

SELF-DIFFERENTIATION IN THE GRAFTED LIMB-BUD OF THE CHICK

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THE purpose of this paper is to describe two specimens resulting from the grafting on to the chorioallantoic membrane of the chick of fragments of embryonic chick limb-buds.

The first specimen was a very small basal piece of the left posterior limb-bud of a four-day chick. It was grafted on to the chorioallantoic membrane of a chick of seven days' incubation.

On the fourth day of incubation of a normal chick the hind limb is no more than an exaggeration of the Wolffian ridge, consisting of closely aggregated mesoblast cells. On the fifth day the limb-bud consists of a projection from the side with an axis equal to about two-thirds the breadth. A condensation of mesenchyme is beginning axially and proximally, but there has been no cartilage formed. The pelvic girdle develops in continuity with the femur, as two outgrowths of the proximal part of the latter, beginning about the sixth day. About the eighth day the pelvis begins to separate from the femur (Johnson).

The specimen to be discussed was left as a graft for five days. It was then fixed. On opening the egg, the graft was found on the inner side of the chorioallantoic membrane suspended from the latter by a stalk, and having very clearly the general shape of a femur. Its length was about .75 cm.

Sections showed that the graft now consisted, no longer of a fragment of undifferentiated limb-bud, but of a highly differentiated and very easily recognisable femur.

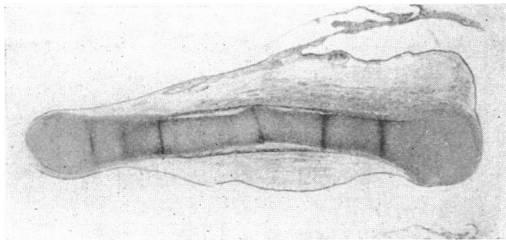


Fig. 1. Longitudinal section of the graft.

Fig. 1 is a photograph of the femur in longitudinal section. The axis consists of well-developed cartilage, more fully differentiated towards the centre, less so at the two ends, where there is less matrix. There are also

more mitotic figures at the ends than in the middle. The central region of the cartilage is surrounded by a double layer of perichondral bone. Fig. 3 shows a high-power view of this bone in process of formation by numerous osteoblasts.

As regards its gross morphology, the specimen is a typical femur. Fig. 2 is drawn from a graphic reconstruction made on squared paper. There is a typical head and trochanter, while the distal end is swollen as though in preparation for the formation of condyles. Situated at the distal end is a small piece of free cartilage in the position of the patella, and there seems to be no reason for doubting that such is its identity, though its shape is somewhat irregular. There is also another small piece of cartilage, to the anterior side of the femur and a little less than one-third the total length of the femur from the proximal end. This probably represents a fragment of displaced pelvis. If so, it is the only piece of pelvis represented in the specimen.

Attached to the right anterior side of the distal end of the femur is some muscle, still in an embryonic condition, and extending to beyond the middle of the femur.

In shape, the cartilage is curved, with the convexity of the curve to the right, just as in the left femur of the adult bird.

It has been said that the specimen was when grafted a very small basal piece of the left posterior limb-bud, and that at this stage the limb-bud is merely an undifferentiated region of mesenchyme cells, with an axial condensation but no more, surrounded by the ectoderm. In the same specimen after five days' life as a graft we find a well-developed femur, both anatomically and histologically highly differentiated, but we do not find any other regions of the limb or limb-skeleton, except a patella and a small piece of cartilage which from its position cannot well represent more distal portions of the limb than the femur, and probably is a fragment of pelvis. Now, if the limb-bud of a chick at four days' incubation were a "harmonious equipotential system," we should expect that a fragment, if it were able to continue differentiation at all, would produce not a femur only, but a whole limb, or would at any rate attempt to do so. The alternative view, that the limb-bud is a mosaic structure, in which the future regions of the limb are predetermined long before they are visibly differentiated, is obviously more in accord with this experiment,

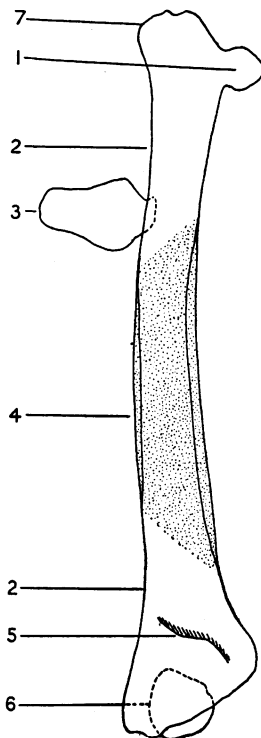


Fig. 2. Graphic reconstruction of grafted femur, viewed from behind.

- (1) Head.
- (2) Shaft of femur.
- (3) Fragment of ectopic pelvis, on opposite side.
- (4) Region at middle of femur, ensheathed in perichondral bone.
- (5) Line of attachment of muscles, on the opposite side of the femur.
- (6) Patella, on opposite side.
- (7) Trochanter.

although, until the experiment has been carried out on a larger scale, it cannot be held to be proved. Spurling's experiments, however, in which he extirpated the entire limb-bud, support this view. He was able to show quite definitely that regeneration did not follow upon extirpation of the whole limb-bud, and that after the operation there developed only those parts of the pelvic girdle which had not been removed. Thus the pelvic girdle is certainly a mosaic as regards its various parts; and the entire girdle-complex and appendicular limb form a mosaic as between these two parts, since the girdle Anlagen do not

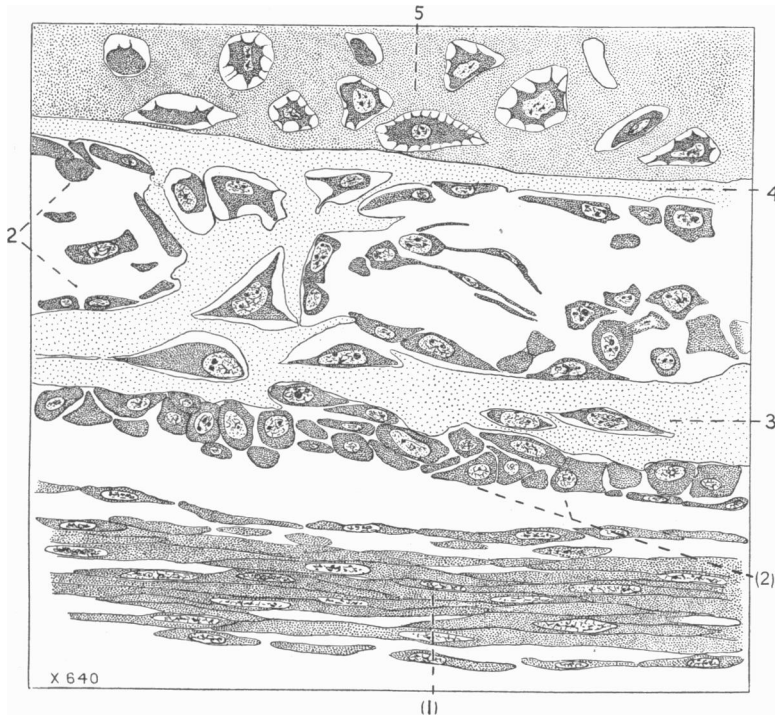


Fig. 3. High power view of perichondral bone of grafted femur.
(1) Periosteum. (2) Osteoblasts. (3) Perichondral bone, outer layer.
(4) Perichondral bone, inner layer. (5) Cartilage.

regenerate the limb axis. But Spurling did not show that the appendicular part of the limb forms a mosaic in itself, because in all his experiments the entire limb-bud was removed. In the experiments which have just been described, however, only a small fragment of the limb-bud was concerned and it only formed part of the limb. It may therefore be provisionally concluded that the limb-bud of the chick of four days' incubation is a mosaic structure in which the potentialities of the various regions are represented spatially, and any region of it is only able to form some or all of that region of the limb for which it was already destined.

It is obviously improbable that the small piece of the limb-bud which was

grafted consisted of the region destined to form femur in its entirety and nothing else. Actually, it is known to be probable that a fragment of the pelvis was included. It is far more probable that either only part of the femur region was included in the graft, and that this part, by regulation, formed an entire femur, or that a small part of the more distal regions of the limb was also included but came to nothing, on account of its small size. Against this latter view is the fact that a small piece of tissue apparently representing a piece of the pelvis was able to differentiate itself to the extent of being able to form cartilage. There seems to be no reason why a small piece of crus should not have done the same thing. Further, it will be seen in Fig. 1 that there is a distinct bend or notch in the femur about the middle of its length, looking as if it had been broken there. This is not seen in Fig. 2 because of course the plane of a graphic reconstruction is at right angles to that of the sections. This bend or notch rather suggests that this may be the point from which the regeneration of the femur proceeded, i.e. that the rudiment of the whole femur region was not included in the graft, but only the rudiment of the part proximal to this notch, the rest having been regenerated. On the other hand, if this were true, one would expect to find a marked difference in the degree of histological differentiation of the two ends of the femur. Such is not the case, both ends being equally differentiated. If this supposition be correct, it is probable that the regenerated end is the distal, since the presence of the piece of cartilage probably representing pelvis would seem to indicate that the proximal end must have been present in the original graft.

This capacity for regulation may at first sight appear to contradict what has been said about the limb's mosaic predetermination. However, if the predetermination is only of whole regions, and fragments of each region can regulate the whole regions, the contradiction disappears. The limb-bud's predetermination is thus not complete in detail, but only in broad outline. It may be called a regional mosaic¹.

This specimen furnishes quite conclusive proof of the ability of the femur to self-differentiate when removed from the proximity of the organs which normally surround it. The question of symmetry hardly comes into this paper, since there can be no question of any influence of the tissues surrounding the limb-bud subsequent to grafting, and we have no information as to what may have happened before grafting. It is important to note that the specimen had its left-handed nature already impressed upon it at the time of grafting.

The work of Peebles should perhaps be mentioned here. She grafted the extremities of fore-limb-buds on to the bases of hind-limb-buds, and *vice versa*, at four days' incubation. She believes that the grafted wing-tip developed as a leg-tip and *vice versa*; but admits that her results were inconclusive. If we accept her view, it would indicate that, even though the limb-buds may be a mosaic structure, yet this is not unchangeable. The work merits repetition. However, Milojević has since repeated the work with regenerating limb-buds

¹ See postscript.

of *Triton cristatus* (young metamorphosed specimens) and finds that Peebles' view is correct, but for the earliest stages of regeneration only. It is not clear, however, from Milojević's preliminary account at what level amputation was carried out. An interesting field for work is here opened up.

Lillie removed the wing-buds of chicks of from three to six days' incubation, and found no case of regeneration.

The second specimen will not be described in detail. Although a certain amount of differentiation has occurred since grafting it has not gone so far as in the previous case, and the form of the specimen is much less regular and more difficult to recognise. It was a piece of either the right or left posterior limb-bud of a four-day chick. The limb-bud of both sides was grafted on to the same membrane, and hence it is not possible, owing to the poor differentiation of the graft, to decide which of the two grafts it is. It was left as a graft for three days only. Histologically it consists of cartilage, still somewhat poorly differentiated, and shading off at the edges into dense mesenchyme (procartilage); this grades again, but in parts with an abrupt transition, into the less condensed mesenchyme of the peripheral tissues of the limb and chorioallantoic membrane. In shape it is very irregular, and it is not possible to recognise particular regions, except that it seems to contain at least a part of the pelvic girdle. The form is too irregular to enable one to say more than this.

SUMMARY

We may conclude from the experiments described that:

(1) The limb-bud of a chick of four days' incubation is a mosaic structure, at least as regards its chief regions, and not a "harmonious equipotential system."

(2) A fragment of the femur-rudiment of a four day chick can undergo regulation and form a complete femur.

(3) Thus the limb-bud at this stage consists of a number of different regions, each predestined to give rise to one segment of the limb only; but each of these regions is totipotent as regards all the parts of the segment to which it is destined to give rise.

It is shown that:

The femur-rudiment of a four day chick can undergo self-differentiation, both anatomical and histological, when grafted on to the chorioallantoic membrane.

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Postscript. Since the above was written, P. Weiss ("Unabhängigkeit der Extremitätenregeneration vom Skelett," *Arch. mikr. Anat. u. Entw. mech.* vol. CIV, p. 359, 1925) has shown that in the regeneration of the limbs of (metamorphosed) *Triton cristatus* each segment of the limb-skeleton will complete itself, but that when a whole bone or set of bones (humerus, shoulder-girdle) is removed entire, they are not replaced; i.e. each segment of the skeleton is toti-potential as regards its own regulation, but the limb-skeleton as a whole is a mosaic of regions with different potentialities. This independently confirms the view we have taken above of the state of affairs in the limb-bud of the four-day chick.