing the length of the longest axis into that of the shortest.) Two sample sets of camera-lucida drawings are shown in Fig. 5. The ratios of axial lengths give a rough measure of the shape of the blastemal section. The ratio of a perfect circle will be 1 and this value will fall towards zero as the blastema becomes progressively more elliptical. Although these blastemas are clearly not perfect ellipses, the measurements are adequate to demonstrate quantitatively the obviously different shapes of forearm and upper arm blastemas (Figs. 5, 6). The clearcut change in shape demonstrated in Fig. 6 must occur during blastema outgrowth when dedifferentiation causes the collapse of muscle.

---

**Fig. 7. Blastemal shape and healing modes.**

(a) A transverse section through the mid upper arm of a normal axolotl. Note the centrally situated humerus (h) and the surrounding muscle blocks (m). The limb appears circular. Magnification: × 147.

(b) A transverse section through the mid forearm of a normal axolotl. Note the two skeletal elements, the anterior radius (r) and the posterior ulna (u) and the surrounding muscle blocks (m). Again, the limb appears roughly circular. Magnification: × 150.

(c) A transverse section taken 430 μm from the distal tip of a MB stage upper arm blastema. The shape is grossly circular. The ratio r of this section is 0.65. Magnification: × 350.

(d) A transverse section taken 400 μm from the distal tip of MB stage forearm blastema from the contralateral limb of the same animal from which the section 1c was taken. The shape is elliptical. The ratio r of this section is 0.1. Magnification: × 280.

(e) The radial healing mode proposed for short arc intercalations in the circular upper arm blastema. (f) The dorsal to ventral healing mode proposed for short arc intercalation in the elliptical lower arm blastema.
Fig. 8. (a) A three-digit double posterior limb formed following immediate amputation of a double posterior upper arm construction. (b) A seven-digit double posterior limb formed following a second amputation of the limb shown in 8a through the forearm region. Note the appearance of digit 1 in the midline, and the larger mass of carpal elements. Scale bar = 1 mm. (c) Diagrammatic representation of the proposed intercalation of anterior circumferential position values brought about by the dorsal to ventral healing mode of the elliptical double posterior forearm blastema.
blocks in the stump, leaving an array of blastemal cells surrounding the central supporting skeletal structure (see Fig. 7a–d). Since there are two such skeletal structures in the forearm lying in parallel across the anterior-to-posterior axis, the shape of this axis is maintained while the dorsal to ventral sides collapse and become closer together, thus forming an ellipse. In the upper arm, by the same argument, the centrally situated humerus supports a more circular blastema.

The consequences of this clear difference in shape between the oval forearm blastema and more circular upper arm blastema are important for a model based on local interactions and intercalation. The healing modes governing which cells contact which during growth of the blastema will be grossly different in the forearm and the upper arm blastemas. Included in Fig. 5 is a series of cell counts which show the number of blastemal cells contacting a thin line drawn along the two transverse axes which were measured. These cell counts are meant only to indicate roughly the likelihood of contact between cells from the extreme ends of these axes, and have not been quantitatively analysed. For example, in Fig. 5a, the section taken 700 μm from the distal blastemal tip of this forearm blastema has 41 cells separating the anterior to posterior extremes yet only 10 lie between the dorsal and ventral sides. In the corresponding section from the contralateral upper arm blastema 37 cells separate the posterior to anterior and 31 cells the dorsal to ventral sides. It seems clear that if local cell contacts govern the establishment of new positional values then the cells from the dorsal and ventral sides of the forearm blastema are more likely to contact than cells from the anterior to posterior sides. By the same reasoning, in the upper arm, cells from different circumferential positions will have a more even chance of contacting. This kind of analysis stresses the importance of the probabilistic nature of cell contacts and intercalation which is governed by healing modes.

Having established that these shape changes exist at the two proximal to distal levels examined, we can return to the regenerative ability of symmetrical limbs. Double posterior symmetrical regenerates expand their patterns when amputated in the forearm by the addition of midline structures. These extra structures will occur when, for example, 12 and 5 contact from dorsal to ventral, and produce 4, 3, 2 and 1 (Fig. 8c), the values normally lying on the anterior limb region (see Fig. 1b; see also Holder et al. 1980). The shape of the forearm blastema ensures that this event will occur frequently. This shape difference also makes a second prediction. If the preferential direction of healing in the forearm is dorsal to ventral, then cells from anterior and posterior sides will only contact rarely. This possibility should ensure that double anterior and double posterior limbs surgically constructed in the forearm will not show the healing time effect which is so clearly seen in the upper arm. Such symmetrical forearms should behave like short-healing-time groups from the upper arm and readily distally transform. This has indeed been shown to be the case by Krasner & Bryant (1980).
Symmetrical limb stumps produce symmetrical blastemas

Taken together, these symmetrical limb experiments demonstrate the central importance of the limb circumference to the process of distal outgrowth. This central role can be understood in terms of the number, arrangement and localized contacts that cells with different circumferential positional values make during the early stages of regeneration. Despite the considerable body of evidence which relates to the role of the circumference (see also Stocum, 1978, 1980b, c), it has been suggested that the healing of tissues in surgically constructed symmetrical limbs is a non-specific phenomenon (Maden, 1981). It is clear that the experiments presented here rely very much on differential directions of healing governed either by wound sites or the shape of amputation planes. The evidence which clearly shows that blastemas derived from double limb stumps bear symmetrical arrangements of circumferential position values comes from grafts where such blastemas are exchanged with comparable-staged blastemas derived from normal asymmetrical limb stumps (Holder & Tank, 1979; Stocum, 1980b). Double anterior and double posterior upper arms amputated after a month of healing form Medium Bud blastemas which are indistinguishable from those formed on normal amputation planes. This in itself indicates that the early phases of regeneration, that is dedifferentiation and early blastemal cell accumulation, are relatively normal in symmetrical limb stumps. Following the exchange of blastemas between symmetrical stumps and normal asymmetrical stumps, two distinct points emerge. Such grafts reliably produce supernumerary limbs when either the symmetrical blastema apposes the asymmetrical stump or when the asymmetrical blastema apposes the symmetrical stump. In addition, these extra limbs emerged at the precise circumferential positions and have the handedness that would be expected from the polar coordinate model (Holder & Tank, 1979). For example, a blastema derived from a double posterior stump produces a supernumerary limb on the anterior side of a normal stump and this supernumerary is of stump handedness. This can only occur if the grafted blastema comprises a set of posterior positional values which interact with the adjacent anterior stump values. That is, the blastema truly is a double posterior construction. The same observation applies to double anterior blastemas. The second important point to emerge from these experiments is that the symmetrical blastemas are not rescued by the asymmetrical stump upon which they are grafted. Once the stage of MB has been reached, these blastemas always cease regenerating and produce distally incomplete structures. Therefore it is clear that blastemas derived from long-healing-time symmetrical stumps are symmetrical in terms of the positional values which they bear and that this symmetrical arrangement of positional values is likely to be responsible for the early cessation of regeneration and growth.
CONCLUSIONS

This paper attempts to draw together the evidence from experiments involving symmetrical limb tissues which strongly indicate that local cell-to-cell interactions and intercalation govern the process of pattern regulation in the regenerating limb. The eventual pattern of structures regenerated from symmetrical or asymmetrical normal stumps depends on three major factors: the number of circumferential positional values present at the amputation plane, the spatial arrangement of these values around the circumference and the constraints of blastemal shape upon the directions of healing which bring cells from specific regions of the circumference together during blastemal outgrowth. The role of local cell contact is further emphasized when cells on either side of a symmetrical midline are prevented from contacting by a wound.

The symmetrical limb experiments discussed in this paper also demonstrate that the circumference of the limb plays a major role in determining the degree of distal outgrowth and the nature of the pattern of structures within the regenerate (see also Stocum, 1980b, c). The importance of local cell interactions and the intimate association between the amount of proximodistal regeneration and the circumferential properties of the amputation plane ensure that the polar coordinate model remains as a powerful theoretical tool with which to further our understanding of epimorphic regeneration and the link between growth and spatial patterning mechanisms.

It is a pleasure to thank Susan Bryant and Patrick Tank for their support in many ways and for sunny days in Irvine which they shared. I am also indebted to Susan Reynolds for all sorts of help with the manuscript and my colleagues in the Anatomy Department at King’s College London for innumerable favours. I also thank Charleston Weekes for his expert technical assistance and his patience, and Philip Batten for the photography. My own work reported in this paper was supported financially by the Science Research Council.

REFERENCES


