J. Embryol. exp. Morph. Vol. 57, pp. 203–217, 1980
Printed in Great Britain © Company of Biologists Limited 1980

Regulation and potency in the forelimb rudiment of the axolotl embryo

By J. M. W. SLACK

From the Imperial Cancer Research Fund Mill Hill Laboratories, London

SUMMARY

Anterior, posterior, dorsal and ventral halves of the pre-bud forelimb rudiment of tail-bud axolotl embryos can all give rise to normal limbs after the complementary half has been removed.

A histological study of the regulation of the posterior half rudiment showed no requirement for mesodermal healing across the gap, and no proliferative zone. The development of the limb bud on the operated side lags behind that of the control bud for several weeks of larval life.

When half limb rudiments and double half limb rudiments were grafted to the head they did not develop unless both the posterior and the dorsal margin were present. Double posterior and double dorsal halves could develop into duplications on this site, single halves formed normal or hypomorphic limbs.

When half limb rudiments were grafted to the flank the anterior halves developed into normal limbs and the posterior halves into duplicate limbs.

The results are interpreted to indicate that at the pre-bud stage the limb rudiment is a homogeneous group of cells with no internal regional subdivisions. Its regulative behaviour is thus similar to that of early embryos and different from the regeneration behaviour shown by adult organs.

INTRODUCTION

The recent upsurge of interest in regeneration, prompted largely by the polar coordinate model of French, Bryant & Bryant (1976), has raised in a sharp way the question of the nature of the pattern-forming mechanisms in the *embryonic* amphibian limb. It is now generally accepted that control of pattern formation in the regeneration blastema depends on some cryptic coding of the different parts of the adult limb which is first laid down during embryonic development, but this does not mean that the mechanisms at work in the two phases of the life-cycle are necessarily the same. In particular it does not mean that the mechanisms of pattern restitution after the removal of tissue are the same, as assumed for example by Bryant & Iten (1976), in their survey of classical work on amphibian limb development.

The words 'regulation' and 'regeneration' are often used interchangeably in the literature on pattern formation. However, it is necessary to distinguish

[1] *Author's address:* Imperial Cancer Research Fund, Mill Hill Laboratories, Burtonhole Lane, London NW7 1AD, U.K.

between several different types of phenomenon when considering such questions and for the purposes of the present paper I shall consider two of them and define the terms accordingly. In this paper 'regulation' will mean the readjustment of the fate map of an undifferentiated primordium in response to the removal of tissue, so that a greater number of structures are formed from the remaining part than would be formed in normal development. 'Regeneration' will mean the reformation of a differentiated organ after a part has been removed.

Although these definitions are made with respect to a single criterion, the state of differentiation of the tissue, the distinction is in practice also associated with two other features: the nature of the pattern elements formed and the relative times of pattern formation and growth.

In classical examples of the regulation of the whole body of early embryos, two complete copies of the whole pattern are formed. This is true of the 2-cell frog embryo (Spemann, 1936), the 2- or 4-cell sea-urchin embryo (Hörstadius, 1973), the avian blastoderm (Lutz, 1949; Spratt & Hass, 1960) and the 2-cell mouse embryo (Tarkowski, 1959). In contrast to this, the division of appendages in vertebrates and arthropods leads to the production of a new set of *distal* pattern elements from both of the complementary cut surfaces so that one half of the organ ends up with a normal pattern and the other half with a mirror duplicated pattern. This is true of the urodele limb (Butler, 1955) and the legs of hemimetabolous insects (Furukawa, 1940).

In embryos which do not grow during development, such as the frog and the sea urchin, the pattern formed from a half embryo is initially half-sized, although the size later increases to normal during the feeding larval phase. It is probably also true for embryos which do grow during development, such as birds and mammals, that when the primary body axis is first formed from a half embryo it is smaller than normal and that it does not catch up in size until the late embryonic stage. Regeneration on the other hand is associated with the initial formation of a blastema in which there is extensive cell division so much of the growth of the regenerate takes place before any new pattern elements have appeared.

The two types of behaviour here called regulation and regeneration do not cover the whole animal kingdom and there are some well-known systems such as hydroids or planarian worms that behave differently. This matter is discussed more fully in another paper (Slack, 1980) in which the terms 'epimorphosis' and 'morphallaxis' are used in their original sense to define different types of *regeneration* and the type of regeneration considered here is designated 'epimorphic monodirectional regeneration'. However, the simple distinction made in the present paper does correspond closely with that made by French *et al.* (1976) between the behaviour of primary and secondary embryonic fields respectively. The primary field in this context means the whole early embryo at the stage when the general body plan is being specified, and the secondary fields are the particular organ rudiments, such as somite plate, kidney or limb rudiments, which make up the general body plan.

In the present paper I report a number of morphogenetic properties of the embryonic limb rudiment of the axolotl and conclude that these fit closely the present definition of regulation and not that of regeneration. To this extent I disagree with French, Bryant & Bryant and instead support Harrison's (1918) description of the amphibian limb rudiment as an equipotential system, although I believe that the polarity of the organ is later imposed by interactions with the surrounding tissues as described in my earlier papers (Slack, 1976, 1977*a*, *b*). In this discussion I suggest a possible relationship between the patterning mechanism active during embryonic development with that active during regeneration.

The embryonic stages used in this work are, in the axolotl, some 3 days prior to the formation of the limb bud. This is why the tissue in question is referred to throughout as the limb rudiment and not as the limb bud. It is important to note that the classical experiments of Harrison and others quoted by Bryant & Iten (1976) were also not performed on limb buds but on so-called 'limb discs', which consisted of the tissues opposite somites 3–5$\frac{1}{2}$, comprising somatic and sphlanchnic mesoderm, part of the pronephric and flank mesoderm, and the overlying epidermis. In my opinion the real limb rudiment is only a part of this limb disc and the disposition of the other tissues must be known in order to interpret the classical experiments correctly.

MATERIALS AND METHODS

All the experiments were performed on axolotl embryos which were obtained either by natural or by artificial matings from animals kept in the laboratory (for artificial fertilization details see Slack & Forman, 1980). If possible the embryos were used at stage 34 (Bordzilovskaya & Dettlaff, 1979), which is the stage at which the body axis straightens, the heart beat starts and the embryos first twitch if poked. Some experiments were, however, done on other stages between 30 and 35.

The embryos were removed from their jelly with sharp forceps and washed in three changes of sterile 1/10 'normal amphibian medium' (NAM, see Slack & Forman, 1980). The operations were carried out under sterile conditions in 1/4 NAM + 1/3000 MS 222 (amphibian anaesthetic, Sandoz A.G., Basel) in petri dishes coated with 1 % agar ('Noble Agar', Difco Laboratories). All the operations consisted of the extirpation of parts of the limb rudiment or their transplantation from one embryo to another. The fragments in question are shown in Fig. 1. The 'anterior half' is the region ventral to the third somite, the 'posterior half' is ventral to the fourth and fifth somites, the 'dorsal half' is ventral to somites 3–5 and extends from the lower edge of the somites to the lower edge of the pronephric bulge, the 'ventral half' extends from here to a level parallel to the ventral limit of the gill bulge. The 'whole rudiment' comprises the whole region covered by these four overlapping halves. It is important to stress that,

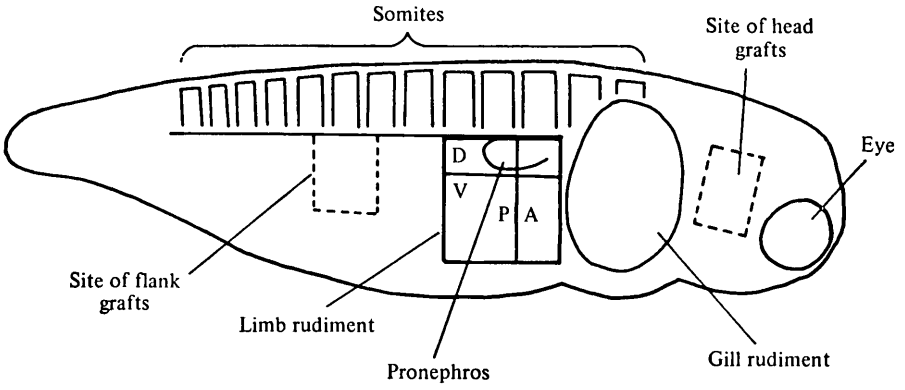


Fig. 1. Diagram of a stage-34 axolotl embryo viewed from the right side showing the positions of the four overlapping halves of the limb rudiment and the sites on the head and flank to which they were grafted.

like the limb discs of classical times, this 'limb rudiment' is a larger region than the real limb rudiment. It includes the pronephros dorsally, a strip of flank tissue posteriorly and a little gill tissue anteriorly. Furthermore the thickened region considered to be the limb rudiment proper is double layered, the outer layer abutting on to the pronephros and the inner layer extending beneath it. Although classically the limb bud is supposed to be derived from the somatic mesoderm, corresponding to the outer layer, it is probable that the inner layer contributes tissue as well.

The grafts were held in place for at least 30 min by small glass bridges made from coverslip fragments. Then the embryos were transferred to individual 5 cm petri dishes containing 1/4 NAM and allowed to develop at 18 °C. After 2 days they were transferred to 1/10 NAM, which was changed every 3 days until the hatching stage was reached. They were then fed daily on brine shrimps until the limb skeleton had developed.

The limbs were then fixed in 4% formaldehyde (10% conc. formalin), 1% $CaCl_2$, 40 mM Tris, pH 7·4, for 4 h or more. They were bleached overnight in Mayer's bleach followed by 20 vol. H_2O_2 in 70% alcohol, then stained for 3 h in 0·1% Alcian green 2GX in acid alcohol, destained in acid alcohol overnight, dehydrated in alcohols and cleared in oil of wintergreen. The limbs are classified structurally as 'normal', 'duplicate', hypomorphic' or 'other'. Duplicate limbs (called reduplications in previous papers) are of variable width and contain two posterior sets of elements related by a central plane of mirror symmetry. Hypomorphic limbs contain less than the normal complement of elements and thus range from limbs lacking one finger down to jointed cartilage spikes. It is not normally possible to say with certainty which structures are present in a hypomorph when it has only one or two digits.

For histological examination embryos were fixed overnight in Zenker's fluid

Table 1. *Development of half limb rudiments* in situ

Fragment	Cases	Growth	Normal	Duplicate	Hypomorphic	Other
Anterior	44	24	18+1 (79%)	0	4	1
Posterior	28	27	25 (93%)	2	0	0
Dorsal	18	14	13+1 (100%)	0	0	0
Ventral	19	9	5 (56%)	0	3	1
Totals	109	74				

(Cases indicated as '+1', in this and later tables, are imperfect in that they bear a supernumerary digit out of the plane of the others).

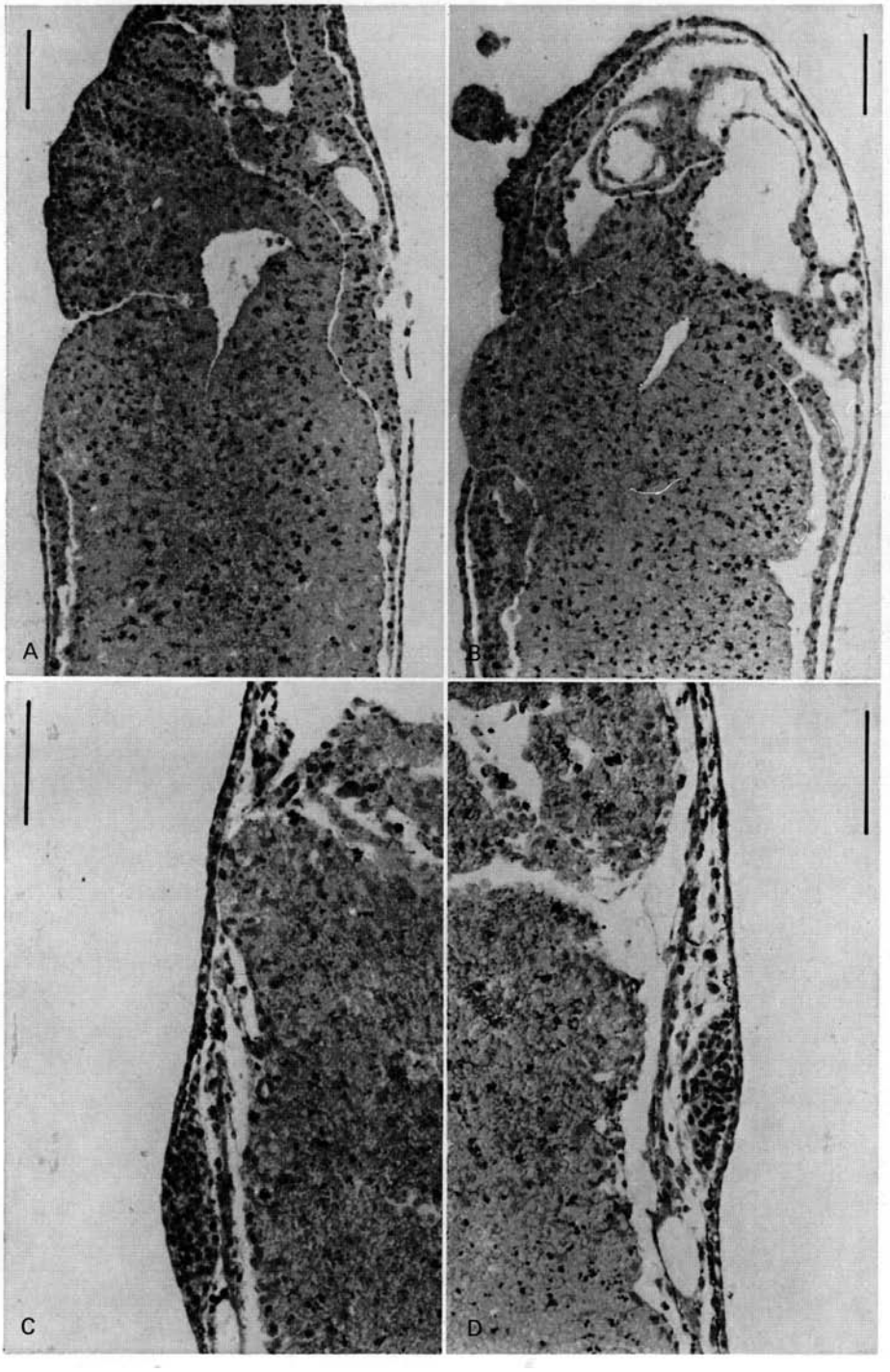
containing 5% acetic acid, washed exhaustively in tap water, demercurified in 35% alcohol containing a little iodine, stained overnight in Grenacher's borax carmine and destained overnight in acid alcohol. They were then dehydrated in butanols, mounted in 56° m.p. wax, sectioned at 10 μm and counterstained with 0·025% naphthalene black in saturated aqueous picric acid.

RESULTS

Extirpations

In a number of embryos the anterior, posterior, dorsal or ventral half of the limb rudiment was removed and the behaviour of the remaining half was studied. The results are shown in Table 1. The dorsal or the posterior half left *in situ* nearly always produced a limb bud and the anterior and ventral halves also did so but with a somewhat reduced frequency. In most cases the bud that appeared on the operated side was smaller than the control bud and this difference persisted for several weeks although eventually the two limbs became of equal size. The schedule of visible differentiation also lagged behind that of the control side. In 85% of cases the limbs which grew from the half rudiments had a normal pattern. There was some tendency for the ventral halves to yield hypomorphic structures, but although two duplications arose from posterior halves there was certainly no fragment which reliably formed a duplication in this situation.

Since the growth of the posterior halves seemed to be the most predictable, a further 32 embryos had the right anterior half rudiment removed at stage 34 and were fixed for histological examination between 1 and 6 days later. Healing was found to occur fairly slowly: usually the epidermis had grown back across the gap by day 3 although there was considerable variation in rate between individual embryos. Where it had not yet healed there was a slight protrusion of the prospective liver through the wound. There was little if any mesodermal continuity across the gap even by day 6. On the control side the two-layered mesoderm of the limb region was clearly visible by day 2, the first signs of a bud by day 3 and an underlay of striated muscle by day 4. On the operated side the same


A
B
C
D

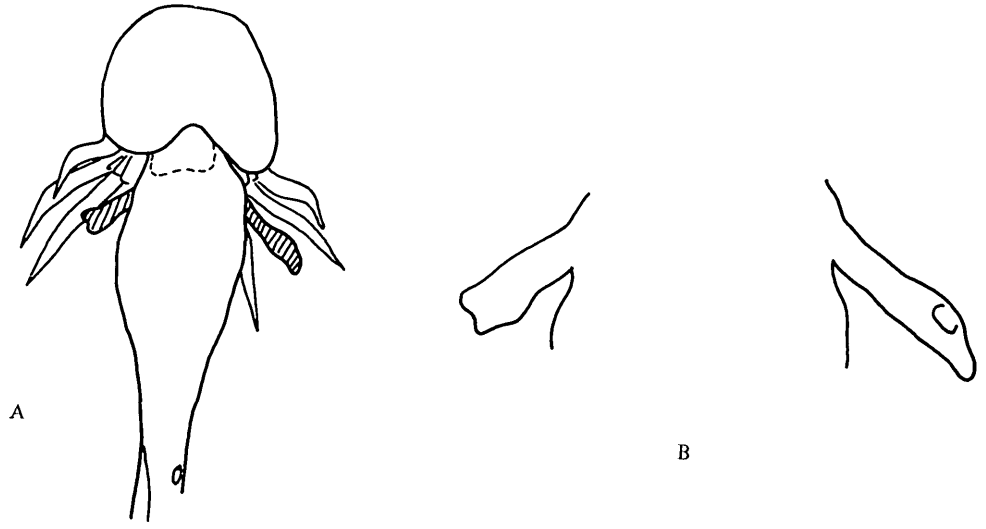


Fig. 3. Camera-lucida drawings of an axolotl larva 25 days after removal of the anterior half of the limb rudiment from the right side. (A) × 12. (B) × 25. The limb on the operated side is still smaller and less well differentiated than the limb on the control side.

sequence was followed with a limb bud appearing at the anterior edge of the remaining mesoderm. Both the pronephric tubules, which normally lie dorsal to the bud, and the muscular underlay were truncated at the level of the wound and here the liver rudiment abutted directly on to the epidermis (see Fig. 2). At no stage was there any sign of a proliferative zone in any of the mesodermal tissues in the vicinity of the wound. This is not really surprising since the limb buds which formed after the extirpations lagged behind those of the control side in their rate of development. There is some variation between cases in the rate at which they catch up but the process is gradual and occurs during and after the visible differentiation of the limb. In Fig. 3 is shown a camera lucida drawing of a larva from which the right anterior half limb rudiment had been removed 25 days previously, showing that the difference in size and developmental stage is still appreciable.

FIGURE 2

Frontal sections of axolotl embryos from which the anterior half of the limb rudiment has been removed on one side (left side of pictures). (A) Fixed after 1 day: shows the mesodermal thickening of the limb region on the control side and a gap on the operated side. (B) Fixed after 2 days: shows appearance of double layered structure in the limb rudiment of both sides, with a persistent gap on the operated side. (C) Fixed after 5 days: shows a limb bud developing from the residual mesoderm on the operated side. The epidermis has healed but there is still a gap in the mesoderm anterior to the bud. (D) The same embryo: control limb bud in a different plane of section showing mesodermal continuity anterior to the bud. Scale bars indicate 200 μm.

Table 2. *Development of complementary pairs of half rudiments*

Operation	Cases	Anterior half					Posterior half					Cases producing >1 limb	Mean digit number
		Growth	Normal	Duplicate	Hypomorphic	Other	Growth	Normal	Duplicate	Hypmorphic	Other		
Ant. ½ on flank Post ½ *in situ*	12	9	7+1	1	0	0	12	11	1	0	0	9	8·8
Ant. ½ *in situ* Post ½ on head	11	5	4	0	1	0	10	3	0	6	1	4	6·0
Ant. ½ *in situ* Post ½ on flank	10	6	3	0	3	0	10	0	6	3	1	6	8·3
Totals	33	20					32					19	

These results are included in the totals of Table 1, 3 and 4 along with other cases.

In microsurgery of embryos the cuts cannot of course be made with absolute precision. Because of this it is sometimes argued that regulation is an illusion and that what has really happened is that rudiment has been left in place in some specimens and removed *in toto* from others. In order to counter this objection I felt that it was necessary to study the potency of both complementary half rudiments from the same embryo. Such experiments are rather vulnerable to losses from disease or accident since two embryos must be reared for each result. However, a certain number of cases were collected and the results are shown in Table 2. It shows the results of three types of transplantation in which half of the limb rudiment was left *in situ* and the other half was transplanted to a different site on a host embryo. The important columns for the moment are the last two which show the number of pairs which produced more structures than the set found in a normal limb and the mean digit number of these pairs. The latter figure can exceed eight (each normal hand has four digits) because some of the duplicates and 'others' had more than four digits. Since the proportion of fragments which formed limbs was quite similar to that found in experiments using non-complementary halves, and since 58 % of the pairs gave more than one limb's worth of structures, the reality of regulation in the anteroposterior axis seems inescapable. Because of the low potency of the ventral halves only two complementary pairs of dorsal and ventral halves were obtained in which something grew from both parts, and in both cases the transplanted dorsal half gave a normal limb and the ventral half gave a small spike. It is not therefore possible to say with confidence that regulation also occurs in the dorsoventral axis; if it does it is certainly more difficult to demonstrate.

Transplantation

A number of experiments were carried out in which each of the four overlapping halves were grafted to the flank or to the head of host embryos. Although other workers have tended to be concerned about the orientation of such grafts with respect to the whole body (e.g. Harrison, 1921; Hunt & Jacobson, 1972) I believe that what is important is the immediate envrionment of the graft. So in all these experiments the halves were orientated harmonically, with all axes parallel to the host axes, except for the double grafts to the head in which the two pieces were placed centre-to-centre so that the axis parallel to the cut was the same for both halves. In spite of the harmonic orientation, different structures were formed in different environments, and this should reinforce the view that polarity and pattern can be influenced by the neighbouring tissues.

In Table 3 are shown the results of implanting various fragments onto the head. The 'whole rudiments' tended to grow with high frequency (87 %) and to form normal limbs. The half limbs grew rather poorly compared to their performance *in situ* and because of this many grafts were done of two similar halves in the hope that an increased cell number would augment the proportion of growths. The posterior halves showed 53 % growth of which half were normal

Table 3 *Development of fragment combinations on the head*

Graft	Cases	Growth	Normal	Duplicate	Hypo-morphic	Other
Whole rudiment	16	14 (88%)	11	1	1	1
Anterior half* and flank strip	18	10 (56%)	7+2	0	1	0
Anterior half	26	0 (0%)	0	0	0	0
2 × ant. half	14	2 (14%)	0	0	2	0
Posterior half	30	16 (53%)	8	0	7	1
2 × post. half	28	14 (50%)	4+1	4+1	3	1
Dorsal half	12	3 (25%)	2	0	1	0
2 × dorsal half	11	7 (64%)	1	4	0	2
Ventral half	10	0 (0%)	0	0	0	0
2 × ventral half	10	0 (0%)	0	0	0	0
totals	175	66				

* This result previously published in Slack (1977*a*).

and half hypomorphic. These hypomorphs tended to look like the posterior parts of the limb but it was difficult to identify the elements with certainty. When two like fragments were combined the percentage growing was not increased but a number of double posterior duplications were formed. This is not surprising because when two posterior halves are joined centre to centre the combination is one of a region of limb rudiment sandwiched between two pieces of flank tissue. This has previously been shown to give rise to duplications in other types of graft (Slack, 1976). In contrast to the behaviour of the posterior halves, the anterior halves showed no growth at all. Even when two like halves were combined, only two small hypomorphs were formed from 14 cases. For comparison the second line of the table shows a result previously published (Slack, 1977*a*) in which an anterior half was grown together with a strip of flank tissue on the head. This gave 55% growth with most of the limbs being normal.

A similar difference of potency was shown between dorsal and ventral halves. The ventral halves showed no growth, either singly or in pairs. The dorsal halves showed 25% growth on their own and 63% growth in pairs, the latter giving rise to some duplications. These were double posterior rather than double dorsal duplications and are not easy to explain since the posterior edges should have been aligned on the fragments. But since it is very difficult to place the halves accurately in such double grafts, it is perhaps possible that lateral movement between the components during healing brings a limb rudiment region in between two pieces of flank and so the duplications arise by the same mechanism.

In summary, the results of transplantations to the head show that the fragments which grow are those containing at least part of the original dorsal and

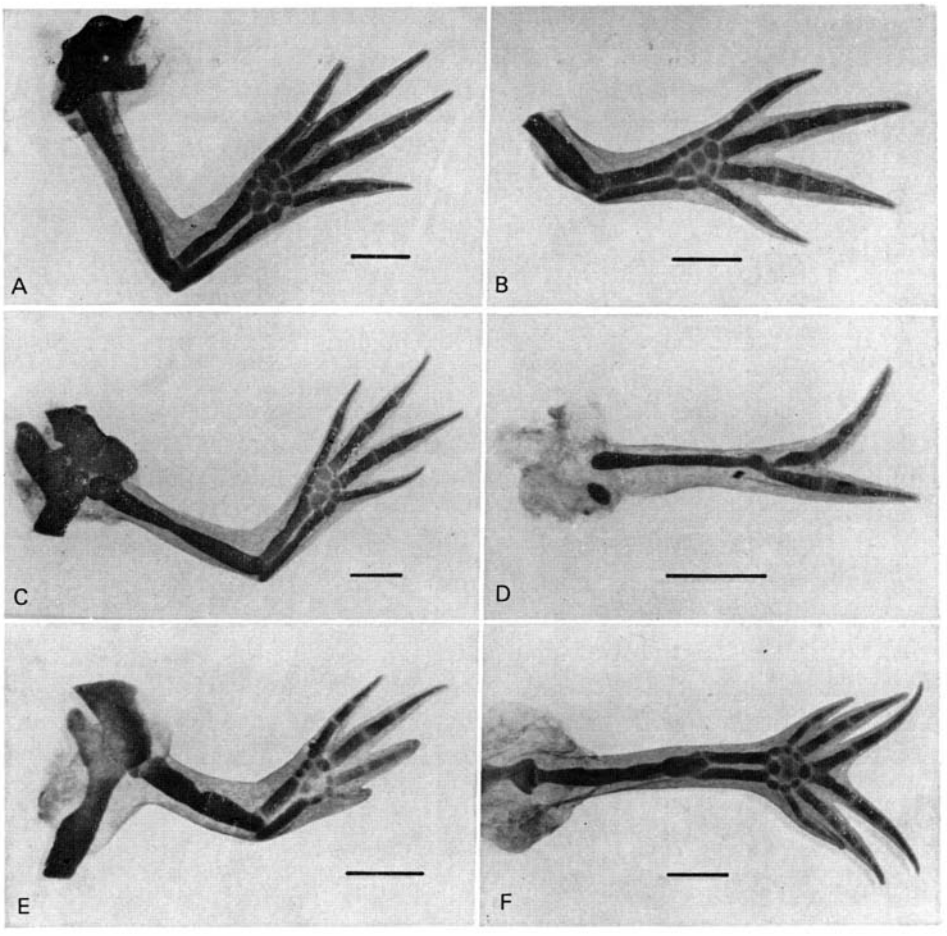


FIGURE 4

Whole mounts of pairs of limbs obtained by allowing half of the limb rudiment to develop *in situ* and the complementary half of the same rudiment at a foreign site. The limbs formed on the donors are on the left and those formed on the hosts are on the right.

(A) posterior half *in situ*.
(B) complementary anterior half on flank.
(C) anterior half *in situ*.
(D) complementary posterior half on head.
(E) anterior half *in situ*.
(F) complementary posterior half on flank.
Scale bars indicate 1 mm.

Table 4. *Development of half rudiments in the flank*

Graft	Cases	Growth	Normal	Duplicate	Hypo-morphic	Other
Anterior half	50	32 (64%)	24+3	5	0	0
Posterior half	32	20 (63%)	0	16	3	1
Dorsal half	11	7 (64%)	2+1	1	3	0
Ventral half	11	0 (0%	0	0	0	0
Totals	104	59				

posterior edges, and the requirement for the posterior can also be met by a strip of flank tissue.

In Table 4 are shown the results of grafting the four overlapping halves to the flank. The behaviour of the halves on this site differs both from that on the head and from that on the normal site. The anterior halves showed quite good growth (64%) forming predominantly normal limbs plus a few duplications. The posterior halves showed the same propensity to grow (63%) but nearly all of them developed into duplications. The dorsal halves showed good growth and formed a variety of structures while the ventral halves formed nothing at all. So, as before, it seems that part of the original dorsal edge is necessary for growth. The posterior edge is not necessary, presumably because flank tissue is present at the site of the graft. The presence of the anterior edge seems to ensure formation of a normal limb while its absence results in a duplication.

DISCUSSION

The results of the extirpations indicate that the true limb rudiment lies in the vicinity of the intersection of the AP and DV cuts of Fig. 1 although perhaps less than half is included in the fragment here called the ventral half. Since the overwhelming tendency is to produce normal limbs rather than hypomorphic ones it seems reasonable to conclude that there is not at this stage any internal subdivision of the rudiment. This experiment has been performed before by Harrison (1918) although with fewer cases and without any complementary pairs or histological analysis. Although he obtained more duplications from the posterior halves the results are broadly similar to the present ones, and seem to confirm his conclusion that the limb rudiment is at this stage an 'equipotential system'.

None of the half rudiments left *in situ* formed significant numbers of duplicates which indicates that division leads not to regeneration and duplication but to the formation of two complete patterns. The histological study of the development of posterior halves *in situ* showed that the healing of the mesoderm across the gap is not necessary for the formation of a limb bud and that no proliferative zone is apparent in the 6 days following the operation during which time a limb

bud is established from the residual tissue. The limbs on the operated side lagged behind the control side in their rate of development although it is not known whether the operated limbs were smaller than control limbs of the same developmental stage since the maximum expected difference is only 20% of linear dimensions. It is probable that size regulation proceeds gradually and simultaneously with cytological differentiation.

So both with respect to the pattern elements formed and with respect to the time of growth relative to pattern formation, the half-limb rudiment behaves like an early embryo rather than like a differentiated limb. According to the definitions presented in the introduction it shows regulation rather than regeneration. It must be concluded that the distinction proposed by French *et al.* (1976) between the primary embryonic field that regulates and the secondary fields that regenerate is not correct and that the real distinction is between the behaviour of undifferentiated rudiments and of differentiated organs.

The results of the transplantations are consistent with the conclusion that the 'whole rudiment' of Fig. 1 includes all of the competent limb tissue plus certain surrounding tissues which have a morphogenetic significance for the limb. These are (i) posteriorly some flank tissue which interacts with the limb rudiment to produce a source of a graded signal controlling anteroposterior pattern (Slack, 1977*b*), (ii) anteriorly some gill tissue which acts as a barrier to the passage of signals, (iii) dorsally some tissue which is necessary for the growth of the limb bud and which may possibly be concerned with its regionalisation in the dorsoventral axis.

It is very striking that the same fragment will produce a different pattern when grown on the three different sites. The results for the anterior and posterior halves are the most clearcut and can be summarized as follows:

Half	Site		
	In situ	Head	Flank
Anterior	Normal	No growth	Normal
Posterior	Normal	Normal or hypomorphic	Duplicate

At first sight the third column may suggest a regeneration/duplication phenomenon but it is clear that an explanation for this result based on the internal dynamics of the limb rudiment would fail to account for the results in the first two columns. However, if we suppose that pattern is controlled by the surrounding tissues then all of them can be explained. The posterior half forms a normal limb *in situ* or on the head because it carries its own strip of flank with it at its posterior edge. It forms a duplicate on the flank because in this situation it has flank tissue on both sides. The anterior half forms a normal limb *in situ* with rather lower frequency than the posterior half because at least a little healing is necessary to reconnect the limb rudiment to the flank tissue. It fails to develop at all on the head because it lacks the flank tissue, but when grafted together with a

piece of flank tissue will form a normal limb (Table 3, line 2). When grafted to the flank it forms a normal limb rather than a duplicate because its own anterior edge is not limb tissue and so is not capable of interaction with the flank.

These results only bear on the state of affairs in the tail-bud embryo prior to the formation of the limb bud. We are still largely ignorant about events in the limb bud itself, although there is some evidence for a zone of polarizing activity similar to that in the chick limb bud which is thought to control the pattern of differentiation in the anteroposterior axis (Cameron & Fallon, 1977).

What, then is the relationship between embryonic development and regeneration? The ability to regenerate, in the sense defined in the introduction, must be acquired during larval growth, and my own view as to the course of events is as follows:

Phase 1. From the regionalization of the mesoderm in the gastrula to the formation of the limb bud. The forelimb rudiment is a homogeneous group of cells. They are in a state of determination such that they must form structures of the forelimb but they are not regionally subdivided. The forelimb is 'determined' with respect to the future arrangement of parts in the transverse axes only in so far as it bears a necessary spatial relationship to the neighbouring parts of the primary body plan, notably the gill and flank rudiments.

Phase 2. The limb bud prior to differentiation. The regional subdivision of the bud occurs with respect to graded signals which operate across the whole extent of the competent tissue. The high points of these gradients are specified by the surrounding tissues.

Phase 3. The differentiated larval limb. The organ is now subdivided into discrete territories each of which bears a stable 'epigenetic coding'. The codings are incremented in steps of one across the limb and bear a one-to-one relationship to the values of the former gradients at each point.

Phase 4. Regeneration. When internal tissues are exposed by surgery they dedifferentiate to form the regeneration blastema. In the blastema the codings are erased and the cells are reprogrammed by signals from the adjacent differentiated tissues, these bearing a one-to-one relationship to the codings represented at the interface.

According to this type of scheme there would have to be two types of positional information. There are the signals that can programme or reprogramme the blastema or the limb bud and there are the stable codings of the differentiated tissues. Unfortunately we have yet to find the biochemical nature of either of them, although we might speculate that ions or small molecules would be suitable for the first type (McMahon, 1974) and proteins or glycoproteins for the second type (Slack, 1980).

REFERENCES

BORDZILOVSKAYA, N. P. & DETLAFF, T. A. (1979). Table of stages of normal development of axolotl embryos and the prognostication of timing of successive developmental stages at various temperatures. *Axolotl Newsletter*, no. 7.

BRYANT, S. V. & ITEN, L. E. (1976). Supernumerary limbs in amphibians: experimental production in *Notophthalamus viridescens* and a new interpretation of their formation. *Devl Biol.* **50**, 212–234.

BUTLER, E. G. (1955). Regeneration of the urodele forelimb after reversal of its proximodistal axis. *J. Morph.* **96**, 265–282.

CAMERON, J. & FALLON, J. F. (1977). Evidence for a polarizing zone in the limb buds of *Xenopus laevis*. *Devl Biol.* **55**, 320–330.

FRENCH, V., BRYANT, P. J. & BRYANT, S. V. (1976). Pattern regulation in epimorphic fields. *Science*, **193**, 969–981.

FURUKAWA, H. (1940). Transplantation experiments on appendages of *Anisolabis maritima* (Dermaptera) I–III. *Jap. J. Zool.* **8**, 479–535.

HARRISON, R. G. (1918). Experiments on the development of the forelimb of *Amblystoma*, a self differentiating equipotential system. *J. exp. Zool.* **25**, 413–461.

HARRISON, R. G. (1921). On relations of symmetry in transplanted limbs. *J. exp. Zool.* **32**, 1–136.

HÖRSTADIUS, S. (1973). *Experimental Embryology of Echinoderms*. Oxford: Clarendon Press.

HUNT, R. K. & JACOBSON, M. (1972). Development and stability of positional information in *Xenopus* retinal ganglion cells. *Proc. natn. Acad. Sci. U.S.A.* **69**, 780–783.

LUTZ, H. (1949). Sur la production experimentale de la poly-embryonie et de la monstruosité double chez les oiseaux. *Archs Anat. microsc. Morph. exp.* **38**, 79–144.

MCMAHON, D. (1974). Chemical messengers in development: a hypothesis. *Science*, **185**, 1012–1021.

SLACK, J. M. W. (1976). Determination of polarity in the amphibian limb. *Nature*, **261**, 44–46.

SLACK, J. M. W. (1977*a*). Determination of anteroposterior polarity in the axolotl forelimb by an interaction between limb and flank rudiments. *J. Embryol. exp. Morph.* **39**, 151–168.

SLACK, J. M. W. (1977*b*). Control of anteroposterior pattern in the axolotl forelimb by a smoothly graded signal. *J. Embryol. exp. Morph.* **39**, 169–182.

SLACK, J. M. W. (1980). A serial threshold theory or regeneration. *J. theor. Biol.* **82**, 105–140.

SLACK, J. M. W. & FORMAN, D. (1980). An interaction between dorsal and ventral regions of the marginal zone in early amphibian embryos. *J. Embryol. exp. Morph.* (in the Press).

SPEMANN, H. (1936). *Embryonic Development and Induction*. Reprinted 1967. New York: Hafner.

SPRATT, N. T. & HAAS, H. (1960). Integrative mechanisms in development of the early chick blastoderm. I. Regulative potentiality of separated parts. *J. exp. Zool.* **145**, 97–137.

TARKOWSKI, A. H. (1959). Experiments on the development of isolated blastomeres of mouse eggs. *Nature*, **184**, 1286–7.

(Received 21 December 1979, revised 16 January 1980)