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EXPERIMENTAL EPIPHYSEAL TRANSPLANTATION

Part III. *The Influence of Age*

By

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INTRODUCTION AND PROBLEM

In two previous papers I have described radiological observations on the survival and growth of the autotransplanted proximal end of the fibula of the rabbit (*Heikel* 1960 (2)) and histological observations on the survival, regeneration and growth of the transplants (*Heikel* 1960 (3)). I concluded that the peripheral parts of the transplanted epiphyseal cartilage survive and that from these parts a new epiphyseal cartilage regenerates, but that the increased cell proliferation necessary for regeneration obviously exhausts the resources of the reserve zone, with the result that there is early cessation of growth. Besides it appeared that there was no difference between transplantations performed at different ages between 10 and 29 days.

In clinical practice it is not always possible to carry out an epiphyseal transplantation at the corresponding age, *i.e.* 1 to 2 years (*Heikel* 1960 (1)). It is therefore important to know to what extent the capacity for longitudinal growth is retained beyond this age, and whether there is an age-limit after which the transplant loses this capacity either wholly or partly.

The object of the present investigation has been to study whether the age at which a growing bone with epiphyseal cartilage is transplanted influences its capacity for longitudinal growth, and, if there is such an influence, to study the way in which age plays a role, and to determine the optimal age for the performance of epiphyseal transplantation.

METHOD AND MATERIAL

The experiments were performed on 163 rabbits aged 10 to 70 days. The results were followed either histologically or radiographically and in a few cases in both ways (Table 1). The afore-mentioned investigations seemed to show that the site of the transplant was immaterial, but that the important premise for its survival and growth was that it should fuse with the host bone. They further showed that in transplantation to the thigh, the transplant often fused to the femur in a rather transverse position, which made radiographic observations difficult. For this reason, in the present work the proximal part of the right fibula was transplanted to the left leg, where it was placed in front of the left fibula. In other respects, the technique of transplantation, of obtaining radiographs and of measuring growth were the same as in the earlier investigations. As no internal fixation was used, the radiological observations in a number of experiments showed that the transplant did not fuse with the host bone. In a few weeks these grafts became atrophic and their epiphyseal line closed. Six of these transplants were found to have grown longitudinally before the closure of the epiphyseal line (from 1 to 3, and in a single experiment 7 mm) but in the majority of these there was no distinguishable growth. None of the experiments in which there was no radiological evidence of bone contact was included in the present series. Furthermore, eight of the radiologically observed animals died or were killed at an early date, and were therefore excluded.

TABLE 1

The Number of Animals of Different Ages and the Kind of Observation.

Age of animal at transplantation of the fibula	Number of animals			
	Histologically observed	Radiologically observed	Radiologically and histologically observed	Total
1½–2 weeks	12	11	–	23
3 weeks	–	9	1	10
4 weeks	22	12	1	35
5 weeks	–	5	–	5
6 weeks	2	20	–	22
7 weeks	21	–	4	25
8 weeks	–	9	3	12
9 weeks	–	9	–	9
10 weeks	14	8	–	22
Total	71	83	9	163

HISTOLOGICAL OBSERVATIONS

80 rabbits were used for this part of the investigation. They were killed 2, 4, 6, 8, 10, 12, 14, 16 and 18 (and in some few experiments 21) days after the transplantation (Table 1). The transplant together with

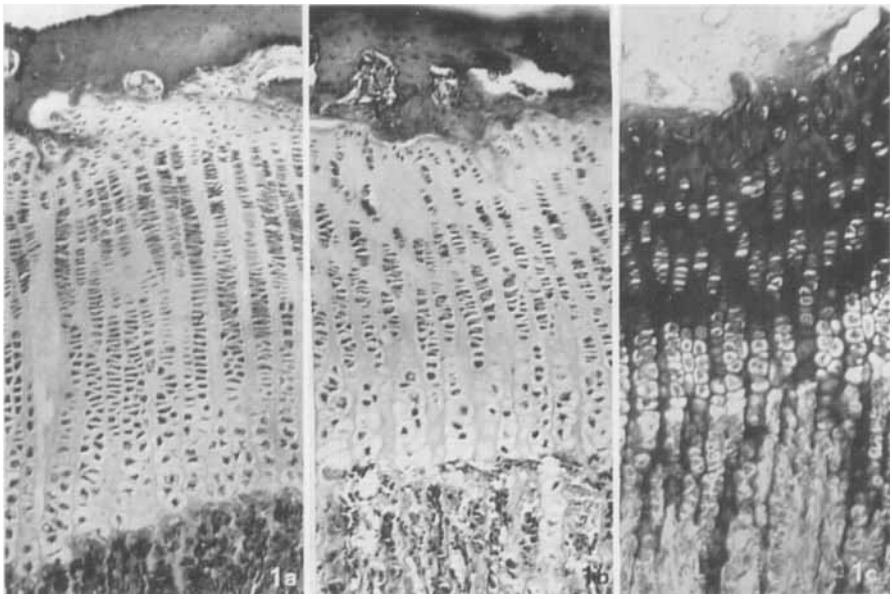


Fig. 1.
Normal epiphyseal cartilage of the fibula of the rabbit. a. at 6 weeks, b. at 10 weeks, c. at 12 weeks. (a and b stained with haematoxylin-eosin, c with toluidine blue).

a part of the host bone was fixed in formalin, decalcified, sectioned and stained either with haematoxylin, toluidine blue or PAS-dye.

For the sake of comparison the epiphyseal cartilage of the left fibula was simultaneously studied in a number of cases. The following normal data should be mentioned; the epiphyseal nucleus of the rabbit fibula becomes visible at the age of 10 to 17 days (*Heikel* 1960 (1)). An epiphyseal plate is demonstrable from the age of about 4 weeks. Initially, however, it is thin, highly perforated and irregular. From the age of about 6 weeks it is 0.2 mm thick, and in parts compact, while in its metaphyseal surface there are cavities occupied by capillaries. The height of the epiphyseal cartilage at the age of 4 to 7 weeks is 0.6 mm and becomes gradually reduced so that at the age of 10 weeks it is 0.5 mm. At the age of 150 to 225 days the epiphyseal line closes (*Heikel* 1960 (1)).

In my previous paper the term "reserve zone" was employed, *Trueta* called the same cell layer "the germinative zone" and since this denomination agrees better with my idea of the function of this zone (see below) I have adopted it in the present study. Here, as in my previous paper, the border between the epiphyseal cartilage and the metaphyseal bone has been called the "metaphyseal border".

After two and four days only degenerative changes were observed

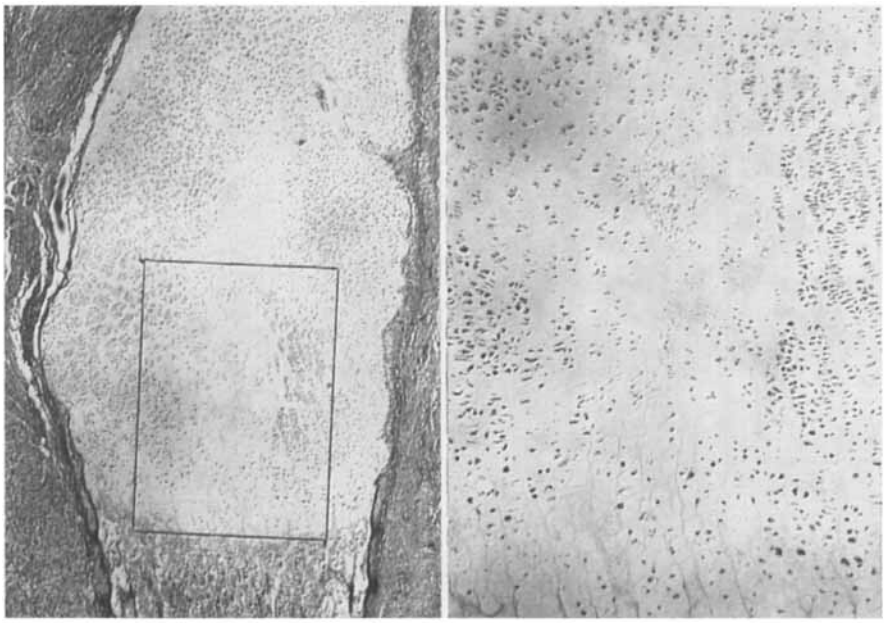


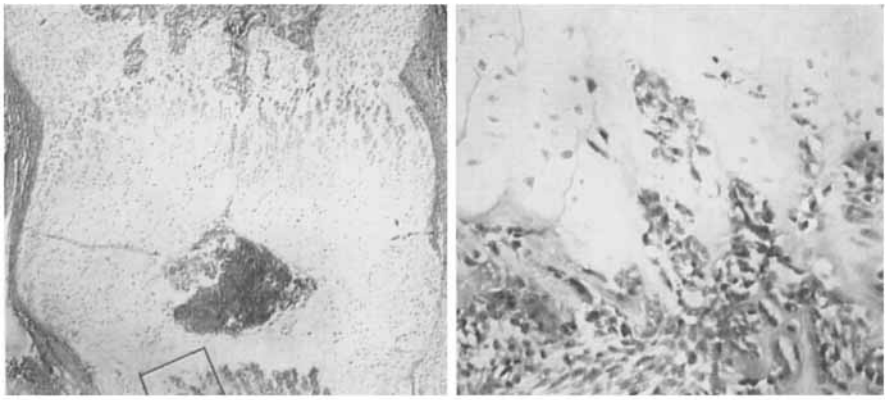
Fig. 2.
Experiment 365. Age group 1½ weeks, observation time 6 days. a. The necrotic area is clearly seen between the well-stained, ca. 0.6 mm wide peripheral parts of the cartilage. b. Magnification of the central part of a. The capillary with shrunken erythrocytes in the necrotic cartilage is seen.

in all preparations and these changes were similar in the different age groups. From the sixth day onwards regenerative phenomena were seen. These varied from one case to another even within the same age group. With regard to the course and degree of regeneration, however, certain differences between the age groups could be traced. In the following, observations on the transplantations carried out at the ages of 1½ to 2, 4, 7 and 10 weeks are recorded.

Transplantation at the Age of 1½ to 2 Weeks.

(At the time of the transplantation no ossification nuclei had yet appeared in the epiphysis).

After six days the border between the central necrotic cartilage and a peripheral zone about 0.6 mm wide, which appeared vital, was comparatively distinct except in the hypertrophic zone, in which colourless shrunken nuclei were to be seen not only in the centre but also near



a b

Fig. 3.

Experiment 361. Age group 1½ weeks, observation time 10 days. a. Note the epiphyseal nucleus, the height of the hypertrophic zone of the almost completely regenerated epiphyseal cartilage and the rupture in the necrotic cartilage. b. Magnification of a. Irregular ingrowth of fibrocytes into the necrotic cartilage is seen.

the surface of the transplant (Fig. 2a). In the necrotic portion of the epiphyseal cartilage, probably at the site where the ossification nucleus would have appeared, capillaries with shrunken erythrocytes (Fig. 2b) and, at the periphery of the cartilage, capillaries penetrating from the surroundings and with living erythrocytes were visible. In the metaphysis there was detritus close to the metaphyseal border. From the surroundings fibroblasts and capillaries had grown into the metaphysis in its external parts, reaching as far as the metaphyseal border, but calcification of the cartilage matrix had not taken place.

After eight days an indentation was observed in the epiphyseal cartilage, which was probably the beginning of the perichondral ring of the ossification groove. On either side of the indentation there were areas with living cartilage cells which seemed to advance towards the centre of the transplant. The necrotic cartilage had (because of this?) been compressed to a low triangular area close to the epiphyseal border and did not seem to reach the surface of the transplant. Within the cartilaginous areas mentioned there were short irregular columns of cartilage cells here and there. In the metaphysis capillaries and streaks of fibroblasts had grown as far as the metaphyseal border but calcification of the cartilage matrix had not taken place.

After ten days there was an irregularly shaped ossification nucleus in the epiphysis but no epiphyseal plate. The height of the epiphyseal

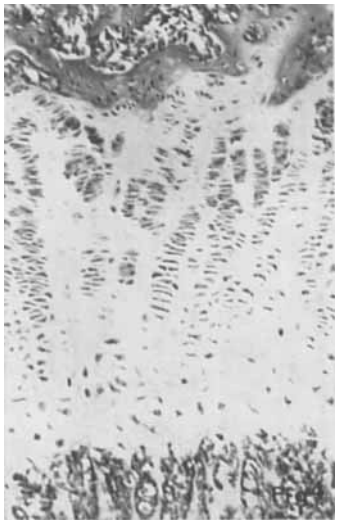


Fig. 4.
Experiment 360. Age group 1½ weeks, observation time 12 days. Beneath the thin epiphyseal plate there is only a thin germinative zone.

cartilage could not therefore be determined, but the distance from the nucleus to the metaphyseal border was about 2 mm (Fig. 3a). Nearest to this there was an area of necrotic cartilage which reached to the periphery of the transplant. From the metaphysis irregular connective tissue spurs had grown into the necrotic cartilage but actual calcification of the matrix had not occurred (Fig. 3b). On the epiphyseal side of this area there was a central cavity filled with erythrocytes, evidently haemorrhage from a capillary which had grown down from the epiphyseal nucleus. On either side of the capillary a new epiphyseal cartilage had regenerated, however, and contact between the nucleus and the site of the haemorrhage seemed to have been cut. The columns of new epiphyseal cartilage were sparse and irregular and their hypertrophic zone very high.

After twelve days the epiphyseal nucleus showed a suggestion of an epiphyseal plate. The entire growth cartilage had regenerated but beneath the epiphyseal plate the germinative zone was very low (Fig. 4) and the columns of cartilage cells were irregular and, in places, sparse. Along the entire metaphyseal border calcification of the intercellular substance of the cartilage had taken place. The primary bone trabeculae in the metaphysis were lined with osteoblast-like cells. About 2 mm from the metaphyseal border there vas a transverse streak of uncalcified remnants of necrotic cartilage which showed the extent of the longitudinal growth. The transplant had fused with the host bone.

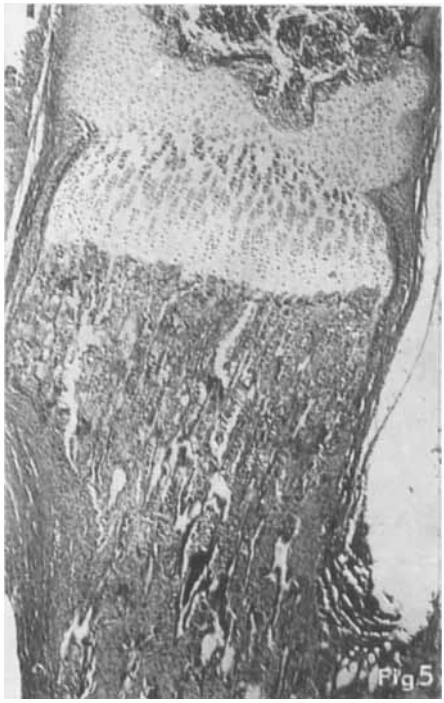


Fig. 5.
Experiment 358. Age group 1½ weeks, observation time 16 days. The regenerated epiphyseal cartilage looks normal, but the central part of the germinative zone is thin. Note the fusion with the host bone (bottom, right).

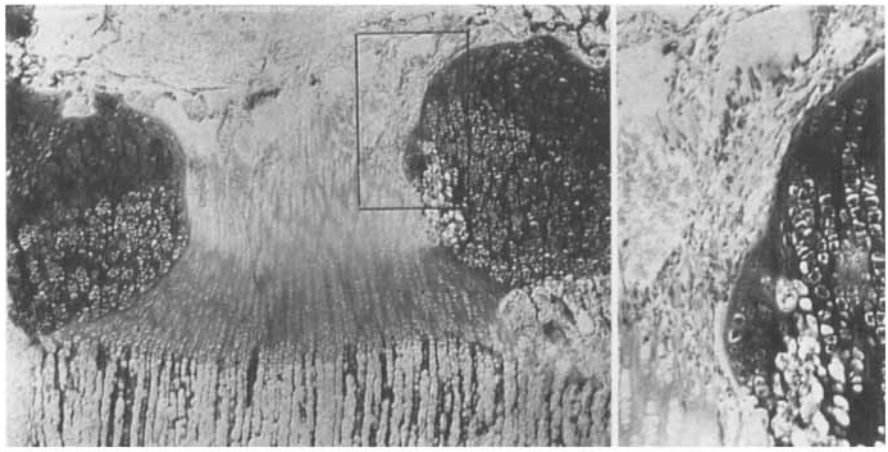
After fourteen and sixteen days the epiphyseal cartilage looked almost normal, except that the central part of the germinative zone was very thin. Between the nucleus and "the ossification groove" there were large areas of undifferentiated cartilage, rich in cells (Fig. 5). At the metaphyseal border ossification seemed almost normal and the graft had fused.

Transplantation at the Age of 4 Weeks.

(At the time of the transplantation the transplants had an ossification nucleus in the epiphysis but the nucleus had no epiphyseal plate. Thus there was a considerable amount of undifferentiated cartilage beside the nucleus).

After six days the height of the epiphyseal cartilage was 1 to 1.1 mm in the centre. Only a border zone of the cartilage 0.3 to 0.7 mm wide appeared vital. Streaks of fibroblasts and capillaries growing into the metaphysis reached the metaphyseal border in the periphery but calcification of the matrix had not occurred.

After eight days epiphyseal cartilage had generated in the peripheral



a b

Fig. 6.

Experiment 276. Age group 4 weeks, observation time 8 days. (Stained with toluidine-blue). a. The mushroom-shaped necrotic cartilage in the centre had a rupture beneath the epiphyseal plate. On both sides there is regenerating epiphyseal cartilage compressing the necrotic part. To the right there is enchondral ossification. Opposite the necrotic cartilage the metaphysis contains no living cells. b. Rupture with erythrocytes and streaks of fibrocytes and capillaries growing from the nucleus into the necrotic cartilage.

areas, and its columns contained a very large number of hypertrophic cells. At the metaphyseal border calcification of the cartilage matrix had set in. In the centre, the necrotic area, evidently as a result of expansion of the regenerating cartilage, had taken on a mushroomlike shape with still longer columns. At the metaphyseal border of the "mushroom" no calcification was visible. Thus the necrotic cartilage was subject to a gradual overgrowth by the peripheral parts of the metaphysis (Fig. 6a). Within the necrotic cartilage, at the border between the germinative and proliferative zones, there was a rupture with haemorrhage from a capillary which had grown down from the epiphyseal nucleus (Fig. 6b).

After ten and twelve days a distinct epiphyseal plate was still missing. From the nucleus capillaries had grown down into the necrotic cartilage. In the border areas of the epiphyseal cartilage regeneration occurred which seemed to have expanded towards the centre, compressing the necrotic area. In the metaphysis capillaries and streaks of fibroblasts had reached the whole metaphyseal border and in the peripheral areas

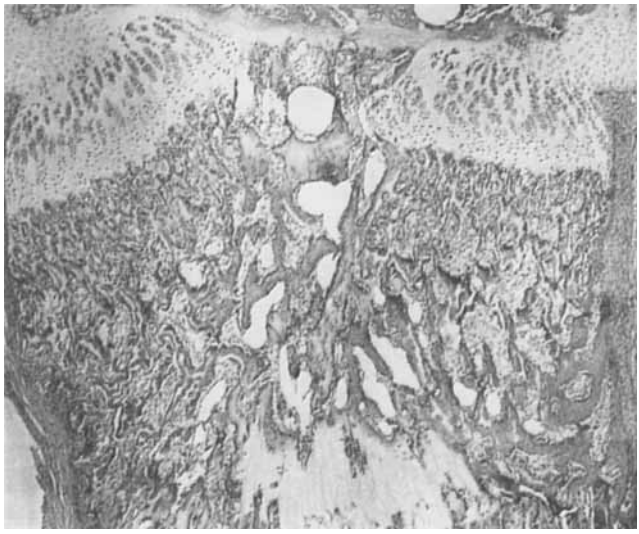


Fig. 7.
Experiement 292. Age group 4 weeks, observation time 14 days. The regenerated peripheral parts of the epiphyseal cartilage seem to diverge from the areas of undifferentiated cartilage at the "corners" of the nucleus. Beneath the central part of the epiphyseal plate there is only necrotic germinative zone. A bony bridge from the nucleus to the metaphysis and in the metaphysis remnants of the necrotic cartilage are seen (bottom).

calcification of the matrix of the regenerated epiphyseal cartilage had taken place.

After fourteen and sixteen days the nucleus had developed an epiphyseal plate which reached close to the surface of the transplant. Beneath the centre of the plate, remnants of the germinative zone of the necrotic cartilage were visible. In the periphery there were large areas of regenerated epiphyseal cartilage which, in the section, resembled two "berets" because the cartilaginous columns diverged from points beneath the "corner" of the epiphyseal nucleus where there were still comparatively large areas of undifferentiated cartilage. At the metaphyseal border of the regenerated epiphyseal cartilage ossification and longitudinal growth had taken place. In the centre, the columnar zone of the necrotic cartilage had been torn loose from the germinative zone and overgrown by the peripheral growth so that the remnants of the necrotic cartilage were lying below in the metaphysis about 2 mm from the metaphyseal border. A partly ossified connective tissue bridge had developed here between the nucleus and metaphysis (Fig. 7). In

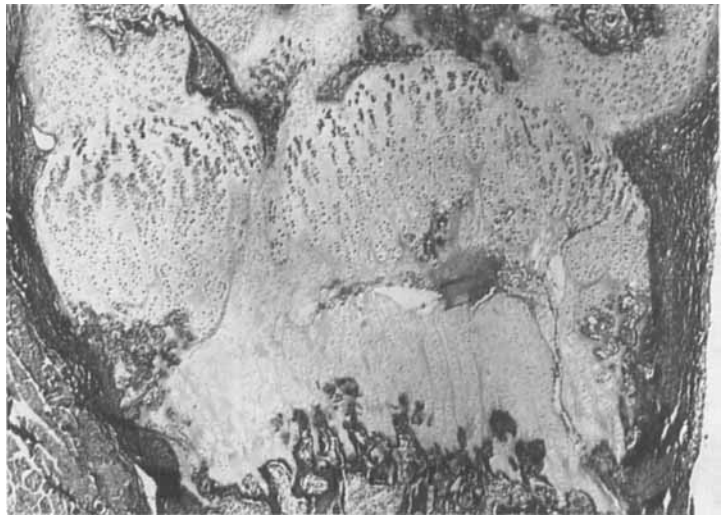


Fig. 8.
Experiment 342. Age-group 4 weeks, observation time 16 days. The necrotic cartilage is not definitely peeled off, but from its metaphyseal side it is broken up by ingrowing connective tissue and capillaries. Beneath the "coners" of the nucleus there is undifferentiated cartilage, and in the central parts there are round, expanding cartilage islets. The regeneration of the epiphyseal cartilage is nearly complete.

other experiments the epiphyseal plate had not yet developed and between the nucleus and the ossification groove, as also centrally in the epiphyseal cartilage, expanding islets of undifferentiated cartilage could be observed (Fig. 8). The necrotic area had not been definitely peeled off and ossification of the regenerated epiphyseal cartilage had occurred within narrow border areas only.

After eighteen to twenty-one days the epiphyseal cartilage had regenerated, with the exception of the germinative zone, which was almost entirely missing in the centre. Here, instead, there was often a bony bridge between the nucleus and the metaphysis. In the metaphysis, at a distance of about 3 mm from the metaphyseal border, remnants of necrotic cartilage were seen.

Transplantation at the Age of 7 Weeks.

(At the time of the transplantation the epiphyseal nucleus had an epiphyseal plate reaching close to the surface of the transplant).

After six days the epiphyseal cartilage was 0.6 mm high. Only within a small peripheral area on the metaphyseal side of the ossification

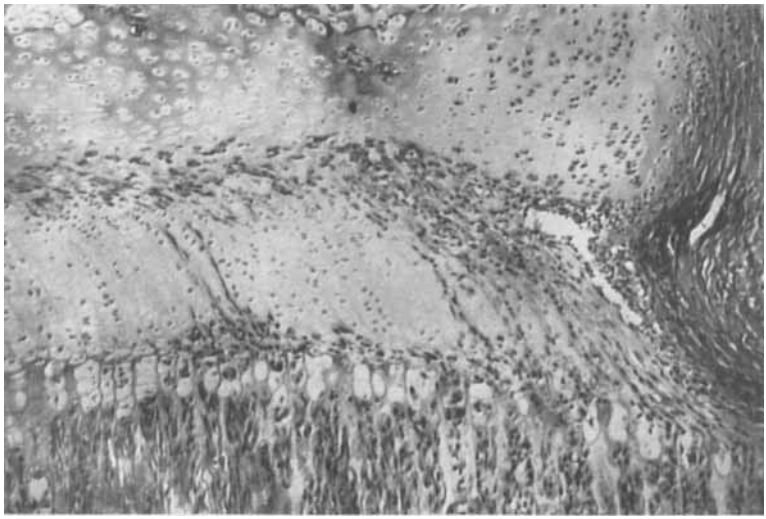


Fig. 9.

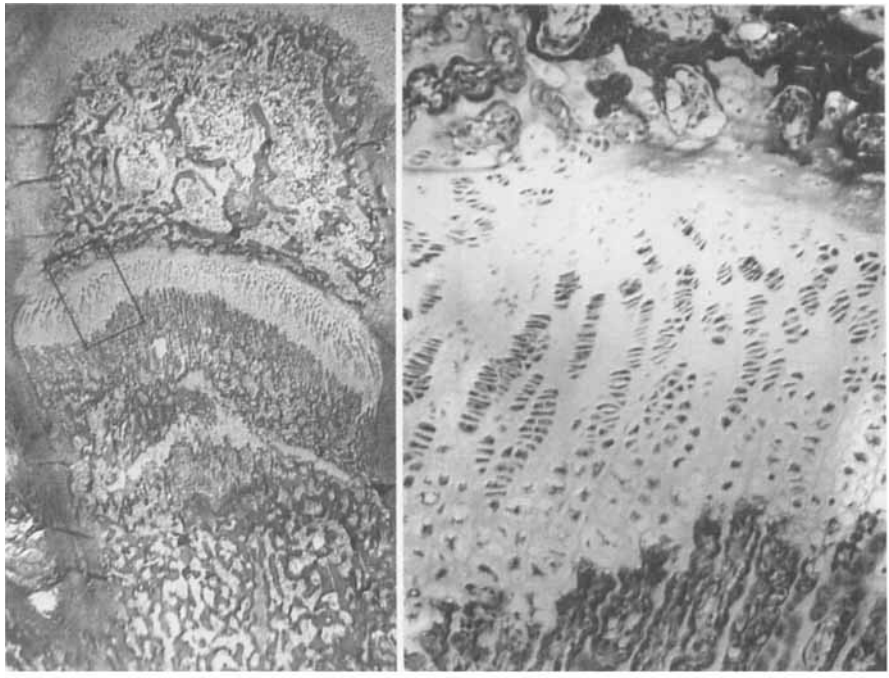
Experiment 349. Age-group 7 weeks, observation time 10 days. Streaks of fibrocytes grow from the surroundings in between the necrotic cartilage (bottom, left) and the regenerated cartilage (top right) and from the metaphysis between the columns of the necrotic cartilage.

groove did the cartilage look vital and there was a suggestion of column formation.

After eight days the regenerated areas were somewhat larger and peripherally the cartilage matrix has ossified to a depth of about 0.2 mm. In other places connective tissue streaks had grown from the surroundings of the transplant in between the regenerated and the necrotic cartilage, which here reached the surface of the transplant. In the epiphyseal nucleus capillaries were seen, in the metaphysis mainly streaks of fibroblasts but also a few capillaries which had grown forward to the metaphyseal border.

After ten days the epiphyseal cartilage was 0.5 to 0.7 mm high. It looked entirely devitalized and there were patches of detritus. In the metaphysis fibroblasts and capillaries had grown to the metaphyseal border except in the centre, where there was a small area with detritus. From the surroundings, connective tissue had grown in between the regenerating and the necrotic cartilage (Fig. 9).

After twelve and fourteen days the epiphyseal cartilage was necrotic with the exception of small regenerating border areas, where ossification had occurred to a depth of 0.2 to 0.4 mm over a maximum width



a b

Fig. 10.

Experiments 356. Age-group 7 weeks, observation time 16 days. a. The epiphyseal cartilage is regenerated and looks fairly normal (cf. Fig. 1 b), remnants of the necrotic cartilage are seen in the metaphysis and there is contact with the host bone (bottom). b. Most of the germinative zone is necrotic.

of 0.7 mm, some overgrowth over the necrotic cartilage having taken place.

After 16 and 28 days the peripheral growth in length had in some experiments resulted in a mushroom shape of the necrotic cartilage and partial overgrowth by the metaphysis. From the latter irregular spurs of connective tissue and capillaries from the sides and from the metaphyseal border had grown into the necrotic cartilage. In other experiments the epiphyseal cartilage had regenerated, except that in the centre of the germinative zone almost all cells looked devitalized (Fig. 10 a and b). The remainder of the necrotic cartilage constituted a transverse streak in the metaphysis about 1½ mm from the metaphyseal border. The transplant had fused to the host bone.

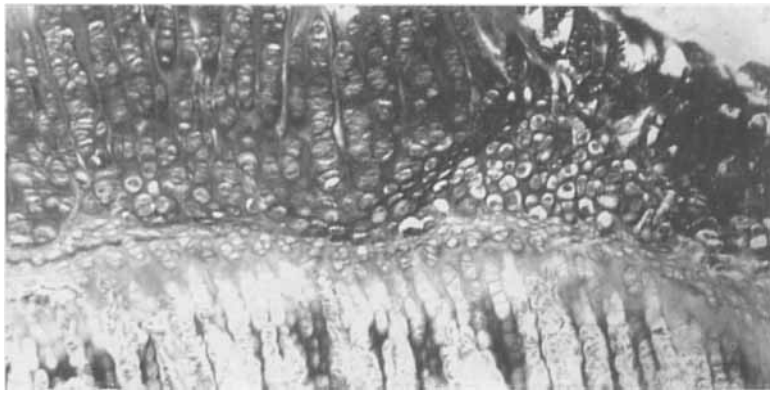


Fig. 11.
Experiment 289. Age-group 10 weeks, observation time 14 days (stained with toluidine-blue, compare with Fig. 1 c). The regenerated cartilage (dark stained, right) is separated from the metaphysis by the (light stained) narrow streak of necrotic cartilage and ossification has not started.

Transplantation at the Age of 10 Weeks.

(At the time of the transplantation the plate of the epiphyseal nucleus reached close to the periphery of the transplant).

After six days the epiphyseal cartilage was 0.5 mm high and looked devitalized except for small triangular border areas. There was an abundance of detritus in the metaphysis and ingrowing capillaries and fibroblasts were no closer to the metaphyseal border than 2 mm.

After eight days the small areas of regenerated cartilage were still separated from the metaphysis by the necrotic cartilage, which reached to the surface of the transplant. In the border areas of the metaphysis, capillaries and streaks of fibroblasts reached as far as the metaphyseal border.

After fourteen days the regenerated cartilage was still separated from the metaphysis (Fig. 11). In the necrotic cartilage there were patches of detritus. From the epiphyseal nucleus capillaries had grown down into the cartilage. From the surroundings, connective tissue had grown in along the metaphyseal surface of the epiphyseal plate. There was an abundance of capillaries in the metaphysis and a lining of osteoblast-like cells on the primary bony trabeculae, but ossification of narrow border areas of the epiphyseal line was visible in a few preparations only.

After twenty-one days the epiphyseal line had fused almost com-

pletely in some preparations, while in others an epiphyseal cartilage 0.05 to 0.15 mm high and without a germinative zone was visible. The metaphysis had fused with the host bone. 2 mm from the metaphyseal border a transverse band of necrotic cartilage was seen.

RADIOLOGICAL OBSERVATIONS

The number of experiments in which bone contact was detected was 72. 63 animals were observed to an age of 100–170 days, and 23 of these to over 170 days, an age at which the growth of the fibula of the rabbit is, as a rule, almost complete (*Heikel* 1960 (1)). In about 3/4 of the experiments the position of the transplant allowed radiographic measurement of the growth (Table 2). It should be noted that in the three youngest groups the percentage of cases in which bone fusion occurred was almost 100, but in the older groups only 50–75. This may have depended on a greater ability of the younger transplants to survive, but may have resulted from the greater technical difficulties of transplantation in the older animals, too.

TABLE 2

The Number of Experiments in which the Transplant Showed Radiological Evidence of Contact with Host Bone.

Age of animal at transplantation of the fibula	Number of animals		
	Total	Observed to an age of at least 100 days	Measurement of growth possible
1½–2 weeks	11	8	8
3 weeks	9	8	8
4 weeks	12	8	8
5 weeks	3	2	1
6 weeks	17	17	15
7 weeks	3	3	2
8 weeks	6	6	3
9 weeks	6	6	2
10 weeks	5	5	0

The mean growth of the transplants, and the extreme variations, are shown in Table 3. After transplantation at an age of 1½–2 weeks the fibula retained in mean 50 and in the most successful experiment as much as 80 per cent of its capacity for longitudinal growth. In the 3-week group the corresponding figures had fallen to 36 and 65, in the

6-week group to 21 and 41, and in the 8-week group to 15 and 27 per cent, respectively.

TABLE 3

The Longitudinal Growth of the Transplanted Fibula in Relation to the Left (Control) Fibula.

Age of animal at transplantation of the fibula	Mean age of the animals at the end of the observation time	Longitudinal growth of the transplanted fibula relative to the growth of the left fibula (%)	
		Mean	Variations
1½–2 weeks	143 days	50	5 – 81
3 weeks	164 days	36	0 – 65
4 weeks	136 days	18	0 – 48
6 weeks	139 days	24	0 – 41
8 weeks	174 days	15	0 – 27
9 weeks	179 days	11	0 – 13

DISCUSSION

A comparison of the four age groups in the histological series shows that re-nutrition of the transplant required two days less in the two youngest groups. This is probably due only to the fact that in these the volume of the transplant was less and the capillaries had to grow a shorter distance, but anyhow it resulted in a promotion of the regeneration of the surviving epiphyseal cartilage. In all experiments the latter comprised a border area of more or less equal width (ca. 0.6 mm) which indicates that survival was made possible through nutrition by diffusion from the surroundings. In experimental lesions of epiphyseal vessels *Trueta* noticed that a border area of the epiphyseal cartilage was supplied by vascular anastomoses around the plate but he did not mention its width. It is possible that it is this area that survives, although at transplantation the anastomoses are cut.

Regeneration of the epiphyseal cartilage was quickest and most complete in the youngest age groups. This might again be due to the comparatively small volume of the transplants in these groups. One cannot, however, disregard the fact that as long as there is no epiphyseal nucleus, or the nucleus is small and its epiphyseal plate does not reach close to the surface of the transplant, the epiphyses contains a large amount of undifferentiated cartilage which might be assumed to be mobilized to replace the necrotic germinative zone. As soon as the epiphyseal plate has developed fully (at the age of about 6 weeks), access to undifferentiated cartilage is highly limited.

Trueta mentions that the origin of the germinative zone is still unexplained. It has, for example, been assumed that cell production takes place at the ossification groove. In none of my preparations has the histological picture suggested this to be the site at which the cartilage cells are produced; on the contrary, there has been some evidence of cell production within the undifferentiated cartilage between the epiphyseal nucleus and the ossification groove (Figs. 6, 7 and 8).

The regenerated epiphyseal cartilage extends towards the centre of the transplant partly through a fan-like divergence of the columns towards the centre of the transplant and possibly through the formation of new columns medially of the first columns. Thus the columnar zone of the necrotic cartilage is compressed from the sides and in the direction of the metaphysis (*Heikel* 1960 (3) and *Trueta* 1960 (6)). Since the columns grow obliquely towards the centre, the germinative zone lags behind and, in the centre of the transplant, is low or entirely missing. Through pressure from the sides concentrated towards the columnar zone of the necrotic cartilage, the columnar zone is torn loose from the germinative zone which remains beneath the centre of the epiphyseal plate. This was most pronounced in the oldest age groups and is the reason why growth soon ceases for want of new formation of cartilage cells. *Trueta* has also described a rupture of the "ischaemic cartilage", usually at the metaphyseal end, in experimental interruption of the nutrition of the epiphysis but he did not mention whether the rupture always took place in the same layer.

The difference in the speed at which nutrition was reestablished on the metaphyseal side does not seem to have been the conclusive factor accounting for the difference between the groups. Even though the calcification of the matrix of the epiphyseal cartilage cannot take place before the capillaries have reached the metaphyseal border, it is evident that only cartilage cells which have undergone the normal degenerative process can be subject to this ossification process. In the older groups the necrotic cartilage had long acted as a barrier between the regenerating cartilage areas and the metaphysis. According to *Trueta,* such a barrier, if persisting for a minimum of 10 days, may become permanent. This was probably the case in experiment no. 289 (Fig. 11). If, however, the barrier is broken by contact between the surviving cartilage and the metaphyseal capillaries either from the beginning or through the growth of the former, ossification and growth in length here occur in the usual way. During the time that passes before ossification sets in, the columns of the epiphyseal plate have grown by about 50 per cent of their normal

length through an increase in the number of hypertrophic cells. This, a fact also observed by *Trueta,* is the reason why the height of the epiphyseal cartilage increases during the first few days after transplantation. When contact has been established with the epiphyseal vessels, these cells evidently rapidly undergo their normal degenerative process and thereafter ossification proceeds rapidly until the regenerated areas of the epiphyseal cartilage have reached a normal height. This explains the short period of rapid growth which the growth curves of the transplants exhibit (*Heikel* 1960 (1), Fig. 10). The necrotic cartilage is thus overgrown and its peeling off is facilitated. If the latter does not occur rapidly enough and if the expansion of the regenerating cartilage towards the centre of the transplant is delayed, the capillaries grow down from the epiphyseal nucleus, followed by connective tissue. This may also happen from the metaphysis, along the border between the regenerating and necrotic cartilage. When contact between the epiphyseal nucleus and the metaphysis is established, a bony bridge is formed through ossification and growth ceases.

The number of experiments observed radiologically is comparatively small and the results vary greatly. From Table 3 one gains the impression, however, that the changes of a transplant preserving 50 per cent or more of its capacity for growth in length—under given experimental conditions—are good if transplantation is carried out at the age of 1½ to 2 weeks, small if transplantation takes place at the age of 3 and 4 weeks and minimal at the age of 7 and 10 weeks.

CONCLUSIONS

The histological observations, which are in good agreement with *Trueta*'s observations on selective interruption of the nutrition of the epiphyseal cartilage partly on the epiphyseal and partly on the metaphyseal side, seem to explain why the transplant stands a good chance of preserving a significant amount of its capacity for growth in length only if transplantation is carried out on a young animal. They cannot explain, however, why the result seems to depend on fusion of the transplant with the host bone. Seemingly, all regenerative processes which determine the degree of retained capacity for longitudinal growth have occurred before or simultaneously with the occurrence of bony fusion. Here biological factors which cannot be identified on the basis of the present experiments may possibly play a role.

What are the practical consequences of the present observations?

If transplantation is carried out at an early age, the transplant would under normal conditions have a long growth period and a comparatively small percentual growth inhibition would result in considerable limitation of the final length. If, on the other hand, transplantation is not carried out until significant growth in length has already taken place, a growth inhibition which, reckoned as a percentage, is smaller need not result in a great final shortening. This can be calculated theoretically on the basis of the figures in Table 3 and the normal growth curve for the rabbit fibula (*Heikel* 1960 (1)), provided that at the time of the transplantation each transplanted fibula is of normal length and has a normal capacity for longitudinal growth. Table 4 shows that if this theoretical calculation is correct, there should be no significant difference between the final lengths of the transplants in the different age groups.

TABLE 4

The Theoretically Calculated Final Length of the Transplanted Fibula at the End of the Growth Period.

Age of animal at transplantation of the fibula	Normal length of fibula at age of transplantation (mm)	Normal longitudinal growth during the remaining growth period (mm)	Observed mean longitudinal growth percentage	Theoretically calculated	
				Mean longitudinal growth of the transplant (mm)	Final length of the transplant (mm)
1½–2 weeks	13	37	50	18.5	31.5
3 weeks	16	34	36	12.0	28.0
4 weeks	19	31	18	5.5	24.5
6 weeks	23	27	24	6.5	29.5
8 weeks	28	22	15	3.5	31.5
9 weeks	30	20	11	2.0	32.0

Under certain experimental conditions, in the rabbit, the most favourable time for transplantation, if the chief stress is laid upon the growth in length of the transplant, is the age of 1½ to 2 weeks, but if the chief stress is laid upon the final length of the transplant, the age of the experimental animal is of no conclusive significance.

To what extent these observations are applicable to clinical conditions and to transplants of larger volume cannot be determined on the basis of the present investigation.

SUMMARY

The proximal part of the right fibula was transplanted to the left leg of 163 rabbits, aged 1½–2, 4, 7 and 10 weeks, respectively. The survival

and longitudinal growth of the transplant was observed radiographically and histologically.

The renutrition of the transplant required two days less in the two youngest groups which promoted the regeneration of the surviving parts of the epiphyseal cartilage. The regeneration was most complete in the youngest groups. This was supposed partly to be a consequence of the fact that as long as the epiphyseal nucleus is small there is a large amount of undifferentiated cartilage in the epiphysis which might be assumed to be mobilized to replace the non-surviving part of the germinative zone. If this part is not replaced—as was the case in the older groups—the longitudinal growth of the transplant soon ceases for want of new formation of cartilage cells.

There seemed to be good changes of a transplant preserving 50 per cent or more of its capacity for longitudinal growth only if the transplantation was carried out at the age of 10–14 days and minimal chances at the age of 7 and 10 weeks.

RESUME

La partie proximale du cotyle droit a été transplanté à la jambe gauche chez 163 lapins âgés de 1½–2, 4, 7 et 10 semaines respectivement. La survie et la croissance longitudinale de la transplantation on été observées radiographiquement et histologiquement.

La renutrition de la transplantation demande deux jours de moins dans les deux groupes d'âge les plus jeunes chez lesquels s'opère la régénération de la partie survivante du cartilage épiphysaire. La régénération a été la plus complète dans les groupes les plus jeunes. Cela a été supposé être partiellement la conséquence du fait qu'aussi longtemps que le noyau épiphysaire est petit, il y a une grande quantité de cartilage non différencié dans l'épiphyse qui peut être mobilisé pour remplacer la partie non survivante de la zone germinative. Si cette partie n'est pas remplacée – comme ce fut le cas pour le groupe plus âgé – la croissance longitudinale de la transplantation cesse rapidement par suite du besoin de nouvelle formation de cellules cartilagineuses.

Il semble qu'il y ait de bonnes chances pour une transplantation qui a conservé 50 pour cent ou plus de sa capacité de croissance longitudinale pourvu que la transplantation soit opérée à l'âge de 10 à 14 jours et des chances minimes à l'âge de 7 et 10 semaines.

ZUSAMMENFASSUNG

Der proximale Teil der rechten Fibula wurde auf das linke Bein von 163 Kaninchen, die 1½–2, 4, 7 und 10 Wochen alt waren, verpflanzt. Das Überleben und Lägenwachstum des Transplantates wurde röntgenologisch und histologisch beobachtet.

Die Wiederernährung des Transplantates erforderte in den beiden jüngsten Gruppen zwei Tage weniger, was die Regeneration der überlebenden Teile des Epiphysenknorpels begünstigte. Die Regeneration war am vollkommensten in den jüngsten Gruppen. Augenscheinlich war dies teilweise eine Folge der Tatsache, dass in einem Stadium in dem der Epiphysenkern klein ist, eine grosse Menge von undifferenziertem Knorpel vorhanden ist, von dem man annehmen kann, dass er mobiliziert wird um den nichtüberlebenden Teil der Wachstumszone zu erstatten. Wenn dieser Teil nicht erstattet wird – wie es der Fall in der älteren Gruppe war – dann hört das Längenwachstum des Transplantates wegen mangelvoller Neubildung von Knorpelzellen bald auf.

Gute Möglichkeiten, dass das Transplantat 50 Prozent oder mehr seiner Fähigkeit zum Längenwachstum bewahrte, schienen nur vorhanden zu sein, wenn die Verpflanzung im Alter von 10–14 Tagen vorgenommen wurde, während die Aussichten im Alter von 7 und 10 Wochen sehr gering waren.

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