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TRANSPLANTATION OF EPIPHYSEAL CARTILAGE AND CRANIAL SUTURE

EXPERIMENTAL STUDIES ON THE PRESERVATION OF THE GROWTH CAPACITY IN GROWING BONE GRAFTS

by

SOINI RYÖPPY

ACADEMIC DISSERTATION

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"— il faut observer sans cesse et réfléchir longuement aux données de l'observation. Réfléchir pour corriger ce que Valéry a appelé l'étonnante inexactitude probable de l'observation immédiate, le faux qui est l'œuvre de nos yeux, car, ajoute-t-il, observer, c'est pour la plus grande part imaginer ce que l'on s'attend à voir. Il y a, en somme, après l'observation, un travail nécessaire de l'esprit qui fournit la matière sur laquelle va s'exercer la recherche expérimentale vérificatrice."

RENÉ LERICHE.

(La chirurgie discipline de la connaissanse. P. 202.)

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Helsinki, October 1964.

S. R.

CONTENTS

I INTRODUCTION

When, at the end of the 19th century, transplantation of bone began to develop into an important method of treatment, the need for a bone graft which would continue its growth after transplantation became evident. A search for suitable material was begun, because in growing bones the replacement of bone defects with the usual transplants resulted in recurrence of the deformity, even when the correction had been temporarily successful.

The experimental results of some pioneering workers as long ago as the 18th century showed that the growth in length of the long bones took place in the epiphyses. Hence, transplantation of the epiphysis should have solved the problem. Experimental work done during the first decades of this century, however, showed that the problem was much more difficult than had been expected. The results of animal experiments have been very inconsistent, and so far they have not provided a very clear picture of the possibilities of transplantation of the epiphyseal cartilage. Yet some results suggest that such transplants may be successful in favourable circumstances. What is the reason for so many conflicting results? What is the maximal growth potential of the graft? It is evident that many of the factors contributing to the successful results are still unknown.

From the point of view of preservation of growth, several clinical attempts to transplant the epiphysis have not been encouraging. On the other hand, the results of other methods of treatment have mostly not been satisfactory, either. The tendency in the treatment of congenital bone defects of the extremities is to begin it as early as possible to prevent secondary deformities. Because of the increasing number of patients in the youngest age group and the increasing standards expected, the problem of the treatment of these cases is becoming increasingly urgent.

The epiphyseal cartilage graft has been the only type of growing bone graft experimented on hitherto. Only autogenous grafts have shown any tendency to continue growth. This limited source of grafting material is one of the disadvantages of the epiphyseal grafts. These considerations led the author to attempt to find a new type of growing bone graft. As one such possibility, calvarial bone including the cranial suture was chosen. The growth of the calvarium takes place at the sutures by proliferation of the sutural connective tissue and successive apposition of bone. This growth is analogous to the endochondral growth of the long bones, but as a histological process it is far simpler. Because the transplantation of connective tissue is relatively easy, it was anticipated that the preservation of growth capacity would be possible. In the previous literature the author has not found any report of the transplantation of cranial sutures in the sense of growing bone transplants.

As regards the clinical applicability of such a procedure, we know that the regenerative capacity of calvarial bone is very considerable in infants. This makes the taking of the grafts possible without risk.

The present work is divided into two parts: the transplantation of the epiphyseal cartilage plate and of the cranial suture. In both these groups the main focus of interest of the study was the persistence of the growth capacity of the transplants. The growth of the grafts was followed radiographically and the specimens were examined by radiological and histological methods.

II TRANSPLANTATION OF EPIPHYSEAL CARTILAGE

REVIEW OF THE LITERATURE

EPIPHYSEAL CARTILAGE PLATE AND THE GROWTH OF LONG BONES

In 1727, *Hales* published the results of an experiment in which he drilled holes in the tibia of the chicken and observed that the growth in length took place at the ends of the bone. By feeding experimental animals with madder, *Duhamel* (1742) was able to show that the bone formed during the madder feeding was coloured red and situated in the metaphysis and in the superficial layer of the diaphysis. He drew the conclusion that longitudinal growth takes place at the end of the metaphysis and growth in thickness by apposition on the diaphysis. *Duhamel* also fixed nails in the legs of growing pigeons and confirmed the observation of *Hales*. *Hunter* (1779), with his well-planned experiments, confirmed the foregoing results. As a conclusion to his experiments with madder feeding he stated that longitudinal growth occurred at the epiphyseal cartilage plate (ECP). He emphasized that a continuous remodelling takes place in bones by resorption and deposition of bone. The experiments of *Flourens* (1842) confirmed the previous conclusions.

Strelzoff (1872), *Gudden* (1874), *Wolff* (1892), *Thoma* (1915) and *Policard* (1930) considered the interstitial growth of bone to be possible under certain circumstances. Others, however, including *Kölliker* (1831), *Humphry* (1861), *Wegner* (1874), *Schwalbe* (1876), *Haas* (1926), *Gatewood* and *Mullen* (1927) and *Troitzky* (1932), were of the opinion that the longitudinal growth of the tubular bones does not take place interstitially, but depends on endochondral growth in the ECP.

Later, the question of interstitial growth became more complicated when several workers noticed that the mass of bone formed after fusion of vertebral bodies could grow in length (*Biscard* and *Musselman* 1940, *Hallock et al.* 1957, *Risser* 1956, *Cleveland et al.* 1957 and *Johnson* and *Southwick* 1960). Yet, as the results of *Johnson* and *Southwick* show, this phenomenon can certainly be explained by factors other than the interstitial growth of bone.

Kornew (1929) placed circular metallic markers in the diaphysis of the long bones of rabbits and dogs and found that the distance between these markers increased continuously during growth. From this observation he draw the conclusion that the growth of long bones is interstitial in character, the epiphyses having only a stimulating and regulating function.

The opinion now generally accepted is that the growth in length of the long bones takes place at the ECP. It could even be said, as *Sissons* (1956) put it: "the rigid nature of calcified bone tissue makes its interstitial growth impossible."

The type of bone formation in the ECP is called endochondral ossification (*Maximow* and *Bloom* 1948, *Ham* 1953, *Sissons* 1956, *Trueta* and *Morgan* 1960). The general histological features of this process were first adequately described by *Müller* (1858). In fact, many different processes, i.e. the interstitial growth, maturation, calcification, death and disintegration of cartilage, and the formation, calcification and destruction of bone, are taking place in this area simultaneously (*Ham*). This is also reflected in the microscopic structure of the ECP, which can be divided into various zones:

The resting zone, or germinal layer, is situated immediately under the bone plate which separates the ECP from the epiphysis. It consists of amorphous material in which scattered cartilage cells are present.

The proliferation zone consists of longitudinal columns of cartilage cells arranged in close order and flattened against each other. In this zone mitotic figures are seen.

The zone of hypertrophic or oedematous or maturing cells is a direct continuation of the columns in the proliferation zone. The cells are large, spherical or cuboid and oedematous, with a central nucleus. The cells of this zone produce phosphatase, which brings about the calcification of the intercellular substance.

The zone of degeneration and calcification. In this zone osteoblasts from the metaphysis invade the disintegrating cartilage cells and deposit bone.

In the intercellular substance, amorphous in the resting zone, the collagen fibres arrange themselves parallel to the columns at the level of the proliferation zone. More distally, the space between the columns becomes very narrow and the calcification of the intercellular substance begins at the level of the last oedematous cells.

The thickness of the ECP is related to its activity. At birth, calcification of the epiphysis has not begun in most bones. When the bone plate forms, the proximal margin of the ECP becomes visible radiologically. In the proximal epiphysis of the fibula of the rabbit this takes place between the tenth and fifteenth days of age (*Heikel* 1959). There is also a constant relation between the number of cells of the ECP and its activity (*Trueta* and *Morgan* 1960). For instance, in the three-month-old rabbit the number of cells in a column of the proliferation zone in the proximal ECP of the tibia is 10—20, but in the vertebral epiphysis only 6—8.

The zone of calcified cartilage, also referred to as the primary spongiosa, is the site of progressive deposition of bone. Distal to it, longitudinal bony trabeculae, which show inclusions of cartilage matrix, are seen. In this, extensive remodelling takes place and this area is called secondary spongiosa.

With age the ECP becomes narrower and the number of cells decrease. The time of fusion of the various epiphyseal plates is different. According to *Heikel* (1959), the fusion of the proximal ECP of the rabbit fibula takes place between 150 and 225 days of age.

In the ECP no perforating vessels, such as might be of importance for the nutrition of the cartilage cells, are found (*Nussbaum* 1923, 1926, *Hurrel* 1934, *Marneff* 1951, *Trueta* and *Morgan* 1960) although some investigators have noted channels in it, the function of which is not clear (*Lexer et al.* 1904, *Schulze* 1933). *Hurrel* has reported the presence of blood vessels traversing the ECP of the human foetus. The vascularization of the ECP has recently been studied in detail by *Trueta* and *Little* (1960) and *Trueta* and *Amato* (1960). According to them, the nutrition of the germinal layer and possibly of most of the ECP, takes place by way of the vessels perforating the bone plate from the epiphyseal side. These vessels are branches of arteries which penetrate the epiphysis close to the capsular insertion near the ECP. The function of the metaphyseal vessels is related to the calcification of the cartilage matrix, the resorption of the degenerated cells and the laying down of lamellar bone. They are of no nutritonal importance even to the hypertrophic cells. Experimental ischaemia of the ECP induced by local arrest of nutrition from the epiphyseal side resulted in disturbance or arrest of growth. The arrest of nutrition from the metaphyseal side caused disturbed growth in only a few cases.

RELATIVE GROWTH OF DIFFERENT EPIPHYSEAL CARTILAGE PLATES

Hales (1727) had already noted that growth was not equal at the two ends of the long bones. *Duhamel* (1742) noticed in his experiments on pigeons that the proximal end of the tibia grew more than the distal end.

After inserting metallic markers in the bones of rabbits and chickens, *Ollier* (1867) came to the conclusion that in the forelimb the epiphyses near the elbow grew very little compared with those situated farther from it. In the hindlimbs, on the contrary, the epiphyses situated near the knee grew the most. *Ollier* drew attention to the fact that the growth ratios of the epiphyses were different in different species of animals. In the rabbit the growth ratio of the proximal and distal ECP of the tibia was 23:13. He also refuted with experimental proof the argument of some previous authors that the distal ECP of the fibula grew more than the proximal one. In his experiments the ratio was the same as in the tibia. On resecting one of the two epiphyses of the long bone, *Ollier* noticed that the remaining epiphysis could grow more than normally, i.e. partially compensate for the growth of the lacking epiphysis.

In 1915, starting from the hypothesis that the nutrient vessel around which the development of the long bone begins retains its original position as the central point of the medullary cavity, *Digby* performed measurements on human long bones. According to him, the growth ratio of the proximal and distal ECP of the tibia was 57:43 and of the ulna 19:81.

Bergmann (1929) measured the growth of different epiphyseal cartilage plates in human subjects, using "the lines of arrested growth", which were seen in radiographs of patients treated with phosphorus-containing cod-liver oil or ultra-violet light. The ratio in both the tibia and fibula was proximal ECP: distal ECP = 15:12.

Using metal markers on dogs, *Bergmann* reported equal growth in the proximal and distal epiphyses of the tibia.

Payton (1933) used madder feeding on pigs, paying special attention to the division between the longitudinal growth of the ECP and that of the epiphysis. When the bone plate between the ECP and the epiphysis has formed, the ECP can no longer contribute to the growth of the epiphysis but the growth in length of the latter may continue by proliferation of the articular cartilage. This must be distinguished from the longitudinal growth of the diaphysis, i.e. of the ECP. The growth ratio between the proximal and distal ECPs was 17:16 in the tibia, and 13.7:16.4 in the fibula. The growth of the proximal epiphysis of the fibula was correspondingly greater.

Using metallic markers on the rabbit, *Silfverskiöld* (1934) found the proximal: distal growth ratio in the tibia to be 14:10. He also noticed the possibility of compensatory growth if the growth of one of the two epiphyseal plates was disturbed.

Bisgard and *Bisgard*, in 1935, placed steel shot in the bones of three goats and obtained for the proximal:distal growth of the tibia the ratio 42:52 and in the foetal period (measured from the lines of arrested growth caused by phosphorus feeding) 49:51.

Heikel (1960), in his experimental work on the rabbit, used *Silfverskiöld*'s values for calculations of the growth of the fibula, taking into consideration that the ECP of the fibula grows less than the proximal ECP of the tibia.

Larsen and *Nordentoft* (1962) analysed "the lines of arrested growth" in three patients. They did not pay attention to the amount of growth, but measurement of the growth from the X-ray pictures published in their paper gives the ratio proximal ECP: distal ECP = 6:7 in both the tibia and the fibula.

Anderson et al. (1963) used lines of temporarily arrested growth, seen at the ends of the diaphyses of long bones of children, for calculation of the growth of different epiphyses. The growth of the proximal end of the tibia between ten and fifteen years of age was 57 per cent of the total tibial growth.

Summary and comments

In many experimental works the longitudinal growth of the epiphysis has been included in the longtudinal growth of the bone. Histologically this is replacement of the interstitially and appositionally growing articular cartilage by bone (*Payton* 1933, *Weinmann* and *Sicher* 1947, *Ham* 1953) and should be distinguished from longitudinal growth of the ECP. Some common features are seen in the relation of the growth between the different epiphyseal plates, but the ratios reported by different authors differ even in the same animal species. The reports on the relative growth of the proximal and distal ECP of the fibula compared with those of the tibia seem to vary.

EXPERIMENTAL TRANSPLANTATION OF THE EPIPHYSEAL CARTILAGE

The history of the transplantation of the epiphyseal cartilage plate (ECP) is closely connected with the history of bone transplantation.

According to *Peer* (1955), the first successful transplantations of bone were performed in the beginning of the 19th century. *Ollier* (1867) was one of the first to examine bone grafts systematically. When, in the rabbit, he resected the epiphyseal end subperiosteally, he observed complete regeneration and considerable growth after the operation. Total excision of the epiphyseal cartilage resulted in the arrest of growth. In the rabbit and the dog *Ollier* transplanted whole bones subcutaneously and into other tissues. He reported that in rabbit the grafts remained viable and in the young rabbits he observed some growth in thickness in the transplanted metatarsal bones. Longitudinal growth was never seen and no proliferation in the ECP either. In young dogs the grafts were resorbed. *Ollier* was the first to divide the grafts into auto-, homo- and heterografts. He considered that part of the cells in autogenous bone grafts remained viable.

Rudnew (1880), *Radzimowsky* (1881) and *Barth* (1893, 1894) concluded that all the cells of the bone graft die and the graft is replaced by host tissue.

The first reported transplantations of the ECP were made by *Helferich* in 1899. On a young rabbit the distal ECP of the ulna, with a thin layer of bone on both sides, was detached and reimplanted. Some amount of growth was observed, but the growth was inadequate and resulted in deviation of the wrist. The material was examined histologically by *Enderlen* (1899). He concluded that the reimplanted ECP retains a large part of its vitality. The cells situated near the perichondrium and the encoche withstood the transplantation best. The central part of the ECP became necrotic. Some days after the transplantation an intracellular oedema of the cartilage cells was noticed, which was also visible macroscopically as a thickening of the ECP.

In the rabbit *Zoppi* (1900) performed both auto-, homo- and heterotransplantations. In autografts he observed formation of new bone and interpreted the result as good from the point of view of preservation of growth. In homografts similar changes were not seen and the heterografts were resorbed. The conclusion drawn by *Galeazzi* (1907 from his auto- and homotransplantations was that growth stopped in most cases, the cells of the ECP died and the ECP became ossified. In some cases there was slight regeneration, but it was so inadequate that growth remained seriously disturbed.

In 1911, *Wrede,* to confirm the clinical experiences of *Lexer,* performed so-called transplantations of a half-joint in rabbits. The whole epiphyseal end of the bone was transplanted, but it never remained viable.

Axhausen (1912) tried to clarify the "absolute transplantability" of the ECP. Using rats and rabbits he transplanted epiphyseal ends of bones to soft tissues of another animal of the same species. In histological examination he noted that a part of the superficially situated cells remained viable. Some ossification was also seen in these parts. *Axhausen* drew the conclusion that the ECP is transplantable in a histological sense, but that regeneration is inadequate for clinical purposes.

Using the metatarsophalangeal joint with a part or the whole metatarsal bone as the graft, *Borst* (1912) performed reimplantations and homogenous transplantations in the rabbit. Growth was seriously disturbed in all cases, but in reimplants he noticed some growth. The homografts did not grow. Concerning his homotransplantations of the knee-joint in the rabbit, *Dalla-Vedova* (1912) reported that in no case had "histological healing" occurred.

In rabbits aged two months, *Rehn* and *Wakabayashi* (1912) homotransplanted the proximal end of the radius to the corresponding site into another animal of the same litter. The fate of the grafts was followed histologically after observation periods of different lengths. There was good union between the graft and the host bone; no central necrosis or cellular oedema of the ECP was seen. After five weeks, changes indicative of arrest of the growth appeared.

The authors concluded, however, that growth continued undisturbed and considered clinical homotransplantation of ECP to be justified.

v. Tappeiner (1913) observed some growth in his reimplants, which consisted of the distal end of the metarsal bone of the dog. The homografts did not grow.

Heller (1914) began his experiments in 1911; in these he endeavoured to ascertain the extent to which the growth in the ECP graft was preserved. On rabbits aged 4—8 weeks, he reimplanted the distal end of the ulna. In none of his cases was normal growth seen; the shortening was 1/13 of the total length of the bone in the most successful cases. In homografts the shortening was more pronounced. *Heller* also made four experiments on goats. In the reimplantation of the distal end of the radius the shortening was 1/3 of the total length of the bone. The results of the histological examination corresponded to those of *Enderlen* in the reimplants. The cells in the homografts, on the other hand, all died.

Minoura (1914) transplanted metatarsophalangeal joints of the rabbit as auto- and homografts into subcutaneous tissue, muscle, liver and the peritoneal cavity. A number of cells in the superificial parts of the graft seemed to have survived. No organized growth was seen even in the autografts, which showed only a kind of scattered proliferation (Wucherung). The graft never became longer, — rather the contrary.

In 1915 and 1916 *Haas* transplanted the metacarpal bones in the dog either whole or split into two. As another type of graft he used the ECP of the same bone. The graft included a thin bone plate on either side of the ECP. Both in reimplantation and in transplantation into the corresponding site in the opposite leg the growth, even in the most successful experiments, was only a few millimetres. Transplantation in two stages to the adjoining metacarpal bone resulted in considerably diminished growth. The author concluded that the ECP is very sensitive as a transplant and that its survival in transplantation is directly related to revascularization.

In none of his auto- and homogenous transplantations of half-joints in rabbits was *Giani* (1916) able to detect any longitudinal growth in the graft. *Pucci* (1916) transplanted the cubital joint as a homograft in the rabbit. The fusion between the graft and the host bone and likewise the function of the transplanted joint were good.

To verify the results of *Rehn* and *Wakabayashi*, *v. Tappeiner* (1916) made similar experiments. He did not distinguish between proximal and distal epiphyseal growth, but only measured the total length of the bone. There was no shortening of the operated bone even in experiments in which the ECP had changed into connective tissue. The author concluded that transplantation had failed in only two cases out of fourteen.

In the rabbit *Segale* (1917) reimplanted both the proximal end of the radius and the metatarsophalangeal joint. He used as a marker the metal wire by which the graft was fixed to the diaphysis. In not a single case was longitudinal growth noticed. In histological examination a number of cells in the peripheral parts of the graft seemed to have survived.

Heller, in 1918, continued more carefully than before. He left only a very thin layer of bone on each side of the ECP. The distal ECP of the ulna of the rabbit, aged 4–6 weeks, was used as the graft. The growth was measured with the aid of metal markers in the diaphysis. In reimplantation, 21 of the total of 27 cases showed only slight shortening. When the same graft was rotated through 180 degrees so that the original epiphyseal surface came to face the metaphysis, growth was more disturbed. In histological examination the author noticed that almost the whole reserve zone often remained viable. In homografts some amount of growth was measured during the first 1–2 months after transplantation; after that the ECP degenerated and disappeared.

The femurs of frog embryos were grown in tissue culture by *Fell* (1929), who noticed that they grew to threefold their original size, preserving their original form grossly.

Fohl (1929) repeated the reimplantation experiments of *Heller* and obtained a good result in four out of seven cases. One similar experiment was made on a monkey. During the observation period of fifteen months growth continued normally without any shortening. From this the authors concluded that the size of the graft was of no decisive importance for the results.

Kornew (1929) transplanted pieces of the diaphysis of tubular bones to the spinous processes in rabbits and dogs and noticed that longitudinal growth had taken place in the grafts. In another group of experiments autogenous pieces of diaphysis were transplanted to segmental defects in the long bones. The distance between the circular metallic markers in the grafts increased. From his findings *Kornew* drew the conclusion that such grafts, as well as tubular bones, normally grow interstitially.

Reschke (1929) considered a pedicle graft to be the safest type of graft. Trying to find the optimal moment for dividing the stylus, he stripped the skin off the foreleg of the rabbit and placed the limb in the subcutis of the abdomen. The division of the stylus before the fourth week resulted in damage to the ECP. This author expressed the opinion that an epiphyseal cartilage graft is unable, even theoretically, to grow more than it would have done in its original site.

The experiments of *Brücke* (1930) were of the same type as those of *Heller* and his results corroborated the conclusions of the latter. *Haas* (1931) also repeated *Heller*'s experiments. He obtained good results only in cases where the ECP was not completely removed with the graft. i.e. the cut lines were not far enough from the ECP. He expressed the opinion that the good results obtained by *Heller* and *Fohl* were due to regeneration of the cartilage cells left on the outside of the graft. *Haas* concluded that the ECP loses its capacity for growth after transplantation.

May (1934) reimplanted the whole radius of the dog. The extraperiosteally removed graft became necrotic, but in the subperiosteally removed graft the ECP regenerated. The graft grew less than 50 per cent of the control.

Selye (1934) removed the distal end of the femur and as the result obtained a new cartilaginous epiphyseal end, the cartilage tissue of which resembled that of articular cartilage. He reported that growth continued normally.

Silfverskiöld (1934) tried to fill the gaps in the previous experimental work and to check the uncertain results. He performed numerous experiments and in different ways arranged transplantations of the ECP. The pedicle graft grew almost normally, but the new bone was thin and not useful for practical purposes. When the ECP graft was transplanted into the joint cavity, the graft was gradually resorbed. In repeating the experiments of *Heller* and *Fohl*, he obtained the same results as these workers. He took great care to ensure that no cartilage celles were left on the outside of the graft. Transplantation of the ECP of the ulna to a segmental defect in the diaphysis of the opposite ulna resulted in gradual ossification of the ECP and no growth was observed. When the same graft was transplanted to its corresponding site in the opposite limb, growth was much more limited than in the reimplants, — in the most successful cases about half of the normal. The homografts did not grow. The author considered the clinical application of epiphyseal transplantation as possible with certain restrictions.

Pereira and *Dupertuis* (1936) transplanted pieces of epiphyseal cartilage one cubic centimetre in size to the diaphysis of the same bone in a young rabbit, a procedure which resulted in the formation of large exostoses.

On reimplantation ("isolement") of the distal ECP of the ulna in the rabbit, *Sousa Pereira* (1937) reported considerable growth. The shortening after twelve months was 3—5 mm. When the graft was rotated through 180 degrees around its transverse axis the shortening was 8—18 mm, the growth of the graft 2—6 mm and the direction of growth towards the diaphysis. The author concluded that the graft continued its function in all cases, but changes which resulted in disturbance of the growth were seen.

Bisgard (1939) took two slices of cartilage of the distal femoral ECP of the goat, and transplanted them to a segmental defect in the diaphysis of the tibia. In three weeks both grafts were ossified. In specimens taken three months later no cartilage cells were seen.

Banks and *Compere* (1941) excised the distal ECP of the femur in the rabbit, which resulted in bony union between the epiphysis and metaphysis. *Hellstadius*

(1947) resected the distal end of the ulna with the ECP and a piece of metaphysis and diaphysis. In one of the extraperiosteal resections good regeneration and considerable growth in length were seen. In all cases of subperiosteal resection the ulna grew significantly in length.

Gordon and *Warren* (1948) used as grafting material dices of cartilage taken from the foetal epiphyses of the rabbit. These were transplanted to cortical defects in the diaphysis of adult animals. No formation of exostoses was seen. The cartilage cells lost their capacity for growth even if they remained viable for long periods (twelve weeks in these experiments). *Lacroix* (1951) transplanted pieces of ECP of a young rat beneath the renal capsule of another animal of the same litter. They grew considerably during the observation period of four weeks, forming new bone.

Herndon and *Chase* (1952) noticed that in the reimplants and homotransplants of the knee joint in the dog a number of bone cells of the reimplants survived. The greater part of the graft became necrotic and was gradually replaced by host tissue.

Using the "Spongiosatest", *Lenz* (1955) concluded that all the bone cells died, but the soft tissue parts of the graft survived at least partially and preserved their osteogenic capacity. As little as two days after transplantation part of the graft made contact with the host circulation. The surviving cells of the graft participated in the process of creeping substitution of the graft.

Peer (1955), in his survey of the literature concerning the transplantation of the ECP, pointed especially to the disagreement among investigators regarding the long-term survival of the ECP after transplantation. He concluded that "the fate of the epiphyseal cartilage in autogenous and homogenous transplants and the capacity of the cartilage to grow are surrounded with considerable doubt."

Using rabbits, *Ring* (1955[1]) examined the regeneration after total and partial excision of the distal ECP of the ulna, and noticed that the ECP could regenerate transitorily in some cases, even after total excision. The ECP became ossified in most cases. The same author (1955[2]) also reimplanted the ECP after rotating it 180 degrees around its transverse axis. Some of the reimplants showed almost normal growth. In the latter group growth was poor, and ceased 6–8 weeks after transplantation. In transplantation of the ECP to the corresponding site in the opposite leg (*Ring* 1955[3]) 5 out of 18 experiments were interpreted as successful. All homografts failed to grow. The author concluded that the application of epiphyseal transplantation in clinical practice was justified, but pointed out that there is no ready source of autogenous epiphyseal cartilage for grafting material.

Betzel in 1956 transplanted tubular bones of newborn puppies to the dorsal musculature of adult dogs and noticed that the grafts were totally resorbed. No formation of new bone was seen.

In the experiments of *Schneider* (1956), where the ECP of the newborn rabbit was transplanted to a segmental defect in the diaphysis of the radius of a young rabbit, enchondral ossification, but no significant growth, was seen in the graft. Bony union was achieved, but the graft seemed to disturb the consolidation rather than to promote it.

In puppies, *Schraiber* (1956) transplanted the proximal end of the fibula to the site of the excised epiphyseal end of one of the bones of the foreleg. The graft included a relatively long piece of diaphysis. The maximal growth of the graft was 133 per cent of its original length. The rapidity of growth depended on the general growth rate of the animal, being greater in an actively growing bone. In microscopic examination the author noticed that all osteocytes and cartilage cells died. A number of cells in the perichondrium, periosteum and epiphyseal cartilage survived. Towards the end of the first month after transplantation the graft was restituted and enchondral growth in the ECP was in process.

Felts (1957) reported that in the transplantation of whole isologous and homologous bones to the subcutaneous tissue on mice aged two days, the growth of the isografts was 80 per cent of the normal, while the growth of homografts ceased 12—16 days after transplantation.

On 2 to 29 days old rabbits *Heikel* (1959, 1960) transplanted the proximal epiphyseal end of the fibula as an autograft to the site of the radius, which had been totally or partially removed. The transplantation was performed as a one- or two-stage operation. The growth was in most cases measured from a piece of metal wire inserted in the diaphysis. In the best cases the longitudinal growth of the graft was 2/3 — 8/9 of the growth of the ulna and about 1/2 – 2/3 of the growth of the control ECP of the fibula. In one series the same type of graft was implanted into the thigh. The growth of this varied between 1/8 and 1/2 of the growth of the fibula. About half the grafts were fused side to side with the femur. The experiments were carried out on animals of different ages, even on very young animals, but no definite correlation between the age of the animals and the growth of the graft was seen. In histological examination, the author noticed that the central part of the ECP underwent necrosis, but a thin peripheral layer of the reserve zone survived transplantation. The ECP regenerated from the surviving parts. This process cut off the region of the necrotic cartilage and islets of this were visible up to 23 days after transplantation in the diaphysis at a distance which corresponded to the growth of the graft as measured on radiograms.

The transplant of *Entin et al.* (1962) consisted of the whole metacarpophalangeal joint of puppies. Most of the grafts healed well at the areas of bone contact. After fifteen weeks, morphological changes pointing to degeneration of the graft appeared. In some experiments the time of observation was sufficiently long for evaluation of long-term results. In these a striking retardation of growth was seen. Destruction of the ECP was noted on microscopic examination of these transplants.

Summary and comments

As many authors have pointed out (*Silfverskiöld* 1934, *Ring* 1955, *Peer* 1955, *Heikel* 1960—61), comparison of the previous investigations is not easy because the techniques in most of them are different. On the other hand, it is not possible to understand the role of the different factors without differently arranged experiments.

In many investigations the possible growth, and especially the amount of growth of the graft, has not been demonstrated in a convincing manner, because the marking or the control methods have been unsatisfactory (*Ollier* 1867, *Helferich* 1899, *Zoppi* 1900, 1902—03, *Galeazzi* 1907, *Borst* 1912, *v. Tappeiner* 1913, *Heller* 1914, *Giani* 1916, *Fohl* 1929, *Kornew* 1929, *Brücke* 1930, *Ring* 1955).

The homograft seems not to be capable of prolonged growth (*Zoppi* 1900, *Galeazzi* 1907, *Wrede* 1911, 1913, *Axhausen* 1919, *Borst* 1912, *Dalla-Vedova* 1912, *v. Tappeiner* 1913, *Minoura* 1914, *Giani* 1916, *Herndon* and *Chase* 1952, *Ring* 1955, *Betzel* 1956, *Schneider* 1956, *Felts* 1957). Some contrary results have been reported (*Heller* 1914, 1918, *Rehn* and *Wakabayashi* 1912, *v. Tappeiner* 1913, *Lacroix* 1951). *Heller*, when repeating his experiments, noticed that only insignificant growth had taken place during the first 1—2 months. *Rehn* and *Wakabayashi* and *v. Tappeiner* used as the graft the proximal end of the radius, which is normally responsible for only about 20 per cent of the whole growth of the bone (*Digby* 1915, *Bisgard* and *Bisgard* 1935). The result was probably interpreted as positive because no markers were used. All later control experiments of this type have been negative. *Lacroix*, in his work, did not measure the amount of growth.

The autograft, and especially the reimplant, seemed in certain instances to show considerable preservation of growth capacity. On this point there are many discrepancies between the results published, the explanation of which is by no means clear in all cases. Some are probably due to misinterpretation and others to differences in technique. The reimplantation and transplantation from one side to the other of the distal ECP of the ulna may evidently give good results, especially if only a thin layer of bone is transplanted with the ECP

(*Heller* 1918, *Silfverskiöld* 1934, *Sousa Pereira* 1937. In these reports the amount of growth, compared with the normal, was not shown in a convincing manner, it is true. The results obtained by *Haas* (1931) for the same type of transplantation were negative, but his material consisted of only a few scattered experiments. The reimplantation of the ECP of the metatarsal and metacarpal bones did not give a positive result from the point of view of preservation of growth (*Minoura* 1914, *Haas* 1915–16, *Segale* 1917).

The proximal ECP of the fibula seems to have given positive results even when a part of the diaphysis was transplanted with it (*Schraiber* 1956, *Heikel* 1960). The ECP, when transplanted to a defect in the diaphysis, did not continue to grow, but became ossified (*Silfverskiöld, Sousa-Pereira, Bisgard* 1939). When transplanted as a free graft to the soft tissues, the ECP did not preserve its capacity for growth (*Ollier, Minoura, Axhausen, Heller, Silfverskiöld*). Only *Heikel* noticed some growth in this type of graft. When the ECP was rotated 180 degrees around its transverse axis, it showed some amount of growth in the opposite direction (*Heller* 1918, *Sousa-Pereira, Ring*). As a pedicle graft the ECP grew almost normally (*Reschke* 1929, *Silfverskiöld*).

In no cases has the transplanted ECP grown as much as it would have done in its original site.

It seems to be histologically established that a part of the cells of the transplanted ECP survive (*Enderlen* 1899, *Segale, Heller* 1918, *Schraiber, Heikel* Some of these cells certainly play a very important role in the regeneration of the graft. The survival of the resting zone of the ECP seems to be of the utmost importance for the preservation of growth capacity (*Heller, Ring, Heikel*).

CLINICAL TRANSPLANTATION OF THE EPIPHYSEAL CARTILAGE

On clinical material *Ollier* (1867) performed a number of resections of epiphyseal ends and whole joints. He reported that subperiosteal resection of the epiphyseal end often resulted in good regeneration and even some longitudinal growth in length of the resected epiphysis.

Zoppi (1902, 1903) reported a case of a 12-year-old patient in whom he had transplanted a piece of the distal epiphyseal end of the fibula to the site of the distal end of the tibia, which had been destroyed by osteomyelitis. The ECP was clearly visible in radiographs 2 months after the operation.

Galeazzi (1907) used a thin plate consisting of the distal ECP of the humerus as a graft. This was in two cases transplanted to the distal end of the shortened radius. The graft necrotized.

Lexer (1908 and 1913), *Borst* (1912), *Küttner* (1913), *Capurro* and *Pedemonte* (1953), among others, used fresh homogenous grafts, which consisted of whole bones, epiphyseal ends or whole joints. In many cases the grafts fused well and no resorption was noticed macroscopically. By 1920, *Lexer* had given up using homografts and recommended only autografts in this type of transplantation. In some cases *Küttner* transplanted a growing epiphyseal end of a monkey to his patients. He reported a case in which the fibula of a monkey had been transplanted to the site of a congenitally lacking fibula. One year and eight months later no resorption was visible radiologically, but there was no growth in the graft. The ECP was clearly visible.

In a child aged 7 years, *v Saar* (1913) transplanted the proximal end of the fibula to the site of the excised distal end of the radius. Regarding the same case, *Heller* (1914) later reported that one and a half years after the operation there was no growth in the graft, although the ECP was radiologically visible.

On the basis of these previous experiences *Axhausen* (1919) regarded the pedicled graft as the only possibility for successful transplantation. In a child, the distal end of whose radius was destroyed by tuberculosis, he transplanted half of the distal end of the ulna to the site of the former in two stages. 4 years later the distal ECP of both the radius and the ulna had grown 9 mm. *Axhausen* performed a similar operation in a case of total defect of the radius. 12 years later he wrote to *Silfverskiöld* (1934) that the cases had "remained good". *Axhausen* also tried to transplant the split distal end of the tibia to the site of the missing fibula. Because of technical difficulties this became a free graft. Six weeks later the graft was removed and examined histologically. Only a number of superficially situated cells of the ECP were viable. *Axhausen* regarded the possibilities of regeneration and continuation of growth as doubtful.

Oehlecker (1922, 1923) reported some growth in the phalanx of a toe which had been used as a substitute for the missing proximal phalanx of a finger. After 7 years, growth of several millimetres had occurred. The growth in pedicled grafts, transplanted according to the procedure of *Nicoladoni*, continued normally in most cases. Reimplantation of the distal end of the radius resulted in cessation of growth in one case. The same author performed 8 free transplantations of the ECP on clinical material and in all of them obtained a negative result. He concluded that the pedicled graft was the only type capable of success.

In a child aged 4 years, *Straub* (1929) transplanted the split distal end of the tibia to the site of a damaged distal end of the opposite tibia. 16 years later it was seen that the graft had grown and the shortening had not increased.

The idea of using a split tibial graft to correct a defect in the fibula was also tried by *v. Assen* (1934). He modified it by leaving some tibiotalar ligaments as a pedicle and obtained normal growth in the graft.

Starr (1945) observed no growth in the proximal end of a fibula transplanted to the site of the missing distal end of the radius.

Wenger (1945) substituted the first metatarsal bone by a fibular graft containing the ECP on a 7-year-old child. The growth of the graft during the following 3-year period was 0.5 cm.

In a child aged 11 years, *Key* (1949) reimplanted the proximal end of the radius. In radiological examination 4 years later the structure and growth of the graft seemed normal.

Barr (1954) reported that the distal ECP of the ulna, which had been transplanted to a defect in the diaphysis of the radius, had grown 2 cm and caused a dislocation of the head of the radius.

Peer (1955) buried a supernumerary toe and finger in the abdominal fat and noticed by palpation and roentgenographic examination 22 months later that the bones and cartilage had retained their structure and that the motility of the joints was good.

Riordan (1955) used a modified method of *Starr* in the treatment of defects of the radius. There was no growth in his 10 fibular grafts. Some increase in length was attributed to the ossification of the epiphysis. He reported that he had seen some cases of other authors in which clear growth was visible. He regarded successful transplantation as possible if the child was young (under 1 year of age) and the graft small enough. *Bunnel* (1955) in this connection expressed the opinion that epiphyseal grafts do not grow, basing his view especially on the results of *Haas*.

Heikel (1959) published the early results of two cases treated by the method of *Riordan*. In one of these some growth was visible $11^1/_2$ months after the transplantation.

Sulamaa (1960, 1963, 1964) transplanted a metacarpal bone to the site of a missing radius and noticed some growth in the graft during the first 2 years after the operation. A gradual ossification of the ECP led to cessation of the growth.

Riordan (1962), on the basis of clinical experience, was of the opinion that large joint transplants will not survive, but that there was a change of survival and continuation of growth if small non-weight-bearing joints or epiphyseal ends are used on young patients.

Spira and *Farin* (1964) transplanted the distal ECP of the fibula on a child aged 9 years to a segmental defect in the diaphysis of the same bone. After eighteen months the ECP proved to be radiologically open, but histological examination revealed complete disorganisation in it. The remnants of the ECP appeared as a transverse band of fibrous tissue and cartilage.

Summary and comments

The continuation of growth in the free epiphyseal cartilage graft seems to have been demonstrated in some cases of clinical epiphyseal transplantation (*Oehlecker* 1922, *Straub* 1929, *Wenger* 1945, *Key* 1949, *Heikel* 1959 and *Sulamaa* 1960). In the cases of *Oehlecker*, *Wenger*, *Heikel*, and *Sulamaa* the growth of the graft was too small to be clinically significant. *Straub* was not able to measure the amount of growth in the graft because he had not used markers. The interpretation of the result was also confused by the persistence of the damaged distal ECP of the tibia. The result of *Key* was not convincing, because the proximal ECP of the radius normally provides only 20 per cent of the whole growth of the radius (*Digby* 1915, *Bergmann* 1929, *Bisgard* and *Bisgard* 1935). It seems that insufficient attention has been paid to the results of animal experiments.

PROBLEMS

1. To what extent is it possible to retain the capacity for growth of the epiphyseal cartilage plate of the fibula in transplantation to the corresponding site in the opposite leg?
2. How do the form and structure of the graft differ from those of the normal epiphyseal cartilage plate after the observation period?
3. How does the lack of bony contact between the graft and the host bone influence the fate of the graft?

MATERIAL AND METHODS

MATERIAL

19 rabbits and 23 mongrel dogs were used as experimental animals. Because of infections and complications during the anaesthesia or the operation, 9 rabbits and 9 dogs were excluded from the material. The age of the rabbits was 22 days and of the dogs between 26 and 60 days. The animals were chosen without regard to sex, size or colour. On some animals more than one experiment was performed.

The types and number of experiments done on the different animals are seen in table 1.

TABLE 1. — *Transplantation of the ECP of the fibula. A group of control experiments is included in the table.*

Type of experiment	Number of experiments Rabbit	Dog
Investigation of normal growth of ECP by using metal markers (control series)		8
Transplantation of ECP to the opposite fibula	8	14
Transplantation of ECP into muscle	6	8
	14	30
Total	44	

OPERATIVE METHODS
(fig. 1)

Both the rabbits and the dogs were anaesthetized with ether. Most of the dogs were given Nembutal 30 mg/kg I.M. as premedication. The operative field was shaved and washed with two antiseptic solutions successively (Rodalon ® and 1 per cent mercurochrome). The habitual aseptic operative technique was used.

Measurement of the proportional growth of the epiphyses of the tibia and fibula. To permit the use of the tibia as a control of the growth of the proximal ECP of the fibula, a group of control experiments was performed on 4 dogs. A transverse metal marker was placed in the diaphysis of both fibula and tibia. The distance of the markers from both epiphyseal lines was measured radiographically during growth.

Transplantation of the ECP into the corresponding site in the opposite fibula. A lateral longitudinal incision was made at the site of the proximal end of the fibula. The proximal end of the fibula was exposed and the peroneal nerve separated from it. The fibula was cut in the metaphysis by sawing with a thin-bladed scalpel. The distance from the cut line to the ECP was 3—4 mm on the rabbit and 4—5 mm on the dog. The epiphysis was either cut about 2 mm proximally from the ECP or detached as a whole with the graft. The freeing was extraperiosteal and extraperichondral. At the distal end of the graft a metal marker was attached and in many cases a short stainless steel wire was inserted into the distal end to give better adaptation to the host bone. This operation was performed successively in the two legs and the grafts were then interchanged. During the procedure the graft was kept in sterile physiological saline solution. The incision was closed by interrupted catgut sutures and the

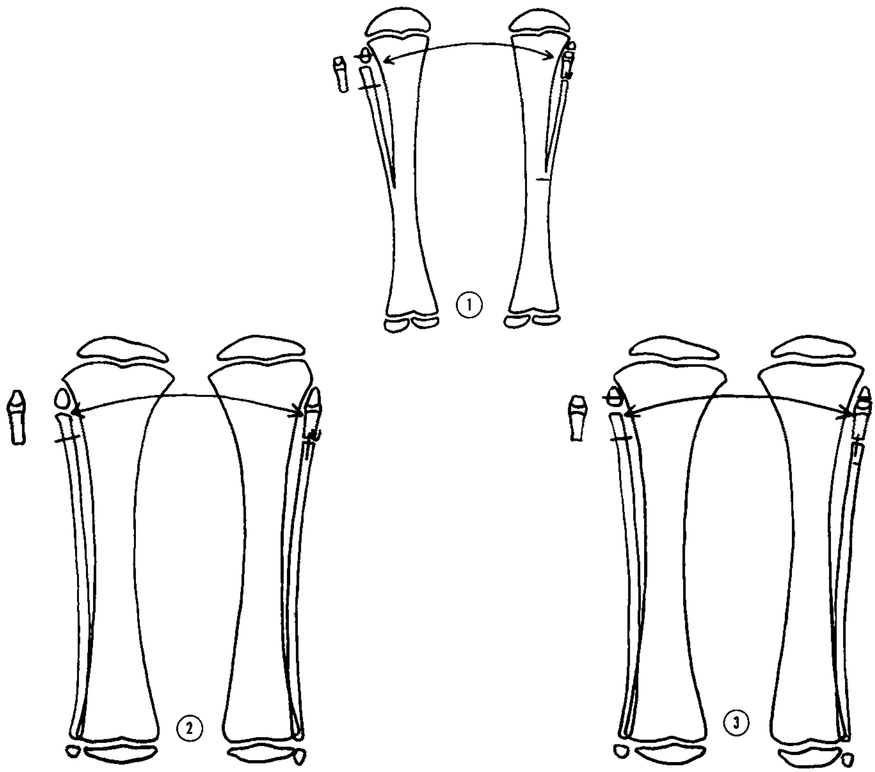


FIG. 1. – *Transplantation of ECP. Operative methods in the rabbit (1) and in the dog (2 and 3). The graft was excised on both sides successively and the grafts were then interchanged. In the pictures only the first part of the procedure is illustrated. Note the marking methods. In the dog two types of graft were tested.*

operative area covered with a plastic film (Nobecutan®). No other bandage or splinting was used.

Transplantation of the ECP into muscle. A graft of the same type as described above was placed between the peroneal muscles of the same leg. In this group the graft was not marked with metal markers.

CONTROL METHODS

The length of the graft was measured during the operation and from the specimen after the killing of the animal. During the observation time the length of the graft was measured from the radiographs. Comparison of the results of the measurements from the graft or specimen and from the radiographs showed that the accuracy of measurement was 0.5 mm.

Transplantation to the opposite side. The control method was different in the rabbit and the dog.

R a b b i t. The distal end of the fibula of the rabbit is fused with the tibia. The growth of the fibula is therefore dependent on only one ECP. As controls both the tibia of the same leg and the fibula of another animal of the same litter were used. The latter control was added, because it was possible that the graft might disturb the growth of the tibia. The distance of the ECP from either the metal marker or the site of fusion of the tibia and fibula was measured. To eliminate the error of a possible shifting of the site of fusion, which has been suspected by *Heikel* (1960), a metal marker was placed in many cases in the diaphysis of the tibia. In fact, it was noticed by this method that the site of fusion could be used with sufficient accuracy as the distal point of measurement.

The growth of the ECP of the tibia is greater than that of the fibula. The result is that during growth the ECP of the fibula moves in a distal direction in relation to the proximal ECP of the tibia. This was allowed for by calculating the average distance between the proximal ECPs of the tibia and fibula in a group of rabbits of different ages (see results, table 2, p. 32) and subtracting the value obtained from the growth of the proximal ECP of the tibia.

D o g. Two types of controls were also used: the growth of the proximal ECP of the tibia of (1) the same leg, and (2) another animal of the same litter. The latter control was added for the same reasons as in the rabbit. In calculating the control value from the growth of the tibia, the average obtained in the control experiments was used (see results, table 3, p. 33). The average value of 51 per cent, which corresponds to the contribution of the proximal ECP to the growth of the tibia, was used in the calculation. The diaphysis of the fibula grows less than that of the tibia. The shift of the proximal ECP of the fibula in relation to that of the tibia corresponds to this difference in growth. The values showing this difference were obtained by the same method as in the rabbit (see results, table 2, p. 32). This difference was divided by two (corresponding approximately to the ratio 51:49).

Transplantation into the muscle. The control methods were the same as in the foregoing group.

RADIOLOGICAL AND HISTOLOGICAL METHODS

X-ray examinations. During the observation period radiological check-ups were made by the usual routine methods. The focal distance was 100 cm. Special attention was paid to ensure that the whole plantar side of the leg was in contact with the film. Some of the animals were anaesthetized with Nembutal before the X-ray examination.

Radiological examination of the specimen. The animals were killed with Nembutal or ether. The tibia and fibula and the free grafts in the muscle were dissected out, examined macroscopically and placed in 5 per cent formalin solution. The radiological examination was made with a special X-ray apparatus adapted for the examination of delicate bone structures (ERK 10/50, made by Messrs. O.Y. Havemann A.B, Helsinki). The voltage and power of the current were modified according to the size and calcium content of the specimens. The photographic data for the radiographs of the most delicate specimens were 20 kW, 10 mA, 20 sec.; focal distance 30 cm.

Histological methods. The specimens were decalcified in 5 per cent nitric acid, embedded in paraffin, cut longitudinally and stained with haematoxylin and eosin for microscopic examination.

RESULTS

NORMAL GROWTH OF THE PROXIMAL ECP OF THE TIBIA AND FIBULA

The results of measurement of the average distance of the proximal ECP of the tibia and the fibula at different ages are seen in table 2. In the rabbit, the distance increased between the ages of 2 and 28 weeks from 3 mm to 8.5 mm. This is the difference in the longitudinal growth of the proximal ECP of the tibia and that of the fibula. In the dog the increase between the ages of 3 and 45 weeks was 6.5 mm, which is the difference in longitudinal length of the diaphyses of the tibia and the fibula.

TABLE 2. — *The distance between the proximal ends of the metaphyses of the tibia and fibula during growth.*

Rabbit											
Age (weeks)	2	3	4	6	8	11	13	17	24	28	
Distance (mm)	3	4	4.5	5.5	6	6.5	7	7.5	8	8.5	
Dog											
Age (weeks)	3	4	8	15	17	22	26	28	30	34	45
Distance (mm)	3.5	4	4.5	5.5	6	7	7.5	8	8.5	9	10

The measurement of the longitudinal growth of the proximal ECP of the tibia and fibula in the dog gave the results seen in table 3. The average growth of the proximal ECP of the tibia was 50 per cent of its total growth. In the fibula the contribution of the proximal ECP was 52.5 per cent. The difference in growth of the diaphyses corresponds to the results seen in table 2, but as the growth in millimetres of the proximal ECP of the fibula and tibia are practically the same, the relative contribution of the proximal ECP of the fibula becomes slightly greater. The value of 51 per cent was used in the calculation, because the greater tendency of the markers to turn in the fibula makes it safer.

TABLE 3. – *The normal growth of the proximal ECP of the tibia and fibula on the dog.*

Animal No.	Age (weeks)	Side	*Tibia* (mm) Length	Growth	Prox. length	Prox. growth	Growth of prox. ECP in %	*Fibula* (mm) Length	Growth	Prox. length	Prox. growth	Growth of prox. ECP in %
271	10	d	77		26			72.5		12.5		
	10	s	77		23.5			72.5		23		
	24	d	128	51	52	26	51	121.5	49	37.5	25	51
	24	s	127.5	50.5	49	25.5	50.5	120.5	48	48	25	52
272	10	d	75		28			70		35		
	10	s	75.5		26			71		12		
	24	d	123	48	52.5	24.5	51	117.5	47.5	60	25	52.5
	24	s	123.5	48	50	24	50	117.5	46.5	36	24	53.5
273	10	d	78		24			73		17		
	10	s	77.5		24			73		22		
	24	d	130	52	50.5	26.5	51	123	50	43.5	26.5	53
	24	s	129.5	52	50.5	26.5	51	122	49	36?	14	(28.5)*)
274	10	d	78		27			73		43		
	10	s	77.5		26.5			72		30.5		
	24	d	130.5	52.5	54	27	51.5	123	49.5	69	26	52.5
	24	s	130	52.5	54	27.5	52.5	122.5	50	57	26.5	53
Average							51					52.5

*) the marker expelled

TRANSPLANTATION OF THE ECP OF THE FIBULA TO THE CORRESPONDING SITE IN THE OPPOSITE LEG IN THE RABBIT

The results are seen in table 4. There was clear growth in 7 out of 8 grafts. In 3 cases the amount of growth was comparable to normal. The graft had fused to the diaphysis in all except one case. Fusion to the epiphysis was noticed in only 3 cases and seemed not to be essential for the preservation of growth. The ECP and the radiological density of bone were interpreted as radiologically normal in all well-grown grafts. A good initial alignment of the graft in the host area seemed to be of significance for the succesful result.

In figures 2 and 3 examples of the experiments are seen.

FIG. 2. — *Transplantation of ECP. Rabbit no. 173. X-ray picture 1 week (1), 9 weeks (2) and 19 weeks (3) after the transplantation. The growth of the graft equalled that of the control.*

TABLE 4. — *Transplantation of the ECP of the fibula to the corresponding site ir*

Animal No.	Age at operation (days)	Time of observation (weeks)	Growth of *graft* (mm)	Growth of ECP of another animal *(contr. I)*	*Growth of graft in %/o of control I*	Growth of prox. ECP of tibia (mm)	Calculated growth of fibular ECP (mm) *(contr.II)*	*Growth of graft in %/o of control II*
112	22	22	19.5	27	72	27.5	24	*83*
171	28	19	10	24	*42*	27	23.5	*43*
172	28	19	10	26	*38*	24	20.5	*49*
173	28	19	24	21	*114*	27	23.5	*104*
323 d	28	29	23	27.5	*84*	28.5	24.5	*94*
323 s	28	29	11	27.5	*40*	25	21	*52*
324 d	28	19	21.5	20.5	*105*	23.5	19.5	*110*
324 s	28	19	0	20.5	*0*	17.5	13.5	*0*

FIG. 3. — *Transplantation of ECP. Rabbit no. 323. The grafts have been interchanged. (1) X-ray picture after transplantation. (2) The specimens. Note the metallic markers for measurement of growth. The growth of the graft was 94 per cent of the normal on the right side and 52 per cent on the left.*

the opposite leg in the rabbit.

Macroscopical and radiological observations					
Fusion of graft to diaphysis	Fusion of graft to epiphysis	Radiol. appearance of ECP	Radiol. density of bone	Initial alignment of graft	Special observations
fused	not fused	open	normal	satisf.	graft slender
fused	not fused	partly fused	decreased	poor	bony prominence at site of fusion with diaphysis
not fused	not fused	partly fused	decreased	unsatisf.	
fused	not fused	open	normal	good	graft slender
fused	fused	open	normal	good	
fused	fused	fused	normal	good	bone bridge between diaphysis of tibia and fibula
fused	fused	open	normal	good	bone bridge as above
fused	not fused	fused	almost 0	poor	graft forms an arch-like bridge on tibial diaphysis

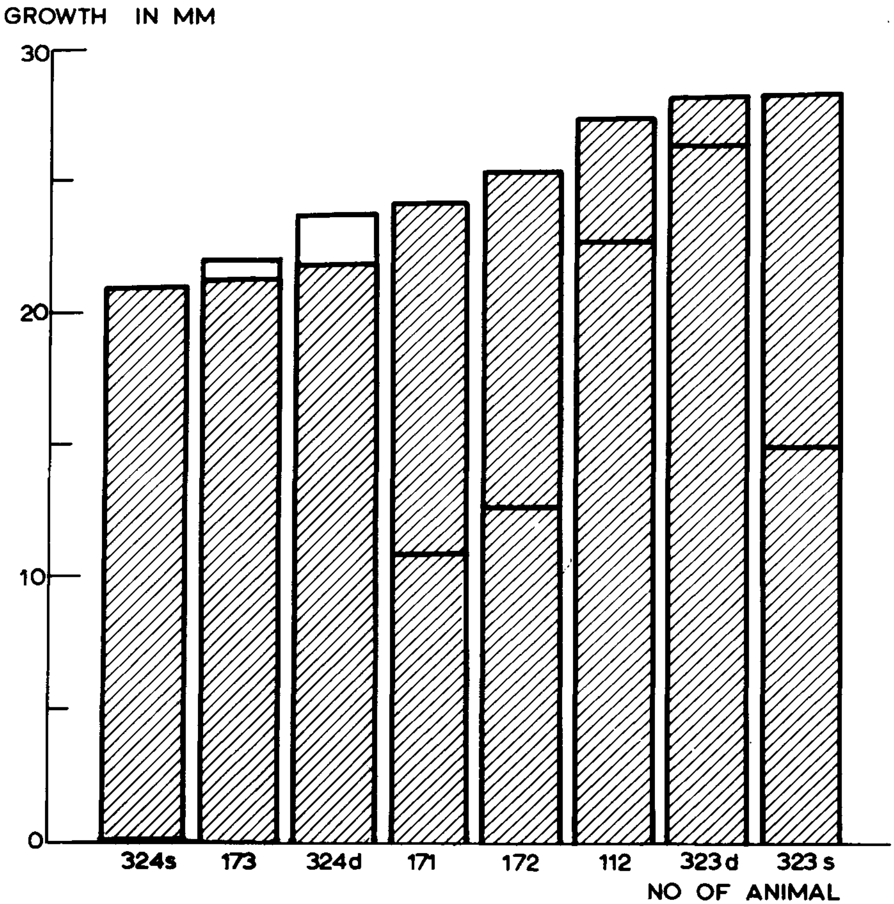


FIG. 4. — *Transplantation of the ECP to the corresponding site in the opposite leg in the rabbit. The growth of the grafts (the level of transversal lines) as compared with the normal growth of the ECP (hatched columns).*

The growth of the grafts as a percentage of the control growth is graphically illustrated in figure 4.

Histological findings

Microscopic examination was made in cases 327 (control), 112, 171, 172 and 173. *Case 327* (control animal, age 33 weeks). See fig. 5. The ECP is regular, and relatively wide. The resting zone is very narrow. For the most part its matrix looks homogeneous, but in places areas of slight granulation are seen. Many of the palisades of the proliferating zone begin very near the bone plate. The number of growing cells in the columns varies between 10—15, the number of hypertrophic cells between 3—7.

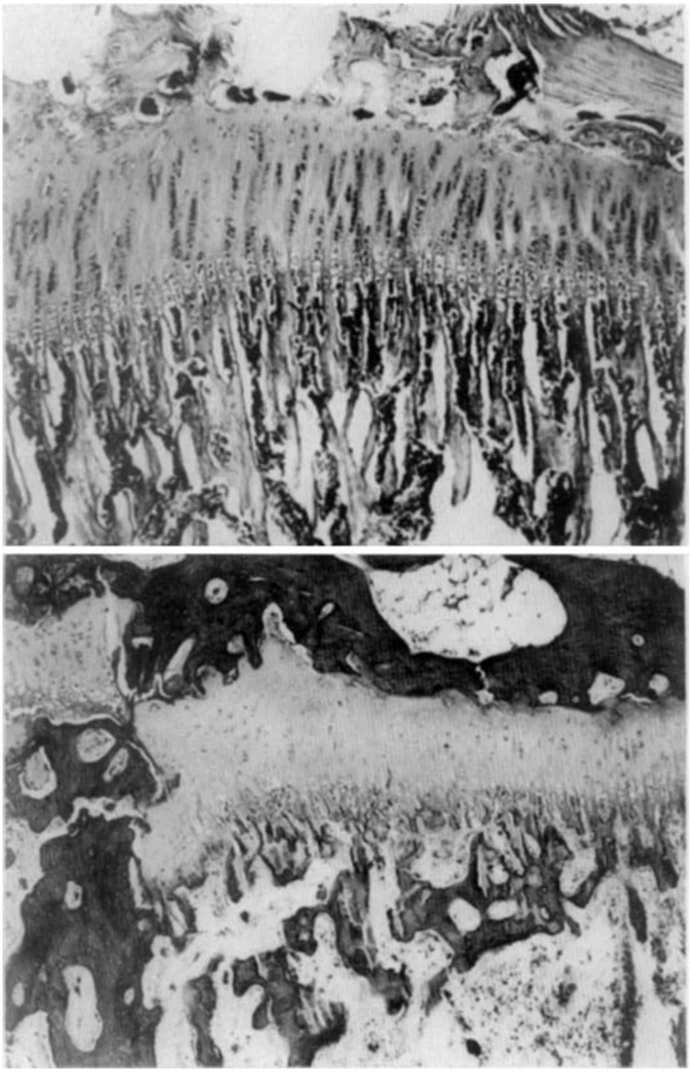


FIG. 5. — *Epiphyseal transplant at the corresponding site in the opposite leg in the rabbit. Above: Normal fibular ECP of a rabbit aged 33 weeks. Case no. 327. Below: The ECP of the transplant in case no. 112. Time of observation 22 weeks. Note the bony bridge between the epiphysis and the metaphysis.*

Case 112 (growth 83 per cent of the control). See fig. 5. The ECP is a little narrower than in the preceding case. Except for the central part, it is mostly fairly regular. The columnar arrangement of the cells is not so clear as in the previous case. The resting zone looks wider than in the control, but areas of granulation or striation are more prominent. The other zones of the ECP are relatively narrow. The zone of bone formation looks thin but otherwise normal. As a prominent feature a marked narrowing is seen in the centre of the ECP. In some sections a bony promontory is seen on both the epiphyseal and metaphyseal surfaces of the plate, separated from each other only by a thin layer of partly ossified connective tissue. The columnar arrangement of cartilage cells in this area is irregular and scattered.

Case 171 (growth 43 per cent of the control). The ECP consists of scattered, short, irregular columns or groups of cartilage cells, separated by deeply and unevenly staining cartilage matrix. The streaked character of the matrix is very striking. The different zones of normal ECP cannot be distinguished. On a narrow area at the metaphyseal border relatively normal formation of bone is seen.

TABLE 5. – *Transplantation of the proximal ECP of the fibula to the corresponding*

Animal No.	Age at operation (days)	Time of observation (weeks)	Growth of *graft* (mm)	Growth of tibia of another animal (mm)	Growth of fibular ECP of another animal (mm) *(contr. I)*	*Growth of graft in % of control I*	Growth of tibia of experimental animal (mm)	Calculated growth of fibular ECP (mm) *(contr.II)*	*Growth of graft in % of control II*
211 d	45	13	7	79.5	39.5	*18*	81	40	*18*
211 s	45	13	10	79.5	39.5	*25*	82.5	41	*24*
212 d	45	13	26	79.5	39.5	*66*	75	37	*70*
212 s	45	13	13.5	79.5	39.5	*34*	75.5	37.5	*36*
221 s	60	11	21.5	69	34	*63*	68	33.5	*64*
221 s	60	11	22.5	69	34	*66*	67.5	33.5	*67*
222 d	60	11	26	69	34	*76*	76	38	*68*
222 s	60	11	16.5	69	34	*49*	74	37	*45*
241 s	23	22	16	97	47.5	*34*	93	45.5	*35*
246 s	23	22	47	97	47.5	*99*	95	46.5	*101*
334 d	26	27	47.5	98	48	*101*	103.5	50	*95*
334 s	26	27	34.5	98	48	*72*	104	51.5	*67*
335 d	26	27	42.5	98	48	*89*	92.5	44	*97*
335 s	26	27	42	98	48	*87*	91	44	*95*

Case 172 (growth 49 per cent of the control). The ECP is thin and irregular and penetrated in the middle by a bony bridge in between the epiphysis and the metaphysis. A thin resting zone is seen only in some areas. The palisades of cartilage cells are scattered, short and separated by a deeply staining, streaked matrix. The zone of bone formation is very narrow or totally lacking.
Case 173 (growth 104 per cent of the control). Compared with case 327, the graft is thin in diameter. The ECP is regular and relatively wide. The resting zone is thin or in places lacking and its matrix stains rather unevenly. The various zones of the ECP are clearly visible, and the columns relatively long, containing 10–15 cells. The zone of calcification and the metaphysis look normal.

site in the opposite leg in the dog.

Macroscopical and radiological observations					
Fusion of graft to diaphysis	Fusion of graft to epiphysis	Radiol. appearance of ECP	Radiol. density of bone	Initial alignment of graft	Special observations
not fused	not fused	fused	greatly decreased	good	
fused	fused	bone bridges	normal	unsatisf.	epiphysis has the form of an hourglass
fused	fused	obligue, bone bridge	uneven in metaphysis	good	angulation at the site of fusion with diaphysis
fused	not fused	partly fused	normal	poor	
fused	[1]	bone bridge	normal	good	
fused	[1]	bone bridge	normal	good	
fused	[1]	bone bridge	uneven in metaphysis	good	
fused	[1]	obligue, bone bridge	normal	satisf.	
fused	fused	fused	normal	poor	
fused	fused	bone bridge	normal	good	
fused	fused	normal	normal	good	epiphysis flat
fused	fused	uneven	normal	good	epiphysis flat
fused	[1]	normal	normal	satisf.	
fused	[1]	normal	normal	good	bone bridge between diaphysis of tibia and fibula

[1] the epiphysis was transplanted as a whole.

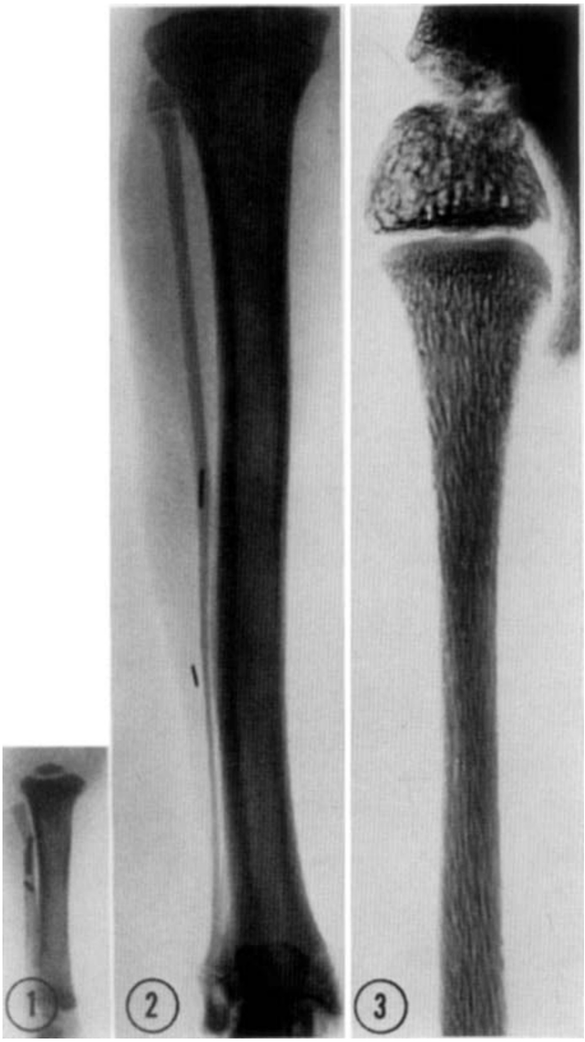


FIG. 6. – *Transplantation of ECP. Dog no. 335 d. X-ray control after transplantation (1) and after an observation period of 27 weeks (2). (3) Enlarged X-ray picture of the specimen. The growth of the graft equalled that of the control.*

The histological structure of the ECP was normal in one case (173), in which the graft had grown as much as the control. In others, changes which differed from the normal were seen. Among these, the narrowness of the ECP and especially of the resting zone, the irregular pattern of the cartilage columns and the bony and connective tissue streaks in between the epiphysis and the metaphysis were the most prominent.

TRANSPLANTATION OF THE ECP OF THE FIBULA TO THE CORRESPONDING SITE IN THE OPPOSITE LEG IN THE DOG

The results of the transplantation of the ECP in the dog are seen in table 5. The growth of the graft was seen in all 14 experiments. In 4 cases the amount of growth was practically normal. Fusion of the graft to the diaphysis had taken place in all except one experiment. Fusion to the epiphysis was evident in 6 cases but had not occurred in 2 out of 8 cases in which the epiphysis had been cut. The ECP was radiologically normal in three cases, which had also grown normally. In all the other experiments some abnormalities, such as unevenness or bony bridges, were seen. The radiological density of the bone was interpreted as normal in most cases. The initial alignment of the graft in the host area was good in the cases where the graft had grown well.

In figures 6, 7, and 8 some examples of the transplantation of the ECP in the dog are seen.

A graphic illustration of the observed growth of the grafts compared with their calculated growth is presented in fig. 9.

Histological findings

In cases 73 (control), 74 (control), 82 (control) ,211 s, 212, 221, 222, 334 and 335 histological examination was made.

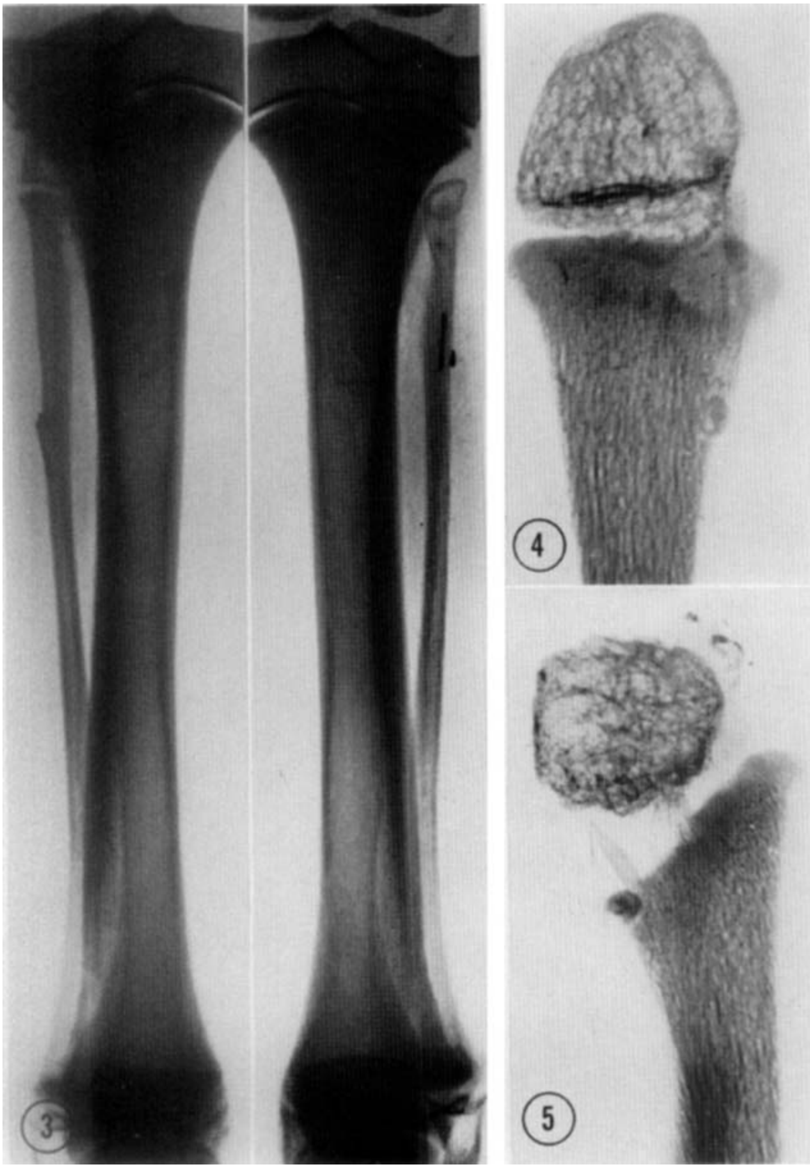
Case 73 (control animal, age 28 weeks). See fig. 11. The ECP is rather thick and its structure is regular. The various zones of the cartilage are clearly distinguished. The resting zone consists of about 1/5 of the thickness of the plate. Its matrix is homogeneous except for some minor areas of slight granulation or streaking. The columns are relatively long, consisting of 10—20 cells. The columns in the zone of intracellular oedema consist of 3—5 cells and the zone of degeneration is of about the same thickness.

Case 74 (control animal, age 28 weeks). The microscopic picture does not differ significantly from case 73.

Case 82 (control animal, age 21 weeks). The ECP is narrower than in the two foregoing cases and more irregular in character. The bone plate is penetrated by numerous blood vessels. In some places the resting zone comprises almost half the thickness of the ECP, but is totally lacking in others. Its matrix shows areas of granulation and weak staining. The columnar zone is relatively narrow and the arrangement of the columns slightly irregular. The zone of intracellular oedema is relatively higher than in the two foregoing cases.

Case 211 s (growth 24 per cent of control). The ECP is of about the same thickness as in case 82 and rather regular. The various zones are clearly distinguished. The resting zone is seen only in places. The cartilage columns are very short and scattered, containing only 6—7 cells. The matrix shows pronounced fibres throughout the ECP. In some sections massive bands of partly ossified connective tissue connect the metaphysis with the epiphysis. The zone of intracellular oedema and that of degeneration, like the metaphysis, look normal.

FIG. 7. – *Transplantation of ECP. Dog no. 222. The grafts have been interchanged. X-ray pictures 5 days (1), 5 weeks (2) and 11 weeks (3) after transplantation. (4) and*



(5) Enlarged X-ray pictures of the specimens. Growth of the graft on the right side 68 per cent and on the left 45 per cent of the control.

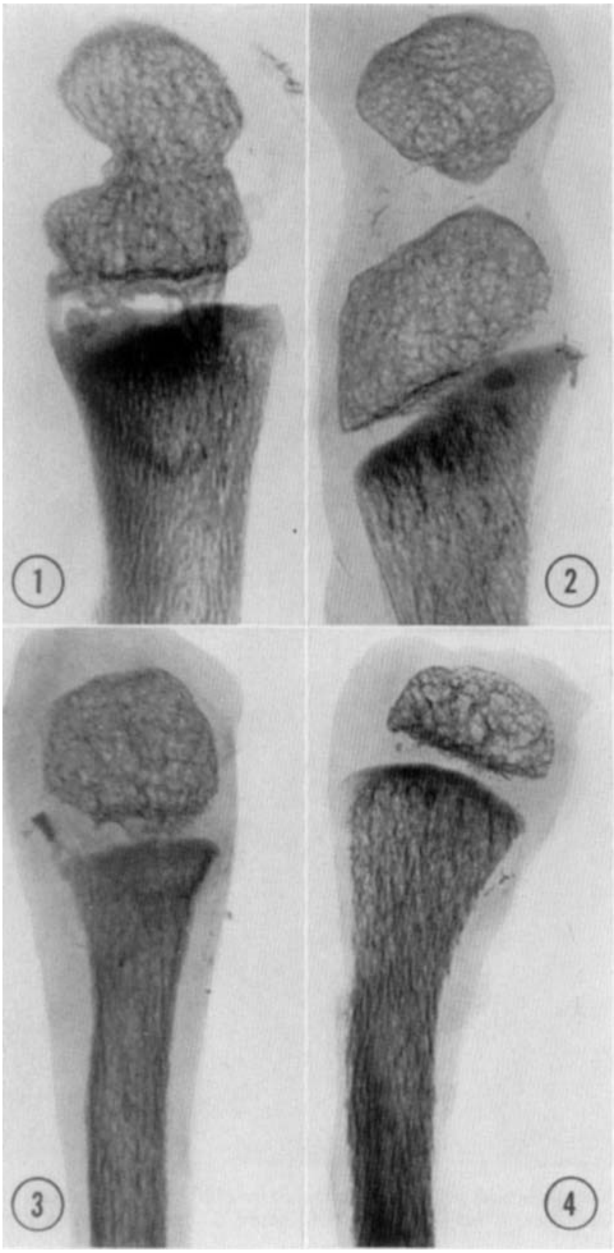


FIG. 8. – *Transplantation of ECP in the dog.* ***Enlarged*** *X-ray picture of the specimen in the cases 211 s (1), 212 s (2), 221 d (3)* ***and*** *221 s (4).*

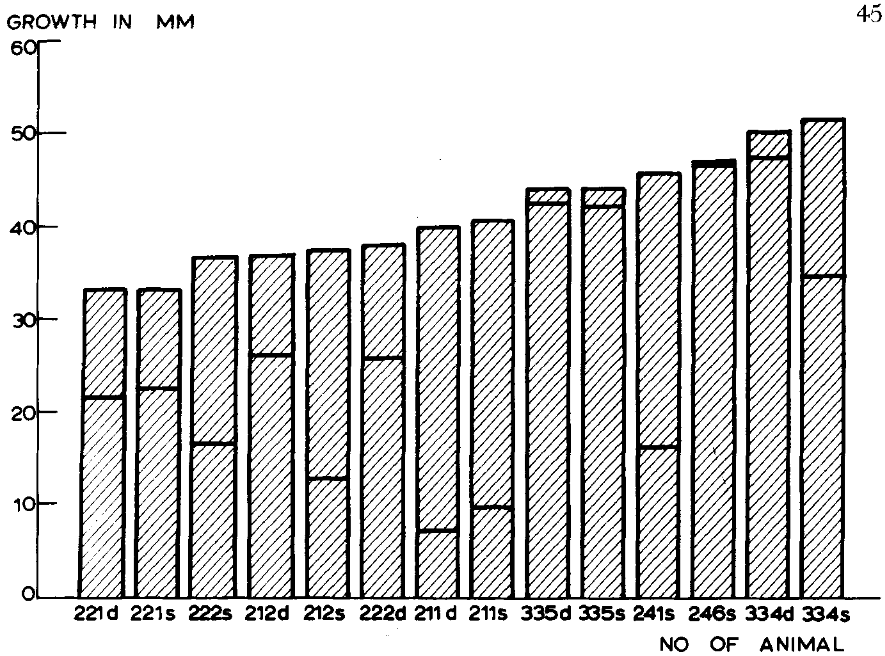


FIG .9. — *Transplantation of fibular ECP to the corresponding site in the opposite leg in the dog. The growth of the grafts (the level of transverse lines) as compared with the normal growth of the ECP (the hatched columns).*

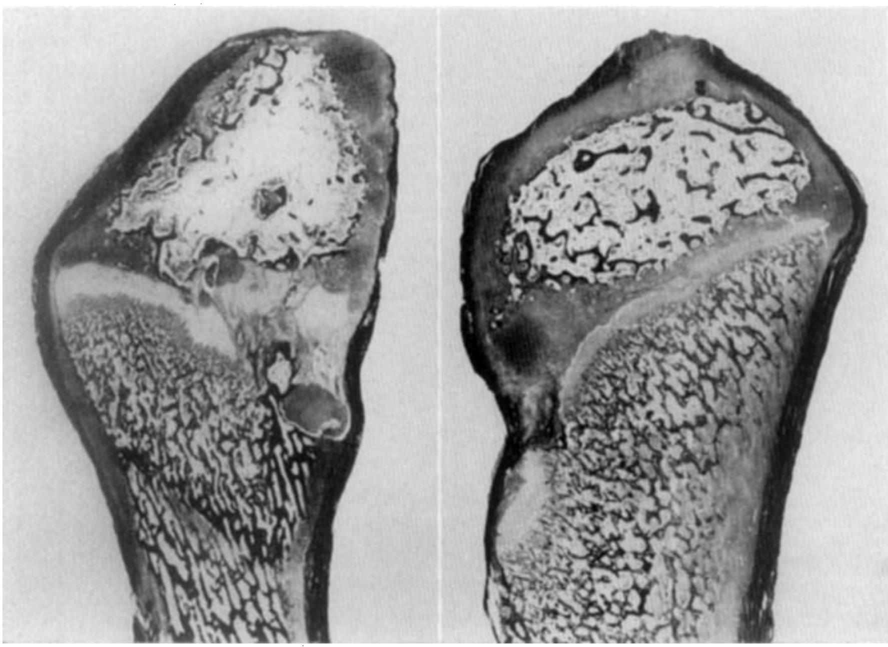


FIG. 10. — *Microscopical picture of the fibular ECP graft at the corresponding site in the opposite leg in the dog. Left: case no. 222 s. Right: case no. 212 d. Note the irregular pattern of the ECP and the ossified bridge traversing it in both specimens.*

Case 212 d (growth 70 per cent of the control). See fig. 10. The thickness of the ECP is about the same as in the foregoing case. Its form is for the most part relatively regular, but it is penetrated by several thin connective tissue bands and one massive, partly ossified bridge. The thickness of the resting zone varies considerably. Except for minor areas of granulation it appears rather amorphous. The cartilage columns are short and, in the areas of traversing connective tissue bands, scatter and diverge from the longitudinal plane. A massive, partly ossified conical bridge of connective tissue is situated near the border of the ECP. The whole plate seems to be inclined to this side and the bony trabeculae in the metaphysis are correspondingly oblique. On the opposite side of the metaphysis very intensive bone resorption is seen.

Case 212 s (growth 36 per cent of control). The thickness of the ECP is about the same as that of the control animal. The form of most of the plate is more or less regular. The resting zone is lacking in many parts; its matrix stains unevenly and shows areas of granulation and streaking. Numerous deeply staining bands of fibres traverse the ECP. In these areas the arrangement of the short palisades of cartilage cells is irregular. Near the lateral border of the ECP a partly ossified bridge of connective tissue, containing a blood vessel, is seen. On the proximal surface of the epiphysis a partly ossified piece of cartilage is seen connected to the epiphysis by cartilage tissue.

Case 221 d (growth 64 per cent of control). The greater part of the ECP is in some sections relatively regular, but very thin. The resting zone is lacking in places and in its stead there is a narrow zone of fibrous tissue. The columns of cartilage cells are very short, consisting of only a few cells. The matrix forms massive, deeply staining bundles of fibres traversing the whole ECP. In some sections connective tissue bands are seen traversing the plate. In one section a broad, partly ossified bridge of connective tissue is seen and in this area the whole structure of the cartilage is disorganized.

Case 221 s (growth 67 per cent of control). In some sections, the ECP looks relatively regular, especially in its peripheral parts. Its structure is very similar to that of the foregoing case, with the exception that the matrix is more homogeneous. The central part of the ECP is in places very irregular, its width varying considerably. The resting zone is mostly lacking. The columnar arrangement is in many places disordered and the plate is traversed by several partly ossified bridges of connective tissue, which contain relatively large blood vessels.

Case 222 d (growth 68 per cent of control). Compared with the control animal no. 82 the ECP is wider. Its form and structure are very regular. In the resting zone areas of fibrous tissue and unevenly staining areas of matrix are seen. In places relatively broad bundles of collagenous fibres traverse the plate. The columnar arrangement of the cells is mostly quite regular and the zone of ossification looks normal. In one section a bridge of collagenous tissue is seen traversing the plate near its peripheral border. In this area a step-like formation in the ECP is seen.

Case 222 s (growth 45 per cent of control). Only about half of the ECP appears relatively normal. In its middle part a massive bridge, consisting of bone, fibrous tissue, blood vessels and scattered cartilage cells, connects the epiphysis and the metaphysis. The rest of the epiphyseal cartilage consists of unevenly staining areas of cartilage, partly separated from each other by fibrous tissue. In this area no regular endochondral ossification is seen. See fig. 10.

Case 334 d (growth 95 per cent of control). The microscopic picture corresponds to that of control animal no. 73. In its central parts the resting zone shows minor areas of uneven staining and granulation and the zone is in places very narrow.

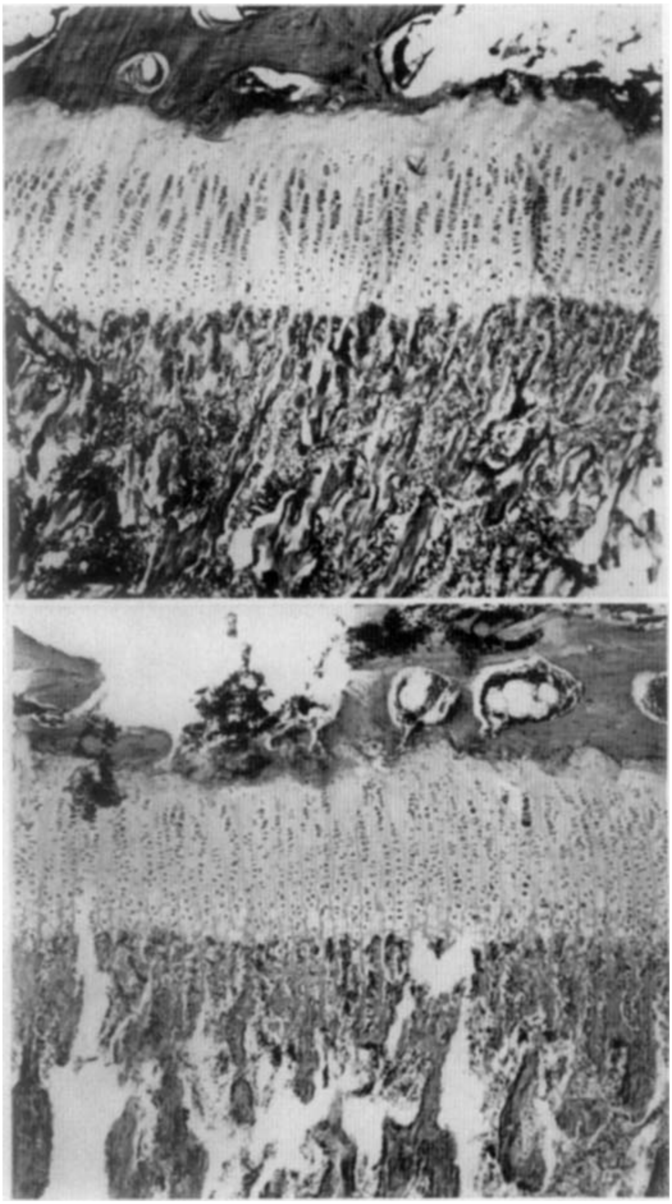


FIG. 11. – *ECP graft at the corresponding site in the opposite leg in the dog. Above: control animal no. 73. Below: case no. 335 d. The microscopic structure of the transplanted ECP corresponds to that of the control animal.*

Case 334 s (growth 67 per cent of control). The histological picture is rather similar to that of the foregoing case, except that the resting zone is lacking in places and in the middle of the ECP a narrow band of collagenous tissue traverses the plate.

Case 335 d (growth 97 per cent of control). See fig. 11. The form and structure of the ECP corresponds to that of control animals nos. 73 and 74.

Case 335 s (growth 95 per cent of control). No significant difference in the microscopic picture compared with the control animals is observable.

The microscopic structure of the ECP was normal in three cases (334 d, 335 d and 335 s). The growth of the graft in these was about as great as that of the control. In all other specimens microscopic examination revealed changes which were not found in control epiphyseal lines of about the same age. Partly or totally ossified bridges of connective tissue were seen traversing the plate in most specimens. In some cases the ECP was very irregular and its structure grossly disturbed.

TRANSPLANTATION OF THE ECP OF THE FIBULA INTO THE MUSCLE

The results of transplantation of the ECP to the calf muscle, both in the rabbit and in the dog, are seen in table 6. In the rabbit some growth of the graft was seen in 6 out of 8 experiments. In the dog growth had taken place in 3 out of 6 experiments. The amount of growth in all of these cases was relatively insignificant. The graft was not found in 2 cases in the rabbit and in 3 cases in the dog. The diaphyseal end of the graft usually became thin and the radiological density of the graft decreased. The graft moved in the distal direction during growth.

By measuring the distance between the ECPs of the tibia and the graft, an attempt was made to study the possible stimulating effect of the former upon the growth of the graft. The results obtained show no definite correlation between the distance and the amount of growth, but it is seen that the two best grown grafts were situated very near the ECP of the tibia after transplantation.

In fig. 12 the development of the graft 253 s, which had grown the most, is seen.

Histological findings

In cases 253, 241 and 246 histological examination of the specimens was made.

Case 253 s. The specimen consists of a piece of bone formed by a peripheral layer of compact bone and a central cancellous layer. From the peripheral compact bone trabeculae are orientated towards the centre. On one side a layer of hyaline cartilage

TABLE 6. – *Transplantation of the ECP of the fibula into muscle on rabbit (R) and on dog (D).*

Animal No.	Age at operation (days)	Time of observation (weeks)	Growth of graft (mm)	Growth of proximal ECP of fibula in control animal (mm)	Growth of proximal ECP of tibia (operated leg) (mm)	Distance of graft from ECP of tibia after operation (mm)	Growth of graft in % of control
R 251 d	19	19	3	31	35.5	7	10
R 251 s	19	19	2 [2])	30	34.5	8	7
R 252 d	19	19	7	30	34	2	23
R 252 s	19	19	2 [2])	29.5	33.5	5	6
R 253 d	19	19	2	29	33	10	6
R 253 s	19	19	9	29.5	33.5	1	27
R 254 d	19	19	0 [1])	32	36	2	0
R 254 s	19	19	0 [1])	32	36	6	0
D 241	23	22	8	49.5	47.5	10	16
D 246	23	22	4 [2])	49.5	48.5	5	8
D 281	16	30	0 [1])	54	52.5	5	0
D 282	16	11	0 [1])	30.5	33.5	19	0
D 283	16	30	0 [1])	54	56.5	10	0
D 284	16	13	3	54	39	4	5

[1]) the graft has been resorbed
[2]) uncertain, because the ECP is not visible.

is seen on the surface of the specimen. No signs of endochondral ossification are visible.

Case 241. See fig. 13. The ECP is relatively regular and wide. The epiphysis is partly ossified. The resting zone is relatively wide. In it blood vessels are seen. In many sections the cartilage columns are arranged in a slightly radiating manner. The primary spongiosa of the metaphysis looks broader than normal.

Case 246. The specimen is composed of a non-ossified epiphysis, a partial ECP and a metaphysis. The ECP only reaches to the middle of the specimen. It is very narrow and no regular cartilage columns are seen. The cartilage cells are arranged in scattered groups, in which the more distally situated cells are oedematous and degenerating.
The rest of the site of the ECP is filled with fibrous tissue.

DISCUSSION

The experiments were performed on both rabbits and dogs, which had both advantages and inconveniences. The data were less uniform; more factors came into play; because of the difference in anatomy more technical practice was

FIG. 12. – *Growth of the epiphyseal graft in the calf muscle. Rabbit no. 253 s. The unmarked graft is seen at the site indicated by the arrow. X-ray picture 6 days (1), 3 weeks (2), 6 weeks (3) and 19 weeks (4) after transplantation. In the same leg a suture transplant, with markers, is seen at the site of the excised fibular ECP.*

needed, etc. Since the earlier experimental investigations have been done almost exclusively on the rabbit, this species was chosen as the chief experimental animal for the sake of comparison. On the other hand, the larger proportions and "more human" anatomy of the leg of the dog offered a valuable addition to the work. Because of the stable anatomical situation and proportionately greater longtudinal growth, the ECP of the fibula, transplanted to the corresponding site of the opposite leg, was suitable for these experiments, where the maximal growth capacity was the object of the study. The selection of these animals, and also of the operative technique and marking procedures, took place after a great number of preliminary experiments.

The routine X-ray methods, though quite adequate for checking the growth of the graft, were seen to be rather unsatisfactory for a study of the structure of the specimen. In this respect the usefulness of the special radiographs was evident.

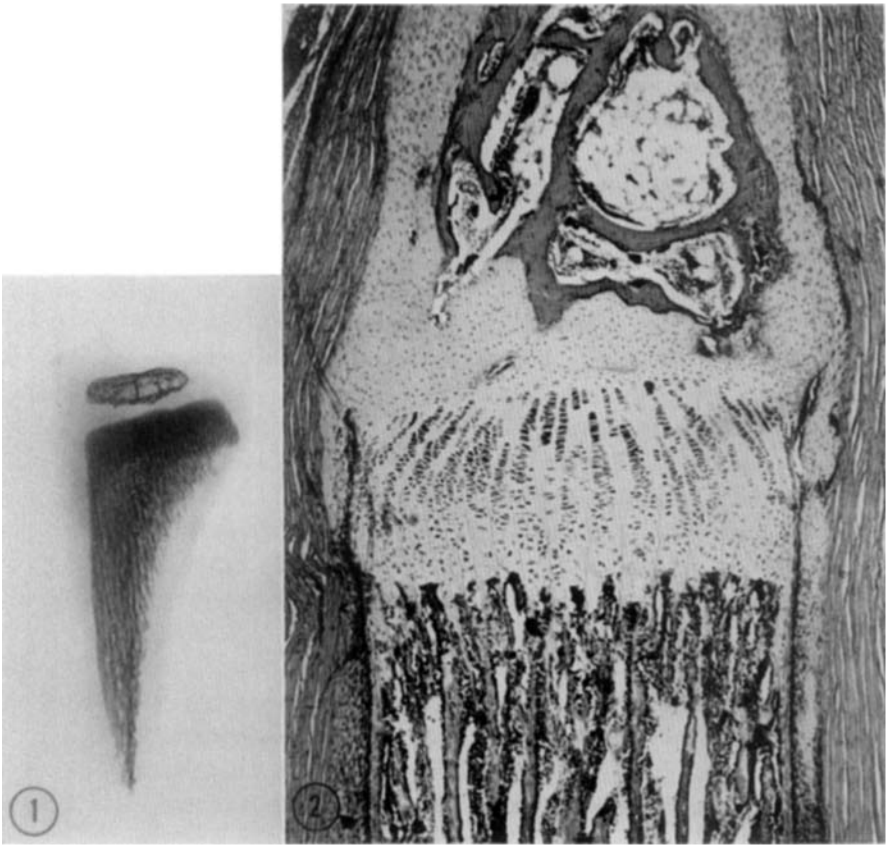


FIG. 13. — *Epiphyseal graft in the calf muscle. Dog no. 241. (1) Enlarged X-ray picture of the specimen. (2) Microscopic picture of the same. The ECP is relatively regular and wide.*

In order to reduce any possible error due to using the tibia as a control of the growth, two types of control were used. In the rabbit, the values obtained from the tibia and from the fibula of another animal of the same litter differed sigificantly. It was evident that the graft had disturbed the growth of the tibia in some cases. In the dog, no difference in the results was noticed. This may be because the dog's fibula, which is not fused with the tibia, did not, at least to the same extent, have a restraining influence on the growth of the tibia.

As was already mentioned, the marking method was chosen after many preliminary experiments. Many investigators have previously used metal markers, but their drawbacks have usually not been pointed out. Some authors (*Silfverskiöld* 1934. *Bisgaard* and *Bisgaard* 1935. *McElvenny* 1963) have mentioned them, however. During growth a continuous remodelling of the bone takes place. As the result of growth the marker situated in the metaphysis gradually moves in the direction of the diaphysis. As the result of remodelling it may become situated beneath the periosteum. The marker situated in the middle of the metaphysis may even become situated in the medullary cavity (*McElvenny*). In the present material it was seen in many cases (see fig. 6) that the transverse metal marker often gradually turned in a longitudinal direction and became situated beneath the periosteum.

Langenskiöld and *Edgren* (1950) noticed that the focal changes caused at the end of the metaphysis by local irradiation gradually moved towards the surface and in the direction of the diaphysis. They concluded that in some layer of the ECP a movement of the cells in the transverse direction occurs. They also presented the hypothesis that the growth of the periosteum could take place by the same mechanism. *Humphry* (1861) was of the opinion that the periosteum grows interstitially. If we suppose that the growth of the periosteum is interstitial, in contrast to the growth of the bone beneath it, the conclusion of *Kornew* (1929) that the tubular bones grow interstitially can perhaps be understood. His markings were circular cerclage wires, the movement of which would correspond to the growth of the periosteum.

In some experiments of the present work the metal marker became situated in the soft tissues outside the bone during growth. Then it usually showed a tendency to move distally more rapidly than would be accounted for by growth of the bone. This could be clearly seen in instances in which the other marker was situated in the bone. This source of error could be eliminated by following the growth radiographically. The marker situated beneath the periosteum seemed not to move in respect to the bone.

In spite of disagreement among investigators regarding the success of epiphyseal cartilage grafts, the experimental results of some authors (*Helferich, Heller, Fohl, Silfverskiöld, Sousa Pereira, Ring, Schraiber, Heikel*) support the view that preservation of the growth capacity is possible. These earlier published investigations did not provide a satisfactory object of comparison for the present work, because of the different methods or because exact results and controls were often not given. The most satisfactory comparison was offered by the works of *Silfverkskiöld* and *Heikel,* whose experiments were well planned and so gave very reliable results. In the most successful cases transplantation of the ECP to the corresponding site on the opposite leg gave, according to *Silfverskiöld,* growth amounting to 50 per cent of the normal. The fibular graft used by *Heikel,* when transplanted to the site of the radius, in the best cases gave 1/2—2/3 of the growth of the corresponding fibular ECP.

In the present work longitudinal growth which was comparable to the normal was seen in some cases in both rabbits and dogs. In the light of the results obtained there should be no doubt that the transplanted ECP can retain its growth capacity and in successful cases grow as much as its origin presupposes. A very striking feature was the significant variation in the amount of growth. This was reflected rather well in the appearance of the grafts at the end of the observation period. Practically all grafts in which growth was clearly disturbed showed macroscopic, radiological and microscopic abnormalities. A considerable amount of growth was seen in some cases where ossified bridges connected the epiphysis and metaphysis. This is in accordance with the results of *Ford* and *Key* (1956), *Sieffert* (1956), *Friedenberg* (1957) and *Johnson* and *Southwick* (1960) who noticed that longitudinal growth could continue in spite of local epiphyseodesis.

The result of transplantation to the muscle corresponded to the result obtained by *Heikel* and showed that the graft may continue growing to a slight extent. This growth was of no practical value, however, because the graft became very thin. *Silfverskiöld* made the same observation in the case of his pedicle graft, which grew almost normally in length. These results confirm the truth of the statement first made by *Roux* (1895) that preservation of function is essential for successful transplantation. The hypothesis that the graft would grow better if its ECP were near the ECP of the neighbouring bone was supported by the two most successful results.

What are the factors which cause disturbance or cessation of growth after transplantation? On what does the marked variation in the results depend? The data of the present study permit only a limited discussion of these problems.

If the survival of the graft cells is considered essential for the preservation of growth, one of the main tasks is the rapid restoration of nutrition. The survival of the cells in the germinal layer seems to be especially important. Transplantation causes a temporary arrest of nutrition. If this interval is too long there is a disturbance or arrest of growth. The time needed for revascularization is influenced by the distance that the new vessels have to grow. *Stringa* (1957) found the rapidity of revascularization in cortical bone to be 3 mm per week. It must be noted, however, that the vessels of the graft may make contact with the host circulation as soon as the new vessels have perforated them (*Peer* 1955). The periosteum of the autogenous bone graft is revascularized about 24 hours after transplantation (*Hammack* and *Enneking* 1960).

Some earlier investigators (*Heller* 1918, *Silfverskiöld* 1934, *Sousa Pereira* 1937, *Ring* 1955) point out that the most successful grafts have been those where the cut line has been near the ECP. In the present work the epiphyseal cut line was near the ECP except in those cases where the epiphysis was transplanted as a whole. No significant difference in the results was noticed between these two groups. No attempt was made to determine the significance of the length of the diaphyseal surface. The distance of the cut line from the

ECP was about the same in the same animal species. A good initial alignment of the graft in the host area seemed to be of significance for the succesful result.

CONCLUSIONS

1. The ECP of the fibula in both the rabbit and the dog may, when transplanted to the corresponding site of the opposite leg, retain its full growth capacity, i.e. the capacity to be expected in its original site. But in spite of great care in technical performance, some not easily definable factors very often cause a disturbance of growth.

2. In successful cases the transplanted ECP does not differ in either appearance or structure from the normal at the end of the observation period.

3. Without bony contact between the graft and the host bone, only insignificant growth of the graft can take place.

III TRANSPLANTATION OF CRANIAL SUTURE

REVIEW OF THE LITERATURE

The membranous bones of the cranial vault develop in the membranous capsule of the brain from the centres of ossification (*Gardner* 1956, *Weinmann* and *Sicher* 1947). When two adjacent bones come into close relation, a suture develops between them, which is thus a remnant of the membranous capsule. The bone so formed consists of immature coarsely fibrillar bone (*Weinmann* and *Sicher*), also called foetal, coarse-fibred or plexus-like (*Weidenreich* 1930) or primary bone ["primäres Knochengewäbe" (*Thoma* 1917–18)]. Gradually an outer and an inner layer of compact bone develop on it. The formation and remodelling of the particular lamellar structure takes place for the most part postnatally (*Gardner*). As the result of these processes the lamellar structure and the Haversian lamellae of the spongiosa develop. By resorptive mechanisms the diploë characteristic of the bones of the cranial vault is formed.

The course of the frontal suture is more or less rectilinear on the inner surface of the calvarium; on the outer surface it very early becomes serrated. Histologically, the suture comprises three layers (*Weinmann* and *Sicher*). The middle layer consists of an irregular feltwork of connective tissue fibres. It is rich in cells. In the middle layer moderate numbers of blood vessels are also seen (*Thoma*). On both sides of the middle layer, adjacent to the bone, are the peripheral layers, which consist of dense connective tissue. The fibres of this continue into the bone as Sharpey's fibres. The suture is bounded by marginal bone (Agger marginalis, *Thoma*). On its margins, scattered or in groups, osteoblasts are seen, which differ very little from the connective tissue cells. The osteoblasts near the bone margins are somewhat flattened, while those farther from the bone are rounder and more isodiametric. At the margins, osteoclasts are also situated in the resorption lacunae. The marginal bone differs from the other cranial bone in being strongly basophilic and containing an abundance of Sharpey's fibres (*Laitinen* 1956). The affinity for basic dyes decreases with age and in an adult animal there is only a thin dark basophilic layer of bone at the suture margin.

In the ossifying suture the number of connective tissue cells and fibres is decreased, the fibres becoming thin and scattered. The suture becomes thinner and by the action of osteoblasts bony trabeculae are formed. Fat cells and weakly staining homogeneous substance and mucous tissue are also characteristic of the ossifying suture (*Thoma, Laitinen*).

The ossification of the suture proceeds, according to *Thoma*, from the inner table; according to *Moss* (1954), it proceeds simultaneously on both sides but the process advances more rapidly on the outer surface. The ossified suture consists of islets of bone separated by cement lines. Cavities are often seen in it. When the bone margins come into contact with each other, a bony mass is formed at the site of the previous suture.

The arteries supplying the parietal and frontal bones come from the middle meningeal, middle temporal and the deep posterior temporal arteries (*Langer* 1877, *Hyrtl* 1859, *Merkel* 1907, *Luschka* 1865, *Rauber-Kopsch* 1940, *Corning* 1949). *Hyrtl* noticed that the rami perforantes of the middle meningeal artery also penetrates the sutures. *Thoma* showed that both the tables of the skull were penetrated by a vascular network transmitted by parallelly situated Volkman's canals. He also saw vessels running from the dura mater straight to the suture and from the suture to the marginal bone and vice versa. Opinion has been divided about the relative importance of the different vascular systems in the nutrition of the bone. According to *Treves-Keith* (1914), the nutrition of the bone is provided mainly from the dura and stoppage of the blood supply from the dura results in necrosis. These statements concern the human calvarium.

Goldhahn (1924) performed experiments on human beings and on the dog by the injection method, and concluded that the relative importance of the vessels transmitted to the bone from the periosteal side was greater in the dog than in man.

The part played by the sutures in the growth of the calvarium has been much discussed (*Brash* 1934, *Weinmann* and *Sicher* 1947, *Christensen et al.* 1960, among others). Some, like *Brash*, have been of the opinion that the growth in the size of the cranium can be explained by surface accretion and surface absorption alone, without any real separating growth in the suture. It is generally accepted that no interstitial growth takes place in the bone (see p. 14). According to *Weinmann* and *Sicher*, "the role of the proliferation of sutural connective tissue in the growth of the skull can be best expressed by saying that the skull, as a whole, enlarges by proliferation of sutural connective tissue". The growth of the individual bones takes place at the suture by apposition. As a histological phenomenon the growth of membranous bones is strikingly parallel to the endochondral growth of the long bones, although it is not so complicated (*Weinmann* and *Sicher*, *Sissons*, 1956).

Laitinen (1956) brought about artifical fusion of the sutures in the rabbit by inserting clips into them. In some instances the clips served as markers and showed that the distance between these and the suture increased as a result of sutural growth.

David (1896) reimplanted a piece of the calvarial bone in the dog and followed its fate histologically. In the second week vessels grew from the fracture line to the graft. The histological picture was normal by the eleventh week. He concluded that the major part of the bone survived reimplantation. Most investigators have been of the opinion, already expressed by *Barth* at the end of the nineteenth century, that all or almost all osteocytes die (*Peer* 1955). There is disagreement among authorities regarding the survival of bone cells after transplantation. *Peer*, in his detailed survey of the literature, came to the conclusion that osteocytes in autogenous grafts in favourable transplantation sites survive as living entities, especially cancellous grafts with open structure or a thin cortex, and in contact with bone.

Opinions also differ as to the significance of the periosteum for successful transplantation. *Vainio* (1948), in his experimental study on the rabbit, came to the conclusion that the periosteum has a vital significance in the regeneration process. In his experiments the periosteal graft of compact bone always regenerated more quickly than an otherwise similar graft without periosteum. According to *Peer*, most investigators now agree that the revascularization of periosteal grafts takes place more quickly but that the periosteum cannot be considered essential for successful transplantation. The periosteum, like the fascia graft, preserves its structure in transplantation and the connective tissue cells remain viable (*Peer*).

The poor regenerative capacity of the calvarial bone in the adult human is well known. In animal experiments, however, it has been noticed that repair of calvarial defects readily occurs, especially in young animals (*Troitzky* 1932, *Simpson et al.* 1953, *Sirola* 1960). Similarly, in children the regenerative capacity of the calvarial bones seems to be much better than in adults (*Glaser* and *Blaine* 1936, *Rowbotham* 1949, *Ingraham* and *Matson* 1954, *Grob* 1957). Relatively large defects of the calvarial bones have been repaired well in infants (*Sirola*). In examining the repair of the defects of calvarial bone in the rabbit and the cat, *Sirola* noticed that if the defect was covered with a polythene film, the suture did not regenerate, even if marked regeneration of the bone took place.

The growth of bone grafts embodying the cranial suture seems not to have been the subject of any previous experimental investigation. In preliminary experiments the author (*Ryöppy* 1961) has made a radiological study of the reimplanted suture graft, and noticed that the suture was able to retain its appearance almost unchanged.

PROBLEMS

1. Does the coronary suture preserve its capacity of growth after reimplantation?
2. Does the coronary suture preserve its capacity of growth when it is transplanted as an autograft to the site of the excised ECP of the fibula? What is the extent of its longitudinal growth compared with
   – the growth to be expected in its original site?
   – the normal growth of the corresponding ECP of the fibula?
3. How does the appearance and structure of the sutural graft change in the new site?

MATERIAL AND METHODS

MATERIAL

59 rabbits and 19 mongrel dogs were used as experimental animals. Because of complications 7 rabbits and 2 dogs were excluded from the material. The age of the rabbits varied between 16–40 days and that of the dogs between 16–60 days. The time of observation varied between 11 and 41 weeks. The animals were chosen without reference to sex, size or colour. On animals of the same litter different types of operations were often performed.

The types and number of experiments on both animal groups are listed in table 7. In addition, a number of different control experiments were performed. These are listed in the same table.

OPERATIVE METHODS
(fig. 14)

The anaesthesia and preparation of the animals for operation were the same as in the transplantation of the ECP.

Metal markers in the calvarium. The aim of the experiment was to study how much the coronary suture grows normally and to test the value of the markers as a control method. On each side of the coronary suture at a distance of about 3–5 mm from it, a hole was drilled through the bone with an injection needle and a piece of metal wire 1–2 mm in length was inserted into it.

Reimplantation. The aim of the experiment was to study whether the suture preserves its growth capacity in reimplantation. A longitudinal incision was

TABLE 7. — *Transplantation of calvarial bone graft containing a piece of coronary suture. A number of control experiments are included in the table.*

Type of experiment		Number of experiments Rabbit	Dog
Transplantation of the suture			
Reimplantation		16	9
Reimplantation with polythene film		6	3
Transplantation to the fibula		32	16
Transplantation to the thigh		8	
		62	28
	Total	90	
Control experiments			
Measurement of normal growth of suture with the aid of metal markers		5	7
Transplantation of calvarial bone to the fibula (without suture)		5	
		10	7
	Total	17	
Total		107	

made on the top of the head. The coronary suture was exposed. At the site of the suture a rectangular bone plate with its periosteum attached was cut with a scalpel in such a way that the suture ran in the direction of the shorter side of the rectangle. The size of the plate in the rabbit was about 6—8 × 4—6 mm and in the dog 9—11 × 7—9 mm. The medial incision was some millimetres from the sagittal suture. In both short sides of the plate a metal marker was inserted. The plate was then reimplanted. No fixation was needed. Closure was made with interrupted catgut sutures and the wound was covered with a plastic film (Nobecutan®).

Reimplantation with a polythene film. The aim was to study whether the suture graft would preserve its growth capacity if it was isolated from the dura. The operation was similar to the reimplantation described above, except that a circular piece of polythene film, cut a little larger than the reimplant, was placed in between the bone plate and the dura.

Transplantation to the fibula. The aim was to study whether the cranial suture, transplanted to the site of the excised ECP of the fibula, would preserve its growth capacity. The bone plate containing the coronary suture was taken and marked as described above. The graft was placed in physiological saline

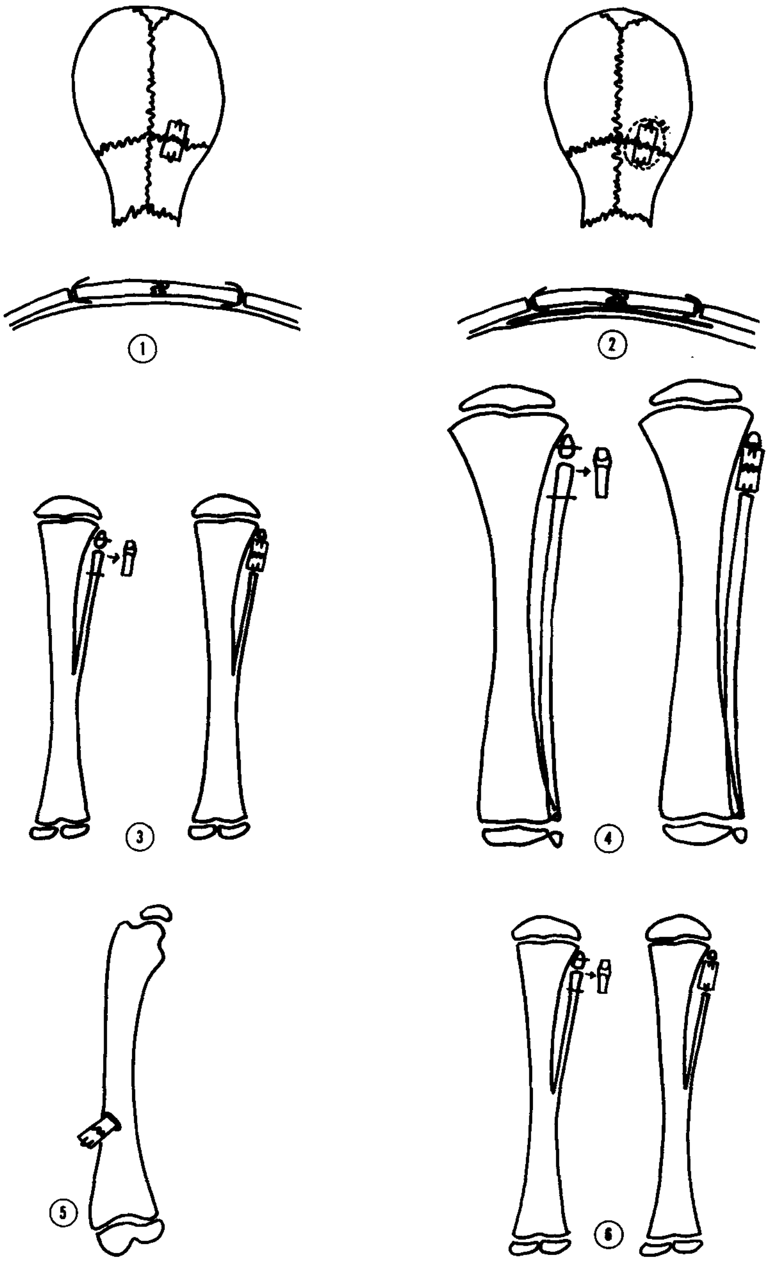


FIG. 14. — *Transplantation of the cranial suture. Operative methods. (1) Reimplantation of the bone plate containing a piece of coronary suture. (2) Reimplantation with polythene film placed in between the graft and the dura. (3) Transplantation of suture graft to the site of the excised ECP of the fibula in the rabbit. (4) The same in the dog. (5) Transplantation into the thigh. (6) Transplantation of a piece of parietal bone to the site of the excised ECP of the fibula (control experiment).*

solution. A piece of bone containing the ECP and part of the epiphysis and metaphysis was resected from the upper end of the fibula by the method described in the transplantation of the ECP. The length of the piece was measured before cutting, and the defect made as long as the suture graft. The sutural graft was placed in the defect in the fibula. No fixation was used in most cases. In some instances the graft was fixed in the diaphysis by inserting a short piece of metal in between the graft and the medullary canal.

Transplantation into the thigh. The aim of the experiment was to study whether the suture graft, placed as a "branch" to the diaphysis of the femur, would preserve its growth capacity. One of the short sides of a suture graft of the same type as above was inserted into a hole made in the cortex in the middle third of the femur. The direction of the graft was at an acute angle distally. Fixation was effected by suturing the muscles over the graft.

Transplantation of calvarial bone of the fibula (without the suture). The aim was to see whether the distance between the metal markers at the ends of the bone plate would remain unchanged if no growth was to be expected. A bone plate of the same size as the suture graft above was removed from the parietal bone and transplanted to the site of the excised ECP of the fibula.

CONTROL METHODS

Reimplantation. The control methods for the rabbit and the dog were different.

R a b b i t. The sagittal length of the cranial vault was measured in a number of rabbits of different ages. For the measurements lateral view radiographs were used. The anterior point of measurement was the ridge in the frontal bone in the middle cranial fossa, and the posterior point was the posterior margin of the parietal bone in the cranial fossa. The curve drawn from these readings (see results, fig. 15, p. 64) was used as the control of the growth of the coronary suture.

D o g. The increase in length of the calvarium was used as the control of the growth of the coronary suture. Because of differences in anatomy (*Sisson* and *Grossman* 1945) the method of measurement was a little different from that used on the rabbit. The measurement was made from the anterior margin of the middle cranial fossa to the posterior point of the posterior cranial fossa.

Because of the great variation in the size of the mongrel dogs, a control method of the type used for the rabbit was considered unsatisfactory. Instead, another dog of the same litter was used as the control animal. This also had the disadvantage that in some cases the difference in size became disturbingly great. In these cases the length of the calvarium of the experimental animal was considered a more reliable control. To get the relation between the increase in the length of the calvarium, measured by this method, and the growth of the coronary suture, control experiments were made in which the metal markers were inserted on both sides of the coronary suture (see results, table 8, p. 63).

Transplantation to the fibula.

R a b b i t. The controls for the sutural growth were made by the same method as in the reimplantation. As controls of the growth of the ECP of the fibula, both the opposite fibula and that of another animal of the same litter were used. The cases in which the latter method was used are mentioned separately in the results. To enable the evaluation of the error of the latter method, the growth of the proximal ECP of the tibia was also measured.

D o g. The growth in length of the calvarium of the same animal, measured and corrected as above, was used as the control. The defect was assumed not to disturb the growth of the calvarium markedly. The control experiments regarding the growth of the fibular ECP were made by the same method as in the rabbit.

Transplantation to the thigh. The method of control of sutural growth was the same as described above for the rabbit.

RADIOLOGICAL AND HISTOLOGICAL METHODS

As a control of the growth of the suture reimplant, a lateral view radiograph was used. The X-ray picture of the specimen was made by placing the cranial vault, cut off after sacrifice of the animal, on the film. For histological examination the specimen was cut in the sagittal plane.

RESULTS

NORMAL GROWTH OF THE CORONARY SUTURE

The results of the measurement of the growth of the coronary suture, measured by the insertion of metallic markers on both sides of the suture, are seen in table 8. The variation of the increase in the distance between the markers is considerable in both the rabbit and the dog. This is probably due to expelling of the markers as the result of remodelling of the bone. Therefore this method could not be accepted as providing a satisfactory control of sutural growth without further study. When the increase in the distance between the markers was compared with the average growth in length of the calvarium, it could be seen that in the rabbit the greatest increase corresponds to the growth in length of the calvarium, i.e. the difference is within the error of measurement, 0.5 mm. Therefore in the rabbit the growth in length of the calvarium, measured by this method, was considered to be a satisfactory control without correction.

In the dog the increase in the distance between the markers did not correspond to the growth in length of the calvarium. Supposing that the greatest increase, 2.5 mm, corresponds to the growth of the suture, the correction of the readings obtained from the growth in the length of the calvarium was obtained by multiplying it by 2.5/5.5. This corresponds to the growth of the coronary suture.

The curve in fig. 15 shows the length of the calvarium of the rabbit at different ages, measured on a number of normal animals. The readings in this curve were used as the control values for sutural growth in the rabbit.

TABLE 8. – *The growth of the coronary suture measured as the increase in the distance between metal markers on either side of the suture. R=rabbit, D=dog.*

Animal No.	Age at operation (days)	Time of observation (weeks)	Distance between markers (mm)			Average growth in length of calvarium in control group (mm)	Growth in length of calvarium (mm)
			Initial	Final	Increase		
R 301	16	41	5	11	6	7	
R 302	16	41	7	13.5	6.5	7	
R 304	16	41	7.5	10	2.5 [1])	7	
R 305	16	41	6.5	8.5	2 [2])	7	
R 306	16	41	6.5	11	4.5 [1])	7	
D 71	41	17	10	12.5	2.5		5.5
D 72	41	17	11.5	13.5	2.5		5.5
D 73	41	17	10.5	11.5	1 [1])		6.5
D 74	41	17	9.5	11.5	2		5
D 81	44	14	10.5	12	1.5		6.5
D 82	44	14	10	?	? [1])		5.5
D 83	44	14	9.5	10	0.5 [1])		7

[1]) one marker expelled.

[2]) both markers expelled

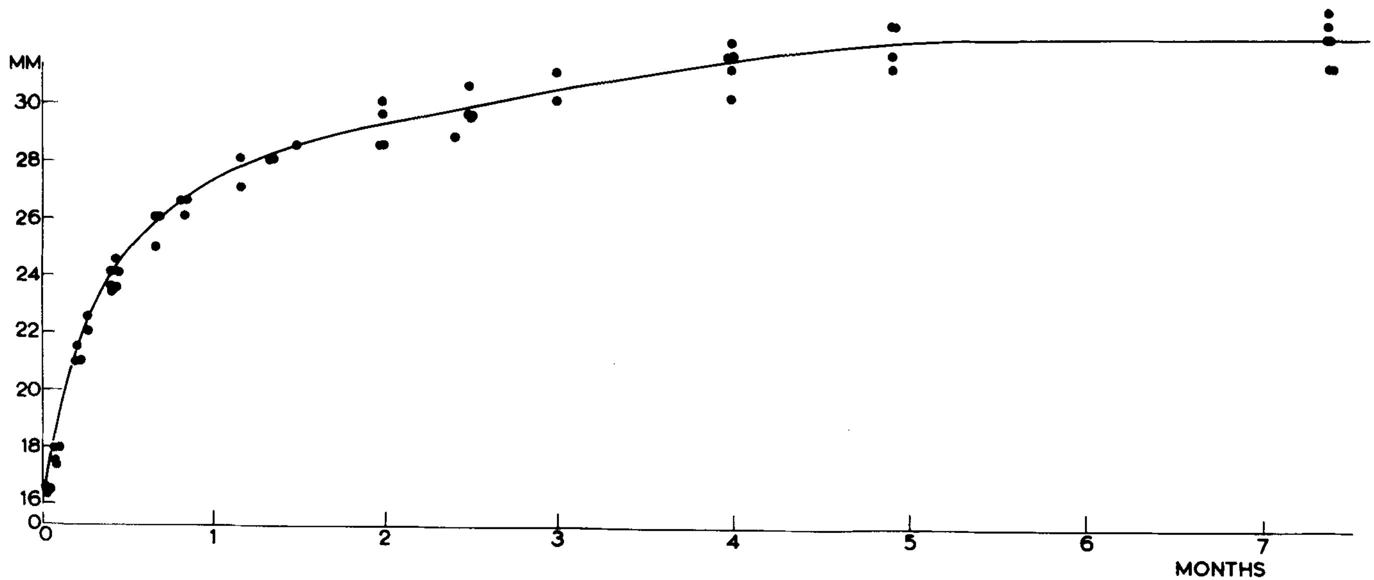


FIG. 15. — *Sagittal length of the calvarium of the rabbit at different ages. The measurements were made on a group of control animals.*

REIMPLANTATION OF THE CORONARY SUTURE ON RABBIT

The results of reimplantation of the coronary suture in the rabbit are seen in table 9. In 10 of the experiments growth of the reimplant was noticed. The amount of growth was comparable to normal in three cases. In the most successful experiments both the radiological appearance of the suture and the radiological density of the graft were normal, as interpreted from the X-ray pictures of the specimens. Bone defects, usually minor ones, were seen at the borders of most grafts, but these did not seem to have disturbed the growth. In fig. 16 two examples of the specimens are seen.

TABLE 9. – *Reimplantation of the coronary suture in the rabbit.*

Animal No.	Age at operation (days)	Time of observation (weeks)	Growth of graft (mm)	Growth of coronary suture in control group (mm)	Growth in % of control	Macroscopical and radiological observations		
						Suture	Bone defects	Radiol density of bone
20	40	7	0	2.5	0	fused	present	normal
21	40	7	0	2.5	0	partially fused	present	uneven
22	40	7	2	2.5	80	patent	present	normal
23	40	7	2.5	2.5	100	patent	absent	normal
24	40	7	2.5	2.5	100	patent	present	normal
25	40	7	0	2.5	0	fused	absent	uneven
26	40	7	3	2.5	120	patent	present	normal
27	30	6	0	3	0	fused	present	uneven
28	30	6	2	3	67	patent	absent	uneven
29	30	6	0.5	3	17	partially fused	absent	uneven
30	30	6	2	3	67	partially fused	present	normal
31	30	6	0	3	0	partially fused	present	uneven
32	30	6	1.5	3	50	partially fused	present	normal
33	30	6	1.5	3	50	partially fused	present	normal
65	19	24	3	6.5	46	fused	present	normal
66	19	24	3.5	6.5	55	patent	absent	uneven

FIG. 16. — *Suture reimplant in the skull of the rabbit. Two examples of the X-ray pictures of the specimens. Left: case no. 23. Right: case no. 21. The metal markers were used for the measurement of growth. Note the bone defect in case no. 21.*

Histological findings

In cases 23 (reimplant and control), 26 (reimplant and control), 327 (control), 24, 25, 28, 30, 32 and 33 histological examination was made.
Case 23 (control, age 13 weeks). The suture appears as an S-shaped, relatively broad structure composed of collagenous tissue. The thickness of the specimen in this section is about 2/3 mm and the breadth of the suture about 1 1/2 mm. The suture is relatively rich in cells and the feltwork of connective tissue fibres appears fairly homogeneous. The fibres are seen continuing to the marginal bone, which is strongly basophilic. In places osteoblasts are seen in abundance at the bone margins. The marginal bone forms long trabeculae which are responsible for the S-shaped form of the suture. Some osteoclasts are seen at the bone margins. In places slight granulation of the collagen fibres is seen. The diploë and the tables are well formed.
Case 26 (control, age 13 weeks). The suture is somewhat more massive and tortuous than in the foregoing case, but its microscopic structure is otherwise similar.
Case 327 (control, age 32 weeks). See fig 17. The thickness of the specimen at the site of the suture is about 1 mm and the length of the area of the suture about 2/3 mm. The course of the suture is not so clear-cut as in the two foregoing cases. The suture is in places interrupted by narrow zones where the edges of marginal bone are almost

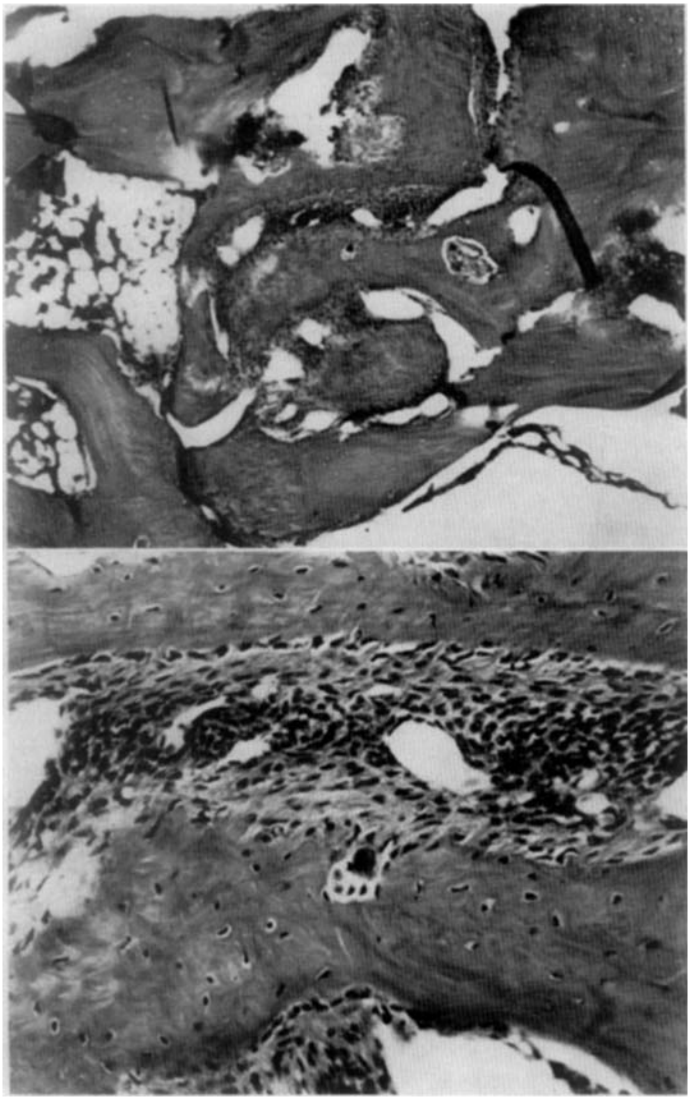


FIG. 17. — *Microscopic structure of the normal coronary suture of the rabbit at the age of 32 weeks. Case no. 327. Below: the structure of the suture at higher magnification. The suture shows areas of fatty degeneration. Note the osteoblast cells lining the suture.*

in contact with each other. Part of the suture tissue is separated in islets. The collagenous tissue shows areas of fatty degeneration and granulation. The number of cells is for the most part diminished in relation to the two foregoing cases. Only in the middle part of the suture are more cells seen in the central layer. The structure of the marginal bone is coarsely fibrillar only in places. In the middle part osteoblasts are seen at the bone margins.

Case 23 (growth 100 per cent of control). The histological picture is very similar to that of the control side, except that the suture looks a little narrower and the number of cells is not so great.

Case 24 (growth 100 per cent of control). No significant difference from the foregoing case is observable.

Case 25 (growth 0). The course of the suture is almost straight across the bone. It is a very narrow, fibrous band, the bone edges on the dural side being almost in contact with each other. The tissue is poor in cells and shows pronounced signs of granulation, fatty degeneration and mucous tissue. Only scattered osteoblasts are seen. The staining of marginal bone, which is lamellar in structure, does not differ from that of the bone in the tables.

Case 26 (growth 120 per cent of control). The suture forms a winding structure, in which isolated islets of bone are visible. It is relatively massive and rich in cells, especially in the central layer. In the bone margins osteoblasts are seen in abundance. Some osteoclasts are likewise seen in the resorption lacunae. The marginal bone is strongly basophilic and coarsely fibrillar. The diploë on both sides of the suture is relatively narrow compared with the tables.

Case 28 (growth 67 per cent of control). The suture is relatively thin, forming only gently winding sinuosities and its course is rather longitudinal. Cells are seen to be rather scanty and the collagenous tissue shows areas of degeneration. Only sparse osteoblasts are situated at the bone margins. The marginal bone is only coarsely fibrillar in places.

Case 30 (growth 67 per cent of control). The suture is in some sections a totally patent, gently sinuous, S-shaped structure. Cells are rather scanty and there are widespread degenerative changes in the collagenous tissue. In places osteoblasts are seen in rows at the bone margins. The marginal bone in these areas is coarse-fibred. In some sections relatively massive bone bridges interrupt the suture, especially towards the dural side.

In three cases, where the graft had grown as much as a normal suture, the microscopic picture did not differ significantly from that of a normal suture at the same age. In other specimens signs pointing to a decreased proliferative activity of the suture were seen.

REIMPLANTATION OF THE CORONARY SUTURE ON DOG

The results of reimplantation of the coronary suture in the dog are seen in table 10.

In 7 of the 8 experiments the growth measured exceeded the error of measurement. In 5 cases the amount of growth was comparable to normal. The

TABLE 10. — *Reimplantation of the coronary suture in the dog.*

Animal No.	Age at operation (days)	Time of observation (weeks)	Growth of graft (mm)	Growth in length of calvarium (mm)	Calcul. growth of coronary suture (mm)	Growth in % of control	Macroscopical and radiological observations		
							Suture	Bone defects	Radiol. density of bone
74	41	22	1.5	5	2.5	60	patent	absent	normal
83	43	14	0.5	7	3	17	fused	absent	increased in suture area
241	23	22	6	11	5	120	patent	absent	increased at suture margins
246	23	22	6	11.5	5	120	patent	absent	normal
281	16	30	6	13	6	100	patent	absent	uneven
282	16	12	5	11.5	5	100	patent	absent	uneven
283	16	30	5	13	6	83	fused	absent	uneven
284	16	30	6	12.5	5.5	109	patent	absent	increased at suture margins

coronary suture at the site of reimplantation was mostly very narrow or ossified. In most cases the other parts of the coronary suture were also already closed except in the area immediately adjacent to the sagittal suture. No bone defects were seen at the borders of the graft. The radiological density on both sides of the suture was increased in 3 cases.

In figures 16 and 17 two examples of reimplantation of the suture in the dog are seen.

Histological findings

In cases 82 (control), 73 (control), 331 (control), 74, 83, 283 and 284, histological examination was made.

Case 82 (control, age 19 weeks). The thickness of the specimen at the site of the suture is 3 mm and the length of the suture area about 6 mm. The course of the suture across the bone is oblique. The suture runs a slightly tortuous course. Its thickness varies considerably, being greatest in the middle and least near the periosteum and the dura. The greater part of the suture is rich in cells, especially the middle layer. Abundant osteoblasts are seen arranged in rows at the bone margins. Osteoclasts are also seen in resorption lacunae. The collagenous fibres appear regular. The marginal bone is strongly basophilic and mostly coarse-fibred. A relatively massive layer of bone separates the suture from the diploë. The outer plate is considerably thicker than the inner one.

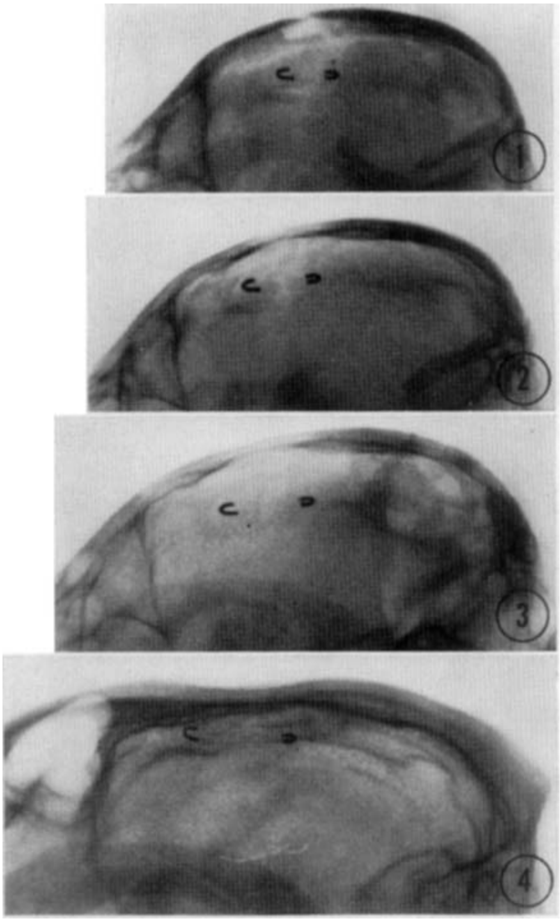


FIG. 18. — *Growth of the reimplanted coronary suture graft in the dog. Case no. 246. X-ray pictures 2 days (1), 2 weeks (2), 4 weeks (3) and 15 weeks (4) after reimplantation. Note the gradual increase in the distance between the markers.*

Case 73 (control, age 28 weeks). The course of the suture is not so oblique as in the foregoing case. The suture cells are less numerous and the layer of coarse fibrillar bone on the two sides of the suture is narrower. The suture is thinner in the middle than in the peripheral parts. Signs of degeneration are seen in the connective tissue. The outer plate is very thick compared with the inner one.

Case 331 (control, age 32 weeks). See fig. 20. The course of the suture is very oblique. It is only very gently sinuous. The thickness of the suture varies little, but it is slightly thinner on the periosteal side. The suture is mostly relatively poor in cells. Osteoblasts are seen in moderate numbers, but in the marginal bone only small remnants of coarsely fibrillar bone are seen and the staining of the marginal bone does not differ much from that of the rest of the bone. No bony bridges traversing the suture are seen.

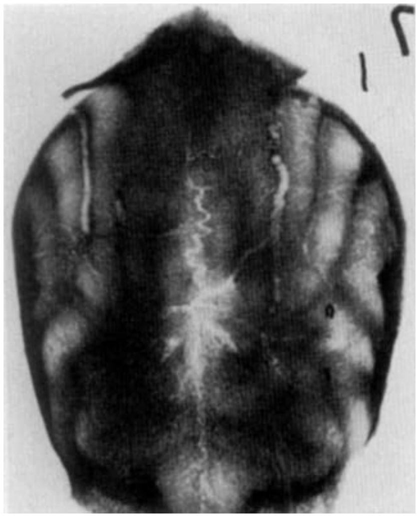


FIG. 19. — *Coronary suture reimplant in the dog. Specimen of case no. 74. On the control side (at the left of the picture) short straight piecces of metal markers are placed on both sides of the suture for measurement of sutural growth. The coronary suture is seen as a very narrow line.*

Case 74 (growth 60 per cent of control). The thickness of the specimen at the site of the suture is 4 mm. The length of the suture area is 3 mm. The suture is mostly of about the same thickness as in case 73, but in some sections there are areas in which the bone edges are almost in contact with each other. Near the outer surface the number of connective tissue cells is less than in the middle and inner parts, where areas with abundant cells are seen. Here numerous osteoblasts are also situated at the bone margins. In places osteoclasts are seen. The suture tissue shows marked changes of fatty degeneration, especially in its outer parts. A coarsely fibrillar type of bone is seen as a thin layer in the middle and inner parts of the specimen. The outer table is very massive.

Case 83 (growth 17 per cent of control). At the site of the suture a narrow oblique band of connective tissue is seen. This is interrupted in many places by transverse bony bridges. In the suture tissue there are only a few scattered cells and no osteoblasts. The marginal bone is lamellar in structure and its staining capacity is the same as that of the more remote bone.

Case 283 (growth 83 per cent of control). The suture forms an oblique, mostly very narrow, gently sinuous structure, which in places is traversed by bony bridges. There are scanty cells and only in places are osteoblasts and coarsely fibrillar bone visible. The structure of the collagenous tissue shows considerable changes of fatty degeneration and granulation.

Case 284 (growth 109 per cent of control). The outer and middle parts of the suture is very oblique, gently winding and thin, in places almost indistinguishable and very poor in cells. A narrow layer of coarsely fibrillar bone is seen on both sides of it. The inner part of the suture is in some sections relatively wide and rich in cells. Osteoblasts are seen at the bone margins, and the marginal bone consists of a relatively wide layer of basophilic coarse-fibred bone.

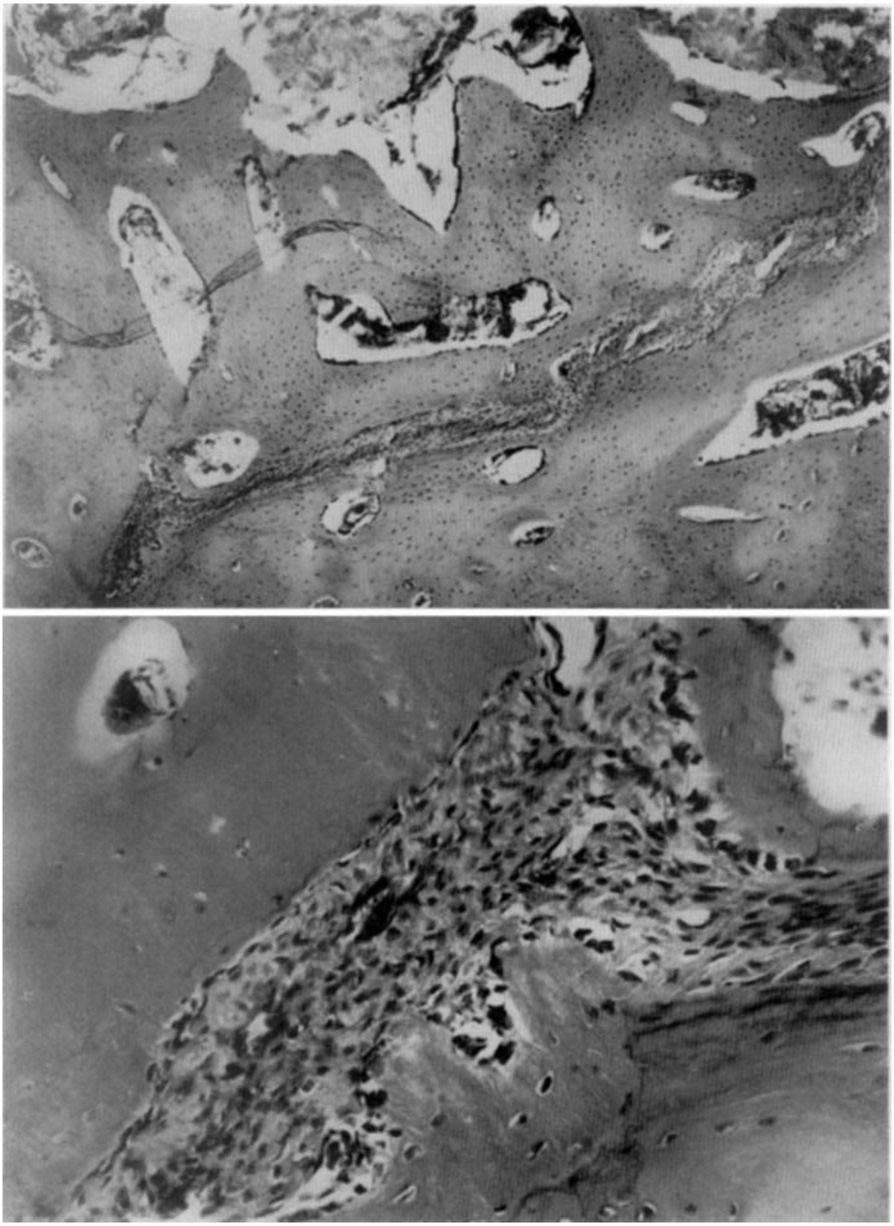


FIG. 20. – *Microscopic structure of the normal coronary suture of the dog at the age of 32 weeks. Case no. 331. Above: the course of the suture is only very gently sinuous. Below: a higher magnification of the suture. A narrow zone of coarse-fibred bone is seen at the suture margins.*

The microscopic picture of the suture in case 284 did not differ significantly from that of the normal suture at the same age. In all other specimens, signs pointing to a decreased proliferative activity, compared to the controls, were seen.

REIMPLANTATION OF THE CORONARY SUTURE WITH POLYTHENE FILM

The results of reimplantation with polythene film, in both the rabbit and the dog, are seen in table 11.

TABLE 11. – *Reimplantation of the coronary suture with polythene film in the rabbit (R) and the dog (D).*

Animal No.	Age at operation (days)	Time of observ-ation (weeks)	Distance between markers (mm)			Growth of control (mm)	Growth of graft in % of control
			Initial	Final	Increase		
R 151	21	22	6	6	0	6	0
R 152	21	22	5	5	0	6	0
R 153	21	22	6	6	0	6	0
R 154	21	22	6	6	0	6	0
R 155	21	6	6.5	6	–0.5	3	0
R 156	21	22	5.5	6	0.5	6	0
D 331	26	27	9.5	14	4.5	6.5	69
D 332	26	27	8	16	8	7	114
D 333	26	27	8.5	12.5	4	6	67

In *the rabbit* no increase in the distance between the markers was noticed in any of the experiments. Bone was formed on the dural side in all cases and thus the piece of polythene was inclosed inside the bone. The new bone was removed before radiography, but the suture was only visible in case *153*. Even in this it was very narrow and the radiological density on either side of it was increased. The radiological density at the site of the suture was uneven in all cases. In case *154* a tiny bone defect was seen on the dorsomedial border of the graft.

In *the dog* the distance between the markers had increased in all 3 experiments. The polythene film was covered with a layer of bone on the dural side. In case *332* a narrow open suture was seen. On both sides of it the radiological density was increased.

Histological findings

In cases 153, 331 and 332 microscopic examination was made.
Case 153. The suture is partly ossified, and partly consists of a narrow band of fibrous tissue, with few cells. The matrix shows pronounced degenerative changes. No osteogenesis is seen. The marginal bone is lamellar and the borderline between the suture and the marginal bone is very sharp.
Case 331. The course of the suture is oblique, running rather straight through the bone. It is relatively poor in cells; hardly any osteoblasts are visible. The matrix shows slight granulation in places. No coarse fibrillar bone is seen in the marginal bone.
Case 332. The suture is a relatively wide, meandering structure, also including some isolated islets of connective tissue. It is rich in cells and the matrix appears homogeneous. Minor blood vessels are seen in the central layer. In places relatively abundant osteoblasts are situated in rows at the bone margins. Most of the marginal bone is coarsely fibrillar in structure.

TRANSPLANTATION OF THE SUTURE TO THE FIBULA IN THE RABBIT

The results of transplantation to the fibula are seen in table 12. In 22 of the 29 experiments growth was demonstrable. In 5 cases the amount of growth was practically as great as that of the ECP of the fibula. When the growth of the graft was compared with that of the corresponding coronary suture in the calvarium, it was noticed that in 20 cases it had grown as much as or more than the latter and in some experiments about 5 times as much. In the most successful experiments the suture at the site of the graft had remained open radiologically, the graft had fused to the diaphysis of the fibula and its radiological density was interpreted as normal. The initial aligment of the graft in the host area was good in all cases where the transplant had grown well. In some experiments the result was poor in spite of good initial alignment. In some cases the first X-ray check-up revealed bowing of the graft at the site of the suture or fracture of the fibula distally of the graft. In 5 cases the site of the markers made the amount of growth measured uncertain. The fusion to the epiphysis did not seem to affect the result. In 3 cases a bony bridge had formed between the graft and the diaphysis of the tibia. The form of the graft changed considerably during the time of observation. The thin plate gradually became a massive shuttle-shaped bone in the middle of which the suture was seen as a kind of transverse junction of irregular form. The radiological structure of the bone in the specimens had a special character. The arrangement of the bone trabeculae somewhat resembled that of the cranial bone, but the trabeculae were arranged more longitudinally than in the latter, and no signs of the diploë were seen.

In figures 21 and 22 two examples of the experiments are seen.

In figure 23 a graphical illustration of the growth of the grafts, compared both with the growth of the normal suture and with that of the ECP of the fibula, is presented.

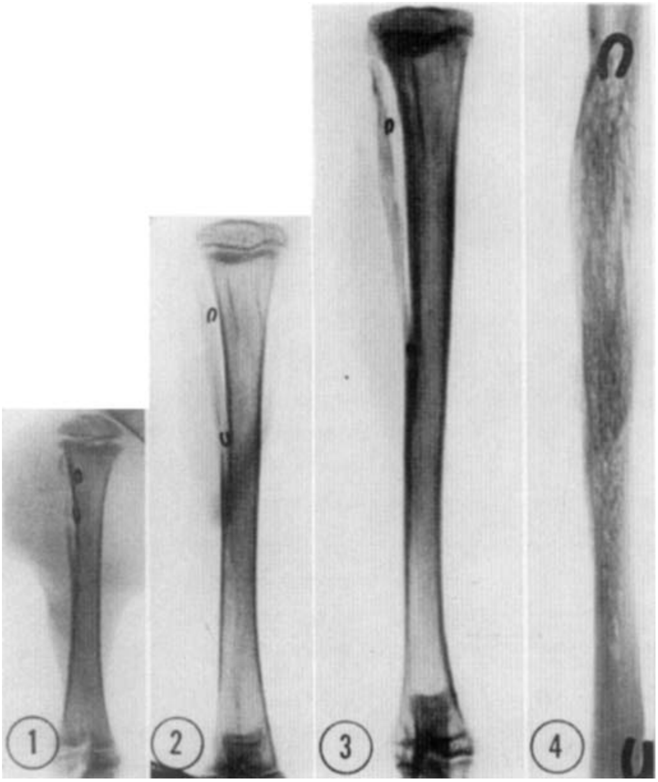


FIG. 21. — *Transplantation of the cranial suture to the site of the excised fibular ECP. Rabbit no. 176. X-ray picture 6 days (1), 9 weeks (2) and 19 weeks (3) after transplantation. Note the gradual increase in the distance between the markers. (4) Enlarged X-ray picture of the specimen. In the middle of it the suture is seen as a light area.*

Histological findings

Cases 114, 171, 172, 173, 174, 175, 176, 252 d, 252 s, 253 d, 302, 304 and 327 were examined microscopically.

Case 114 (growth 8 per cent of control epiphyseal growth). The thickness of the specimen is 4 mm and the length of the suture area is also 4 mm. The suture forms a very tortuous fibrous structure, in which the bone spicules of the marginal bone are situated in between the bends and bony islets are included in the suture tissue. The connective tissue is relatively poor in cells and shows considerable degenerative changes. Osteoblasts are seen abundantly in some of the margins of the bony trabe-

TABLE 12. — *Transplantation of the suture to the site of the excised ECP of the*

Animal No.	Age at operation (days)	Time of observation (weeks)	Growth of *graft* (mm)	Growth of coronary *suture* in control group (mm)	*Growth in % of sutural growth*	Growth of proximal ECP of tibia (mm)	Growth of ECP of control fibula (mm)	*Growth in % of epiphyseal growth*
112	22	22	0.5	6	*8*	26.5	23.5*	*2*
114	22	22	3.5	6	*58*	26	27	*8*
171	28	19	0	5	*0*	26	26 *	*0*
172	28	19	20	5	*400*	24	28.5*	*71*
173	28	19	28	5	*560*	27	23.5*	*119*
174	28	19	0	5	*0*	28.5	25	*0*
175	28	19	0	5	*0*	30.5	26.5	*0*
176	28	19	24.5	5	*490*	25	21.5	*114*
251 d	19	19	5.5	6	*92*	35.5	32.5*	*17*
251 s	19	19	5.5	6	*92*	34.5	32.5*	*15*
252 d	19	19	8.5	6	*142*	34	32.5*	*26*
252 s	19	19	12.5	6	*240*	33.5	32.5*	*38*
253 d	19	19	29.5	6	*492*	33	32.5*	*91*
253 s	19	19	14.5	6	*241*	33.5	32.5*	*45*
254 d	19	19	24	6	*400*	36	32.5*	*74*
254 s	19	19	1.5	6	*25*	36	32.5*	*5*
301	16	43	10	6.5	*154*	40	33.5*	*30*
302	16	43	6.5	6.5	*100*	39	35 *	*19*
304	16	43	24.5	6.5	*377*	42.5	37	*66*
305	16	43	7	6.5	*108*	38	33.5	*21*
306	16	43	8.5	6.5	*131*	44	35	*24*
311 [1)]	37	25	0	4	*0*	22.5	22	*0*
312 [1)]	37	25	0	4	*0*	19.5	20	*0*
313 [1)]	37	25	0	4	*0*	11	24	*0*
325	22	30	6.5	6	*108*	27.5	23.5	*28*
326	22	30	6.5	6	*108*	29.5	28	*23*
327	22	30	26.5	6	*442*	33.5	30.5	*87*
328	22	30	19.5	6	*325*	24	20.5	*95*
329	22	30	26	6	*433*	24	28	*93*

* another animal of the same litter

1) the initial length of the graft 18—19 mm, i.e. about three times as much as in the standard grafts.

fibula in the rabbit.

Suture	Fusion of graft to epiphysis	Fusion of graft to diaphysis	Radiol. density of bone	Initial alignment of graft	Special observations
	Macroscopical and radiological observations				
not visible	fused	not fused	uneven	poor	fracture in fibula, both markers expelled
not visible	not fused	fused	increased	unsatisf.	fracture in fibula, graft fused side to side with tibia
not visible	not fused	not fused	decreased	poor	
patent	fused	fused	normal	satisf.	
patent	fused	fused	normal	good	
patent	fused	fused	uneven	satisf.	suture-like formation distally from distal marker
not visible	not fused	fused	increased	poor	graft broken at the suture
patent	fused	fused	normal	good	
narrow	not fused	not fused	decreased	poor	fracture in fibula
not visible	not fused	not fused	decreased	unsatisf.	
not visible	fused	not fused	decreased	satisf.	
narrow	fused	fused	decreased	satisf.	distal marker at suture
patent	not fused	fused	decreased	good	the specimen is very thin
patent	not fused	fused	decreased	good	
patent	not fused	fused	normal	good	bone bridge between fibula and tibia
patent	not fused	fused	increased	satisf.	fracture in fibula, proximal marker at suture
patent	fused	fused	normal	unsatisf.	distal marker expelled
narrow	not fused	narrow bridge	normal	unsatisf.	bowing at suture after transplantation
patent	not fused	fused	normal	poor	bone bridge between graft and tibia
partly fused	not fused	not fused	normal	poor	
not visible	not fused	not fused	decreased	good	
not visible	[2]	not fused	normal	poor	
not visible	[2]	fused	increased	unsatisf.	marked bowing of tibia
not visible	[2]	fused	increased	satisf.	marked bowing of tibia; bone bridge between graft and tibia
not visible	fused	not fused	normal	good	
not visible	fused	fused	increased	satisf.	slight bowing of tibia
patent	not fused	fused	normal	good	
patent	fused	fused	normal	good	
patent	not fused	fused	normal	good	

[2]) the epiphysis totally removed; fusion of the graft to the metaphysis of the tibia.

FIG. 22. – *Suture transplant at the site of the excised ECP of the fibula in the rabbit. Case no. 327. (1) X-ray picture after transplantation. (2) The specimen. The left side was used as a control. The growth of the graft was 87 per cent of that of the fibular ECP. (3) Enlarged X-ray picture of the specimen. Note the suture in the middle of the graft.*

culae. In others, the number of osteoclast cells in the resorption lacunae is considerable. Only small areas of coarse-fibred bone are seen. The diploic cavities on both sides of the suture are very wide. The bone of the specimen is cancellous.

Case 171 (growth 0). The specimen is very osteoporotic. In the middle of it some thin streaks of fibrous tissue, at places interrupted by bony bridges, are seen traversing the bone. These contain very few cells and collagen fibres. No signs of osteogenesis are visible.

Case 172 (growth 71 per cent). The thickness of the specimen is 3 mm and the suture area is 7 mm in length. Throughout its length the suture consists of bends, in between

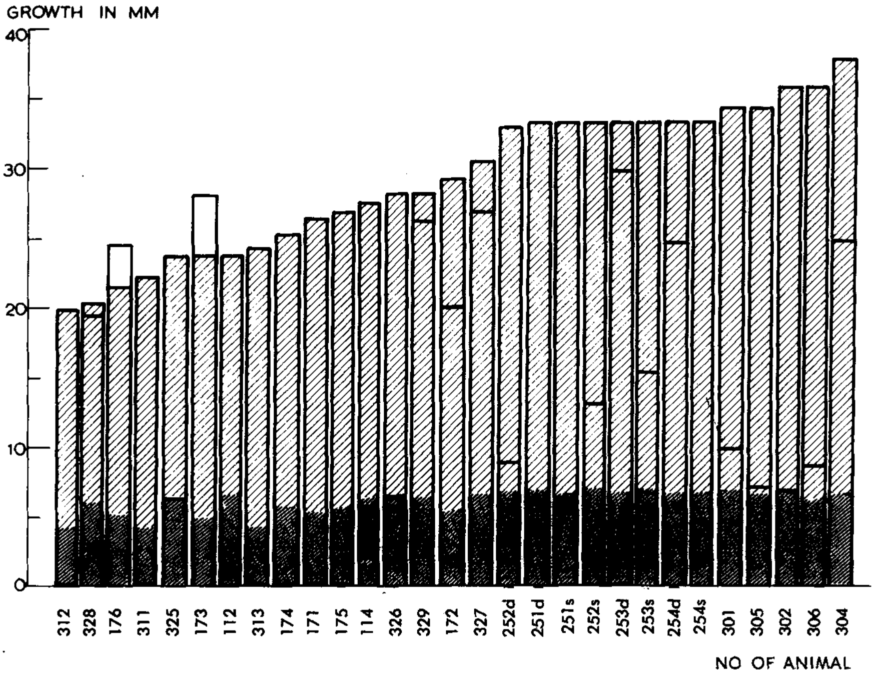


FIG. 23. — *Transplantation of the coronary suture to the site of the excised ECP of the fibula in the rabbit. The growth of the grafts (level of the transverse lines) as compared with the normal growth of the suture (dark columns) and with that of the fibular ECP (hatched columns). In two cases the graft had grown more than the ECP control.*

which long, narrow bony islets are situated. The suture tissue is rich in cells and collagen fibres, which are seen to continue into the bony trabeculae as Sharpey's fibres. The fibroblasts in the peripheral layer of the suture have a round and rather large nucleus. At the margins of the trabeculae abundant osteoblasts are seen arranged in rows. Over large areas apposition of coarse-fibred bone on the trabeculae is seen to have occurred. The bone of the specimen is cancellous and the cavities on both sides of the suture are rather wide and numerous. No tables or cortical layers of bone are seen.

Case 173 (growth 119 per cent). The specimen consists of a piece of bone with a defective epiphysis at one end. This has fused to the graft. No signs of an ECP are visible. At a distance of about 15 mm from the epiphysis a suture is seen, the structure of which is very similar to that of the foregoing case. In between the epiphysis and the suture, the structure of the bone resembles that of a normal diaphysis of the fibula.

Case 174 (growth 0). In the area between the marks no signs of the suture are visible. The bone is rather osteoporotic. At a distance of 20 mm from the proximal end of the specimen a relatively massive suture-like structure is found, where a rather intensive process of ossification is seen.

Case 175 (growth 0). No remnants of the suture are visible in the specimen, which consists of a piece of mostly cancellous bone with a wide medullary cavity.

Case 176 (growth 114 per cent). The thickness of the specimen is 2.5 mm. The length of the suture area at its centre is 8 mm. This is a network of massive longitudinal windings of suture tissue, where the spaces between the bends are filled with long, narrow bony trabeculae. Intensive ossification is seen at the margins of the trabeculae. Most of the bone in the trabeculae is coarse-fibred, but that more remote from the suture or in the central parts of the trabeculae is lamellar. In places intensive resorption of bone is also seen. The bone of the specimen is cancellous without any proper compact superficial layer or medullary cavity.

Case 252 d (growth 26 per cent). The graft is extremely osteoporotic. In the middle of it a patent, narrow, winding suture is seen with scanty cells and connective tissue fibres. A narrow zone of immature bone is seen on either side of the suture.

Case 252 s (growth 38 per cent). The microscopic features of the specimen correspond to those of the foregoing case, but the suture is more massive, and moderate ossification is seen at the margins of the bony trabeculae. The bone of the specimen is very osteoporotic.

Case 253 d (growth 91 per cent). The thickness of the graft is 3 mm and the length of the suture area 6.5 mm. The histological picture is similar to that in case 176. The cavities of the spongiosa are relatively large and numerous.

Case 302 (growth 19 per cent). The greater part of the suture is ossified, and only sparse connective tissue bands or islets are seen at the site of it. In places in the central parts of the specimen the suture is not ossified, but shows areas of granulation and fatty degeneration. Small groups of osteoblasts are seen, but all the marginal bone is lamellar. The cavities of the spongiosa are relatively small.

Case 304 (growth 66 per cent). The suture corresponds to a normal coronary suture in the skull at the age of 30 weeks, but is slightly more winding. It shows pronounced degenerative changes. The number of cells and connective tissue fibres is relatively low. Large blood vessels are seen in the middle layer. Osteoblasts are situated in rows at the bone margins. The border between the suture and the marginal bone is very narrow and stains basophilically. The marginal bone is lamellar.

Case 325 (growth 28 per cent). The histological picture does not differ significantly from that in case 302.

Case 327 (growth 87 per cent). See fig. 24. The thickness of the graft is 2.5 mm and the area of the suture is 5.5 mm in length. The histological structure of the suture differs only insignificantly from that in case 176. The suture is more massive.

In experiments in which the graft had grown as much as the ECP of the fibula, its microscopic picture was very different from that of a normal suture at the same age. It was a network of massive longitudinal "hairpin bends" of the suture tissue, in which the spaces between the bends were filled with long, narrow bone trabeculae. The length of this area was up to 8 millimetres. The suture showed signs of great proliferative activity. Compared with these cases the microscopic picture of the suture in the other specimens showed various degrees of ossification or decreased proliferative activity.

FIG. 24. – *Microscopic structure of the suture graft in the fibula of the rabbit. Case no. 327. Above: the suture forms a series of longitudinal bends, the spaces between which are filled with long, narrow bone trabeculae. Below: higher magnification of the same. The suture is rich in cells and abundant osteoblasts are seen at the bone margins. The marginal bone is coarsely fibrous.*

TRANSPLANTATION OF THE SUTURE TO THE FIBULA IN THE DOG

The results of the transplantation of the suture to the site of the excised ECP of the fibula in the dog are seen in table 13. An amount of growth which exceeded the error of measurement was seen in all 14 experiments. In case 81 it was quite insignificant and in case *283 d* the result was uncertain because the marker had been expelled. In 5 experiments the growth was more than 50 per cent of that of the corresponding ECP of the fibula, in the two most successful cases about 75 per cent of it. Compared with the normal growth of the coronary suture in the skull, it was even 6—7 times as great. At the end of the observation time the suture was radiologically open in most cases. The fusion of the graft with the epiphysis seemed to bear no relation to the result. Fusion

TABLE 13. — *Transplantation of the suture to the site of the excised proximal ECP*

Animal No.	Age at operation (days)	Time of observation (weeks)	Growth of *graft* (mm)	Calcul. growth of coronary *suture* (mm)	*Growth in % of sutural growth*	Growth of proximal ECP of tibia (mm)	Growth of proximal ECP of control fibula (mm)	*Growth in % of epiphyseal growth*
71	41	22	20.5	3	*683*	27	26.5	*77*
72	41	22	20.5	3.5	*585*	29.5	28	*73*
73	41	22	16.5	3	*550*	36.5	37.5	*44*
81	43	15	1	3.5	*29*	17	19	*5*
82	43	15	13.5	2.5	*540*	19	24	*56*
241	23	22	28.5	5	*570*	47.5	49.5*	*58*
246	23	22	30.5	4.5	*677*	48.5	49.5*	*62*
281 d	16	30	26	6	*433*	52.5	54 *	*48*
281 s	16	30	23	6	*383*	54.5	54 *	*43*
282 d	16	13	14.5	5	*290*	33.5	30.5*	*48*
282 s	16	13	13.5	5	*270*	33.5	30.5*	*44*
283 d	16	30	6.5 1)	6	*(108)*	56.5	54 *	*(12)*
283 s	16	30	18	6	*300*	54	54 *	*33*
284 d	16	30	21	5.5	*382*	39	54 *	*39*
284 s	16	30	19	5.5	*345*	44	54 *	*35*

* another animal of the same litter

1) the marker expelled

to the diaphysis of the fibula had taken place and the radiological density was interpreted as normal in almost all cases. The initial alignment of the graft in the host area was good in most experiments. The unsatisfactory position of the markers made the result uncertain in 2 cases. The transplant, originally a flattish plate, had been transformed to a shuttle-shaped, and in many cases very massive, piece of bone. There was some variation in the final form of the grafts. The structure of the bone differed from that of either the cranial bone or the fibula. The bone of the specimen consisted of cancellous bone, where the criss-crossing trabeculae were rather longitudinal. The form of the suture in the specimens varied significantly.

Some examples of the growth of the transplants and of the radiological appearance of the specimens are seen in figures 25 and 26.

of the fibula in the dog.

Macroscopical and radiological observations					
Suture	Fusion of graft to epiphysis	Fusion of graft to diaphysis	Radiol. density of bone	Initial alignment of graft	Special observations
patent	not fused (fused to tibia)	fused	normal	good	suture very oblique
patent	fused	fused	normal	good	
patent	not fused (fused to tibia)	fused	increased	good	distal marker at the suture
not visible	fused	fused	normal	unsatisf.	defect in fibula too long
patent	fused	fused	normal	good	
patent	fused	fused	normal	good	
patent	fused	fused	normal	good	
patent	fused	fused	normal	good	
patent	fused	fused	normal	good	
patent	not fused	fused	normal	good	
patent	fused	fused	normal	good	
patent	not fused (fused to tibia)	fused (with angulation)	increased	satisf.	graft bowed at suture; proximal marker at suture
patent	not fused	fused	normal	good	
patent	fused	fused	normal	unsatisf.	graft bowed after transplantation
patent	fused	fused	normal	good	

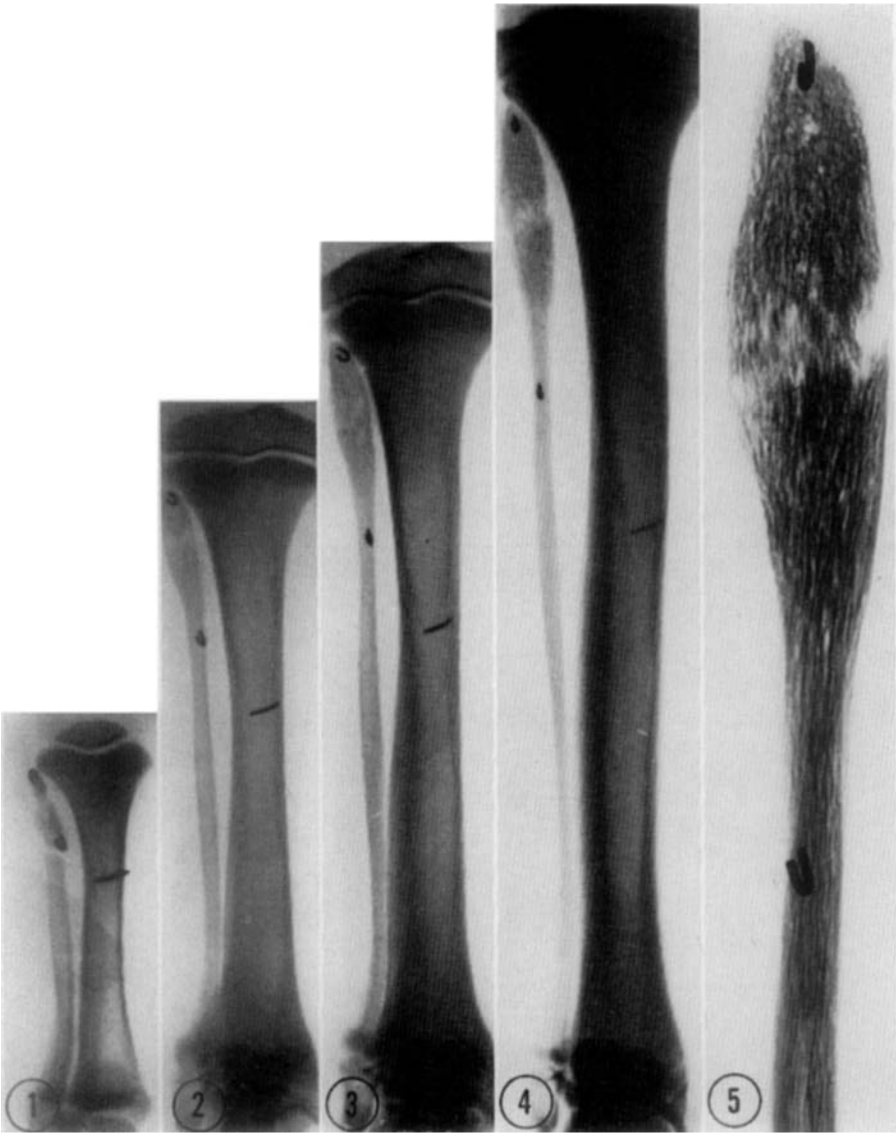


FIG. 25. — *Growth of the suture graft in the site of the excised proximal ECP of the fibula in the dog. Case no. 241. X-ray picture 2 weeks (1), 7 weeks (2), 11 weeks (3) and 22 weeks (4) after transplantation. Note the gradual increase in the distance between the markers. (5) Enlarged X-ray picture of the specimen. Note the form of the graft and of the suture.*

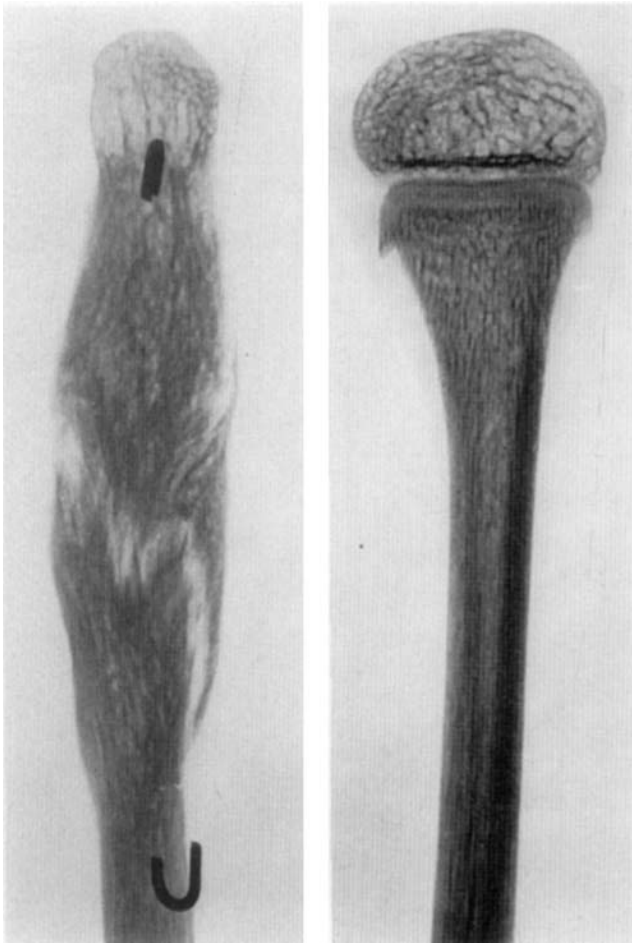


FIG. 26. — *Coronary suture graft at the site of the excised ECP of the fibula. Dog no. 82. Enlarged X-ray picture of the specimen (left) and the control fibula (right).*

In figure 27 a graphical illustration of the growth of the suture transplants in the dog, compared with the growth of both a normal coronary suture and that of the ECP of the fibula, is presented.

Histological findings

Cases 71, 81, 82, 241, 246, 281 d, 281 s, 283 d, 284 d and 284 s were examined microscopically.

Case 71 (growth 77 per cent of control epiphyseal growth). The thickness of the specimen is 4 mm and the suture area is 4 mm in length. The suture forms a complex

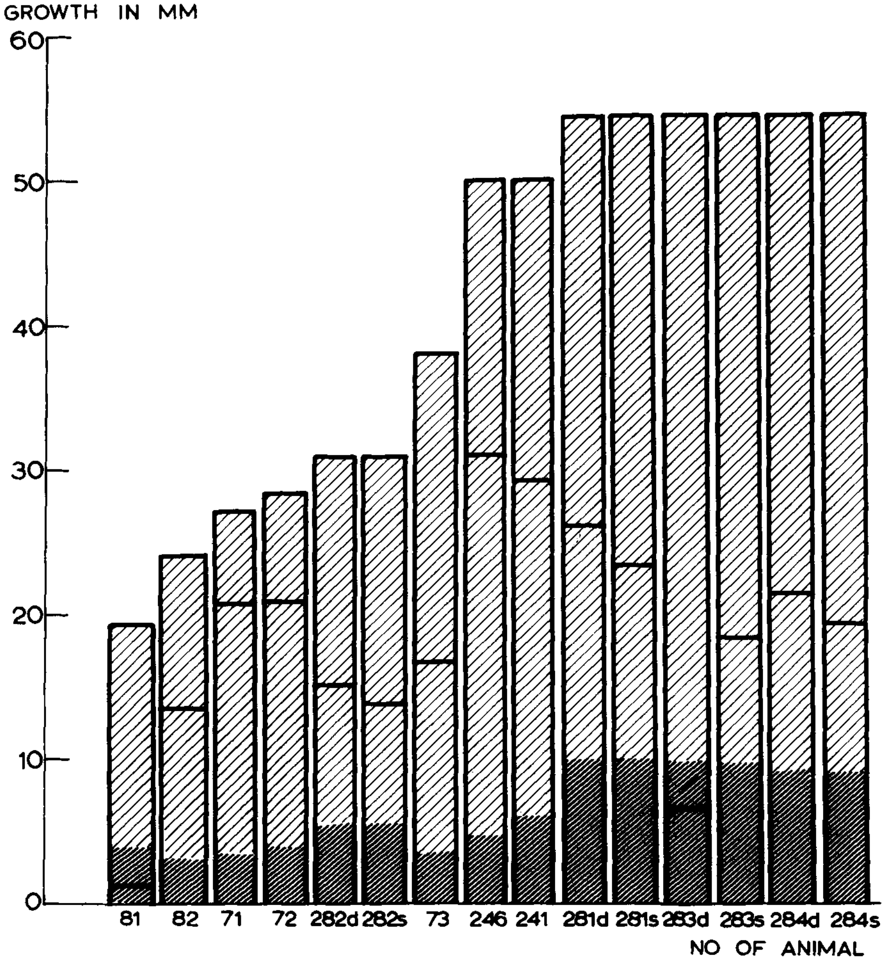


FIG. 27. — *Transplantation of the coronary suture to the site of the excised ECP of the fibula in the dog. The growth of the grafts (level of the transverse lines) as compared with the normal growth of the suture (dark columns) and with that of the fibular ECP (hatched columns).*

structure, in which the broad meanderings enclose relatively elongated bony islets and are separated from each other by the bone spicules of the marginal bone. The connective tissue is relatively rich in cells, but the matrix shows marked degenerative changes. Osteoblasts are seen in abundance, but the blood vessels are relatively few. A rather broad area of the marginal bone is coarsely fibrillar. In places numerous osteoclasts are seen in resorption lacunae both at the suture margins and on the outer and inner margins of the bone. The bone is cancellous; no tables or diploë are to be seen.

Case 81 (growth 5 per cent). No signs of the suture are found. The specimen consists of cancellous bone, at the end of which a layer of hyaline cartilage is visible.

Case 82 (growth 56 per cent). The suture is a complex, massive, winding structure, 6 mm in breadth. There are cells and fibres in abundance. Widespread degenerative changes are seen in the connective tissue. The central part of the larger areas of suture tissue are ossified. Moderate blood vessels are seen in the central layer. The marginal bone is mostly coarse-fibred.
Case 241 (growth 58 per cent). The thickness of the specimen is 7 mm. The suture is a very massive, winding structure 5 mm in breadth. The thickness of the streaks of of connective tissue varies a lot, being very great in places. The cells and fibres are abundant, and the degenerative changes not so marked as in the foregoing case. The cells in the peripheral layers are very large and round, and osteoblasts are abundant. The marginal bone is coarse-fibred. The bone is cancellous-lamellar. See fig. 28.
Case 246 (growth 62 per cent). In form and structure the suture does not differ significantly from that in the preceding case. At the top of the specimen a partly ossified epiphysis is seen fused with the graft.
Case 281 d (growth 48 per cent). The suture with its bends running almost longitudinally across the section is 6 mm in breadth. The thickness of the separate streaks varies considerably. In some places they are almost indistinguishable, in others very broad and rich in cells. Osteoblasts are relatively abundant. The marginal bone is formed mostly by a narrow area of coarse-fibred bone.
Case 281 s (growth 43 per cent). No significant difference from the foregoing cases is seen in the histological picture. The marginal bone is in places lamellar up to the suture.
Case 283 d (growth 12 per cent). Across about half the thickness of the specimen a thin, complex, winding suture is seen. Over the rest all that is visible are scattered remnants of the suture, separated from each other by bone.
Case 284 d (growth 39 per cent). The suture forms a winding structure, mostly of thin streaks of connective tissue, across an area 8 mm in breadth. The lamellar bone between the streaks shows an abundance of basophilic cement lines and the streaks are separated from each other by relatively massive areas of bone.
Case 284 s (growth 35 per cent). The microscopic picture is very similar to that in the foregoing case, except that only small areas of suture tissue are seen. Most of it shows a picture of an ossified suture. In places, isolated round areas of degenerative connective tissue are seen. The bone is cancellous, with relatively small cavities.

The microscopic picture of the grafts which had grown the most revealed a complex, massive, meandering suture formation, in which the longitudinal "hairpin-bends" of connective tissue were separated from each other by long, narrow bone spicules of the marginal bone. This formation showed great osteogenic activity compared to the normal suture at the same age. In the grafts that had grown least various degrees of ossification of the suture were seen.

TRANSPLANTATION OF THE SUTURE TO THE THIGH

The results of transplantation of the coronary suture to the diaphysis of the femur in the rabbit are presented in table 14.

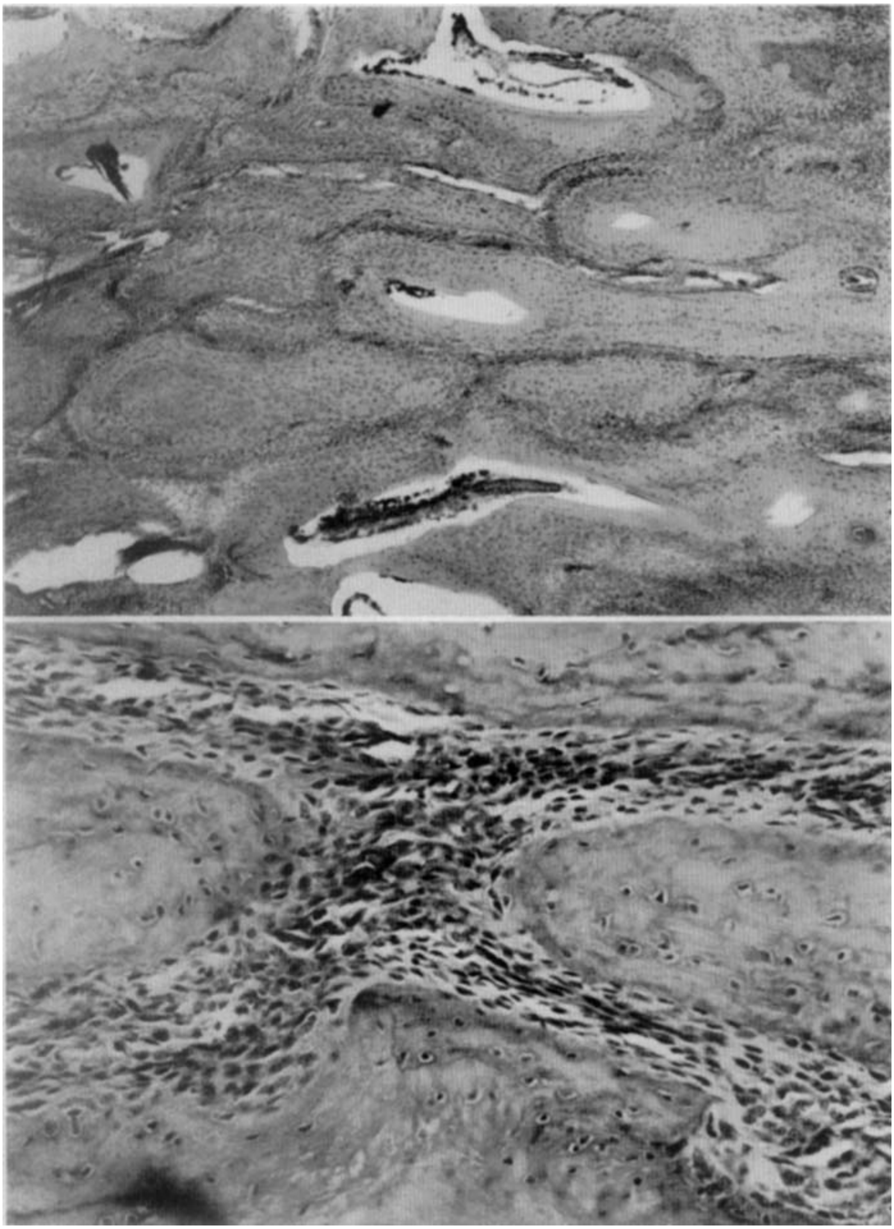


FIG. 28. – *Microscopic structure of the suture graft in the fibula. Dog no. 241. Above: the suture forms a massive structure, in which the meanderings of the suture tissue enclose bony islets and are separated from each other by bony spicules. Below: higher magnification of the same. The suture tissue is rich in cells and signs of intensive osteogenesis are seen at the bone margins.*

TABLE 14. – *Transplantation of the suture to the diaphysis of the femur in the rabbit.*

Animal No.	Age at operation (days)	Time of observation (weeks)	Growth of graft (mm)	Average growth of coronary suture in control group (mm)	Growth of graft in % of control sutural growth	Macroscopical and radiological observations		
						suture	fusion of graft to femur	special observations
61	18	26	(5)	6.5	77	not visible	not fused	distal marker expelled
62	18	26	2	6.5	30		fused	proximal half fused to femur
63	18	26	9	6.5	138		not fused	
64	18	26	0	6.5	0		not fused	
251 d	19	19	0	6.5	0		fused	proximal half fused to femur
251 s	19	19	(12)	6.5	184		side to side	
252 d	19	19	(5)	6.5	77		fused	fracture in femur, good union
252 s	19	19	0	6.5	0		side to side	exostosis at site of graft
253 d	19	19	10	6.5	154		side to side	distal marker expelled
253 s	19	19	(2)	6.5	31		not fused	both markers expelled
254 d	19	19	(6.5)	6.5	100		side to side	distal marker expelled; exostosis
254 s	19	19	(5.5)	6.5	80		side to side	distal marker expelled

In 9 of the 12 experiments the distance between the markers had increased. In only 2 cases (*62* and *253* d) had a branch-like formation developed; the growth of which corresponded to the increase in the distance between the markers. In experiment *63* the transplant, which had not fused with the femur, showed significant growth. In all the other 6 experiments either one or both of the markers had been expelled from the bone, and in most cases lay beneath the periosteum. The graft was usually fused side to side with the femur and only slight signs of it were distinguishable. The increase in the distance between the markers could not be interpreted as growth of the graft. In no case was an open suture found.

In figures 29 and 30 two examples of the experiments are presented.

TRANSPLANTATION OF A PIECE OF CALVARIAL BONE WITHOUT SUTURE TO THE FIBULA

The results of the transplantation of a piece of calvarial bone, which did not include the suture, to the site of the excised proximal ECP of the fibula are

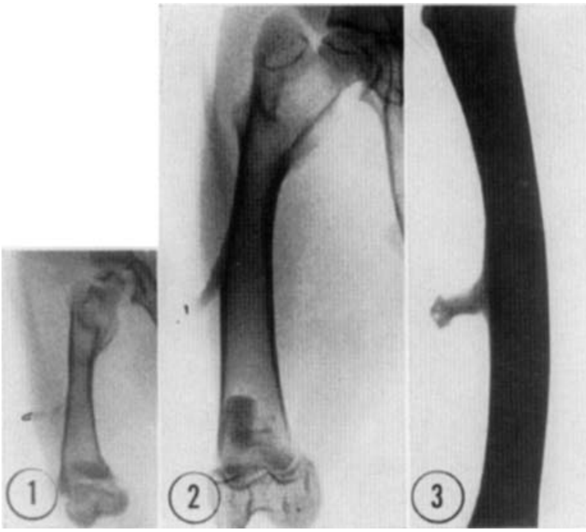


FIG. 29. — *Suture graft at the diaphysis of the femur. Rabbit no. 62. X-ray picture 6 days (1) and 10 weeks (2) after transplantation. (3) The specimen. A branch-like formation has developed, the growth in length of which was rather insignificant.*

FIG. 30. — *Suture graft at the diaphysis of the femur. Rabbit no. 251 s. The graft gradually fused side to side with the femur. The increase in the distance between the markers could not be interpreted as growth of the graft.*

presented in table 15. In no case was any growth of the graft noticed. In the case *301* the distance between the markers had increased, but this resulted from the expelling of one of the markers from the bone (see fig. 31). The result in all cases was either a bone defect in the fibula or a curved bridge from the fibula to the diaphysis of the tibia. In figure 31 an example of this type of transplantation is seen.

TABLE 15. – *Transplantation of a piece of calvarial bone without suture to the site of the excised ECP of the fibula in the rabbit.*

Animal No.	Age at operation (days)	Time of observation (weeks)	Distance between markers (mm)			Growth of fibular ECP (mm)	Growth of graft in % of epiphyseal growth
			Initial	Final	Increase		
301	16	41	5.5	12	6.5	33.5 [1])	19 [2])
302	16	41	6.5	3.5	–3	35 [1])	0
303	16	41	6.5	6.5	0	37 [1])	0
321	28	29	7	7	0	28.5	0
322	28	29	7.5	7.5	0	24	0

[1]) control animal of the same litter
[2]) one of the markers expelled

DISCUSSION

In reimplantation experiments in both the rabbit and the dog, the distance between the markers, inserted at the margins of the graft, had increased in some cases in a measure which corresponded to the normal growth of the suture. In spite of the fact that only very slight variations in operative technique were possible the proportion of these cases was only 3/16 in the rabbit but 5/8 in the dog. The result shows that preservation of growth is possible but, on the other hand, that growth is very easily disturbed.

Bone defects in the rabbit were often seen at the margins of the graft, but in the dog such defects were not found. *Sirola* (1960) noticed that the repair of the calvarial defect was prevented if the dura mater was excised from the area of the trephine opening. It is possible that minor lesions of the dura, often manifested by slight haemorrhage at the surfaces during the operation, could have caused the defects. Because of the larger proportions of the dog such lesions could be better avoided in this animal. No correlation between the occurrence of bone defects and the amount of growth was observed.

When the suture was transplanted to the site of the excised ECP of the fibula, it grew, in the most successful cases, many times as much as the suture normally

FIG. 31. — *Transplantation of calvarial bone without suture to the site of the excised ECP of the fibula. Rabbit no. 301. X-ray pictures made during the observation period reveal the shift in the position of the markers, which is not caused by the growth of the graft. A long defect has formed at the site of the fibula.*

grows in the skull. In the rabbit this ratio was in some cases 5:1 and in the dog 7:1. This can be regarded as a sign of an enormous potential capacity for growth. The result is biologically very interesting, for instance when it is compared with the results of epiphyseal transplantation. In the first part of the present work it was shown that the ECP graft may grow as much as would be expected in its original site. In earlier investigations by numerous authors the ECP graft, when transplanted to another bone, has undergone only 2/3 of that growth, at the most. This amount of growth, if it were possible to obtain it in clinical material, would certainly be sufficient in many cases, and certainly no one would try to produce a new femur instead of a lacking one by using a metatarsal bone as transplant. However, this comparison, although of theoretical interest, does not give very much information about the usefulness of the suture graft as a substitute for the ECP. From this point of view the comparison between the growth of the graft and that of the ECP is more useful.

In some experiments on the rabbit the growth of the suture graft in the fibula was as great as that of the corresponding ECP of the fibula. In two cases it grew even more than the control ECP. In case *173* this result may have been caused to the control, which was the fibula of another animal of the same litter. In case *176* the growth of the graft was distinctly greater than that of the ECP of the opposite fibula. As was shown in connection with the epiphyseal transplant, the distance between the ECP of the tibia and the fibula increases during growth. Therefore, in fact, a really successful suture graft should give as much growth in length as the ECP and epiphysis combined. In table 2 (p. 32) it is seen that the longitudinal growth of the epiphysis during the corresponding time is 3.5 mm. This means that the "ideal" growth of the graft is 25 mm, i.e. 0.5 mm more than the graft had grown.

The initial alignment of the graft in the host area seemed to be, as could be waited, of importance for the end result.

On the dog the suture graft gave 3/4 of the growth of the ECP at most. The cause of the difference in results in the rabbit and the dog is not quite clear. To understand the relative importance of the different factors possibly involved, the course of histological events which are common to all autogenous bone transplants must be borne in mind. The fact that the bone graft, which includes the suture, has been capable of growing must signify that the osteogenic cells of the suture have either survived or been replaced by host cells. According to the literature, it is probable that some, at least, of the connective tissue cells survive transplantation. This also applies to the bone cells. From the transplantation of the ECP we know that the survival of the germinal cells of the ECP seems to be essential for the preservation of growth capacity. Applied to the transplantation of the suture, it means that its success is dependent on the survival of the cells situated in the central layer of the suture.

The survival of the graft cells, on the other hand, depends on the rapidity of restitution of nutrition, i.e. revascularization. In the present work the relative importance of the different pathways of revascularization of the suture reimplant was examined by placing a polythene plate in between the graft and the dura. In the rabbit no growth occurred in such a graft. This confirms the statement of *Treves-Keith* that the nutrition supplied from the dura is more important than that coming from the periosteum. In the dog the results was somewhat unexpected, because the growth of the reimplant did not seem to be markedly disturbed. This could be interpreted as meaning that the vascularization from the periosteum is relatively more important in the dog than in the rabbit.

The size of the graft may to some extent account for the difference in the results in the rabbit and the dog. One might suppose that the revascularization of the larger graft in the dog would take longer than that in the rabbit. This dependence has not been demonstrated before, however. Moreover, the results of the epiphyseal transplantation in the first part of the present work speak against it.

It is difficult to say whether the growth of the transplanted suture in the most successful experiments on the dog, about 7 times as much as its normal growth, signifies its maximal growth capacity. At all events, to give equally good results as in the rabbit, the suture would have had to grow 9—12 times as much as it normally grows.

The differences in the anatomy of the fibula between the rabbit and the dog were manifested in some phenomena noticed during growth. When the growth of the graft was disturbed in the rabbit, the shortening of the fibula corresponded to the disturbance of the growth. It could also cause curving and shortening of the tibia, if it had fused to the epiphysis of the latter. In the dog disturbance of the growth of the tibia was uncommon. Besides that, the shortening of the fibula often bore no relation to the disturbance of the growth of the graft, which was explained by the compensatory growth of the distal epiphysis. This is clearly seen, for instance, in figure 25 (p. 84). The phenomenon of compensatory growth has previously been noticed in animal experiments, e.g. by *Ollier* and *Silfverskiöld.*

The special character the acquired by suture graft after transplantation to the site of the ECP can be regarded as a manifestation of functional adaptation. Its development could perhaps be understood in terms of the normal development of the serrate suture. The formation of this is caused by localized apposition of bone at the two bone margins *(Weinmann & Sicher).* If the same phenomenon were to continue very intensively and for some length of time it is conceivable that the dents might grow much longer. The suture in the grafts had a greatly increased number of "hairpin bends", as if the suture had been "stretched". In fact, the forces acting on it were the same as those that normally act on the ECP of the fibula. The mechanical forces acting on the graft exerted perhaps tension rather than pressure, but this could be of no special significance, because they were physiological.

The suture, when once adapted to its new position, seemed to withstand the local mechanical stress well. The fibula is naturally comparatively favourable as a host area, because the bending forces and weight-bearing in it are not heavy. However, an example of the fact that, as a host area, it does not essentially differ from the ulna, for instance, is provided by the following experiment performed on the dog (fig. 32):

At the age of 26 days a suture graft was transplanted to the site of the excised distal ECP of the ulna. A tiny piece of the epiphysis was left in situ. After 26 weeks it was seen that the graft had grown enormously and been able totally to prevent the ulnar deviation of the carpus which results if growth is insufficient. True, this result was partly due to concomitant shortening of the radius. The function of the limb was quite perfect.

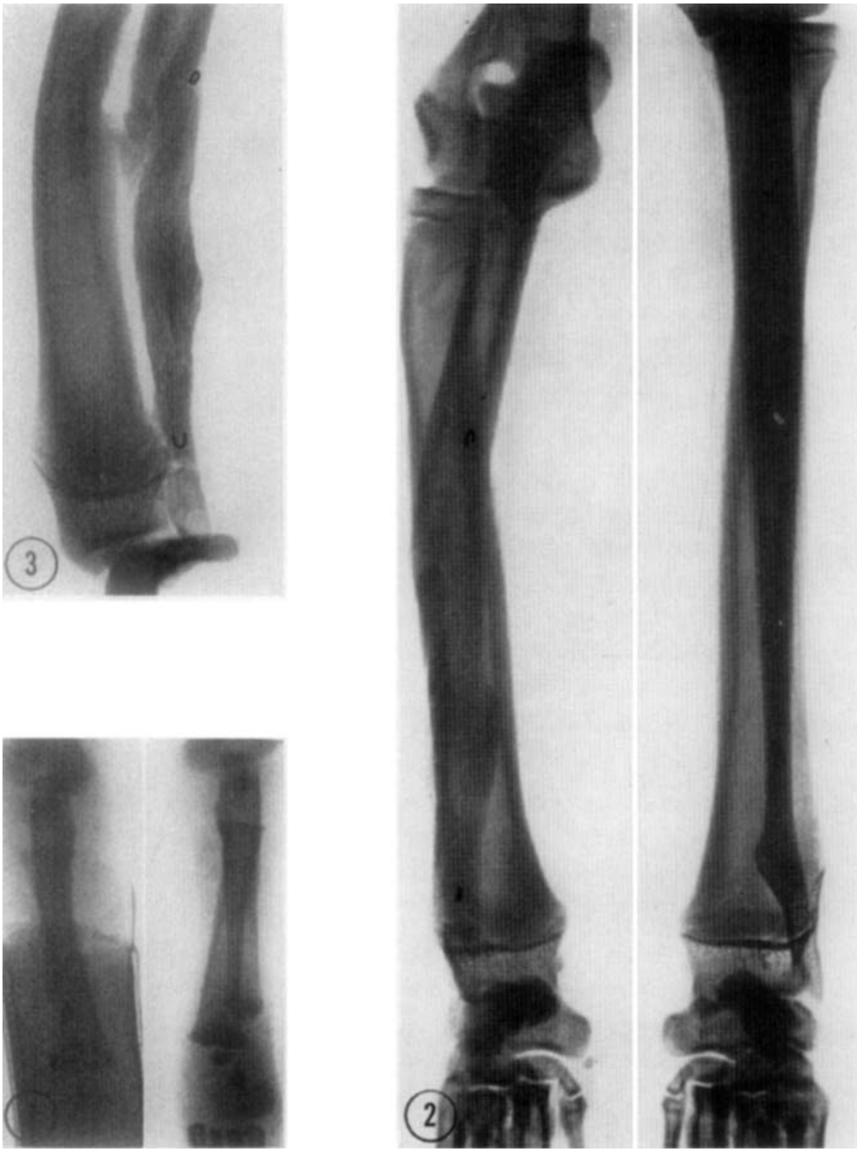


FIG. 32. — *Transplantation of the suture to the excised distal ECP of the ulna in the dog. X-ray pictures 2 days (1) and 26 weeks (2) after transplantation. Note the increase in distance between the markers and the normal position of the carpus. (3) X-ray picture of the specimen.*

CONCLUSIONS

1. The coronary suture of the rabbit and of the dog may, after reimplantation, continue growth normally in favourable circumstances, but the growth of the graft is very easily disturbed.

2. The suture graft, when transplanted to the site of the excised ECP of the fibula, preserves its growth capacity in both the rabbit and the dog. In the rabbit the suture graft may grow as much in length as the corresponding ECP of the fibula normally grows, which corresponds to a 5-fold growth compared with that of the normal suture. In the dog, in the most successful cases, the suture graft grew 3/4 of the growth of the corresponding ECP of the fibula. This is about 7 times as much as the suture normally grows in the skull.

These results signify that the suture has a potential growth capacity many times greater than is revealed in its normal site.

3. The adaptation of the suture graft to its new environment is striking, as is manifested by the considerable changes in its form and structure.

SUMMARY

The main focus of the present work was the preservation of growth in autogenous growing bone grafts. This problem is of clinical interest, because a bone graft capable of growing after transplantation would be of value in the treatment of bone defects in growing long bones. Two types of grafts were used: epiphyseal cartilage and cranial suture.

Transplantation of epiphyseal cartilage. Besides the growth capacity, the importance of the bony contact between the graft and the host bone was also studied. Young rabbits and dogs were used as experimental animals. On them a transplantation of the proximal ECP of the fibula to the corresponding site in the opposite leg or to muscle was performed.

The growth of the graft was followed by X-ray check-ups. The specimens were examined both radiologically and histologically. The growth of the graft was compared with the normal growth of the ECP. Both on rabbit and on dog the amount of growth was in some experiments as great as that of the control, but more or less disturbed in the majority of cases. The microscopic structure was normal in cases where the graft had grown well.

The results were discussed in the light of the literature. The following conclusions were drawn:

1. The ECP of the fibula may, when transplanted to the corresponding site in the opposite leg, retain its full growth capacity. Yet, in spite of great care in technical performance, some not easily definable factors very often cause a disturbance of growth.

2. In successful cases the transplanted ECP does not differ from the normal in either appearance or structure at the end of the observation period.

3. Without bony contact between the graft and the host bone, only insignificant growth can take place in the graft.

Transplantation of cranial suture. In the second part of the work some features of a new type of growing bone graft were studied. This was considered to be motivated because the clinical results of the transplantation of epiphyseal cartilage have not been very encouraging and also because there is a very limited source of autogenous grafting material of this type.

The transplantation properties of the cranial suture were studied by reimplanting a bone plate including the suture and by transplanting it either to the site of the excised proximal ECP of the fibula or to the shaft of the femur. In addition, a number of control experiments of various types were performed. Young rabbits and dogs were used as experimental animals. The growth of the grafts and the structure of the specimens were studied by the same methods as in the first part of this work.

The growth of the reimplanted suture was compared with the normal growth of the suture. The growth of the suture graft, transplanted to the fibula or to the thigh was compared both with the normal growth of the suture and with that of the fibular ECP.

The results were discussed from various points of view. The following conclusions were drawn:

1. The coronary suture may, after reimplantation, continue growth normally, but the growth of the graft is easily disturbed.

2. The suture graft, when transplanted to the site of the excised ECP of the fibula, may preserve its growth capacity. In the rabbit the suture graft grew as much in length as the corresponding ECP normally grows, which corresponds to a 5-fold growth compared with the normal growth of the suture. In the dog the graft grew in some experiments 3/4 of the growth of the corresponding ECP of the fibula. This is about 7 times as much as the suture normally grows in the skull.

The results signify that the suture has a potential growth capacity many times greater than is revealed in its normal site.

3. The adaptation of the suture graft to its new environment was striking, as was manifested by the considerable changes in its form and structure.

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CORRECTIONS

Page 13: for "*Wolff* (1892)" place "*Wolff* (1870)"

Page 13: for "*Kölliker* (1831)" place "*Kölliker* (1875)"

Page 13: for "*Humphry* (1861)" place "*Humphry* (1779)"

Page 13: for "*Biscard*" place "*Bisgard*"

Page 21: for "*Pereira* and *Dupertuis*" place "*Sousa Pereira* and *Dupertuis*"

Page 52, line 14: for "*Humphry* (1861)" place *Humphry* (1779)"

Page 53, line 9: for "Sieffert" place "Siffert"

Page 101, line 6: for "1914" place "1916"

Page 101: in between the lines 15 and 16 add "*Gudden:* Experimentaluntersuchungen über das Schädelwachstum. München 1874. Quoted by *Thoma.*"

Page 105: in between the lines 26 and 27 add "*Strelzoff:* Beiträge zur normalen Knochenbildung. – Zbl. med. Wissensch. *10:* 449, 1872."

Page 106: in between the lines 19 and 20 add "*Wolff, J.:* Ueber die innere Architektur der Knochen und ihre Bedeutung für die Frage von Knochenwachstum. – Arch. Path. Anat. *50:* 389, 1870."