J. Embryol. exp. Morph. 98, 237–249 (1986)

The effect of replacing different regions of limb skin with head skin on regeneration in the axolotl

PETER WIGMORE* AND NIGEL HOLDER
Department of Anatomy and Human Biology, King's College London, Strand, London WC2R 2LS, UK

SUMMARY

Head skin was used to replace different halves of limb skin from the upper and lower arms of axolotls. Replacement of upper arm posterior skin caused the regeneration of a high proportion of single-digit limbs while replacement of dorsal, ventral or anterior skin caused only minor defects to the normal skeletal pattern. When dorsal or ventral skin was replaced, however, regenerates often lacked dorsal or ventral muscle. Results from the lower arm were different in that replacement of any half of limb skin failed to cause defects either in the skeletal or muscular pattern. These results are used in conjunction with previous work (Wigmore & Holder, 1985; Wigmore, 1986) to suggest that posterior skin is essential for regeneration of the anteroposterior axis and dorsal and ventral skin is necessary for the differentiation of the muscle pattern in regenerates from the upper arm. In the lower arm no localized region of skin appears to be essential for regeneration of the normal pattern and the patterning mechanism may have a different spatial organization.

INTRODUCTION

A technique enabling different regions of the limb to be isolated using pieces of skin from the head has been used previously to show major differences in the regenerative capacity of posterior and anterior tissue in the upper arm (Wigmore & Holder, 1985). Specifically, stumps containing posterior tissue were able to produce normal 4-digit limbs while stumps containing only anterior tissue regenerated only single digits. In the lower arm this difference between anterior and posterior tissue was less marked and no half of the limb could produce the complete pattern. All lower arm halves produced between 2 and 3 digits with the pattern of skeletal structures in some cases ending abruptly part way across the anteroposterior axis. In addition to the skeletal pattern the dorsoventral axis was assayed using the pattern of muscles (Maden, 1980, 1982; Maden & Mustafa, 1982*a*; Holder & Weekes, 1984). The presence of dorsal or ventral tissue in the regenerate appeared to be dependent on the presence of dorsal or ventral tissues in the stump. Stumps lacking either of these tissues, whether in the upper or lower parts of the limb, produced regenerates deficient in dorsal or ventral muscle. These results left

* Present address: Department of Anatomy, University of Aberdeen, Marischal College, Aberdeen, UK.

Key words: axolotl, regeneration, limb, skin, muscle, pattern, *Ambystoma mexicanum*.

several questions unanswered. In particular, which components of the stump enable the regeneration of the normal pattern of skeletal and muscular tissues.

It has been known for some time that the dermis of the skin exerts a powerful influence on limb regeneration. Rotation of skin cuffs (Carlson, 1974, 1975) or the movement or implantation of limb dermis to different positions on the limb (Lheureux, 1972, 1975; Settles, 1978; Tank, 1981; Rollman-Dinsmore & Bryant, 1984; Maden & Holder, 1984) causes the production of additional structures in the regenerate while stumps composed of double posterior skin regenerate double posterior limbs (Slack, 1980, 1983; Maden & Mustafa, 1982*b*). Recently flank skin has been used to replace different regions of limb skin in the upper arm of the newt (Tank, 1985); the results showed regional variations in the effect of the graft, with grafts to the ventral side of the stump producing the highest proportion of hypomorphic limbs. Less well studied is the effect of skin on the underlying pattern of muscles. However, transplantation of dorsal or ventral skin has been shown to cause the production of abnormal patterns of dorsal and ventral musculature (Maden & Mustafa, 1982*b*).

Autografts of head skin heal rapidly onto the limb and several experiments have demonstrated that skin from this region has no effect on pattern formation during limb regeneration. Unlike limb skin, head skin does not restore the regenerative ability of irradiated limbs (Umaski, 1938; Lazard, 1967) and implants of head skin into limb stumps do not affect the pattern regenerated (Tank, 1981). The technique of using head skin to isolate different parts of the limb and prevent the incorporation of tissues from different circumferential regions can be extended to examine the role of different regions of the skin in limb regeneration. In particular this paper deals with the role of posterior skin in producing normal regenerates from upper arm posterior half stumps. Additionally, the role of skin in regeneration from upper and lower arm amputation planes is compared together with the influence of skin on the muscle pattern in the regenerate. The results demonstrate a clear anteroposterior asymmetry in the ability of skin regions to control pattern regulation in the upper arm, which is absent in the lower arm. In the upper arm dorsal and ventral skin is seen to affect the muscle pattern although again this effect is not seen in the lower arm.

MATERIALS AND METHODS

All experiments were carried out using axolotls (*Ambystoma mexicanum*), 10–15 cm in length, spawned in the colony at King's College. Animals were kept in tap water in individual containers and fed on raw heart.

Experimental procedure

Animals were operated on whilst anaesthetized with MS222 (Sigma) and allowed to recover in the dark at 10°C for 2 days. Operations consisted of the removal of half the circumference of limb skin (either posterior, dorsal, ventral or anterior) for a distance of 3–4 mm and its replacement with a similar-sized strip of skin from the forehead. The wound in the forehead was found to be covered by epidermis within 24 h and so no attempt was made to treat it. The head skin was sutured into place and the limb amputated immediately through the middle third of the humerus for upper arm operations and the middle third of the radius and ulna for lower arm operations (Fig. 1A). The stumps were viewed end on under a dissecting microscope to check the position of the graft. Controls consisted of carrying out the same procedure as described above but without grafting any head skin onto the limb.

In order to investigate further the role of posterior skin in upper arm regeneration, anterior half upper arm stumps were constructed as described in Wigmore & Holder (1985). This operation involved the removal of all posterior soft tissues and the suturing of a strip of head skin to the medial wound surface. Posterior skin was then separated from the removed posterior half of the limb and sutured to the medial surface of the anterior half stump, distal to the head skin. The limb was then immediately amputated through the posterior skin graft and the middle third of the humerus. This produced a stump with a complete circumference of limb skin together with all the soft tissues on the anterior side of the limb and the humerus but deficient in the subdermal tissues of the posterior side (Fig. 1B). It has been shown previously that anterior half stumps constructed in this way but without posterior skin consistently regenerate only single digits (Wigmore & Holder, 1985).

All experimental animals were checked for retention of the graft every few days for the first few weeks after the operation. Operated animals were left for 60 days after which time they were reanaesthetized and the regenerates amputated, fixed in Bouin's solution, dehydrated and stained with Victoria Blue (Bryant & Iten, 1974) to show the skeletal pattern. *Camera-lucida* drawings were made of all regenerates. Limbs were then wax embedded and serial sections were

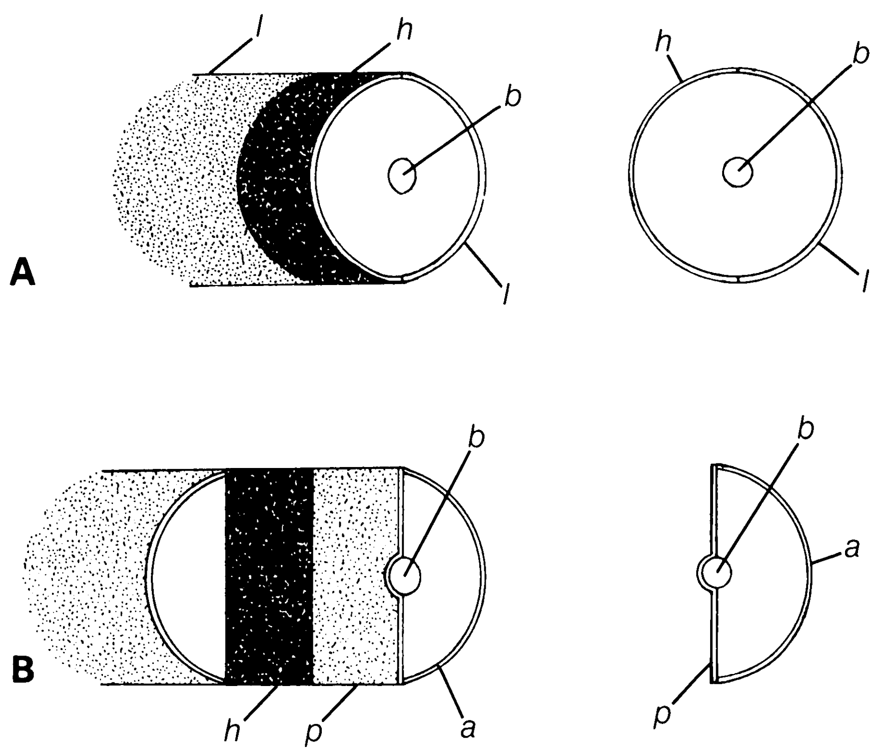


Fig. 1. Diagram of the two types of operations performed. The left-hand drawings show the position of head skin and limb tissues while the right-hand drawings show the stump viewed end on. (A) Grafts of head skin replacing different halves (posterior, anterior, dorsal or ventral) of limb skin. (B) Construction of an anterior half stump by removing all posterior tissues and grafting head skin to the medial surface of the limb. Posterior limb skin was then added distal to the head skin. *b*, humerus; *l*, limb skin; *h*, head skin; *p*, posterior skin; *a*, anterior skin.

cut at 10 μm and stained with haematoxylin and eosin. These sections were used to check the position of the graft and to analyse the muscle pattern.

The muscle pattern was examined at two levels, the proximal metacarpals and the midforearm. In normal limbs at the level of the proximal metacarpals, ventral muscle is continuous across all digits while dorsal muscle, the extensores digitorum breves, occurs as discrete crescents over each metacarpal (Maden, 1980, 1982; Maden & Mustafa, 1982*a*; Burton, Holder & Jesani, 1986). There is also a greater concentration of Leydig cells in the dorsal epidermis at the metacarpal level. At the midforearm level, the dorsoventral axis can be determined from the overall muscle pattern and the presence of the pronator quadratus (pq) muscle which runs from a spine on the ventral side of the ulna towards the radius (Holder & Weekes, 1984; Burton *et al.* 1986).

RESULTS

Upper arms

Posterior skin replaced by head skin

Eight upper arm stumps with this operation were constructed (see Table 1). The majority of regenerates (five) produced only a single digit and had a proximodistal skeletal pattern consisting of a single forearm bone, two carpals, a metacarpal and two phalanges (Fig. 2). One stump failed to regenerate and the remaining two

Table 1. *Analysis of the number of digits regenerating after different halves of skin have been replaced by head skin*

Skin replaced	No. of limbs produced	No. of digits regenerating 5	4	3	2	1	No regeneration	Mean no. of digits
Upper arm								
Posterior half	8		1	1		5	1	1·5
Anterior half	8		8					4·0
Dorsal half	12		9	2		1		3·6
Ventral half	12		7	2	1	2		3·2
Anterior half limbs with posterior skin	9	1	7	1				4·0
Lower arm								
Posterior half	10	1	8	1				4·0
Anterior half	6		6					4·0
Dorsal half	10	2	8					4·2
Ventral half	10		10					4·0

Fig. 2. Victoria-Blue-stained whole mount showing the skeletal pattern regenerating from a right-hand upper arm with posterior skin replaced by head skin. Only a single digit has regenerated. Bar, 0·25 cm.

Fig. 3. Victoria-Blue-stained whole mount showing the skeletal pattern regenerating from a left-hand upper arm with dorsal skin replaced by head skin. Digit **4** and the posterior carpals are missing. Bar, 0·25 cm.

Fig. 4. Transverse section at the metacarpal level of a 3-digit regenerate from a stump with dorsal skin replaced by head skin. Only ventral muscle is present (arrows). *D* and *V* indicate the dorsal and ventral sides of the limb, respectively. *s* is a vascular sinus found on the ventral side of the hand. Bar, 1 mm.

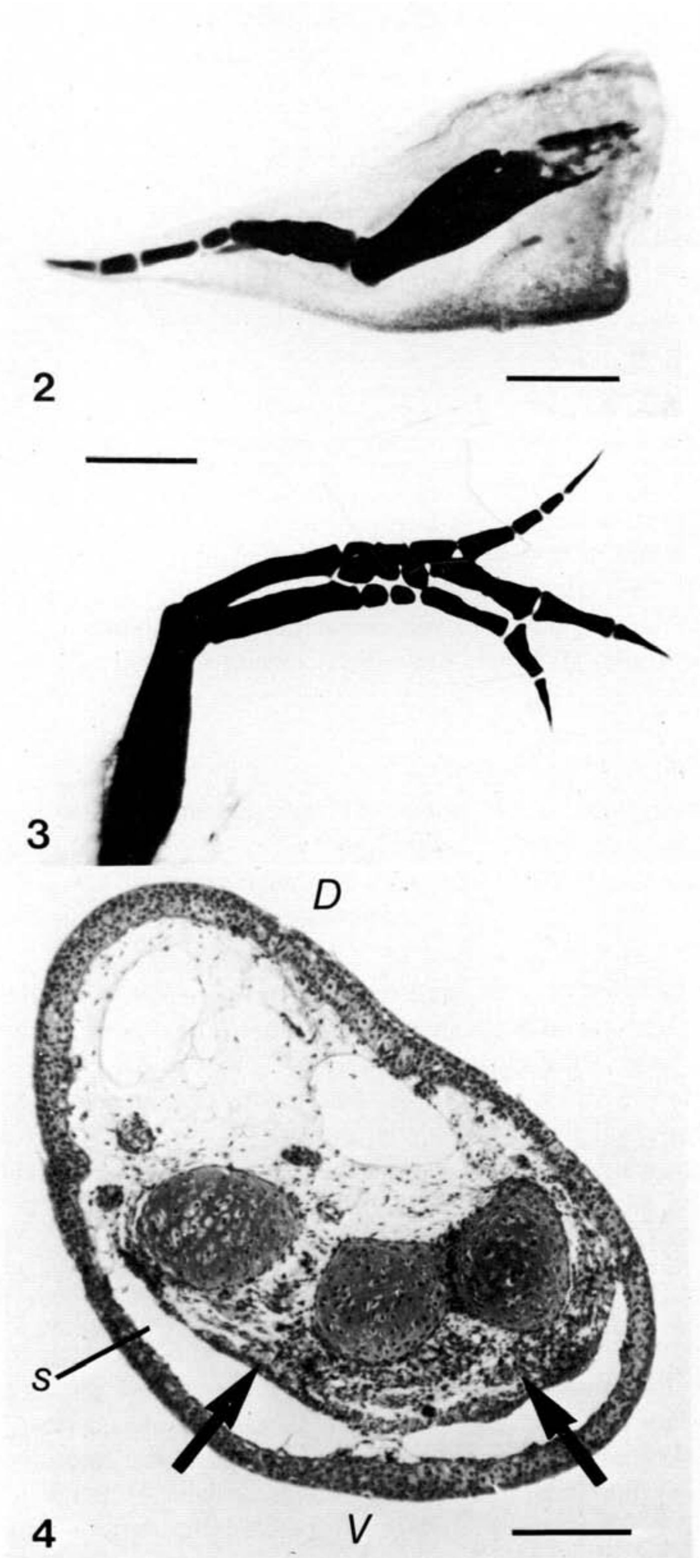

2
3
D
s
4
V

Table 2. *Analysis of the muscle pattern at the metacarpal level in regenerates after different halves of skin have been replaced by head skin*

Skin replaced	No. of limbs produced	No. of multidigit limbs	No. with normal muscle	No. lacking ventral muscle	No. lacking dorsal muscle
Upper arm					
Posterior half	8	2	1		1
Anterior half	8	8	8		
Dorsal half	12	11	8		3
Ventral half	12	10	6	4	
Anterior half limbs with posterior skin	9	9	9		

With one exception, all lower arm regenerates were normal.

regenerates were 3- and 4-digit limbs. Neither of these limbs were normal, both showing reduction or loss of the most posterior digit or carpals.

Sections of these last two limbs showed the 4-digit limb to have a normal muscle pattern while the 3-digit limb lacked dorsal muscle at both metacarpal and forearm regions (see Table 2). Single-digit limbs cannot be analysed for their muscle patterns.

Anterior skin replaced by head skin

In contrast to the posterior side of the limb, all stumps where anterior skin was replaced regenerated normal 4-digit limbs with both radius and ulna. Sections of these limbs showed them to have a normal muscle pattern.

Dorsal skin replaced by head skin

Eleven out of twelve limbs with this type of operation had either 3 or 4 digits (see Table 1) but several limbs showed fusion between digits (four cases) and both 3-digit limbs lacked the most posterior digit (Fig. 3). All regenerates, with the exception of that with a single digit, had both radius and ulna. Sections of the regenerates showed that three limbs (one with 4 digits, and two with 3 digits) lacked any muscle on the dorsal side of the metacarpals (Fig. 4; Table 2). At the forearm level, one of these lacked dorsal muscle, and of the other two, one was double ventral (having ventral muscle on both sides of the limb) and the other was normal.

Ventral skin replaced by head skin

This group of twelve limbs showed the greatest spread of results and the lowest mean digit number after those with posterior skin replaced (see Table 1). The three limbs with 2 or 3 digits showed loss of posterior skeletal structures (Fig. 5) and one 4-digit limb lacked the most posterior carpals. All limbs, including those with only a single digit, had both radius and ulna. Four limbs (a 4-digit, two 3-digit and a 2-digit limb) lacked ventral muscle in the metacarpal region (dorsal half limbs, Fig. 6; Table 2) but only one of these lacked ventral muscle at the forearm level. The remaining limbs had a normal muscle pattern (the single-digit limbs could only be scored at the forearm level).

Upper arm anterior half limbs with posterior skin

These limbs differed from those described previously in that the deeper soft tissues had been removed from the posterior side of the upper arm and the stump was made into an anterior half limb by sealing the medial surface with head skin. Posterior upper arm skin was then added to the medial surface, distal to the head skin (Fig. 1B). All but two of the nine regenerates from these stumps had a normal skeletal pattern with 4 digits (see Table 1; Fig. 7). The 5-digit limb included a serial duplication of digit **3** while the 3-digit limb lacked digit **4** and had only a single forearm element.

Sections of these limbs showed a normal pattern of muscles at both metacarpal and forearm levels.

Lower arms

In contrast to the upper arm, all regenerates from lower arm operations had at least 3 digits (see Table 1) and the majority, irrespective of the position of the head skin, had 4. Minor abnormalities were noted in regenerates from stumps where posterior skin had been replaced. These showed some loss of posterior skeletal structures, for example loss of digit **4** resulting in a 3-digit limb or reduction in size of posterior digits and carpals. In one case digit **4** had three phalanges while the 5-digit regenerate appeared to include a serial duplication of digit **3**. This was also the case in both 5-digit regenerates from stumps with dorsal skin replaced. This type of operation also produced two limbs with digit **4** having three phalanges. Several limbs showed branching at the phalangeal level.

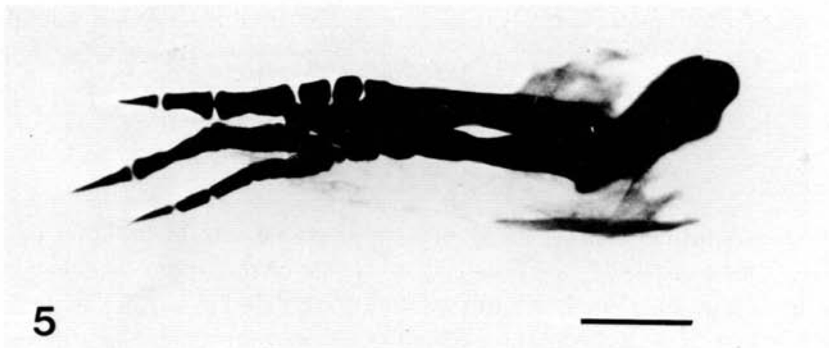
The muscle pattern of all lower arm regenerates was normal at both metacarpal and forearm levels with the exception of one regenerate from a stump with posterior skin replaced; this limb lacked ventral muscle at both levels.

Controls

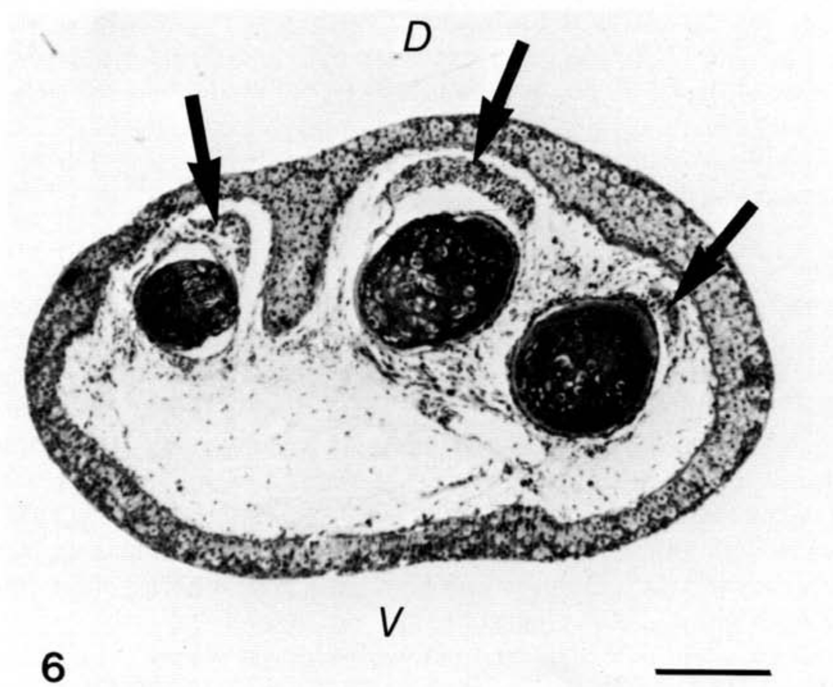
Five control limbs were constructed for each type of operation in both upper and lower arms (40 limbs in all). All regenerates from control limbs produced 4 digits with a basically normal skeletal pattern. Six regenerates had an extra basal carpal between the fused basal carpal one and two and basal carpal three. Sections of control regenerates at both metacarpal and forearm levels showed a normal pattern of muscles.

DISCUSSION

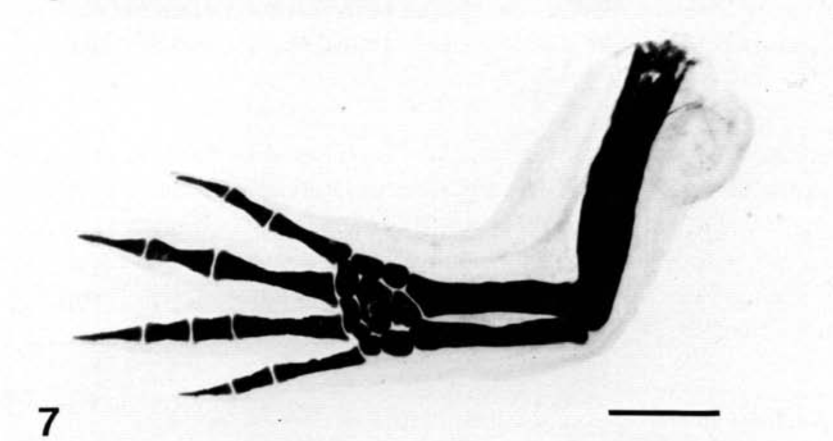
The results presented here support the conclusions drawn from previous work on regeneration from half limb stumps that demonstrated that the regenerative



5



6



7

ability of the limb shows regional variations (Wigmore & Holder, 1985; Wigmore, 1986). In these studies normal limbs regenerated from upper arm half stumps containing posterior tissue but not from stumps composed of only anterior tissues. This difference between posterior and anterior parts of the limb was much less marked in the lower arm, but posterior halves still produced the highest mean digit number of all four quadrants of the limb. The present work was carried out to investigate these differences further not only by regions of the limb but also by eliminating the influence of a single tissue, the limb skin, from particular parts of the limb circumference.

The greatest effect after replacement of half the limb skin with head skin was in the posterior half of the upper arm. Removal of this skin resulted in the regeneration of a high proportion of single digits. This occurred despite the fact that it was only 50 % of the skin that had been replaced and the rest of the limb remained intact. The single digits obtained from these stumps are virtually identical to those regenerating when the whole of the posterior half was removed to produce anterior half stumps (Wigmore & Holder, 1985) and suggests that the cause of the hypomorphic regenerates in those experiments was due solely to the absence of posterior skin from the stump.

The importance of the posterior skin was demonstrated directly by its ability to rescue anterior half stumps which normally only regenerate single digits (Wigmore & Holder, 1985). This result indicated that, in the upper arm, the only essential component of the stump for producing more than a single digit is the posterior skin. This conclusion is consistent with the construction of symmetrical limbs composed of both skin and deep tissues (Stocum, 1978; Tank & Holder, 1978; Holder, Tank & Bryant, 1980). In these experiments double anterior stumps produced single digits and double posterior stumps regenerated double posterior multidigit limbs. When only the skin was made symmetrical and the deep tissues were left intact (Maden & Mustafa, 1982*b*), stumps bearing double posterior skin again regenerated multidigit, double posterior limbs. However, limbs symmetrical for anterior skin regenerated normal 4-digit limbs in an apparent contradiction of the principle that posterior skin is necessary for the formation of a normal anteroposterior axis in the upper arm. This result is puzzling as all of the tissues present have been shown to be inessential to pattern formation (present work and Wigmore & Holder, 1985). The only unique feature of this type of stump is a discontinuity between the deep posterior tissues and the overlying anterior skin

Fig. 5. Victoria-Blue-stained whole mount showing the skeletal pattern regenerating from a right-hand upper arm with ventral skin replaced by head skin. Similar to that in Fig. 4 the posterior digit and carpals are missing. Bar, 0·25 cm.

Fig. 6. Transverse section at the metacarpal level of a 3-digit regenerate from a stump with ventral skin replaced by head skin. Only dorsal muscle is present (arrows). *D* and *V* indicate dorsal and ventral sides of the limb. No vascular sinus is present on the ventral side of the hand. Bar, 1 mm.

Fig. 7. Victoria-Blue-stained whole mount showing the skeletal pattern regenerating from a right-hand upper arm anterior half limb with posterior skin sutured distal to the head skin. Bar, 0·25 cm.

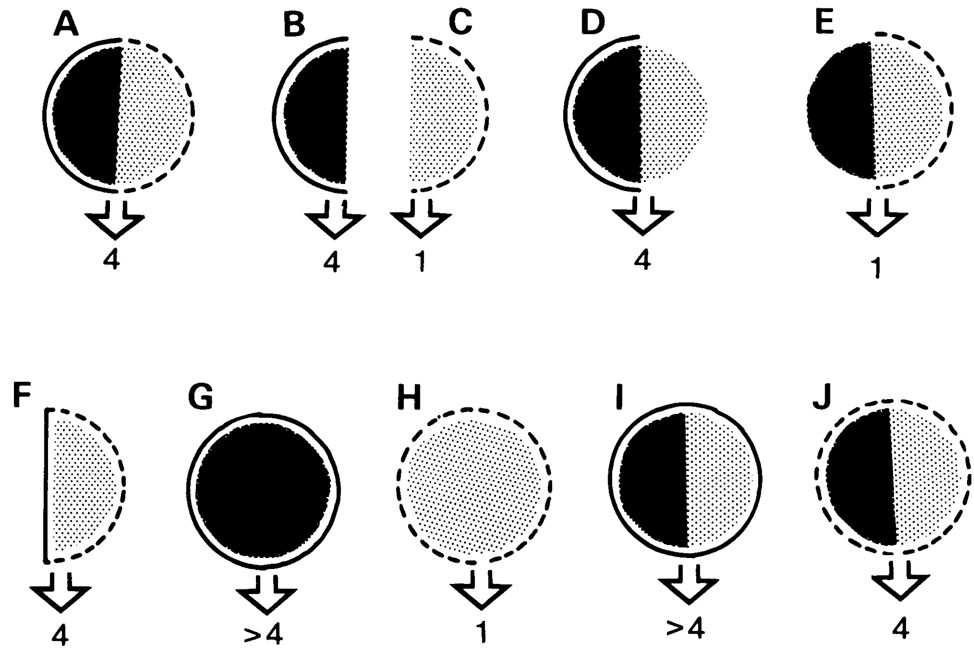


Fig. 8. Diagram of the end on view of the stump from upper arm amputations showing different combinations of tissue created in the present work and by previous authors (see text for references). Solid line indicates posterior skin, broken line anterior skin. Dark shading represents deep posterior tissue and light shading deep anterior tissue. Regions of the stump circumference not shown covered by either posterior or anterior skin are covered by head skin. Numbers under each diagram indicate the commonest number of digits regenerating from each type of operation. (A) Normal limb. (B) Posterior half limb. (C) Anterior half limb. (D) Anterior skin replaced with head skin. (E) Posterior skin replaced by head skin. (F) Anterior half limb with posterior skin on its medial surface. (G) Stump double posterior for both skin and deep tissues. (H) Stump double anterior for both skin and deep tissues. (I) Stump double posterior for skin only. (J) Stump double anterior for skin only.

and it must be concluded that this enables the pattern-forming mechanism to function. The role of tissues that appear to be inessential to regeneration is unclear but certainly in the case of anterior tissues they do contribute large numbers of cells, mainly to anterior structures of the regenerate, when normal stumps are amputated (Rollman-Dinsmore & Bryant, 1984; Tank, Connelly & Bookstein, 1985). The present results and those of the authors mentioned above are shown in diagrammatic form in Fig. 8. As can be seen with the one exeption mentioned previously, only stumps bearing posterior skin show consistent regeneration of more than a single digit.

In contrast to the upper arm, replacement of posterior lower arm skin did not prevent the formation of normal 4-digit limbs. At this level, posterior skin does not appear to be essential for pattern regulation. Posterior lower arm skin is, however, known to have morphogenetic properties as stumps bearing double posterior skin regenerate double posterior limbs (Slack, 1980, 1983; Maden & Mustafa, 1982*b*).

Isolation of half lower arms (Wigmore, 1986) showed that no half could regenerate the complete pattern of the limb. It can be concluded therefore that while the information for pattern formation is concentrated in the posterior half of the upper arm, at distal levels the information is more spatially separated. This conclusion is further supported by the observation that, in contrast to the upper arm, lower arm double posterior and double anterior stumps produce approximately equal amounts of symmetrical outgrowth (Krasner & Bryant, 1980).

In previous experiments where dorsal and ventral halves of the limb were isolated (Wigmore & Holder, 1985; Wigmore, 1986) or symmetrical double dorsal or double ventral stumps were constructed (Burton *et al.* 1986), limbs deficient in dorsal or ventral muscle often regenerated. In the light of these results the present replacement of dorsal or ventral skin was designed to examine the role of skin on pattern regulation in the dorsoventral axis. Replacement of dorsal or ventral skin in the upper arm resulted in the formation of limbs deficient in muscle on the side of the limb where the limb skin had been replaced. This effect is probably not due to direct inhibition of muscle formation by head skin because, as described below, similar operations on the lower arm produced limbs with a normal muscle pattern. In the upper arm the production of the underlying muscle pattern appears to be dictated by the overlying dermis and in the absence of dorsal or ventral skin, muscle on that side of the limb fails to form. This conclusion is supported by the results of Maden & Mustafa (1982*b*) where stumps symmetrical for dorsal or ventral skin produced, in a proportion of cases, regenerates with double dorsal or double ventral muscle. The mechanism by which the muscle pattern is controlled in the axolotl is at present unknown but in the chick limb (Chevallier, Kieny & Mauger, 1977; Chevallier & Kieny, 1982), it is the connective tissue of the limb that dictates the pattern of the different muscles. It is possible that fibroblasts from the dermis of the axolotl skin enter the blastema during regeneration and exert a similar control.

The majority of stumps with dorsal or ventral skin replaced regenerated 4-digit limbs. Since these stumps always contained some posterior skin, this is the expected result if the presence of posterior skin enables the regeneration of multi-digit limbs. Similarly removal of posterior or anterior skin leaves approximately 50 % of dorsal and ventral skin *in situ* which would appear to be sufficient for the regeneration of a normal muscle pattern.

In the lower arm the results of dorsal and ventral skin replacement were different in that all regenerates had normal muscle patterns and, from the distribution of Leydig cells, the overlying skin also appeared normal. This result was unexpected as removal of dorsal or ventral halves of the lower arm (Wigmore, 1986) produced limbs deficient in dorsal or ventral muscle similar to those formed in the upper arm. The results obtained here are, however, consistent with the construction of lower arm stumps bearing symmetrical dorsal or ventral skin (Maden & Mustafa, 1982) where, despite the symmetry in the skin, normal asymmetric muscle patterns were regenerated. It is unclear whether this result indicates a difference in the patterning mechanism in upper and lower parts of the limb or is due to a difference in their response to grafted tissue.

It is a pleasure to thank Stephen Wilson, Gavin Swanson and Paul Martin for many useful comments on this work and Liz Delaney for her dedicated technical assistance. This work was supported by an SERC project grant to Nigel Holder.

REFERENCES

BRYANT, S. V. & ITEN, L. E. (1974). The regulative ability of the limb regeneration blastema of *Notophthalmus viridescens*: experiments *in situ*. *Wilhelm Roux Arch. devl Biol.* **174**, 90–101.

BURTON, R., HOLDER, N. & JESANI, P. (1986). The regeneration of double dorsal and double ventral limbs in the axolotl. *J. Embryol. exp. Morph.* **94**, 29–46.

CARLSON, B. (1974). Morphogenetic interactions between rotated skin cuffs and underlying stump tissues in regenerating axolotl limbs. *Devl Biol.* **39**, 263–285.

CARLSON, B. (1975). The effects of rotation and positional change of stump tissue upon morphogenesis of the regenerating axolotl limb. *Devl Biol.* **47**, 269–291.

CHEVALLIER, A., KIENY, M. & MAUGER, A. (1977). Limb–somite relationship: origin of the limb musculature. *J. Embryol. exp. Morph.* **41**, 245–258.

CHEVALLIER, A. & KIENY, M. (1982). On the role of the connective tissue in the patterning of the chick limb musculature. *Wilhelm Roux Arch. devl Biol.* **191**, 277–280.

HOLDER, N., TANK, P. W. & BRYANT, S. V. (1980). Regeneration of symmetrical forelimbs in the axolotl *Ambystoma mexicanum*. *Devl Biol.* **74**, 302–314.

HOLDER, N. & WEEKES, C. (1984). Regeneration of surgically created mixed-handed axolotl forelimbs: pattern formation in the dorsal–ventral axis. *J. Embryol. exp. Morph.* **82**, 217–239.

KRASNER, G. & BRYANT, S. V. (1980). Distal transformation from double half forearms in the axolotl. *Devl Biol.* **74**, 315–325.

LAZARD, L. (1967). Restauration de la regeneration de member irradies d'axolotl par des greffes heterotipiques d'origines diverses. *J. Embryol. exp. Morph.* **18**, 321–342.

LHEUREUX, E. (1972). Contribution a l'étude du role de la peau et des tissus axiaux du membre dans le déclenchement des morphogenèses régénératrices anormales chez le triton *Pleurodeles waltlii* Michah. *Annls Embryol. Morph.* **5**, 165–178.

LHEUREUX, E. (1975). Nouvelles données sur les rôle de la peau et des tissus internes dans la régénération du membre du triton *Pleurodeles waltlii* Michah. *Wilhelm Roux Arch. devl Biol.* **176**, 285–301.

MADEN, M. (1980). Structure of supernumary limbs. *Nature, Lond.* **286**, 803–805.

MADEN, M. (1982). Supernumary limbs in amphibians. *Am. Zool.* **22**, 131–142.

MADEN, M. & HOLDER, N. (1984). Axial characteristics of nerve induced supernumerary limbs in the axolotl. *Wilhelm Roux Arch. devl Biol.* **193**, 394–401.

MADEN, M. & MUSTAFA, K. (1982*a*). The structure of 180 degree supernumerary limbs and a hypothesis of their formation. *Devl Biol.* **93**, 257–265.

MADEN, M. & MUSTAFA, K. (1982*b*). Axial organisation of the regenerating limb: asymmetrical behaviour following skin transplantation. *J. Embryol. exp. Morph.* **70**, 197–213.

ROLLMAN-DINSMORE, C. & BRYANT, S. V. (1984). The distribution of marked dermal cells from small localized implants in limb regenerates. *Devl Biol.* **106**, 275–281.

SETTLES, H. (1978). Supernumerary regeneration caused by ninety degree turning of limb skin in adult *Notophthalmus*. *Growth* **42**, 297–307.

SLACK, J. M. W. (1980). Morphogenetic properties of the skin in axolotl limb regeneration. *J. Embryol. exp. Morph.* **58**, 265–288.

SLACK, J. M. W. (1983). Positional information in the forelimb of the axolotl: properties of the posterior skin. *J. Embryol. exp. Morph.* **73**, 233–247.

STOCUM, D. L. (1978). Regeneration of symmetrical hindlimbs in larval salamanders. *Science* **200**, 790–793.

TANK, P. W. (1981). The ability of localised implants of whole or minced dermis to disrupt pattern formation in the regenerating forelimb of the axolotl *Ambystoma mexicanum*. *Am. J. Anat.* **162**, 315–326.

TANK, P. W. (1985). Pattern regulation during regeneration of limb stumps bearing partial circumferences of flank skin in the newt, *Notophthalmus viridescens*. *J. exp. Zool.* **233**, 73–81.

TANK, P. W., CONNELLY, T. G. & BOOKSTEIN, F. L. (1985). Cellular behaviour in the anteroposterior axis of the regenerating forelimb of the axolotl *Ambystoma mexicanum*. *Devl Biol.* **109**, 215–223.

TANK, P. W. & HOLDER, N. (1978). The effect of healing time on the proximodistal organisation of double half forelimb regenerates in the axolotl. *Devl Biol.* **66**, 72–85.

UMASKI, E. (1938). The regeneration potencies of Axolotl skin studied by means of exclusion of the regeneration capacity of tissues through exposure to X rays. *Bull. biol. Med. exp. URSS* **6**, 141–145.

WIGMORE, P. & HOLDER, N. (1985). Regeneration from isolated half limbs in the upper arm of the axolotl. *J. Embryol. exp. Morph.* **89**, 333–347.

WIGMORE, P. (1986). Regeneration from half lower arms in the axolotl. *J. Embryol. exp. Morph.* **95**, 247–260.

(*Accepted 18 July 1986*)