

EXPERIMENTAL EPIPHYSEAL TRANSPLANTATION

Part I

Roentgenographic observations on survival and growth of epiphyseal transplants

By

HENRIK V. A. HEIKEL

INTRODUCTION AND PROBLEM

As sequels of congenital defect or injury of the growth zone of a bone, deformities may arise, the treatment of which often presents intricate problems. Attempts have been made to replace a missing or deficient epiphyseal cartilage with a graft (*Lexer, Küttner, Axhausen, van Assen, Straub, Starr, Riordan* and *Bintcliffe*, among others), but the results have not been encouraging. Only in cases of pedunculated and in a few cases of non-pedunculated autotransplantation has growth of the graft been observed. Moreover, the results have not always been convincingly documented.

In congenital aplasia of the radius, *Starr* and *Riordan* replaced the missing radius with an autotransplant consisting of the proximal end of the fibula but were not able to demonstrate longitudinal growth of the graft. Before epiphyseal transplantation can possibly be accepted as an orthopaedic method of treatment, the prospects of obtaining a satisfactory result must be studied as far as is possible.

The object of the present investigation is to study: (1) to what degree the epiphyseal cartilage can retain its capacity for longitudinal growth at autotransplantation, and (2) whether the stage of ossification of the epiphyseal nucleus of the graft at the time of transplantation affects the results obtained.

TABLE 1
*Growth of the Graft in Relation to the Normal (Estimated) Longitudinal Growth of
 Experimental Epiphyseal*

	Helferich	Rehn & Waka- bayashi	v. Tap- peiner	Obata	Haas (1915)	Heller (1919)
Reimplantation	partial		(n)	h	(0)	7/8
Autotransplantation				h		
Homotransplantation ..		(n)	(0)	0		1/5 1/2

(n) = almost normal, h = very reduced.

HISTORY

On the basis of a review of the literature on experimental epiphyseal transplantation and personal experiments, *Silfverskiöld* (1934) found that homo- and heterotransplantation, as well as autotransplantation into soft tissues, of the growth zone of a long bone are of no practical value and that in clinical cases only autotransplantation to bone can be considered. Neither does transplantation of pieces of epiphyseal cartilage to soft tissues or to defects in the long bones result in growth, worthy of mention, as experiments by *Bisgard*, *Gordon* and *Warren*, and by *Urist* and *McLean* have shown. *Ring* demonstrated that though the epiphyseal plate regenerates after excision of the reserve zone with a lamella of metaphyseal bone and even after excision of the entire epiphyseal plate with lamellae of both metaphyseal and epiphyseal bone, longitudinal growth will continue only if the reserve zone has been left intact. Nor has any evidence appeared to challenge the thesis established by *Heller* in 1919 that a condition for the preservation of the capacity of longitudinal growth is that the graft is firmly attached to normal spongiosa of the host bone or to the richly vascular marrow of a young bone.

With the guidance of the measures reported and proceeding from *Ollier's* and *Silfverskiöld's* observations that in the rabbit the distal growth zone of the ulna is responsible for five-sixths of the total longitudinal growth of this bone, I estimated the growth of the graft in relation to normal growth as given in some of the published experimental series (table 1). It is difficult to make comparisons, however, in view of the wide variation in experimental conditions. A feature in common to all the experiments on epiphyseal transplantation published to date is that the bones chosen for transplantation have been ones in which ossification of the epiphyseal nucleus was relatively far advanced.

METHOD AND MATERIAL

Sixty rabbits, 2 to 29 days old, were employed. No attention was paid to sex or to the number of animals in the litter, which varied from one to nine.

The following experiments were made:

1. One-stage autotransplantation of the proximal end of the fibula to the site of the radius. The radius was dissected extraperiosteally

TABLE 1
the Epiphyseal Growth Zone in Question as Stated in Previously Reported Series of Transplantation to Bone.

Fohl	Haas (1931)	May	Silfver- skiöld	Ring	Shraiber
7/8-11/12	1/2	(n)	5/6		(= length of the transplant)
	0		2/3	2/3-3/4	
	0		0	1/2-2/3	

(0) = almost nil, 0 = no growth.

and totally removed, the resulting condition corresponding to that in total aplasia of the radius (A), or the proximal epiphysis was left behind, a condition resembling partial aplasia of the radius (Ap) being produced. The proximal end of the fibula was dissected extra-periosteally, freed from the tibia and cut some millimetres distally of the epiphyseal plate, being then transplanted to the site of the radius, its diaphysis close to that of the ulna and its epiphysis as close to the carpus as possible, fixation being achieved only by suture of the skin wound.

2. The same in two stages: In the first stage the radius was removed and in the second the fibula was transplanted and fixed by suturing of muscles and skin.

3. Autotransplantation of the proximal end of the fibula to the thigh: The fibula was dissected as described above and implanted, with its epiphyseal end distal, into the thigh, the periosteum of the femur having been opened. The graft was fixed by sutures of muscle and skin.

All operations were carried out under local anaesthesia; the suture material was 5/0 Ethicon catcut in an atraumatic needle. No exterior immobilization was applied. Transplantation to the site of the radius was performed on the right side, the left being used as a control. In some animals one fibula was transplanted to the site of the radius and the other to the thigh, in some bilateral transplantation to the thigh was performed. In some experiments labelling of the ulna and/or the graft was achieved by introducing small pieces of stainless steel wire into holes drilled into the diaphysis with a thin needle.

Complications: At or shortly after the operation, epiphyseolysis or fracture of the ulna occurred in some animals. This healed in two cases without any perceptible effect upon the experimental result, while in the remaining cases there was wound rupture and infection.

The epiphyseal cartilage of the graft was damaged in two cases and infection occurred in two cases in connection with labelling of the graft. At two-stage transplantation the placing of the graft in the intended position was difficult, as a consequence of scar formation and malposition occurring after removal of the radius, and of the larger size of the transplant. Owing to various complications, a total of 14 animals were excluded from the experimental series.

Observation: The experimental results were followed by means of radiographic examinations carried out at irregular intervals. In this way observations could be made at many different ages of the animals without expersing any animal to frequent irradiation. On the other hand, the determination of exact time limits for the phenomena observed was rendered difficult by this method. The focal distance at the examinations was constant, 94 cm. The animals were not anaesthetized for the radiographic examination but were held manually. It was therefore not possible each time to achieve exactly the same exposure but, on the other hand, loss of animals owing to complications following anaesthesia were avoided.

Measurement on radiograms was made with a tape-measure and accuracy to within 0.5 mm. was aimed at. The longitudinal growth was mesured as follows:

Growth of the ulna: The increase in lenght as measured from the centre of the distal metaphyseal border of the ulna to the distal contour of the incisura semilunaris (the "coronoid process"), or to the label in the ulnar diaphysis.

Growth of the graft: The increase in distance from the centre of the metaphyseal border to the diaphyseal end of the graft or to the label in its diaphysis respectively, or, after the graft had fused with the ulna, to the coronoid process or to the label introduced into the ulna.

Control measurements carried out on radiograms of animals in which both the ulna and the graft had been labelled and had fused showed, on the one hand that the distances from the two labels to the coronoid process had remained constant, whilst on the other the distances from the two labels to the metaphyseal border of the graft had increased parallely during the growth of the animal. As a criticism of measurement of the growth of unlabelled grafts, which had not fused with the ulna, it might be contended that an appearant increase in length could occur through an osseous outgrowth from the diaphyseal end of the graft. It should be possible, however, to exclude this source of error since, with a single exception (which will be de-

scribed later), no increase of the distance from the label to the diaphyseal end was observed in the labelled cases, the increase occurring only in the distance between the label and the growth zone.

One-stage autotransplantation of the proximal end of the fibula to the site of the radius (experimental series II)¹ (Table 2):

TABLE 2
Autotransplantation of Fibula to the Site of the Radius in One Stage.

No. of Exp.	Age of animal (days)			Defect produced	Epiphyseal nucleus of transplant radiologically demonstrable at transplantation	No. of days after transplantation between which:		Growth in length of transplant (mm)		Growth in length of ulna (mm)		Approximate relation between growth in length of transplant and ulna during entire period of observation	No. of demonstrating figure
	at removal of radius	at transplantation of fibula	at end of period of observation			Occurrence of osseous contact between transplant and ulna was observed	it was observed that growth in length of the transplant had set in	at time when first observed	During remainder of period of observation	From transplantation until it was observed that growth in length of transplant had set in	During remainder of period of observation		
16	3	3	136	A	—	10-17	0-10	1	14	1.5	19	3/4	
24	5	5	68	Ap	—	14-21	14-21	1	11	9	10	2/3	1
26	6	6	49	Ap	—	20-32	0-20	3	7	7	9	2/3	
27	6	6	80	Ap	—	20-32	0-20	3	9	5	13	2/3	
28	11	11	214	Ap	—	9-24	9-24	1	13	9.5	14	< 2/3	
29	11	11	188	A	—	0-9	9-24	6	17	9.5	20	> 2/3	
30	11	11	188	Ap	—	9-24	9-24	7.5	18	12	20	5/6	3
33	10	10	69	Ap	—	8-23	8-23	8	9	10	9	8/9	2
37	15	15	214	Ap	—	6-18	6-18	4.5	20	7	22	> 4/5	
13	2	2	136	Ap	—	—	11-18	3	18	5.5	19	7/8	
14	2	2	171	Ap	—	—	1-11	1.5	15	3	13	1/1	
15	3	3	50	Ap	—	—	3-10	2	13	3	13	1/1	
25	5	5	49	Ap	—	—	0-14	1.5	10	5	10	< 4/5	4
31	10	10	212	Ap	—	—	1-8	1	19.5	2	26	3/4	
34	13	13	214	Ap	—	—	7-22	4	15	9	14	4/5	
35	13	13	214	Ap	—	—	7-22	2	15	11	19	> 1/2	5
36	15	15	238	Ap	—	—	6-18	3	19	6	19	> 4/5	
38	17	17	238	Ap	+	—	4-16	1	12	7	15	> 1/2	
39	17	17	136	Ap	+	—	4-16	2.5	12	7	14	2/3	

(A = total absence of radius,
Ap = partial absence, i.e. the proximal radial epiphysis left behind).

¹ In experimental series I, the radius was removed with a view to reproducing the defect in aplasia of the radius. These experiments are reported in my thesis (*Heikel, H. V. A.: Aplasia and Hypoplasia of the Radius. Studies on 64 Cases and on Epiphyseal Transplantation in Rabbits with the Imitated Defect. Acta Orthopaedica Scandinavica, suppl. 39. Ab Tilgmann OY, Helsingfors, 1959.*)

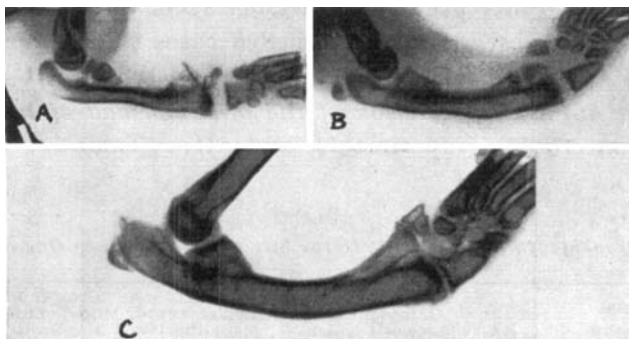


Fig. 1.

Experiment 24: A 14, B 21 and C 65 days after the transplantation of the proximal end of the fibula to the site of the radius. The graft has fused into the shape of a Y with the ulna, its diaphysis is wide and longitudinal growth has been comparatively good.

Of 23 animals operated on at the age of 2 to 17 days, 4 were excluded owing to various complications. The remainder were observed for a period of 44 to 238 days. In all animals examined 1 to 2 weeks after the operation, blurring of the contours and a reduced opacity of the graft were observed. *After 1½ to 3 weeks, there was an increase in the opacity and in the width of the metaphysis and a minimum increase in length of 0.5 mm.* The epiphyseal nucleus, which, at the time of transplantation, was visible in two animals only, became radiologically demonstrable within normal time limits (as determined by Heikel) or was only slightly delayed. In three experiments (nos. 24, 26 and 27) there was apparently epiphyseolysis of the graft and while the original diaphysis was pushed aside and gradually resorbed, a new strong metaphysis grew out of the epiphyseal cartilage (Fig. 1A—C). As can be seen from Table 2, measurable growth of the transplant was observed on the eighth day at the earliest and on the twenty-fourth day at the latest, but, on the other hand, the fourteenth day was the latest date at which no growth had yet been observed. On the basis of this it may be assumed that the growth of the transplant set in, as a rule, during the second and, at latest, during the third week after transplantation.

In eight experiments, the graft fused with the diaphysis of the ulna and in one with a "radial rudiment" growing from the remaining proximal radial epiphysis. The time at which contact between the bones could be radiographically established is seen from Table 2. Since in

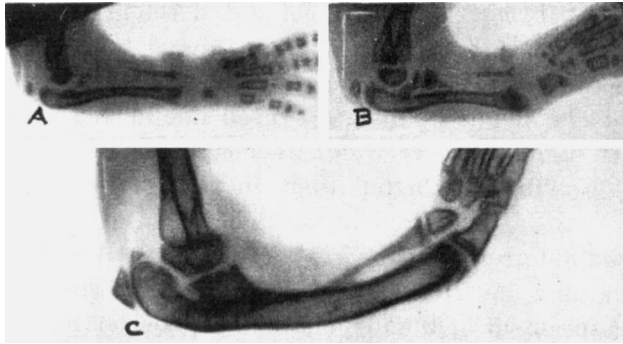


Fig. 2.

Experiment 33: A 1, B 8 and C 57 days after the transplantation of the proximal end of the fibula to the site of the radius. The diaphyseal end of the graft is in contact with the ulna, its diaphysis is thin, the longitudinal growth has been good.

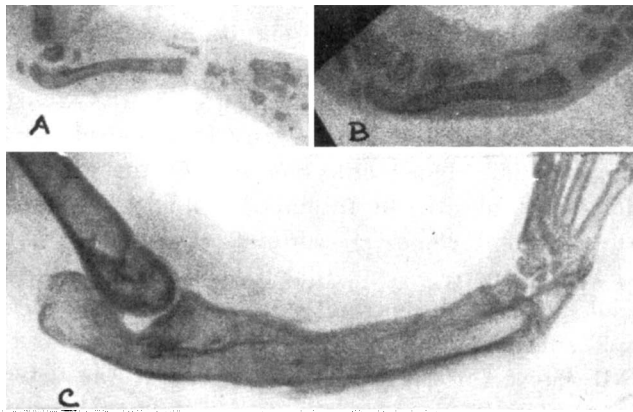


Fig. 3.

Experiment 30: A immediately, B 9 and C 177 days after the transplantation of the proximal end of the fibula to the site of the radius. The diaphysis of the graft has fused side to side with the ulna (through the agency of the bone formation visible in B in the soft tissue?), its longitudinal growth has been good.

nine animals shadows in the soft tissues indicated new formation of bone (from periosteal remnants left behind at dissection of the radius?) the contact may have been formed from this callus.

In three experiments (nos. 24, 28 and 29), the diaphyseal end of the graft fused broadly with the ulnar diaphysis, in the shape of a Y: The diaphysis of the graft seemed wide, its bony structure was good and, though initially poor, its longitudinal growth, after contact had occurred, was good (Fig. 1). In experiments 16, 27 and 33, there was

narrow contact between the diaphyseal end of the graft and the ulnar diaphysis: The diaphysis of the graft was thinner but its total growth in length somewhat greater than in the former experiments (Fig. 2). In experiments 26, 30 and 37 the graft fused side to side with the ulnar diaphysis: Its diaphysis was wide, its bony structure good and its longitudinal growth was better than in the previous two groups (Fig. 3).

The longitudinal growth of the graft was compared with the normal growth of the ulna and fibula. The former comparison was made by means of measurement of the displacement in the relative relation between the diaphyseal end of the graft and the coronoid process of the ulna, on the one hand, and between the distal epiphyseal lines of the graft and the ulna on the other. It is seen from Table 2 that *the growth of the graft* in the nine experiments described above *varied between (about) 2/3 and 8/9 of that of the ulna*. (It should be observed, however, that after removal of the radius, the longitudinal growth of the ulna was considerably inhibited. I shall deal with this point in greater detail elsewhere). In one experiment the graft grew in the same rate as the ulna until the epiphyseal line fused, which happened earlier in the graft than in the ulna. The epiphyseal line of the graft fused even before the age of 4½ months in animals 13 and 16 and before the age of 6 months in animal 28, in six animals which were observed for less than 3 months, there was no fusion at all, and in the remaining animals it took place at the normal time (according to *Heikel*).

The growth curves for the transplants were compared with the normal growth curve for the fibula of the rabbit (as determined by *Heikel*). Since the longitudinal growth of the fibula varies considerably, such a comparison is necessarily rather rough. The growth curves of the graft run level for one to two weeks after the transplantation, thereafter rising for two to four weeks even more steeply than the normal curve, then flattening out and definitely ceasing to rise at the age of 150 to 200 days. In those experiments which were observed for a period of at least 4 months *the growth of the graft varied between 1/3 and 2/3 of the normal longitudinal growth of the fibula at a corresponding age*.

Ten grafts did not fuse with the ulna but grew obliquely past the ulnar diaphysis, or remained parallel with it (Figs. 4 and 5). In these experiments the diaphysis of the graft was, as a rule, thinner and the osseous structure poorer than in those earlier described. The longitudinal growth varied between (about) 1/2 and 1/1 of that of the ulna

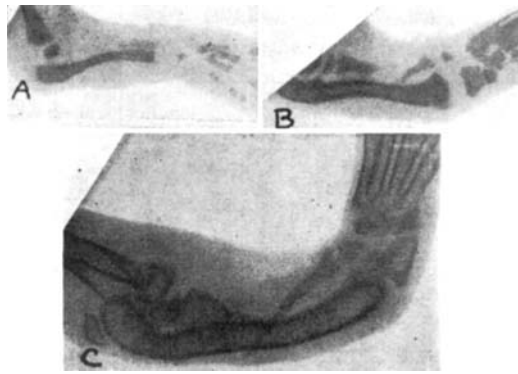


Fig. 4.

Experiment 25: A immediately, B 14 and C 49 days after the transplantation of the proximal end of the fibula to the site of the radius. The diaphysis of the graft is not in contact with the ulna, growing obliquely past the ulnar diaphysis.

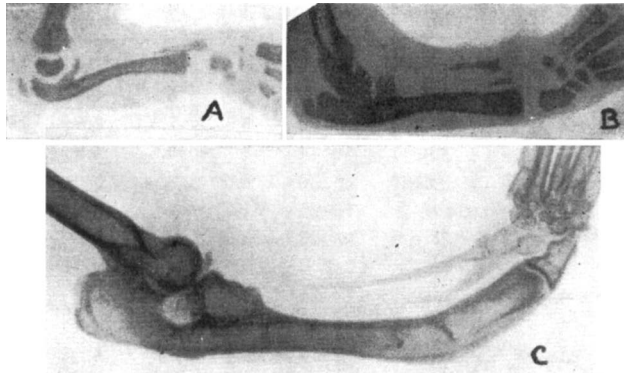


Fig. 5.

Experiment 35: A immediately, B 7 and C 175 days after the transplantation of the proximal end of the fibula to the site of the radius. Despite the presence of newly formed bone in the soft tissues, the graft has not fused with the ulna, its diaphysis is thin, and its epiphyseal line is still open at the age of 175 days.

but, as compared with the normal growth of the fibula, was somewhat less than in the experiments in which the graft had fused with the ulna. Fig. 10 gives, among other curves, the growth curves of the graft with the best growth with (no. 37) and without (no. 36) fusion with the ulna in experimental series II.

Two-stage autotransplantation of the proximal part of the fibula to the site of the radius (experimental series III) (Table 3):

TABLE 3
Two-Stage Autotransplantation of the Fibula to the Site of Radius.

No. of Exp.	Age of animal (days)			Defect produced	Epiphyseal nucleus of transplant radiologically demonstrable at transplantation	No. of days after transplantation between which:		Growth in length of transplant (mm)		Growth in length of ulna (mm)		Approximate relation between growth in length of transplant and ulna during entire period of observation	No. of demonstrating figure
	at removal of radius	at transplantation of fibula	at end of period of observation			Occurrence of osseous contact between transplant and ulna was observed	it was observed that growth in length of the transplant had set in	at time when first observed	During remainder of period of observation	From transplantation until it was observed that growth in length of transplant had set in	During remainder of period of observation		
44	6	10	106	A	(+)	0-14	5-14	2.5	17	8	19	>3/4	6
54	4	17	203	A	—	15-32	5-15	5	13	3	12	>1/1	
60	4	17	229	A	+	73-104	0-12	2	13.5	4	19	2/3	9
65	6	23	82	A	+	0-15	0-15	1	5	6.5	8	<1/3	
66	7	21	135	A	+	6-17	6-17	1.5	2	5	6	1/3	
67	3	14	214	A	—	0-9	0-9	1	18	1	22	4/5	
68	3	14	179	A	—	9-24	0-9	1	14	1.5	*)	*)	
69	4	23	127	A	+	0-15	0-15	1	4	4.5	10	1/3	
71	4	19	179	A	+	5-19	5-19	1.5	8.5	7	11	>1/2	7
72	4	19	127	A	+	5-19	5-19	2	5	4	*)	*)	8
77	3	10	168	A	—	7-25	7-25	5.5	7.5	6.5	14.5	2/3	
84	3	16	77	A	+	0-11	0-11	1	9.5	4.5	*)	*)	
87	4	21	77	A	+	6-20	6-20	4.5	8	3	7	>1/1	
58	4	11	41	A	—	—	13-25	1	1	6.5	4	1/5	
63	6	15	116	A	—	—	8-23	3	0.5	3	17	1/6	
64	6	23	70	A	+	—	—	—	—	—	10.5	0	
76	3	21	119	A	+	—	14-31	1	—	8	6.5	~0	
73	3	21	119	A	+	14-31	6-14	1	—	4	8	~0	
52	3	17	138	A	—	—	5-15	3.5	6.5	4	19	>2/5	
59	4	11	200	A	—	—	0-16	0.5	8.5	1	22	2/5	
62	6	15	135	A	—	—	8-23	0.5	0.5	7.5	16.5	~0	
82	3	27	108	A	+	—	0-17	1	—	3	11	~0	
85	3	23	71	A	+	—	0-17	1	7	5.5	6	2/3	
88	3	25	68	A	+	—	—	—	—	—	13	0	
89	3	25	83	A	+	—	15-25	2	—	6	3	1/5	
90	3	29	63	A	+	—	0-16	2	—	6	4	1/5	
93	4	20	70	A	+	—	9-25	3.5	3	7.5	5	1/2	

(A = total absence of radius,

Ap = partial absence, i.e. the proximal radial epiphysis left behind,

*) cannot be measured owing to marked curvature of the ulna).

Of the 37 experimental animals whose radius was dissected at the age of 3 to 6 days and whose fibula was transplanted (7 to 27 days later) at the age of 10 to 29 days, 10 were excluded owing to various complications. The remaining 27 animals were observed for a period of 41 to 229 days. In 21 of these experiments the ulna and the graft were labelled, in 2 the ulna alone and in 7 the graft alone.

In 17 experiments (the first ones in Table 3), the first postoperative radiographic examinations showed that the graft lay intramuscularly and parallel to or with its proximal end obliquely against the ulnar diaphysis. In 10 experiments, the graft proved to lie wholly or partially subcutaneously or diverging in a proximal direction from the ulna. The prospects of further longitudinal growth would therefore a priori be assumed to be poor. Small diffuse shadows occurred in 10 animals at the site of the removed radius but only in one animal near the graft. Their role in the formation of contact with the ulna can therefore be considered less than in experimental series II.

The epiphyseal nucleus of the graft, which in nine experiments was not visible at the time of transplantation, appeared at the normal time in all but one transplant, yet the longitudinal growth of the latter was just as good as that of the others. It will be seen from Table 3 that *the longitudinal growth of the graft began* at approximately the same time after transplantation as in experimental series II, or *within the second or, at latest, third week*. In one single experiment no growth could be observed. The epiphyseal line of the graft fused (entirely or almost entirely) at the age of 2 to 3 months in one, at 4 months in six and between the ages of 4½ and 7 months in three animals, i.e. earlier than normal in a strikingly large number of animals.

Thirteen of the transplants which had maintained a satisfactory position, and only one of the others fused with the ulna. Table 3 shows the time at which fusion was observed. In experiment 44, fusion occurred in the same way as in experiment 24, the diaphysis of the graft was wide, the osseous structure was good and its longitudinal growth was about half the normal and only a little less than that of the ulna. (Fig. 6). In experiments 65, 67, 68, 69, 71, 72, 84 and 87 the diaphyseal end of the graft fused with the proximal part of the ulnar diaphysis, growth and structure varied and *the longitudinal growth was 1/5 to 1/2 of the normal* (Figs. 7 and 8). In three of these experiments, an angular curvature occurred in the ulna which rendered exact measurement of ulnar growth impossible (Fig. 8). In experiments 54 and 60, there was only a narrow contact between the transplant and the ulnar

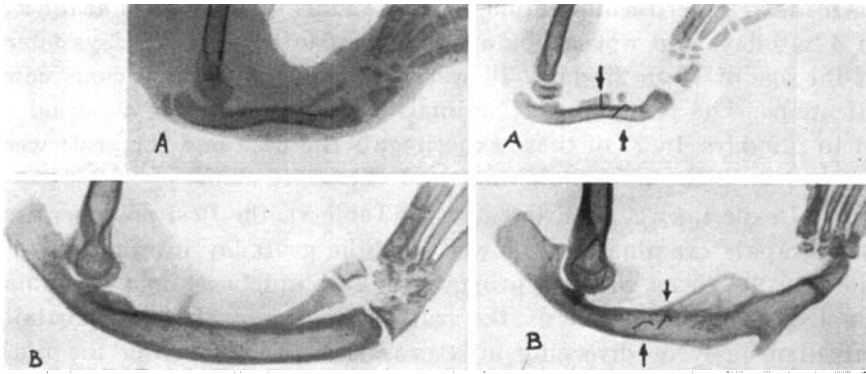


Fig. 6.

Fig. 6.

Fig. 7.

Experiment 44: A 14 and B 108 days after the transplantation of the proximal end of the fibula to the site of the radius. The graft has fused with the ulna into the shape of a Y (cf. fig. 1).

Fig. 7.

Experiment 71: A immediately and B 160 days after the transplantation of the proximal end of the fibula to the site of the radius. The diaphysis of the graft has fused side to side with the ulna; its longitudinal growth has been poor. The arrows indicate the metal wire labels in the diaphysis of the ulna and the graft.

diaphysis; the diaphysis of the transplant was thin but its *growth was 3/7 to 2/3 of the normal* (Fig. 9). In two experiments the graft fused side to side with the ulna (nos. 66 and 73), but longitudinal growth was insignificant. In experiment 77, in which the growth of the graft was good, the epiphyseal plate of the graft turned and gradually came to lie parallel to the ulnar diaphysis. Subsequently, continued growth led to enormous thickening of the bone. Fig. 10 shows the growth curve for the transplant growing best (no. 54) and for that growing least (no. 66).

Four grafts the positions of which were considered satisfactory and nine of the others did not fuse with the ulna. Only four of these exhibited growth worthy of mention, while in most of them growth ceased very soon.

Autotransplantation of the proximal part of the fibula to the thigh (experimental series IV).

This transplantation was performed on 35 rabbits, 6 to 29 days old. Since the graft could not be fixed parallel to the femur, the radiograms could not be uniformly estimated. The growth of the graft was therefore estimated on the basis of (approximate) measurement of the

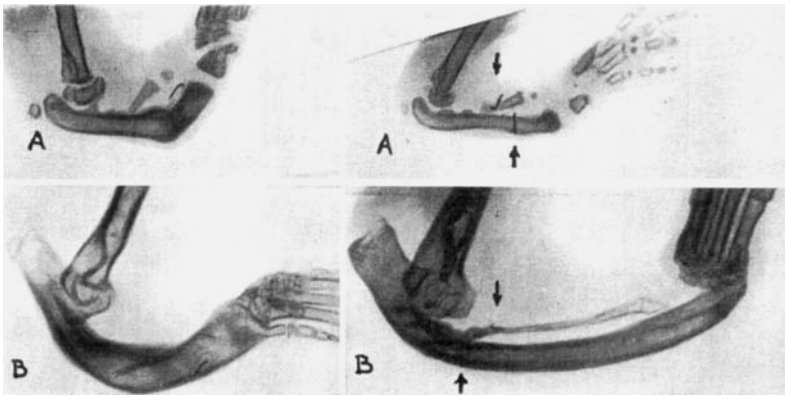


Fig. 8.

Fig. 8.

Fig. 9.

Experiment 72: A immediately and B 82 days after the transplantation of the proximal end of the fibula to the site of the radius. Before transplantation, there had been a fracture of the ulna which had healed, forming an angle. The diaphysis of the graft has fused with the ulna, but longitudinal growth has been poor.

Fig. 9.

Experiment 60: A immediately and B 221 days after the transplantation of the proximal end of the fibula to the site of the radius. The diaphyseal end of the graft has a narrow contact with the ulnar diaphysis, its diaphysis is thin and, at the age of 221 days, its epiphyseal line has fused. Longitudinal growth has been good. The arrows indicate the metal wire labels in the diaphysis of the ulna and the graft.

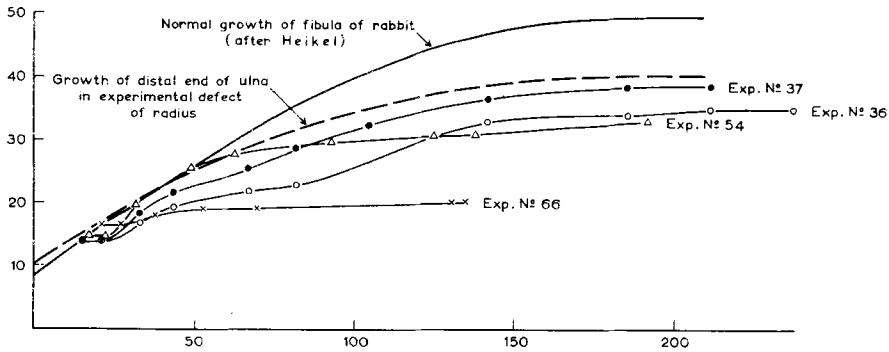


Fig. 10.

Growth curves for some of the grafts belonging to series II and III, compared with the normal growth curve of the fibula and the growth curve for the distal ulnar epiphysis after removal of the radius. To facilitate comparison, the length of each graft at transplantation was assumed to be equal to the length of the fibula at the same age.

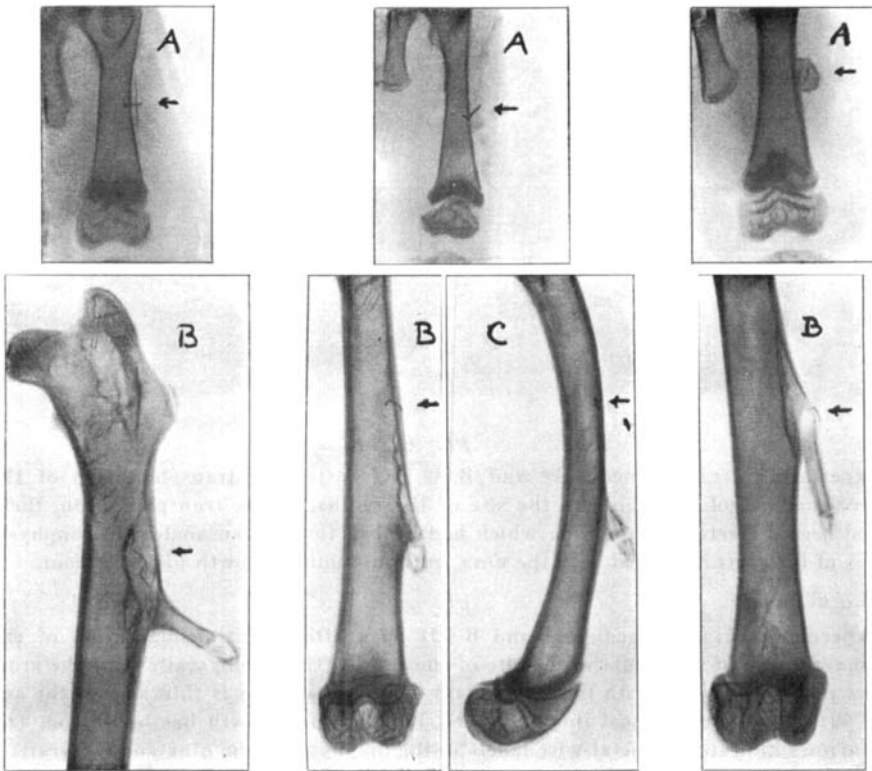


Fig. 11.

Fig. 12.

Fig. 13.

Fig. 11.

Experiment 75: A immediately and B 168 days after the transplantation of the proximal end of the fibula to the thigh. The graft has fused with the femur into the shape of a Y, forming an angle of 60° , its diaphysis is wide, the corticalis is well developed and longitudinal growth moderate. The arrow indicates the metal wire label in the diaphysis of the graft.

Fig. 12.

Experiment 62: A immediately, B and C 135 days after the transplantation of the proximal end of the fibula to the thigh. The diaphysis of the graft has coalesced with that of the femur, its epiphysis is free. Growth has been better than in experiment 75. The arrow indicates the metal wire label in the diaphysis of the graft.

Fig. 13.

Experiment 73: A immediately, B 119 days after the transplantation of the proximal end of the fibula into the thigh. The diaphyseal end of the graft has fused with the femur, into an acute angle; longitudinal growth has been moderate. The arrow indicates the metal wire label in the diaphysis of the graft.

graft itself at the transplantation and measurement on post mortem radiograms. The period of observation varied between 41 and 218 days.

Twenty-seven grafts fused with the femur. The diaphyseal end of 9 of these fused with the femur at an angle which in 8 cases exceeded 40° . All these grafts had a thick diaphysis with good osseous structure but *only in 3 grafts was the longitudinal growth $1/3$, in the remaining grafts $1/8$ to $1/4$ of the normal* (Fig. 11). The epiphyseal line of one graft had fused at the age of only 34 days, and of four at the age of $5\frac{1}{2}$ to 6 months. Eighteen grafts fused side to side with the femur, 4 of them in such a way that the metaphysis growing out from the epiphyseal cartilage of the graft was gradually incorporated with the femoral diaphysis and labelling of the graft alone made measurement of the growth possible (Fig. 12). *The longitudinal growth was $1/3$ to $1/2$ of the normal*. In three experiments the diaphysis of the graft remained free (Fig. 13). In one of these the growth was $1/3$ of the normal. In the other it could not be measured, since the graft had not been labelled. In the remaining experiments the graft was incorporated in a bony mass so that, insofar as there was any growth, the degree and type of this could not be estimated.

Eight grafts did not fuse with the femur. Two of them were resorbed, five grew insignificantly and the diaphyseal end of one extended like an elephant's trunk beyond the greater trochanter in a proximal direction. In this experiment there was no increase in the distance from the label in the graft to the epiphyseal line but only at the diaphyseal end of the graft (!) This was assumed to depend on ossification in the fascia lata induced by the transplant and was not regarded as longitudinal growth in the proper sense.

DISCUSSION AND CONCLUSIONS

At autotransplantation of the proximal end of the fibula to the site of the radius in 2 to 29 day old rabbits, signs of atrophy of the graft were observed during the first postoperative week, but the graft survived and began to grow in length after 1 to 2 weeks. During 2 to 4 weeks the growth was quicker than normal, then becoming slower and gradually ceasing. At one-stage transplantation, this took place simultaneously or slightly earlier than the cessation of the physiological growth of the animal, but at two-stage transplantation considerably earlier, as a rule. At one-stage transplantation, the longitudinal growth of the graft was, on average, greater than at transplantation

in two stages. The reasons that might account for this are many. The fresh granulation tissue which is formed between the graft and the ulna at one-stage transplantation and possibly a simultaneous bone formation from periosteal remnants, might perhaps assist in renutrition of the graft and favour contact with the ulna. When transplantation is performed in two stages, the richly vascularized granulation tissue already present in the site might conceivably favour renutrition of the transplant, while, in some cases, ossification of the *callus* tissue, which has already set in, malposition, traction by the scar and muscular atrophy after the first operation may have an unfavourable effect. The good results obtained in experiments in which the epiphyseal cartilages of the graft and the ulna happened to lie on the same level, are suggestive of a stimulating effect, emanating from the ulnar epiphyseal cartilage, upon the epiphyseal cartilage of the graft.

At one-stage transplantation of the proximal end of the fibula to the site of radius, growth occurred independently of whether the graft had fused with the ulna or not and in the most favourable cases was as much as 2/3 of the normal. At transplantation in two stages growth practically never occurred unless the graft had fused with the ulna when it was, as a rule, only about 1/2 or, exceptionally, a bare 2/3 of the normal.

At autotransplantation of the proximal end of the fibula to the thigh in 6 to 29 day old rabbits, the graft grew only if it fused with the femur and its longitudinal growth was only 1/3 to 1/2 of the normal. This seems to suggest that the surprisingly favourable results of transplantation to the site of the radius were (partly?) due to the fact that the graft in these animals occupied the site of a normally occurring bone and therefore had a physiological task.

Within the different series *no definite correlation between the age of the experimental animals and the growth of the graft* could be established and it is therefore not warrantable to attribute the good results of one-stage transplantation to the site of radius to the lower average age of the animals in this series of experiments.

These experiments, some of which were carried out on younger animals and with a *graft less ossified than the grafts employed in earlier experiments on epiphyseal transplantation*, thus verify previous experience that transplantation into soft tissues is of no practical value. On the other hand, they show that firm primary fixation of the graft in contact with cancellous bone or vascular bone marrow is not absolutely essential for the preservation of the capacity for longitudinal growth.

The experiments show that *at autotransplantation in young animals, the epiphyseal cartilage can retain a considerable capacity for longitudinal growth (1) even if the graft, besides the epiphyseal plate, comprises a comparatively large amount of metaphyseal and diaphyseal bone (2) even when the contact between the graft and the host bone occurs secondarily as a fusion between the diaphysis of the graft and cortical bone at the site of implantation, and (3) apparently independently of whether the epiphyseal nucleus of the graft, at the time of transplantation, has become radiographically visible, or not.*

SUMMARY

The capacity for growth of an epiphyseal graft (the proximal part of the fibula) which at autotransplantation has not been primarily fixed to a bone, and its dependence on the degree of incipient ossification in the epiphyseal nucleus of the graft, was experimentally studied in 60 rabbits aged 2 to 29 days.

At transplantation into the site of the radius, which was dissected extraperiosteally and removed, half the grafts did not fuse with the ulna (Figs. 4 and 5). Yet their longitudinal growth during the period of observation (49 to 238 days) was almost as great as the growth of those grafts which secondarily fused with the ulna (Figs. 1 to 3), or about 1/2 to 2/3 of the normal growth of the fibula at a corresponding age (see also Fig. 10).

At transplantation to the site of the radius removed 7 to 27 days earlier, the grafts, which during a period of observation of 41 to 229 days had secondarily fused with the ulna, increased in length by about 1/5 to 1/2 (in exceptional cases 2/3) of the normal growth of the fibula (Figs. 6—9 and Fig. 10), while those that had not fused with the ulna grew only insignificantly.

At autotransplantation to the thigh, practically no growth could be observed in the grafts which had not secondarily fused with the femur, while the longitudinal growth of those with bony contact with the femur was 1/3 to 1/2 of the normal (Figs. 11—13).

In no experimental series could the degree of the retained capacity of longitudinal growth be proved to depend on whether, at the time of transplantation, ossification of the fibular epiphyseal nucleus had set in or not.

The investigation shows that under the experimental conditions here described an epiphyseal graft may retain as much as half its ca-

capacity for longitudinal growth without primary, but provided there is secondary, contact with bone at the site of implantation, and despite the fact that, besides the epiphyseal plate, the graft also comprises a piece of metaphyseal and diaphyseal bone some millimetres in length.

RESUME

La capacité de croissance d'une greffe épiphysaire (extrémité proximale du peronée qui à l'autotransplantation n'a pas été fixée primairement à l'os, et sa dépendance du degré d'ossification débutante dans le noyau épiphysaire de la greffe a été étudiée expérimentalement chez 60 lapins âgés de 2 à 29 jours.

Parmi les transplantations à la place du radius, disséqué extrapériostalement et enlevé, la moitié des greffes ne se sont pas soudées avec le coude (fig. 4 et 5). Leur croissance longitudinale pendant la période d'observation (49 à 238 jours) était presque aussi grande que la croissance des greffes qui se soudèrent secondairement avec le coude (fig. 1 à 3) soit à peu près $1/2$ à $2/3$ de la croissance normale du fémur à un âge correspondant (v. également fig. 10).

Dans la transplantation à la place du radius enlevé 7 à 27 jours plus tôt, les greffes qui au cours d'une période d'observation de 41 à 229 jours se soudèrent secondairement avec le coude, se sont accrues en longueur de $1/5$ à $1/2$ (dans des cas exceptionnels de $2/3$) de la longueur normale du fémur (fig. 6-9 et fig. 10), tandis que chez celles qui ne se soudèrent pas avec le coude, la croissance a été insignifiante.

Dans l'autotransplantation à la cuisse, on n'a pratiquement pas observé de croissance dans les greffes qui ne se soudèrent pas secondairement avec le fémur, tandis que la croissance longitudinale de celles ayant un contact avec le fémur était $1/3$ ou $1/2$ de la normale (fig. 11-13).

Dans aucune série expérimentale, il n'a été trouvé que le degré du défaut de capacité de croissance longitudinale était dépendant du fait qu'au moment de la transplantation, l'ossification du noyau du peronée avait commencé ou non.

L'enquête montre que dans les conditions expérimentales décrites ici, une greffe épiphysaire peut conserver même la moitié de sa capacité de croissance longitudinale sans contact primaire, mais à condition d'un contact secondaire avec l'os à l'endroit de l'implantation et malgré qu'à côté des plaques épiphysaires, la greffe contenait également un morceau d'os métaphysaire et diaphysaire de quelques millimètres de longueur.

ZUSAMMENFASSUNG

Die Wachstumsfähigkeit eines epiphysären Transplantat (des proximalen Teiles der Fibula), die bei der Autotransplantation primär nicht an einem Knochen fixiert wurde, und ihre Abhängigkeit von dem Grade der beginnenden Verknöcherung im Epiphysenkerne des Transplantates, wurde experimentell an 60 Kaninchen im Alter von 2 bis 29 Tagen studiert.

Bei der Transplantation an die Stelle des Radius, der extraperiostal präpariert und entfernt wurde, vereinten sich die Hälfte der Transplantate nicht mit der Ulna (Fig. 4 und 5). Ihr Längenwachstum während der Beobachtungszeit (49 bis 238 Tage) war jedoch fast ebenso gross als das Wachstum der Transplantate, die sich sekundär mit der Ulna vereinten (Fig. 1 bis 3), oder ungefähr $\frac{1}{2}$ bis $\frac{2}{3}$ des normalen Wachstums der Fibula in einem entsprechenden Alter (siehe auch Fig. 10).

Bei der Transplantation an die Stelle des Radius, der 7 bis 27 Tage vorher entfernt worden war, nahmen die Transplantate, die während einer Beobachtungszeit von 41 bis 229 Tagen sekundär mit der Ulna verwachsen waren, um ungefähr $\frac{1}{5}$ bis $\frac{1}{2}$ (in Ausnahmefällen um $\frac{2}{3}$) des normalen Wachstums der Fibula (Fig. 6–9 und Fig. 10) an Länge zu, während jene, die sich nicht mit der Ulna vereinigt hatten, nur unbedeutend wuchsen.

Bei der Autotransplantation zum Oberschenkel, konnte an Transplantaten, die sich nicht sekundär mit dem Femur vereinigt hatten, fast keinerlei Wachstum beobachtet werden während das Längenwachstum von jenen mit knöchernem Kontakt mit dem Femur $\frac{1}{3}$ bis $\frac{1}{2}$ des Normalen war (Fig. 11 bis 13).

In keiner der experimentellen Reihen konnte bewiesen werden, dass der Grad der beibehaltenen Längenwachstumsfähigkeit davon abhängig war ob die Verknöcherung des fibularen Epiphysenkernes bereits eingenommen hatte oder nicht.

Die Untersuchung zeigt, dass ein Epiphysentransplantat unter den hier beschriebenen experimentellen Bedingungen die Hälfte seiner Längenwachstumsfähigkeit ohne primären Kontakt beibehalten kann, vorausgesetzt, dass es zum sekundären Kontakt mit dem Knochen an der Einpflanzungsstelle kommt. Dies geschieht auch selbst wenn das Transplantat, abgesehen von der Epiphysenplatte, ein Stück der Metaphyse und Diaphyse von einiger Millimeter Länge umfasst.

The references will be published in connection with a following paper: Experimental Epiphyseal Transplantation, Part II, Histological observations.