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THE EFFECT OF SUBPERIOSTEALLY
IMPLANTED AUTOGENOUS WHOLE-THICKNESS
SKIN GRAFT ON GROWING BONE

An Experimental Study

BY

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PREFACE

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INTRODUCTION

The Clinic for Orthopedics and Traumatology, University Central Hospital, Helsinki concentrated in the 1950s upon the study of the properties of skin and its use as material in reconstructive surgery. Four comprehensive publications on the question have already been completed on the initiative and under the direction of Professor K. E. KALLIO, chief of the Clinic. The first of these, an investigation carried out by KIVILAAKSO in 1955, discussed the use of whole-thickness skin to replace tendon and muscular tissue. He found that the skin graft developed tissue which resembled tendinous tissue both grossly and microscopically. The experimental study published by KETTUNEN (1958) was concerned with the use of skin as interposition material in arthroplasty of the hip joint of cat. He established that cartilaginous metaplasia takes place in the connective tissue serving as interposition material. The third worker was JOKINEN (1958) who studied the tensile strength of the whole-thickness skin graft used to repair tendon and ligament defects. He found that skin tendon met the tensile strength requirements of tendons and ligaments although it was not fully as strong as normal tissue. For the clinical use of skin Professor K. E. KALLIO has developed a method of his own, skin arthroplasty; the method has been applied at our clinic in 147 operations between 1954 and today on different joints. The fourth work published, in 1958, was ERKKI KALLIO's "Skin Arthroplasty of the Hip Joint and Corresponding Alloplastic Methods in the Light of a Clinical Study".

Another aspect awaiting experimental investigation was the influence of subperiosteally implanted skin graft on tibia of young growing rabbit and whether it causes changes in the longitudinal rate of growth. This task, the fifth investigation into the properties of skin, he entrusted to the present writer.

SURVEY OF THE LITERATURE

Transplantation of skin into tissues

REVERDIN and MAYOR (1887) were the first to investigate traumatic epithelial cysts. They were subsequently studied by e.g. PELS-LEUSDEN (1905) and WIEN & CARO (1934). KING (1933) suggested that the cysts originated from the epithelium of sweat glands. PEER and PADDOCK (1937) conducted human experiments by placing pieces of skin under the subcutaneous tissue after they had removed the epidermis as extensively as possible. They then studied samples taken at intervals of 7 days to 12 months. Small cystic cavities had developed from the epidermis during the first 2 months. The cystic cavities were preserved throughout the observation period, but they were not lined with epithelium. The sebaceous glands and hair follicles had disappeared, but the sweat glands were preserved. STONE ET AL. (1959) divided the cysts according to etiologic factors into congenital and post-traumatic. Congenital cysts were generally situated under the scalp, the post-traumatic in the hands, fingers and soles of the feet.

Epithelium has also been inserted experimentally in the long bones. YANO (1940) placed pieces of the skin of rabbit embryo in the medullary cavity of the tibia of adult rabbits. In 105 days a pearl-like formation developed. WOLFE and DIAMOND (1956) placed an autogenous mucosal tissue strip in the medullary cavity of rabbit femur. 12—56 days after the implantation the samples showed almost complete necrosis in the epithelium of the implant but new epithelium grew from basal cells. Not a trace of the implant was found in one animal. The implant showed a marked vascularised, fibrocollagenous scar reaction. It was surrounded by a zone of regenerated bony trabeculae. In the connective tissue there were giant cells of foreign body type. The longest observation period was 70 days.

Epidermic cysts have been found in the bones of the end phalanges of the fingers, usually after a trauma. FISHER ET AL. (1958) have recently

reviewed the literature on these cysts. Inclusive of the 3 cases reported by these authors, a total of 36 cases have been published so far. These cysts lack the appendicular tissues of the skin. Giant cells of foreign body type are often present in the periphery of the cyst.

LOEWE (1913) used skin to replace the tendon defect or to strengthen hernia plastic. He published his clinical material in 1929. It comprised 100 patients on whom skin had been used in reconstructive surgery. His results were good and he came to the conclusion that the transplant healed and united well with tissues. REHN (1914) used skin whose epidermis had been removed to replace the extensor tendon of a finger. He later published several papers on the use of skin for a diversity of purposes. He found that when the transplant was subjected to function it was transformed in 6 weeks into a tissue resembling tendon, and he adopted the term "functional metaplasia". According to MAIR (1945), it was not necessary to remove the epidermis when skin was used in hernia plastic. KIVILAAKSO (1955), in his experimental study, concluded that the organism tried to destroy or isolate that part of whole-thickness skin graft which is of ectodermal origin, while transforming the tissue which had developed from mesoderm into an organic part of itself. KETTUNEN (1958) used skin as interposition material in hip joint arthroplasties on cats and observed that the pressure and function of the joint destroyed the epidermis during the first weeks of observation. No epidermal cysts developed.

It has been stated that the skin is very plastic material which can be used also for reconstructive surgery of tissues inside the body. When the transplant is subjected to function or tension, as in replacing a tendon or ligaments, the connective tissue of the dermis transforms according to the situation into a fibrotic tissue resembling the original tissue, and the epidermis disappears. When skin is used e.g. in arthroplasties the preserved connective tissue undergoes functional metaplastic development into cartilage. When subjected to pressure the epidermis disappears quickly, but if the transplant is functionless in the tissue an epidermal cyst develops.

From the outset, it was to be expected that an epidermal cyst would develop in the experiments. The literature, however, provided no suggestion of how it would form in the bone tissue from the subperiosteally implanted skin graft or of what physiological influence it would have. The point aroused special interest when it had been found that the skin grafts had a stimulating effect on bone growth.

Growth of long bones

Before introducing the present investigation, a brief discussion of bone growth in general and especially attempts to stimulate longitudinal bone growth might be in order.

Long bones grow endochondrally from the epiphyseal cartilages. According to TRUETA (1957) epiphyseal cartilage grows both appositionally and interstitially. It grows in width through the function of the cells of the perichondrium, while the actual longitudinal growth takes place in epiphyseal cartilage interstitially with the cartilage cells dividing and swelling. This process is then accompanied by degeneration of the cartilage cells, vascularisation of degenerated cartilage by blood vessels and connective tissue from the adjacent shaft while osteoblasts form bony tissue in the framework of unresorbed cartilage (SISSONS 1956). The final architecture of the bone is determined by its moulding which occurs through equilibrated bone resorption and bone formation.

As long ago as 1727 HALES established that bones grow at the ends. He punched two holes through the middle of the tarso-metatarsal region of a young chicken and found two months later that the holes were equally far apart from one another as before. DU HAMEL (1739—1743) studied bone growth of different species of animals by using intermittent madder feeding and marking bones simply by piercing or placing silver wires into bone corticalis. He found that the bones grew in length principally from epiphyseal cartilages and in thickness principally through the periosteum, forming new layers of bone around the shaft. OLLIER (1867) placed metal marks in the tibia of rabbits, doves and chicken a few days old and established that the distance between the marks remained unchanged although the bone had grown as much as three times longer in the interval. In some chicks in which the mark had been placed at the extreme end of the diaphysis the distance increased 0.5—1.5 mm. Ollier did not attach any significance to this exceptional, small error as detracting from the rule “*qui est le non-allongement du tissu osseux*”. Madder was also used to study bone growth by JOHN HUNTER (1760—1770) and later on by BRASH (1934) and PAYTON (1931). ARIES (1941) made corresponding studies using the alizarin red obtained from madder. Since the discovery of roentgen rays bone growth has been studied by placing metal marks on the bones and observing the position of the marks roentgenologically at fixed intervals. These tests have been performed by HAAS (1926),

GATEWOOD and MULLEN (1927), SILFVERSKIÖLD (1934), and BISGARD and BISGARD (1935). Accidental metal poisoning in some cases made it possible to study the growth of bone with the substance marking the bone. This was the case, for instance, in VAHLQUIST'S (1943) material of two children under 2 who had been given bismuth. The modern method is to use autoradiography for investigations of bone growth. Mention may be made of the work of LEBLOND ET AL. (1950) which confirmed the finding of all the above studies that bone grows solely from epiphyseal cartilage. Based on the original idea put forward by RANVIER (1875), POLICARD (1941) introduced a theory that growth occurs chiefly at the point of junction of the perichondrium and epiphyseal cartilage, with the latter itself participating in the growth process to no greater extent than preventing the formation of an empty space in the middle of the bone when the growing diaphysis and metaphysis push the epiphyses apart. This theory was accepted also by LANGENSKIÖLD and EDGREN (1950). HELLSTADIUS (1947) resected extra-periosteally the distal epiphysis and epiphyseal cartilage of rabbit ulna and found that the bone grew distally. He took it that bone was capable of longitudinal growth interstitially even without epiphyseal cartilage. LACROIX (1951) repeated the experiments and Hellstadius's observation on the longitudinal growth of bone, but observed that an epiphyseal cartilage regenerated and stated that he would hold to his opinion that the longitudinal growth of bone is not possible without epiphyseal cartilage. DÜBEN and GELBKE (1955) carried out similar experiments on puppies. They considered that it was not possible to speak of actual longitudinal growth in Hellstadius's experiments since the treated ulna remained considerably shorter than the control. The lengthening of the bone was in fact due only to bone regeneration at the end of the stump.

DU HAMEL (1743) and OLLIER, (1867) found that bone growth was not symmetrical from both epiphyses but that different epiphyses participated in the growth process in a different manner. GILL and ABBOT (1942) collected the results of different authors in a table showing the percentual growth from the different epiphyses and the method used by each investigator. According to Gill and Abbot, the following percentages are sufficient in practice:

Femur — proximal end 30 per cent, distal end 70 per cent

Tibia — proximal end 55 per cent, distal end 45 per cent

Epiphyseal lines also close at different times. The epiphysis from which the bone grows least closes earlier. When growth has ceased the epiphyseal cartilage is resorbed and substituted by bone trabeculae.

Some physiological and pathological factors affecting bone growth.

In addition to hereditary factors, many physiological and pathological factors affect longitudinal bone growth. According to TRUETA (1957), apart from circulatory factors, natural strain and pressure are the most important factors regulating the growth of bone. It is a generally acknowledged fact that growth is slowed down by inactivity. HOWELL (1917) examined the effect of muscular activity on bone growth by severing the right side brachial plexus in young dogs and found that the limb of the side so treated remained shorter than the control limb. Limb growth is also retarded in post-poliomyelitis paralysis of the limb. According to the HÜTER-VOLKMANN (1862) theory an abnormal increase in pressure retards bone growth whereas reduced pressure or even traction accelerates bone growth. WOLFF (1892) stated, conversely, that increased pressure accelerates bone formation and a decrease in pressure furthers the resorption of bone. MÜLLER (1922) resected one bone from the antebrachium, thus subjecting the epiphyseal cartilage to greater pressure. Growth was slowed down, lending support to the Hüter-Volkmann theory. According to THOMA (1907), pressure on the epiphyseal cartilage must be 6.6 g/sq.mm. to stimulate the growth of the long bones of man. STROBINO ET AL. (1952), again, calculated that a minimum pressure of 37 g/sq.mm. is necessary for any considerable retardation of longitudinal growth. They employed calves as test animals. SMITH and CUNNINGHAM (1957) placed round the epiphyseal cartilage of calves a traction device which pulled the epiphysis away from the metaphysis. They observed that the epiphyseal cartilage increased in breadth but were unable to pronounce definitely concerning the effect of traction on the longitudinal growth of the tibia.

Metabolic factors are of great significance for bone growth. According to SISSONS (1956) disturbed nutrition, e.g. in connection with a chronic disease, may interfere with longitudinal growth. If proliferation of cartilage and osteoblastic bone formation are equally affected, the result is retardation or cessation of growth. If cartilage proliferation continues while osteoblastic bone formation is disturbed, primary spongiosa is not replaced by bone. If, on the other hand, cartilage proliferation ceases and osteoblastic bone formation continues an ever-thickening bony layer originates in the metaphysis, and if the growth normalises from this a transverse line remains in the bone for a long time as evidence of the previous slow-down in longitudinal growth.

The present author observed (1959) that the bone length of rabbits which have recovered from coccidiosis was shorter than the average.

Certain avitaminous conditions cause more specific changes than the general nutritional deficiency mentioned above. BARNICOT and DATTA (1956) quoted Mellanby's experiments according to which vitamin A is a specific chemical controller of osteoblastic and osteoclastic activity. The exact nature of this control, however, is not known. BOURNE (1956) postulated that vitamin C deficiency inhibits the synthesis of both collagenous fibres and bone matrix. vitamin D deficiency produces rickets. Behind the changes provoked by rickets is, according to HARRIS (1956), deficient calcification of bone matrix which is especially apparent in newly-formed bone close to the epiphysis.

According to SISSONS (1956), nutritional factors, inclusive of vitamins, form the background to optimal bone growth while hormonal factors are more responsible for control of bone growth. Hormones especially affect the "maturation" of bones, i.e. the time at which centres of ossification appear and the time epiphyseal cartilages disappear. Overproduction of the growth hormone of the hypophysis causes gigantism or acromegaly, whereas the absence of hormone causes dwarfism. ASLING and EVANS (1956) stated that the growth of bones was retarded after hypophysectomy or thyroidectomy. Bone growth could be restored to normal in hypophysectomised rats by giving them sufficient quantities of growth hormone. No other hormone showed this ability. The maturation of bones also slowed down following hypophysectomy or thyroidectomy. ASLING and EVANS (1956) mentioned concerning the interaction of hormones that thyroid hormone increased the effect of the growth hormone while adrenocorticotrophic hormone diminished it. It was also obvious that testosterone increased and estrogen decreased the effect of the growth hormone. It is a known fact that parathyroid extract elevates the calcium concentration of the blood and promotes the resorption of bone. SILBERBERG and SILBERBERG (1956) studied the effect of steroid hormones on bone. They held that hypofunction of the ovaries retarded skeletal development and decreased ossification. Overdosage of these hormones resulted in retardation of growth and accelerated maturation of the bone. Hypofunction of the testes caused a condition similar to that of hypofunction of the ovaries. All stages of bone development were retarded and maturation was delayed. While small doses of androgenous 17-ketosteroids stimulated, large doses delayed or suppressed linear growth. In addition, androgenous 17-ketosteroids had a stimulating effect on the development of bones in that they promoted condensation of

the ground substance and ossification. According to Sissons (1956), testosterone had a stimulating effect on epiphyseal growth and its effect was synergistic with that of pituitary growth hormone. ACTH and cortisone retarded bone growth or might even bring it to a complete standstill.

Stimulation of longitudinal bone growth.

(a) *Trauma to metaphysis or epiphysis. Periosteal detachment.* OLLIER (1867) was the first to detach the periosteum from the greatest part of the tibial diaphysis in rabbits, thus accelerating the growth of bone so that the tibia lengthened 2–5 mm in three months. WU and MILTNER (1937) performed periosteal stripping on 22 rabbits aged 5–8 weeks in five different ways: (1) by detaching the periosteum from the region of the tibial shaft as a whole; (2) by detaching the periosteum from the distal part of the tibial shaft; (3) by detaching the periosteum from the proximal part of the tibial shaft; (4) by detaching the periosteum from the anterior part of the tibia leaving the nutrient artery intact; (5) by detaching the periosteum from the proximal part of the tibial shaft and the distal part of the femur. The maximum disparity obtained by these workers was 6 mm in three months. Acceleration of longitudinal bone growth was provoked in all groups. The mean length increase for each group was not given, only the individual values. I have calculated the means for the different groups from these results; (1) 3 mm, (2) 2.8 mm, (3) 4.5 mm, (4) 1.2 mm, (5) tibia 2.2 mm and femur 1.8 mm. LACROIX (1951) detached the tibial periosteum of 8 rabbits aged 35 days from the region of the two middle fourths of the bone. Three animals died in 65–70–153 days, the others were sacrificed 235 days postoperatively. The length inequality was 1.0–2.3 mm in seven animals and under 1 mm in the eighth. A spindle-shaped shadowing, due to accelerated osteogenesis, was observed roentgenologically around the diaphysis of the operated animals from the second week. This shadow was rapidly absorbed. Maximal lengthening was achieved by the 21st day and the disparity remained equal in size to the end of the observation period. PEASE (1952) stripped the tibial periosteum of a girl of 11 at and the same time inserted tibial bone segments under the periosteum. The measure caused some acceleration in longitudinal growth but the effect was transient. BRODIN (1955) stripped the periosteum from the proximal half of the tibial shaft and from the area of the proximal metaphysis in 44 rabbits; the epiphyseal line he left intact. The nutrient

artery was spared by leaving the periosteum at the nutrient foramen. The operation was followed by a progressive increase in growth from the distal epiphyseal cartilage on the experimental side and an almost corresponding inhibition of growth from the proximal epiphyseal cartilage. LANGENSKIÖLD (1957) stripped the periosteum of the tibial shaft of 13 rabbits aged 14—36 days and wound a plastic film subperiosteally around the shaft. This produced growth stimulation of as much as 2—3 mm even in 8 rabbits. Considerable callus formation was demonstrable in several cases and in some instances sequestration without manifest infection under the film in the diaphysis. FREJKA and FAIT (1957) stripped the periosteum from the tibia of 28 children until 2 cm from both epiphyseal cartilages, avoiding the nutrient artery. They also sawed 3—6 longitudinal incisions in the tibial cortex. The best result was achieved when the operation was performed at the age of 6—8 years. The authors stimulated a 1—3 cm increase in the length of the operated tibia. When they published their report the first patients were already 20 years of age. BERTRAND and TRILLAT (1948) stripped the diaphyseal periosteum of 23 guinea pigs, performed bone trepanation on one guinea pig and injected turpentine into the marrow cavity of 3 animals. Detachment of the periosteum alone caused a 7—9 per cent increase in length after 3 months. Injections of irritants often caused serious symptoms; 2 animals died immediately; a third sustained a fracture which consolidated spontaneously, but even in this case there was no length inequality within 2 months. The same authors, after periosteal stripping of the tibial shaft in animal experiments, performed the same operation on 4 children aged 5—10. They summarised their findings in the statement that the present technique can effect a lengthening of 1—3 cm in the shorter limb.

b. Other traumas. FERGUSON (1933) drilled a hole in the middle of the epiphyseal line and the bone in 16 children and scraped the bone marrow off with a knife or curette. He produced an increase in length of 0.2—3.1 mm in 3—5 months. KISHIKAWA (1936) drilled several small holes in the tibial shaft of rabbit. Growth increased 1.77 per cent compared with the control side in 4 weeks. WU and MILTNER (1937) carried out the same procedure on 6 rabbits but failed to cause any notable stimulation in growth in 3—6 months. COMPERE and ADAMS (1937) drilled 3 holes through the femur of 3-week old rabbits, but found no acceleration of growth in 48—77 days in the six rabbits that survived. They also fractured the tibia of 21 rabbits and established

growth stimulation in the broken bone as long as the healing process lasted. In the same work they reported a material of 8 children from whom tibial bone grafts were removed for spondylodesis. Longitudinal growth was accelerated in all but one of these eight subjects. The stimulation ceased in all of them as soon as the bone defect healed. HUTCHISON and BURDEAUX (1954) drilled a hole with a diameter of 6.25 mm transversely across the distal metaphysis of the left femur, 2.5 cm proximally from the distal epiphyseal line, in 10 dogs. Only 5 of the 10 animals showed an increase in length, average 0.77 per cent. Three animals showed no difference in femoral length, and 2 animals showed a decrease in femoral length averaging 1.2 per cent. SCHÄFFER, (1951) drilled 2–3 holes in the femur and tibia of 5 children. Three of them had chondrodystrophy, 1 bilateral epiphyseal growth disturbance and the fifth a congenital femoral defect. The reaction varied. There was no reaction in 1 subject, but in general the length of the stimulated limb increased 2 cm annually.

c. Foreign bodies or injected substances in the vicinity of the epiphyseal line. MEISENBACH (1910) inserted in the epiphyseal line of rabbit various agents such as sterile water, sterile graphite nails, staphylococcal vaccine, iodine tincture, carbolic acid, alcohol, formalin. He found microscopically that osteogenesis accelerated especially after formalin injection. TROUT (1915) examined 10 rabbits by inserting a vanadium metal screw into the metaphysis close to the epiphyseal cartilage at the age of 4–6 weeks. In 4 rabbits the screw shortened the limb 0.5 to 2 cm. No changes were established in 6 rabbits. In addition to these animals, the author rejected 12 rabbits in which roentgenological examination revealed that the screw was lodged in the epiphyseal cartilage and 7 animals which developed wound infections. A suspension of staphylococci and streptococci cultured from chronic osteomyelitis and kept at 53–60° C for one hour was injected into the tibial metaphysis of young dogs by GUERRIERO (1927). The test series consisted of 10 dogs. No effect was observed on the longitudinal growth of the tibia. The metaphysis showed a pronounced post-injection bony regeneration. BOHLMAN (1929) drilled holes close to the femoral epiphysis of guinea pigs and inserted in them a great variety of materials, employing four animals for each geant. The materials used were iron pegs, copper pegs, resinous or greasy pitchpine pegs boiled in paraffin, pegs of freshly extracted resin, absorbent cotton soaked in balsam of Peru, vaccine paste of staphylococcus aureus, asphalt pegs, gum camphor, menthol

crystals, thymol crystals, pine pegs without pitch or autogenous bone pegs and ash ivory pegs. None of these agents provoked longitudinal bone growth. On the contrary, the affected limb was often found to be shortened after 85 days. There was around the implant a fibrocellular capsule which sometimes had epithelioid and giant cells. The capsule was surrounded by regenerated bone. The marrow cells were closely packed and even necrotic cells were found. The bone structure revealed irregularity. KISHIKAWA (1936) injected staphylococcus albus suspension or turpentine into the bone marrow of the knee joint of young rabbits. With minor exceptions he found 0.95 per cent acceleration of bone growth in 6 weeks and 5.07 per cent in 16 weeks. He did not mention the number of test animals he used. WU and MILTNER (1937) inserted cotton, gauze, paper, wood and iron shot in the upper metaphysis of the tibia of 12 rabbits aged 5–8 weeks. There were no changes in tibial length during an observation period of 1–3 months. BERTRAND and TRILLAT (1948) stimulated human bone growth by 6 or 8 injections of blood 1–2 times a week into the knee joint of the shorter limb. The final results are not yet known but in 6 cases the growth retardation was stabilised. In 2 cases they produced a gain of 6 mm in 5 months. HAAS (1958), placing the materials in different ways, inserted iron, copper, aluminium and black oxide of manganese in the region of the epiphyseal line of rabbit radius. He succeeded in producing acceleration of growth in only one test, the one in which copper was inserted in the epiphysis and iron in the metaphysis. Retardation of growth was established in one test in which oxide of manganese caused a destructive reaction in the epiphyseal line. The author also placed a pituitary growth hormone implant near the epiphyseal cartilage, but established no effect on longitudinal growth. v. LANGENBECK (1869) inserted iron nails in canine tibia and femur. The operated hind leg gained 1 cm in length in 3 1/2 months. CHAPCHAL and ZELDENRUST (1948) placed steel, copper, zinc and iron plugs in the metaphysis in the distal part of femur and the proximal part of the tibia of dogs, guinea pigs and rabbits (mostly the last). The total of the test animals employed was 32. The authors found it possible with metal and especially iron nails to produce a reaction stimulating longitudinal bone growth. The results, however, were always uneven and uncertain. As the procedure also caused bone deformities they regarded measures of this type as unsuitable for clinical use. PEASE (1952) inserted ivory, vitallium, vanadium, brass or rust-proof steel nails in the metaphysis of 7 children. Stimulation of a varying degree was produced in every case. There were no serious deformities.

PEASE (1956) preferred the following method to stimulate growth: an aluminium net was placed in the medullary cavity of the metaphysis and around the bone a mesh of rust-proof steel; the meshes were fixed with screws through the bone. CHARPENTER and DALTON (1956) inserted iron nails in the epiphyses of 30 human patients. The maximal gain in growth was 3.1—6.25 mm. The authors were of the opinion that the measure might be suitable in some cases in which the patient is under 6 years of age and shows a growth discrepancy of less than 3.8 cm following poliomyelitis. JANSEN (1957) used ivory nails clinically for growth stimulation in 68 patients. The results were minimal. The only effect seen was during the first few postoperative months. HERNDON and SPENCER (1953) implanted copper plugs in the femoral metaphysis of rabbits 3—4 weeks old as close to the distal epiphysis as possible. Animals whose epiphyseal cartilage was damaged were eliminated from the material. The authors finally accepted only 10 animals for the investigation. In 3 rabbits the femur was lengthened by 0.23 cm, in 6 it was shortened by 0.23 cm and in 1 animal the femurs remained equally long. The observation period was 7 months. BLOUNT (1956) achieved a gain of 5—10 mm in 6 postoperative months in 81 patients whose femur and tibia were stimulated. The methods used were extensive periosteal stripping, making a hole in the cortex, curettage of the medullary cavity and insertion of ivory pieces in the bone. KRAMER (1955) stimulated longitudinal growth in the bones of the lower extremity of 12 children by inserting in the femoral and tibial metaphyses iron plugs and brass screws and by drilling multiple holes. The author observed no differences in the effect of these measures. The results ranged from a stimulation of 1.25 cm to a retardation of 6.4 mm. He assumed the acceleration of growth to be due to hyperemia caused by the periosteal irritation of the measure. KRAMER believed that "prolonged stimulation could be best accomplished by elevating the diaphyseal periosteum and placing absorbable foreign material such as chromic catgut under the periosteum. Foreign material that is buried in the bone itself does not cause stimulation of the epiphyseal plate". WILSON and PERCY (1951, 1956) placed various metals in canine metaphysis and concluded that the electrolysis thus developing caused bone lengthening in the region of the growing epiphysis in several cases. They did not regard the procedure as a reliable method of pre-determining growth stimulation. Copper and constantan gave the best result.

d. Obstructing the medullary cavity. KÖNIGSWIESER (1925) achieved some growth stimulation after filling the medullary cavity with camphor and silver nitrate. TRUETA (1953) studied an extensive osteomyelitic child material and found acceleration of growth in the cases in which the medullary cavity was blocked by the process. Growth evened off after "remedullisation". He placed duralumin and vitallium in the marrow cavity of 20 rabbits and, in another series, ligated the nutrient artery, stripped the periosteum and inserted a bony piece in the marrow cavity. He made no mention of the number of animals in this latter series nor gave any accurate results, but contented himself with stating that continuous interruption of the circulation of the nutrient artery and the periosteum could be used to produce considerable acceleration in bone growth. TRUETA attributed longitudinal growth to continuous interruption of the circulation of the nutrient artery and periosteum in consequence of which the epiphyseal cartilage receives a greater quantity of blood via the perforating veins of the metaphysis. TRUETA (1957) supplemented his previous observations by stating that he had stimulated tibial and femoral growth both experimentally and clinically by obstructing the medullary cavity with bone. He found that autogenous fresh bone became absorbed too rapidly to be of use. Autogenous bone that had been boiled for 5—10 minutes or homogenous or heterogenous bone, also boiled, were more effective as their reabsorption lasted for several months, sometimes even for over a year. STÅHL (1957) obstructed the medullary cavity with banked bone chips, stripping the periosteum simultaneously over a distance of 7—8 cm. He performed this operation on 5 children with shortening of the leg after poliomyelitis. He established growth stimulation in all the cases. The best results were achieved in children on whom lumbar sympathectomy had also been performed. In the best case the growth discrepancy was reduced by 3 cm in 3.5 years. LANGENSKIÖLD (1957) obstructed the medullary cavity of the tibia by fibular graft in rabbits aged 14—36 days. With this operation he combined periosteal stripping and he wrapped the diaphysis subperiosteally in plastic film. He achieved a growth acceleration of 1—2 mm after an observation period of 8 weeks.

e. Venous stasis. HELFERICH (1887) stimulated longitudinal growth in a girl of 16 and a boy of 10 by means of stasis. In the girl the discrepancy of 3 cm was reduced by 1 cm and the boy's discrepancy of 3.5 cm was reduced by 2 cm. SCHÜLLER (1889) used tourniquet to treat 2 patients with limb shortening following poliomyelitis. He achieved a lengthening

of 1 cm in the course of 3 months. HARRIS and McDONALD (1936) ligated the femoral vein of 3 sympathectomised sheep, but without affecting longitudinal growth. KISHIKAWA (1936) produced stasis in rabbits by means of an elastic band or by ligating the femoral vein. Growth was accelerated in all the cases. There was a gain of 1.77 per cent in 8 weeks compared with the control side. Details were not reported. WU and MILTNER (1937) used various methods to produce stasis of the circulation in the tibia of 12 rabbits. The procedures adopted were: ligation of the femoral artery or the femoral vein, stripping the soft tissue from the periosteum but not near the epiphyseal line. These measures failed to accelerate longitudinal growth. SERVELLE (1948), in experiments on dogs, observed that ligation of deep veins accelerated longitudinal growth by 2.6–7 per cent in the femur and the tibia. The material consisted of 7 dogs. He recommended for limb-lengthening either ligation of the deep veins or arteriovenous anastomosis. DICKINSON (1953), experimenting with 8 puppies aged 6 weeks, ligated both external iliac vein near the inguinal ligament and the popliteal vein, and injected sodium morrhuate into the distal portion. The procedure failed to influence bone growth. HUTCHISON and BURDEAUX (1954) conducted experiments on 17 dogs applying a tourniquet to the limb. They observed growth stimulation in the epiphyses situated distally from the tourniquet. After the hind legs had been affected by stasis, the authors established a 1.45 per cent growth increase in the tibiae and a gain of 0.52 per cent in half of the femurs. They considered that the success of all growth acceleration methods depends on the degree of venostasis produced.

f. Arteriovenous anastomosis. HARRIS and McDONALD (1936) achieved arteriovenous communication between the femoral artery and the femoral vein in a sympathectomised sheep. There was no effect, however, on longitudinal growth. JANES and MUSGROVE (1950) did arteriovenous anastomosis in ten dogs between the iliac veins. After 3–15 months, the femur of the operated side was longer than the control in 9 animals and the tibia in 6. HIERTONN (1957) performed side-to-side anastomosis in 5 children between the femoral artery and femoral vein, and ligated the femoral vein proximally from the fistula. The growth of the shorter extremity was accelerated considerably. The author intends later to close the fistulae and recanalise the circulation.

g. Sympathectomy. CANNON ET AL. (1929) removed the entire sympathetic trunk on one side in kittens and BAQO (1930) did the same on young rats. They did not observe any growth stimulation on the operated side. SIMON (1930), using rabbits as test animals, had the same result as these two authors. HARRIS (1930) performed sympathectomy on a child, whereupon the growth inequality decreased in 2 years from 3.8 to 2.5 cm. HARRIS and McDONALD (1936) performed unilateral sympathectomy on 10 kittens, 12 puppies and 8 sheep and found no length inequalities. They also reported on 3 patients upon whom unilateral, the left side, sympathectomy had been performed at the age of 2—4 years for Hirschsprung's disease. In each of these 3 patients the left lower extremity grew 1.9—2.5 cm longer than the right. The authors also performed lumbar sympathectomy on 46 patients who had undergone leg shortening. The length inequality decreased in 21 cases, remained unchanged in 8 and increased in 17 cases. KISHIKAWA (1936) removed the lumbosacral ganglion from young dogs and established slight growth stimulation. BARR ET AL. (1950) performed sympathectomies on the shorter limb of patients with limbs of unequal length following poliomyelitis. The discrepancy increased in 13 patients, remained unchanged in 1 and decreased in 9 postoperatively. The authors considered that lumbar sympathectomy could be employed in the treatment of minor length inequalities, but for a discrepancy of any magnitude it might be best to supplement it with or substitute other methods. After this statement, HARRIS indicated in the discussion that followed that the material published by him in co-operation with McDONALD proved that in 20 per cent of the patients the effect on growth was sufficient to produce a notable decrease in the length inequality.

h. Measures affecting the circulation of the bone itself. OLLIER (1867) ligated the nutrient artery. The measure had no influence on the longitudinal growth of the bone. TRUETA (1953) obstructed the nutrient artery of the tibia of 20 rabbits without producing any change in the rate of bone growth. The procedure does not cause permanent ischemia of the diaphysis as the circulation is rapidly restored. BROOKES (1957) obstructed the nutrient canal of the femur in 23 rabbits 24 hours of age. He measured the length of the femurs roentgenologically 30—75—90—120—150 days postoperatively. After an initial shortening the discrepancy was equalised. At the end of the observation period, however, there was a 3 per cent shortening of the operated tibia compared with normal.

i. The effect of diathermy and ultrasonics. WILSON and THOMPSON (1939) reported from the Hospital for Ruptured and Crippled that diathermy has been applied to the epiphyses in the region of the knee of the shorter limb of a small number of paralytic patients. In the majority of the cases there was no further increase in the length inequality of the extremities. WISE ET AL. (1949) studied the effect of diathermy on the region of the knee joint of 50 growing albino rats. The authors applied a wave length of 8 metres or 11 cm. In several instances there was injury to the tissue and disturbance of longitudinal bone growth. The magnitude of the tissue damage was not always in ratio to the degree of growth disturbance. The authors regarded diathermy as contraindicated in children. BUCHTALA (1949) reported that 8 watts applied for 5 minutes 4 or 6 times caused acceleration of growth along the growth line. It also provoked tibial periostitis which he did not regard as an indication of trauma but as stimulation of growth. He warned against ultrasonic treatment for children. DE FOREST ET AL. (1953) treated 9 dogs and 29 rabbits with ultra sound. According to their studies, ultrasonic energy does not cause acceleration of longitudinal bone growth. This energy has a destructive effect on the epiphyseal cartilage of growing bone. Individual tolerance of ultra sound varies greatly. Ultra sound causes a shortening of the tibia and femur, but it also produces irreversible changes in the knee. Unless ultrasonic energy can be more accurately administered its use on growing human bones is not desirable. BERTRAND and TRILLAT (1948) found a wave length of 11 cm to be helpful in stimulating a limb, but their results (5 patients) are not convincing.

OWN INVESTIGATION

The Problems

It will have been seen from the review of the literature that a number of different methods for the stimulation of longitudinal bone growth have been propounded. Judging by the literature, detachment of the diaphyseal periosteum has proved to be the most serviceable of the methods of stimulating bone growth. The object of the present investigation is to ascertain what effect subperiosteal transplantation of skin graft in growing bone has on the longitudinal growth of bone.

The problem was considered under the following headings:

1. Does an autogenous whole-thickness skin graft implanted between the periosteum and the cortex after detachment of the periosteum affect longitudinal growth of the tibia in growing rabbit?

2. Does the stimulation of growth possibly induced occur as an increase in the activity of the proximal or distal cartilage or both epiphyseal cartilages?

3. Do roentgenological changes -- and if so of what type -- originate after a certain period of observation as a result of transplanted skin graft?

4. What macroscopic and microscopic changes can be demonstrated in surgically treated tibia after a certain period of observation?

Material and methods

Test animals

Rabbits were used as the test animals. They were of mixed breeds, chiefly white, and of both sexes. The litter was kept in the same hutch until the animals were weaned at 2 months of age. After weaning, the young were first housed two to a hutch but from the age of 4 months placed in individual hutches. The usual routine of the animal house of the Clinic was observed in the feeding: the rabbits received hay and dry bread daily, and fresh water ad libitum from drinking bottles. The rabbits of the first series, A, were permitted water from drinking vessels. In the summer their diet was supplemented with fresh grass and vegetable, e.g. cabbage leaves and cauliflower.

The tests were carried out in order of series. Series A was operated first, then series B, series C and finally series D. Series A was operated within the shortest period, in the middle of summer, when the majority of the young were c. 2 months old. A coccidiosis epidemic now broke out and killed 27 young in the course of a few weeks. As an epidemiological preventive measure when the epidemic was over, isolation and disinfection were performed by moving the survivors temporarily to other hutches and washing their old hutches with soap and water. Drinking bottles were placed in the hutches and the vessels used up to then were removed. The following tests were performed with a longer interval than the earlier tests in order to have as few young 6–8 weeks as possible in the animal house at any one time. Coccidiosis is a disease to which rabbit young of this age particularly are prone.

Table 1 shows the distribution of the fatalities from coccidiosis between the different groups of series A. In group A₁ (skin graft under the periosteum) 6 young died at the age of 7–8 weeks (Nos. 6, 9, 11, 12, 15 and 19), and 2 at 10 weeks (Nos. 7 and 16). Rabbit No. 5 of the same group died at the age of 20 weeks. In the A₃ group (plain detachment) 5 animals died at the age of 6–7 weeks (Nos. 24, 27, 29, 31 and 35) and 1 at 9 weeks (No. 28). In the A₄ group (skin graft in the medullary cavity) 4 rabbits died at 7–8 weeks (Nos. 39, 40, 42 and 43), 2 at 9–10 weeks (Nos. 41 and 45) and 1 at the age of 12 weeks (No. 38). Five animals died at the age of 8 weeks in the growth control group (Nos. 128, 129, 130, 131 and 132).

When the tests were completed the rabbits were sacrificed by injecting a soporific dose of "Nembutal Veterinary" (Abbot) into their auricular vein. After they had fallen asleep 10 ml of air was injected.

Table 1

Type of operation and number of animals used

Series A. Large detachment of diaphyseal periosteum extending to the metaphyseal area, and

	Number of animals	Died during the observa- tion period
Group A_1 Implantation of skin graft under the periosteum	21	9
Group A_2 Implantation of subcutaneous tissue under the periosteum	2	
Group A_3 Plain detachment	13	6
Group A_4 Implantation of skin graft into the bone marrow cavity	12	7
	48	22

Series B. Small diaphyseal detachment of the periosteum, and

Group B_1 Implantation of skin graft under the periosteum	21	
Group B_2 Implantation of subcutaneous tissue under the periosteum	18	
	39	

Series C. Large detachment of diaphyseal periosteum extending beyond the epiphyseal line, and

Group C_1 Implantation of skin graft under the periosteum	17	
Group C_2 Implantation of subcutaneous tissue under the periosteum	16	
Group C_3 Plain detachment	7	
	40	

Series D. Operation as in Group A_1 , but in addition both tibias marked with an X-ray positive mark

<i>Growth Control Group.</i>	8	5
Total number of young growing rabbits in series	143	(27)

Operative technique

The operations were performed in the operating theatre of the animal house under as aseptic conditions as possible. The young were operated on at the age of 2—4 weeks. The last and oldest to be operated on were the animals of series D, the age of 6 weeks. The operation was performed under local anesthesia of the right leg, the left leg serving as a control. Before surgery the leg and a small area of the abdominal skin were denuded of fur by means of soap and razor. The skin was cleaned with alcohol. The operative field was anesthetised with 0.5 per cent "Xylocain-Exadrin" (Astra) solution. A longitudinal incision c. 2 cm long was made extending to the bone surface in the superio-medial half of the leg. The periosteum was then detached carefully from the surface of the bone with a narrow, sharp elevator. The site and extent of the periosteal area detached differed in the four series. The areas of detachment for the periosteum are illustrated in Figs. 1—3. An extensive diaphyseal detachment extending to the area of the metaphysis was made in series A, care being taken not to damage the epiphyseal cartilage. A similar detachment was performed in series C, but it was extended to the epiphyseal area. Series D underwent a similar operation to series A, but, in addition, a small X-ray positive gutta-percha mark (Farnadent) was placed in the middle of the bone in a cavity made by dentist's drill in the cortex of the diaphysis. The mark was placed in the right tibia via the incision made for detachment of the periosteum. The mark was inserted in the left tibia by drilling through a separate incision about 1 cm long through the periosteum a similar cavity to that in the right tibia. This incision was sutured in the same way as the incision in the right tibia. The epiphyseal line showed a paler shade through the tissues and hence was generally easy to distinguish. In series A, C and D the periosteum was detached around the tibia. In series B the aim was to make a small detachment and fit the implant into the resulting pocket. Care was taken in the detachment procedure not to injure the nutrient artery and vein. Skin or subcutaneous tissue implanted under the periosteum was taken from the abdominal area prepared for the purpose. A square incision of c. 4×4 mm was made in the skin, within which the skin was removed as far as the subcutis. When subcutaneous tissue was employed as implantation material a simple low incision was made, the wound was spread and from the exposed subcutaneous tissue was taken a piece the size of the corresponding piece of skin. The tissue used in the experiments was always

Area of detached periosteum in the four series.



Series A and D: Large detachment of diaphyseal periosteum extending to the metaphyseal area.

Fig. 1



Series B: Small diaphyseal detachment of the periosteum.

Fig. 2



Series C: Large detachment of diaphyseal periosteum extending beyond the epiphyseal line.

Fig. 3

autogenous. The graft was implanted in each case on the boundary between the metaphysis and the diaphysis, generally on the lateral aspect of the bone. The periosteum was sewn with 0000 catgut knots. Four or five knotted sutures of the same catgut were used for the skin. Aqueous penicillin (Novo), 10,000 units, was injected into the operative field and the wound was sprayed with "Nobecutan" (Bofors). For 5 postoperative days the rabbits were given combined penicillin-streptomycin solution "Mycipen paediatric" (Orion); the dihydrostreptomycin dose was 0.0125 g/kg/day and the penicillin dose 20,000 units/kg/day.

R o e n t g e n o g r a p h i c t e c h n i q u e

Bone length was measured roentgenographically by a modification of BRODIN's method (1955). The rabbit was placed on a rack, (Fig. 4) its extended legs fastened to the knobs with a strip of gauze. Once in this position, the rabbit kept quiet during roentgenography without anesthesia. Roentgenography was made with the X-ray tube 2 m from the object, generally at 100 mAs 53 kV, using nonscreen film "Kodirex" (Kodak). Before roentgenography the animal was positioned to give the most symmetrical possible antero-posterior projection of the tibiae on the film. The intention was to measure the bones at the age of 2--3, 12 and 24 weeks. If the animal was not killed after that time it was roentgenographed again at the age of 30 or 52 weeks. Additional roentgenographs were made of certain litters at intervals between these times.

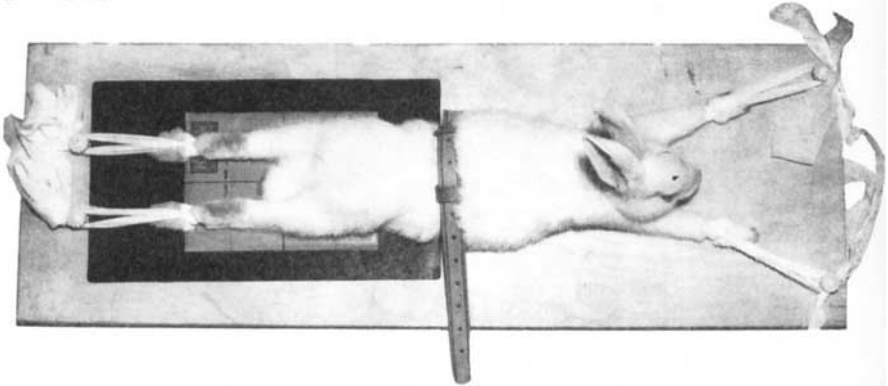


Fig. 4. The rabbit fixed on the rack for roentgenography.

The distance from the eminentia intercondyloida tibiae to the tip of the medial malleolus measured on the viewing table with a calliper was taken as the length of the bone. The measurement was made 3 times and the value recorded was the mean of these 3 measurements.

In series D the distance from the above-mentioned fixed points to the proximal point of the mark inserted in the middle of the bone was also measured. Here again the mean of 3 measurements was the value recorded.

Error of longitudinal measurement

The potential sources of error of measurement of length were:

1. Measuring error
2. Error due to the variability of projection
3. Blurring of the image

It was generally possible to measure the exact antero-posterior projection of the bones, but in some cases the projections varied slightly and this caused an additional error.

Under-exposure produced blurred images. Although the rabbits generally kept immobile, they sometimes moved during an exposure and thus caused a blurring of the image.

The final error of the measurement of length discrepancy was ± 0.1 mm.

Planigraphy

Planigrams were made of some lesions observed in ordinary roentgenograms by using a precision planigraph (Elema) working on the rotation principle. The best image was obtained using a source of radiation 0.3×0.3 mm focal spot 40 mAs and 74 kV. The layer in question was 4 cm and the roentgenogram was made with the rabbit on the rack.

The same planigraph was used to take an enlarged roentgenogram, 1.4 : 1, of the lesion in the right tibia of rabbit No. 8 while the rotation mechanism was kept fixed (Fig. 35).

Histological methods

Samples from the lesion in the right tibia of the sacrificed rabbits were taken by means of a cutter disc fixed to a dentist's drill. In series A, B and C the samples were usually taken from the proximal epiphyseal cartilages of both tibiae. In series D samples were taken as a rule from both the proximal and the distal epiphyseal cartilages of both tibiae of the animals. The samples were fixed in neutral 10 per cent formalin and decalcified in 32 per cent (v/v.) EDTA solution (pH 7.2—7.4) at c. 37°C. After decalcification the samples were dehydrated in an ascending alcohol series (50 per cent, 70 per cent, 80 per cent, 95 per cent and absolute alcohol) and passed through benzol into paraffin in the usual way. The samples were then kept for about a week in paraffin

in an incubator at 63 °C. An ordinary sliding microtome was used to make sections c. 5–6 μ thick. Hematoxylin (Weigert) — van Gieson was used as the staining method.

The height of the zones of epiphyseal cartilage was measured by the method of UOTILA and KANNAS (1952). The equipment described by TALA (1952) was employed in the measuring. Using the same micro-projector, the section of epiphyseal cartilage was projected a distance of 62.5 cm onto paper in $\times 275$ magnification and the boundaries of the zones were drawn. Two fields of vision were drawn for each cartilage; the fields selected were as symmetrical as possible on both sides about 1/2 of the distance from the longitudinal axis of the bone to the periphery. The section was cut in the frontal plane of the cartilage. The projection of each epiphyseal cartilage was measured at 3 points, in the middle and 7 cm in either direction from the middle towards the periphery. This gave 6 measurements for each epiphyseal cartilage. From these 6 values was calculated the mean which is given here as one observation. The mean deviation was 0.12 cm.

Statistical methods *)

Error of measurement

To determine the exact measuring error, each rabbit was roentgenographed 10 times, and between each exposure was freed and then fastened again. Each bone was then measured in the usual way 3 times. The deviations were calculated mathematically. The following results were obtained:

The mean deviation of an individual longitudinal measurement $s = 0.1$ mm.

The measuring accuracy of one observation (mean of 3 measurements)

$$\frac{s}{\sqrt{3}} = 0.06 \text{ mm}$$

Measuring accuracy of the difference between the observations of the right and the left tibia, i.e. discrepancy in length, was

$$\sqrt{2} \frac{s}{\sqrt{3}} = 0.08 \sim 0.1 \text{ mm}$$

Development of length discrepancy

To facilitate treatment, the mean \bar{x} , the standard error of an individual length inequality s_x and the standard error of the mean

*) CRAMÉR'S book (1956) was used as the basis of the statistics.

s_x^- were calculated from the observations $x_1, x_2, x_3 \dots x_n$ made in each group at a certain age.

$$\bar{x} = \frac{\sum x_i}{n}$$

$$s_x = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n - 1}}$$

$$s_x^- = \frac{s_x}{\sqrt{n}}$$

By multiplying by the coefficient t_5 taken from the t -distribution table of "Student", 95 per cent confidence limits were obtained for the means

$$\bar{x} \pm t_5 \cdot s_x^-$$

The results for 143 animals were included in the material (Table 1). It also included the animals which died in the course of the investigation to the extent that observation material on them was available. Series A included 48, series B 39, series C 40, series D 8 and the growth control group 8 animals.

Although the rabbits were radiographed as far as possible in even weeks, this scheme could not always be observed. Since there were insufficient measurements in some weeks, successive results were combined in series A, B and C in order to standardise the treatment of the material. For instance, in group A₁ when the first weighted mean age of Table 3 was 3 weeks, there were 12 observations made at the age of 2, 6 at the age of 3 and 8 at the age of 4 weeks. Other discrepancy means in series A, B and C were combined in a similar way. It was not necessary to combine the results in series D and the means were calculated from measurements made at the age of 6, 8, 12 and 16 weeks.

Comparison between the different groups

The difference of the means D and the standard error of the difference s_D were calculated in the comparison of the means of the different groups

$$D = \bar{x}' - \bar{x}''$$

$$s_D = \sqrt{\left(\frac{1}{n'} + \frac{1}{n''}\right) \frac{(n' - 1) s_x'^2 + (n'' - 1) s_x''^2}{n' + n'' - 2}}$$

Results

After the animals had recovered from the dizziness caused by Zylcain-Exadrin, they began to move almost immediately. The rabbits operated on favoured their right hind leg slightly for the following 2—3 days but then moved freely on all four legs. Despite the operation, there was no disturbance in their normal weight gain. The operation wounds healed excellently and there was no wound infection in a single case.

Effect on longitudinal growth

Individual length discrepancies

To clarify the individual tibia length discrepancies after the rabbits had reached adulthood the values were tabulated separately (Table 2). This table gives the length of the left, i.e. the control tibia and the discrepancy between this and the right tibia in the animals that reached adulthood. The measurements were made when the animals were c. 24 weeks old.

If a roentgenographically observed lesion was present in the tibia, this is also noted in Table 2. This point will be discussed in greater detail on p. 55.

The postoperative development of tibia length discrepancy

To illustrate the difference between the growth of operated tibia and that of the contralateral limb of the same animal, the development of the mean length discrepancy of the operated right tibia and the control (left) tibia in the different groups of the series A, B and C was tabulated (Table 3). The results given in the table are also presented graphically (Figs. 5—14). In addition to the curve for the means, the graphs also show the 95 per cent confidence limits for the means. The abscissa gives the age in weeks.

Series A. The periosteum was detached from the area of the proximal diaphysis in series A. In the first group of this series, A₁, a skin graft was implanted under the detached periosteum.

Table 2. Length of the left tibia and discrepancy between both tibias measured at about 24 weeks age, and the occurrence of possible bone cyst in the operated right tibia

Series	Group	No. of rabbit	Litter	Length of left tibia in mm	Discrepancy in mm	x denotes a cyst
Series A Large detachment of diaphyseal periosteum extending to the metaphyseal area and	Group A ₁	1	1	97.1	+ 2.8	x
	Implantation of skin graft under the periosteum	2	1	96.7	+ 3.3	x
		3	1	99.9	+ 3.1	x
		4	1	103.5	+ 2.2	x
		8	5	98.7	+ 1.7	x
		10	7	103.7	+ 0.8	x
		13	9	106.7	+ 0.5	x
		14	9	102.3	- 0.8	x
		17	11	103.8	+ 1.3	x
		18	11	101.8	+ 1.5	x
		20	13	96.9	- 0.1	x
	21	13	93.9	+ 6.3	x	
	Group A ₂	22	26	97.8	0.0	
		23	26	94.1	+ 0.3	
	Group A ₃ Plain detachment	25	2	94.4	- 1.2	
		26	2	93.4	- 0.7	
		30	7	99.8	- 0.4	
		32	9	108.7	- 0.5	
		33	10	99.8	+ 0.5	
		34	11	99.8	+ 0.5	
		36	13	82.5	- 1.5	
Group A ₄ Implantation of skin graft into the bone marrow cavity	37	2	93.9	+ 1.0	x	
	44	9	103.1	+ 1.4	x	
	46	11	100.8	+ 3.5	x	
	47	12	97.1	+ 0.8		
	48	13	89.9	- 0.3	x	

Series	Group	No. of rabbit	Litter	Length of left tibia in mm	Discrepancy in mm	x denotes a cyst	
Series B Small diaphyseal detachment of periosteum and	Group B ₁ Implantation of skin graft under the periosteum	49	14	109.4	- 0.6		
		50	15	105.1	- 0.8		
		51	15	104.6	+ 0.2	x	
		52	15	107.2	- 0.4	x	
		53	15	109.1	0.0	x	
		54	16	97.7	+ 1.1		
		55	16	101.5	- 0.6	x	
		56	16	102.3	- 1.0		
		57	16	101.3	- 0.1		
		58	17	87.5	- 0.3		
		59	17	96.0	- 0.4		
		60	17	85.1	- 1.5	x	
		62	17	97.6	- 1.1		
		63	17	96.1	+ 0.2		
		64	18	104.0	+ 0.6		
		65	18	106.8	+ 0.7		
		66	18	100.2	+ 0.1		
		67	19	96.8	+ 1.0		
		68	19	99.8	0.0		
	69	19	96.0	- 0.1			
		Group B ₂ Implantation of subcutaneous tissue under the periosteum	70	14	106.6	- 0.8	
			71	15	104.2	0.0	
			72	15	106.2	- 0.2	
			73	15	105.0	- 0.1	
			74	16	99.2	+ 0.1	
			75	16	102.2	- 1.4	
			76	16	100.4	- 0.4	
			77	17	84.4	- 0.2	
			78	17	88.0	0.0	
			79	17	94.2	+ 0.9	
			80	17	91.5	- 0.1	
			81	18	98.2	+ 0.6	
			82	18	108.2	+ 0.4	
			83	18	101.1	- 0.6	
			84	19	104.2	- 0.6	
	85		19	103.7	- 0.9		
	86		19	102.4	+ 0.9		
	87		19	103.2	+ 0.2		

Series	Group	No. of rabbit	Litter	Length of left tibia in mm	Discrepancy in mm	x denotes a cyst	
Series C Large detachment of diaphyseal periosteum extending beyond the epiphyseal line and	Group C ₁ Implantation of skin graft under the periosteum	88	20	97.9	- 1.8	x	
		89	20	97.0	- 2.0	x	
		90	20	96.6	- 4.0	x	
		91	21	108.3	- 0.7	x	
		92	21	103.3	- 1.5	x	
		93	22	106.4	- 0.6	x	
		94	22	102.2	+ 0.3	x	
		95	23	105.0	- 2.3	x	
		96	23	100.6	- 2.4	x	
		97	23	101.6	- 3.2		
		98	24	100.3	- 2.3	x	
		99	24	94.9	- 1.3		
		100	24	98.3	- 0.4		
		101	24	97.9	- 1.0		
	102	25	106.2	- 3.3			
	103	25	102.5	- 1.8	x *)		
	104	25	99.4	- 1.0	x *)		
		Group C ₂ Implantation of subcutaneous tissue under the periosteum	105	20	97.3	- 1.7	
			106	20	97.3	- 1.4	
			107	20	97.3	- 1.5	
			108	21	111.5	- 1.0	
			109	21	107.7	- 2.4	
			110	21	110.9	- 2.0	
			111	22	104.0	- 1.6	
			112	22	101.3	- 1.3	
			113	23	104.5	- 2.1	
			114	23	103.5	- 1.3	
			115	24	99.5	- 1.5	
			116	24	97.7	- 1.4	
			117	24	94.0	- 0.6	
		119	25	102.0	- 0.9		
		120	25	99.5	- 2.2		
		Group C ₃ Plain detachment	121	20	99.7	- 1.6	
	122		20	95.7	- 3.6		
	123		21	110.0	- 2.0		
	124		22	102.0	- 1.9		
	125		22	101.1	- 1.9		
	126		24	95.8	- 1.1		
	127		24	96.7	- 1.1		

*) a cyst was found at the age of 10 weeks but not at 22 weeks.

Table 3. Development of tibia length discrepancy in different groups

Group	Mean age in weeks	Number of measurements	Discrepancy in mm				Discrepancy per mille			
			Mean	Standard error of the mean	Confidence limits		Mean	Standard error of the mean	Confidence limits	
A ₁	3	26	0.1	0.1	0.0	0.2	3.0	1.2	0.5	5.5
	6	28	0.6	0.1	0.4	0.7	9.0	1.3	6.3	11.7
	9	19	0.9	0.2	0.6	1.3	12.7	2.2	8.1	17.3
	12	13	1.6	0.2	1.2	2.0	20.2	2.4	14.9	25.5
	17	28	2.2	0.2	1.9	2.6	24.6	2.0	20.6	28.6
	25	13	1.6	0.5	0.5	2.7	16.5	5.3	4.9	28.1
	52	3	1.4	0.4	-0.2	3.1	13.7	3.5	-1.5	28.9
A ₂	3	4	0.2	0.1	-0.1	0.5	5.0	2.6	-3.5	13.5
	12	6	0.5	0.1	0.3	0.7	6.1	1.0	3.5	8.7
	22	4	0.1	0.1	-0.1	0.4	1.3	0.8	-1.3	3.9
A ₃	3	21	0.2	0.1	0.0	0.4	4.5	1.7	0.9	8.1
	6	16	0.5	0.1	0.2	0.8	9.1	2.2	4.4	13.8
	9	7	0.7	0.2	0.2	1.3	10.4	3.3	2.2	18.6
	13	7	0.0	0.1	-0.3	0.4	0.0	1.8	-4.5	4.5
	18	9	0.1	0.2	-0.3	0.5	0.6	1.9	-4.9	5.1
	26	7	-0.5	0.3	-1.2	0.2	-5.4	3.2	-13.3	2.5
	53	5	-1.0	0.4	-2.1	0.0	-10.8	4.2	-22.4	0.8
A ₄	3	21	0.1	0.1	-0.0	0.2	2.5	1.7	-1.0	6.0
	6	15	0.7	0.2	0.2	1.1	11.4	3.6	3.8	19.0
	10	7	0.6	0.2	0.1	1.1	7.7	2.4	1.8	13.6
	13	4	0.6	0.8	-1.9	3.1	6.5	9.7	-35.1	48.1
	22	11	1.1	0.4	0.1	2.0	10.8	4.4	0.9	20.7
	50	6	0.8	0.5	-0.5	2.0	7.8	5.0	-5.0	20.6
B ₁	3	21	0.0	0.2	-0.1	0.1	1.1	1.2	-1.5	3.7
	12	21	0.1	0.5	-0.1	0.3	1.3	1.4	-1.6	4.2
	16	15	0.0	0.8	-0.5	0.4	-0.1	2.2	-4.9	4.7
	24	26	-0.1	0.7	-0.4	0.2	-1.2	1.4	-4.0	1.6
	52	3	-0.7	0.5	-1.9	0.6	-6.0	3.0	-18.9	6.9
B ₂	3	18	0.0	0.1	-0.1	0.2	1.1	1.4	-1.9	4.1
	12	18	0.1	0.1	-0.1	0.4	1.4	1.3	-1.3	4.1
	16	15	0.1	0.1	-0.2	0.3	0.6	1.1	-1.8	3.0
	24	25	-0.2	0.1	-0.4	0.1	-1.8	1.1	-4.1	0.5
	52	3	-0.4	0.4	-2.2	1.5	-3.3	3.9	-20.2	13.6

Group	Mean age in weeks	Number of measurements	Discrepancy in mm				Discrepancy per mille			
			Mean	Standard error of the mean	Confidence limits		Mean	Standard error of the mean	Confidence limits	
C ₁	2	18	0.1	0.0	0.0	0.1	1.2	1.2	- 1.2	3.6
	11	21	0.1	0.2	-0.4	0.5	1.1	2.5	- 4.2	6.4
	24	17	-1.7	0.3	-2.3	-1.1	-17.1	2.7	-22.9	-11.3
	30	8	-0.8	0.3	-1.5	-0.2	- 7.9	2.5	-14.1	- 1.7
C ₂	2	16	0.1	0.1	0.0	0.2	3.1	1.9	- 0.9	7.1
	11	19	0.1	0.1	-0.2	0.3	1.4	2.1	- 3.0	5.8
	24	15	-1.5	0.1	-1.8	-1.3	-14.8	1.2	-17.4	-12.2
	30	10	-2.2	0.2	-2.7	-1.7	-19.8	1.9	-24.2	-15.4
C ₃	2	7	0.1	0.1	-0.1	0.3	2.9	2.1	- 2.2	8.0
	11	9	-0.2	0.2	-0.8	0.4	- 2.0	3.1	- 9.2	5.2
	24	7	-1.9	0.3	-2.7	-1.1	-18.9	3.4	-27.3	-10.5
	30	6	-2.0	0.1	-2.3	-1.8	-19.2	0.8	-21.1	-17.3
Growth Control	3	15	0.0	0.1	-0.1	0.1	0.3	1.7	- 3.3	3.9
	8	7	0.2	0.1	0.0	0.3	3.0	1.3	- 0.1	6.1
	19	6	-0.1	0.1	-0.3	0.2	- 0.5	0.9	- 2.8	1.8
	52	3	-0.1	0.1	-0.4	0.2	- 1.0	0.6	- 3.5	1.5

Development of tibia length discrepancy in group A₁

Large detachment of the periosteum and implantation of skin graft under the periosteum.
(Discrepancy = the length of right operated tibia less the length of left tibia)

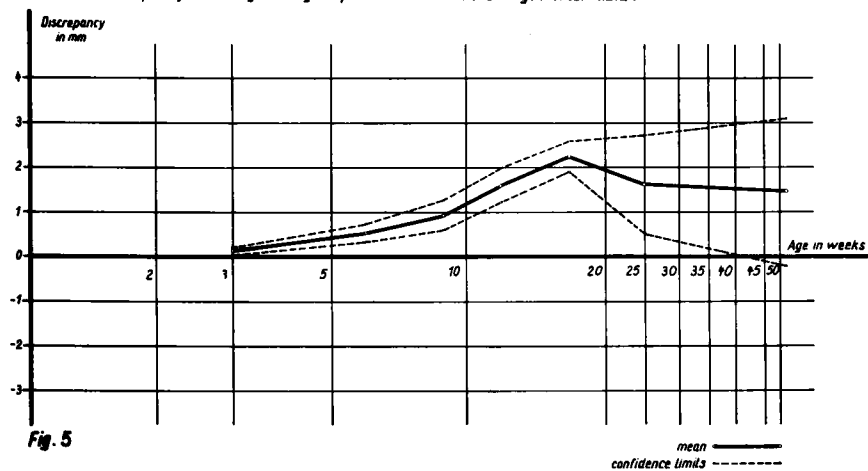


Fig. 5

As will be seen from Fig. 5, the animals in this group showed a distinct tibia length discrepancy in favour of the operated leg. The difference

was 0.6 mm at 6 weeks and the maximal discrepancy, 2.2 mm, was seen at 17 weeks. It was then reduced a little to 1.6 mm for 25 weeks and 1.4 mm after a year. The confidence limits of the results were broader at 52 weeks, but for that age data were available on 3 animals only.

Group A₂, besides detachment of the periosteum, had implantation of subcutaneous tissue under the periosteum.

Development of tibia length discrepancy in group A₂

Large detachment of the periosteum and implantation of subcutaneous tissue under the periosteum

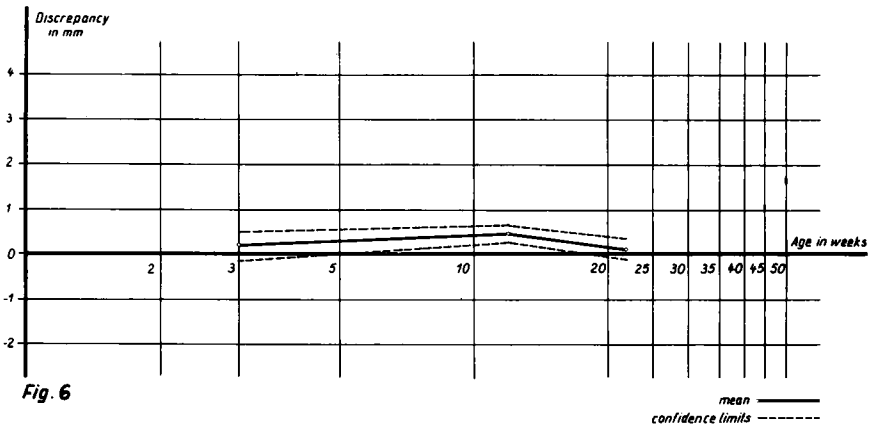


Fig. 6

Fig. 6 shows that there was a small mean length discrepancy, 0.5 mm, in favour of the operated limb at the age of 12 weeks. At 22 weeks the discrepancy had already been reduced to 0.1 mm and consequently the initially small inequality obtained in this series gradually diminished further. The results differ statistically significantly from 0 only for the age of 12 weeks.

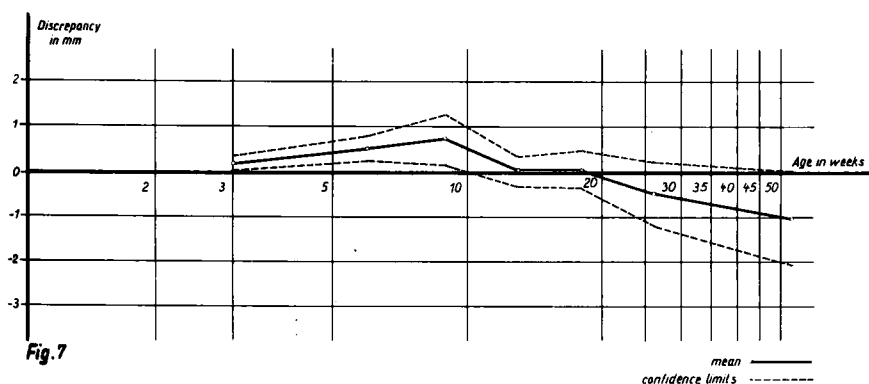
Detachment of the periosteum was the only measure performed in group A₃.

As will be seen from Fig. 7, group A₃ showed stimulation of growth of the order of 0.5 mm in favour of the operated leg at the age of 6 weeks and a maximal discrepancy of 0.7 mm at 9 weeks. At 26 weeks the operated leg was 0.5 mm shorter than the control and at 53 weeks 1.0 mm shorter than the control. However, the difference from 0 was statistically significant only at 6 and 9 weeks.

Group A₄ was a control group in which the skin graft was implanted into the bone marrow cavity.

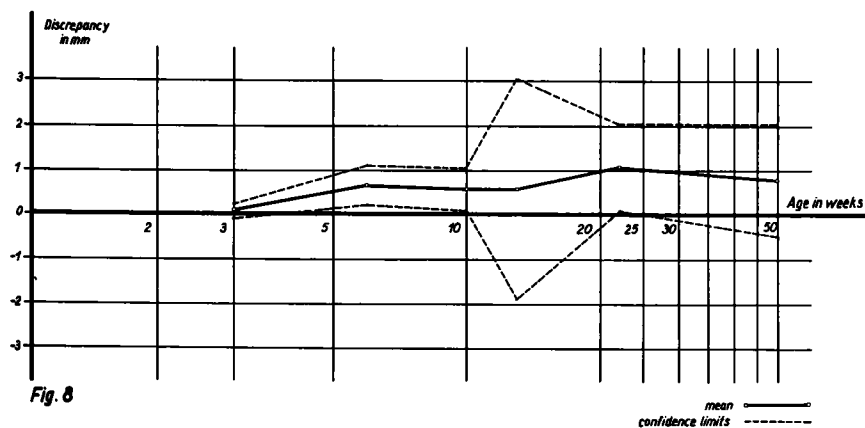
Development of tibia length discrepancy in group A₃

Large detachment of the periosteum.



Development of tibia length discrepancy in group A₄

Large detachment of the periosteum and implantation of skin graft into the bone marrow cavity.

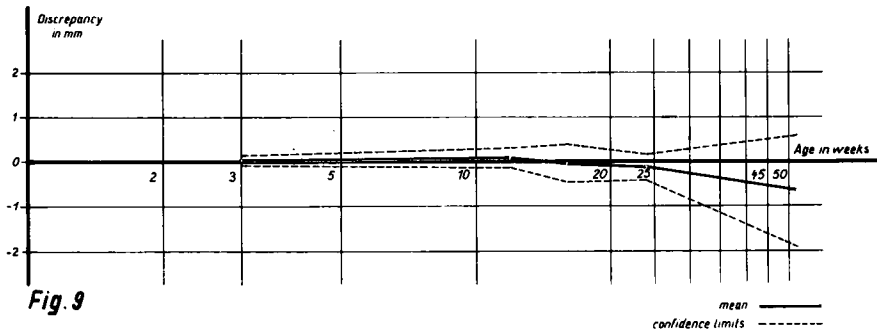


According to Fig. 8, group A₄ displayed a small mean stimulation in growth, but owing to the varying irritation effect of the transplant and partly also to the paucity of the material (seen especially from the range of the confidence limits at 13 weeks) the results differed significantly from 0 only at the ages of 6 and 10 weeks.

Series B. Series B was subjected only to minimal diaphyseal detachment of the periosteum close to the boundary between the metaphysis and the diaphysis. The implant was fitted into a periosteum pocket of equal size. In group B_1 the implanted material was skin.

Development of tibia length discrepancy in group B_1

Small detachment of the periosteum and implantation of skin graft under the periosteum



As will be seen from Fig. 9, no statistically significant differences occurred in this group although the mean length discrepancy at the age of 62 weeks was negative, i.e. -0.7 mm.

In group B_2 subcutaneous tissue was employed as the implantation material.

Development of tibia length discrepancy in group B_2

Small detachment of the periosteum and implantation of subcutaneous tissue under the periosteum

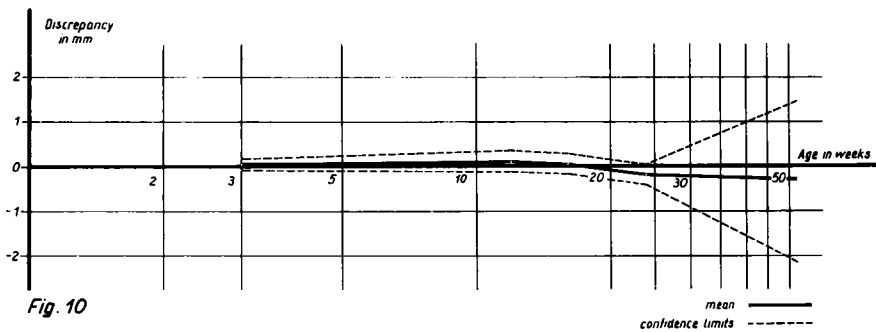


Fig. 10 shows that no statistically significant differences were established in this group. At 52 weeks the tibia length discrepancy was negative, i.e. -0.4 mm.

Series C. In series C the periosteal detachment was made in the same way as in series A, i.e. by detaching the periosteum over a large area of the proximal diaphysis, except that the detachment was extended to the area of the perichondrium lining the epiphyseal cartilage.

In group C₁ a skin graft was implanted under the periosteum after detachment.

Development of tibia length discrepancy in group C₁

Large detachment of diaphyseal periosteum extending beyond the epiphyseal line and implantation of skin graft under the periosteum.

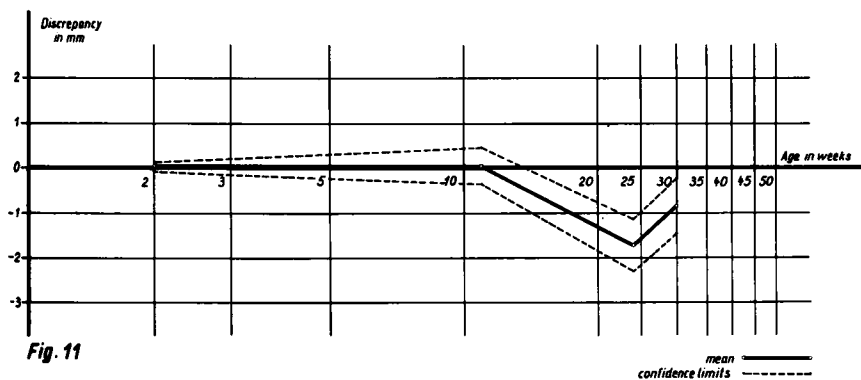


Fig. 11

As will be seen from Fig. 11, no mean length discrepancy occurred in group C₁ at 11 weeks. At 24 weeks the operated leg was 1.7 mm shorter than the left leg and at 30 weeks the length inequality was -0.8 mm. Here, again, the operated leg was clearly shorter than the control.

In group C₂ subcutaneous tissue was implanted under the periosteum.

As will be seen from Fig. 12, no mean length discrepancy was established at 11 weeks of age. At 24 weeks a distinct shortening, -1.5 mm, of the operated leg was observed and at 30 weeks the leg was even shorter, -2.2 mm.

Detachment of the periosteum was the only measure performed on group C₃. It was carried out in the same way as for the other groups in series C.

Development of tibia length discrepancy in group C₂

Large detachment of diaphyseal periosteum extending beyond the epiphyseal line and implantation of subcutaneous tissue under the periosteum.

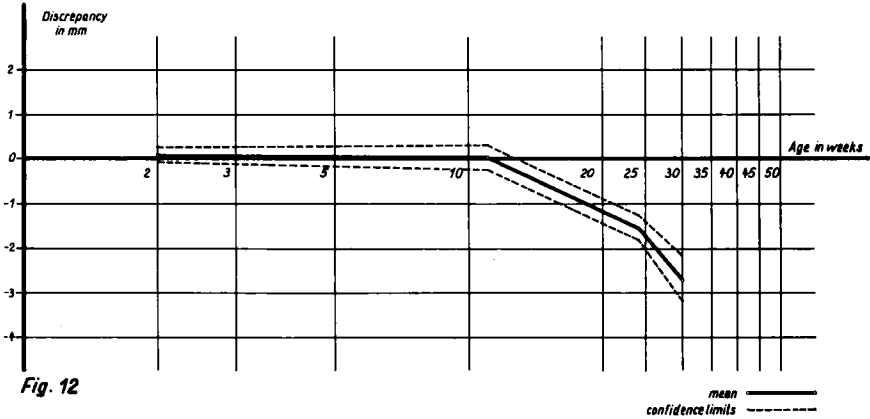


Fig. 12

Development of tibia length discrepancy in group C₃

Large detachment of diaphyseal periosteum extending beyond the epiphyseal line.

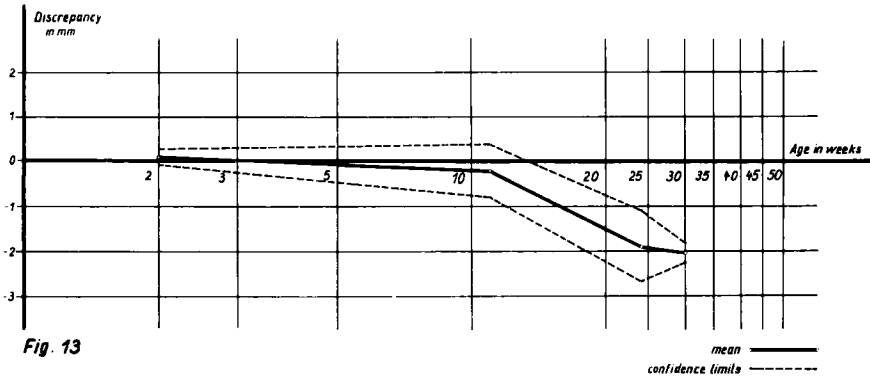
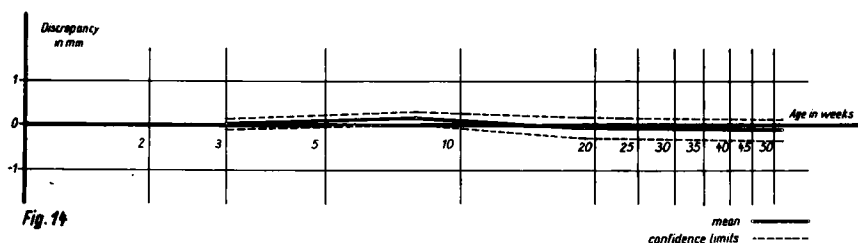


Fig. 13

Fig. 13 shows that no statistically significant length discrepancy was observed in this group at the age of 11 weeks. At 24 weeks the operated leg was 1.9 mm shorter than the control. At 30 weeks the shortening was more pronounced and the discrepancy was 2.0 mm.

No operation was performed on the animals of the growth control group. It was included merely to establish that rabbits show no spontaneous length inequalities in the tibias. In fact no statistically significant differences were found, as can be seen from Fig. 14.

Development of tibia length discrepancy in growth control group



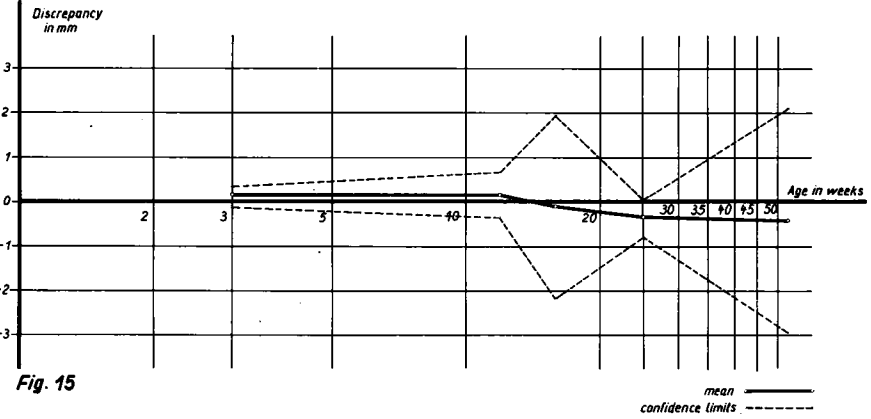
As the length of the tibias varied considerably in young rabbits of the same age, the length discrepancies were calculated per mille of the length of the control bone. These per mille values are given in Table 3. Comparison between them and the absolute differences in terms of mm shows no essential differences between the graphs plotted for these values.

Table 4. The development of tibia length discrepancy in subgroups B_{1a} (cyst formation) and B_{1b} (no cyst formation)

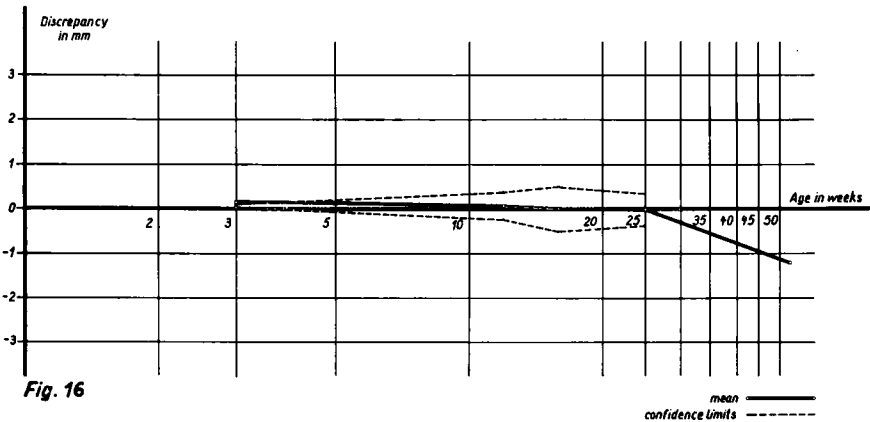
Group	Mean age in weeks	Number of measurements	Discrepancy in mm		
			Mean	Standard error of the mean	Confidence limits
B_{1a}	3	6	0.1	0.1	-0.1 0.4
	12	6	0.2	0.2	-0.4 0.6
	16	4	-0.1	0.5	-2.2 2.0
	25	8	-0.3	0.2	-0.8 0.1
	52	2	-0.4	0.2	-2.9 2.1
B_{1b}	3	15	0	0.1	-0.1 0.1
	12	15	0.1	0.1	-0.2 0.4
	16	11	0	0.2	-0.5 0.5
	24	18	0	0.2	-0.4 0.4
	52	1	-1.2		

Table 15 on p. 56 shows that the incidence of roentgenologically demonstrable cyst varies in the different groups. In order to establish the effect of the cyst on growth, group B_1 was divided into two subgroups B_{1a} and B_{1b} . Subgroup B_{1a} included the animals which developed a cyst, subgroup B_{1b} the animals with no cyst in the tibia. Subgroup B_{1a} thus represents principally the influence of the cyst, for the periosteal detachment was minimal. Subgroup B_{1b} represents the corresponding detachment control for in these cases the graft had slipped out from under the periosteum. The results are given in Table 4.

Development of tibia length discrepancy in subgroup B_{1a}
Cyst formation.



Development of tibia length discrepancy in subgroup B_{1b}
No cyst formation.



The confidence limits in Table 4 are always on both sides of zero which indicates that there was no significant stimulation of growth in either subgroup. The results are given graphically in Figs. 15 and 16 where, it will be seen, the O-line is not intersected by the confidence limits.

As mentioned on p. 42, the same finding was made for the B₁ group as a whole.

Analysis of factors changing rate of growth

To analyse the effect of the various measures on the longitudinal growth of rabbit tibia, a comparison was made between the means of the length discrepancy at the same age in the different groups. The comparison was performed by calculating the differences of the corresponding means and the confidence limits of the difference.

The growth-stimulating effect of the skin graft. Groups A_1 and A_3 were compared first in order to establish whether the implantation of a skin graft under the periosteum increases the stimulation produced by detachment of the periosteum.

Table 5. Comparison of discrepancy means of groups A_1 and A_3

Groups	Mean age in weeks t	Difference of the means \pm its standard error in mm $D \pm s_D$	Confidence limits in mm		Degrees of freedom
			Ci		f
$A_1 - A_3$	3	-- 0.1 \pm 0.1	- 0.2	0.1	45
	6	0.0 \pm 0.2	- 0.3	0.3	42
	9	0.2 \pm 0.3	- 0.4	0.8	24
	12	1.6 \pm 0.3	1.0	2.2 v)	18
	17	2.2 \pm 0.3	1.5	2.8 v)	35
	25	2.1 \pm 0.7	0.6	3.6 v)	18
	52	0.4 \pm 0.6	- 1.0	1.8	6

The symbol v) used in Tables 5-10 and 14 indicates a statistically significant deviation of the differences from 0.

It will be seen from Table 5 that significant differences appeared between these groups and that the implantation of skin graft under the periosteum increased materially the stimulating effect of its detachment on bone growth.

The growth-stimulating effect of subcutaneous tissue. Table 6 compares group A_2 with group A_3 for the additional stimulating effect of implanting subcutaneous tissue under the periosteum after large detachment.

Table 6. Comparison of discrepancy means of groups A_2 and A_3

Groups	t	$D \pm s_D$	Ci		f
$A_2 - A_3$	3	0.0 \pm 0.2	- 0.4	0.4	23
	12	0.5 \pm 0.2	0.1	0.8 v)	11
	22	0.3 \pm 0.4	- 0.4	1.0	18

For details of symbols used in Tables 6, 7, 8, 9, 10 and 14, see Table 5.

It appears from Table 6 that a statistically significant difference occurred at 12 weeks. Consequently, subcutaneous tissue implanted under the periosteum stimulated longitudinal growth slightly more than simple detachment of the periosteum.

Skin contra subcutaneous tissue as growth-stimulant. The next two groups compared were A_1 and A_2 , to ascertain whether the effect on longitudinal growth differed when skin or subcutaneous tissue was implanted under the periosteum after large detachment.

Table 7. Comparison of discrepancy means of groups A_1 and A_2

Groups	t	$D \pm s_D$	Ci	f
$A_1 - A_2$	3	-0.1 ± 0.1	- 0.3 0.2	28
	12	1.1 ± 0.3	0.6 1.7 v)	17
	22	1.5 ± 0.8	- 0.5 3.5	15

Table 7 shows a significant difference between the means of the groups at the age of 12 weeks, with 95 per cent probability. The effect of the skin graft was thus more appreciable than that of subcutaneous tissue.

The growth-stimulating effect of cyst. Subgroups B_{1a} and B_{1b} were compared for differences. In one of them a cyst had formed in the bone, in the other the graft implanted under the periosteum had slipped from under the periosteum and there was no cyst formation.

Table 8. Comparison of discrepancy means of subgroups B_{1a} and B_{1b}

Groups	t	$D \pm s_D$	Ci	f
$B_{1a} - B_{1b}$	3	0.1 ± 0.1	- 0.1 0.3	19
	12	0.1 ± 0.2	- 0.4 0.6	19
	16	-0.1 ± 0.5	- 1.2 1.0	13
	25	-0.3 ± 0.3	- 0.9 0.2	24

It will be seen from Table 8 that there was no significant difference between these subgroups.

The role of the size of the periosteum in growth-stimulation (in the presence of a cyst) Groups A_1 and B_{1a} were compared to establish whether large detachment of the periosteum is a necessary additional measure to cyst

formation for stimulation of longitudinal growth. Group A_1 represents a situation in which a cyst had formed in the bone and where the periosteal detachment was large.

Table 9. Comparison of discrepancy means of groups A_1 and B_{1a}

Groups	t	$D \pm s_D$	Ci		f
$A_1 - B_{1a}$	3	0.0 ± 0.1	- 0.2	0.2	30
	12	1.4 ± 0.3	0.9	2.0 v)	17
	16	2.4 ± 0.5	1.4	3.3 v)	30
	25	2.0 ± 0.7	0.6	3.4 v)	19
	52	1.8 ± 0.5	0.2	3.5 v)	3

Table 9 shows that the means differed significantly from 0. In fact, large detachment of the periosteum is clearly necessary to produce stimulation. A cyst alone does not cause stimulation.

The effect of trauma on epiphyseal cartilage. Series C was different from all the other groups in that the detachment of the periosteum was extended to the area of the perichondrium lining the epiphyseal cartilage. In group C_1 skin was implanted under the periosteum, in C_2 subcutaneous tissue and in C_3 the only measure was detachment of the periosteum. The results obtained, again, differed from those of the other groups. All groups of the C series, unlike the case in series A and B, showed a distinct and similar retardation of growth at the age of 24 weeks. This was obviously because the operative trauma sustained by the epiphyseal cartilage exerted a retarding effect on its growth. At 30 weeks the C_1 curve rose but the curves for C_2 and C_3 fell still further. Table 10 gives the limit values for the differences in question to ascertain whether C_1 differs at this point statistically significantly from groups C_2 and C_3 .

Table 10. Comparison of discrepancy means of groups C_1 and C_2 : C_1 and C_3

Groups	t	$D \pm s_D$	Ci		f
$C_1 - C_2$	30	1.3 ± 0.4	0.6	2.1 v)	16
$C_1 - C_3$	30	1.2 ± 0.3	0.5	1.9 v)	12

Table 10 shows that C_1 differed statistically significantly from groups C_2 and C_3 at 30 weeks. Hence the cyst formation in C_1 group diminishes the growth retardation resulting from trauma to the proximal epiphyseal cartilage.

To summarise the results of the measurements, large detachment of the periosteum is the basis of growth-stimulation operations. A cyst originating in the bone from whole-thickness skin implanted under the periosteum causes stimulation of longitudinal bone growth if the periosteum has been detached from the area of the proximal diaphysis of the tibia without damaging the epiphyseal cartilage. Cyst formation in the bone does not produce growth-stimulation without detachment of periosteum. Large detachment of the proximal diaphyseal periosteum causes only slight growth stimulation. The implantation of a graft of subcutaneous tissue under extensively detached periosteum provokes a slightly greater growth stimulation than simple periosteal detachment on the same scale.

Small detachment of the periosteum on the boundary between the metaphysis and the diaphysis does not stimulate growth even if skin or subcutaneous tissue is implanted under the periosteum.

If periosteal detachment is extended to the area of the epiphyseal cartilage, growth is retarded at 12—30 weeks of age. If a skin graft is implanted under the periosteum, however, the final shortening of the operated limb is not as marked as if detachment is the only measure or subcutaneous tissue is implanted under the periosteum.

Growth from the different epiphyseal cartilages

The purpose of the experiments of series D was to establish the degree to which the growth of tibia and the growth stimulation induced by surgery were distributed between the proximal and distal epiphyseal cartilage. Differing from the preceding series, the animals of series D were operated on at the age of 6 weeks. The operative procedure was the same as for group A₁: large detachment of the periosteum and skin implanted on the boundary between the metaphysis and the diaphysis. To follow the growth from the different epiphyseal cartilages, a small X-ray positive gutta-percha mark was inserted during the operation in the cortex in the middle of the bone diaphysis. This small mark in the bone did not affect longitudinal growth, as has been shown by e.g. GATEWOOD and MULLEN, BISGARD and BISGARD, and BRODIN.

Table 11 shows the development of the length discrepancy of the right and left tibia of the animals of series D. The analysis is similar to that for the other series in Table 3.

Table 11. Development of tibia length discrepancy in series D

Age in weeks	Number of measurements	Discrepancy in mm			
		Mean	Standard error of the mean	Confidence limits	
6	8	0.1	0.1	— 0.1	0.2
8	8	0.6	0.1	0.2	0.9
12	8	1.1	0.2	0.6	1.5
16	8	1.1	0.3	0.4	1.8

Table 11 shows a distinct and statistically significant growth stimulation in series D at 8, 12 and 16 weeks of age. The result corresponded to that of group A₁.

Table 12. Distribution of the growth between the proximal and distal epiphyseal cartilage in series D. (8 rabbits)

Age intervals	Tibia	Proximal				Distal					
		Mean in mm	Standard error	Confidence limits		Per cent	Mean in mm	Standard error	Confidence limits		Per cent
6 — 8	Right	5.5	0.2	5.1	6.0	52.1	5.1	0.2	4.6	5.5	47.9
	Left	5.5	0.2	5.1	5.9	53.7	4.7	0.2	4.2	5.2	46.3
8 — 12	Right	8.9	0.2	8.4	9.4	58.0	6.5	0.3	5.8	7.1	42.0
	Left	8.9	0.2	8.4	9.4	60.2	5.9	0.3	5.1	6.7	39.8
12 — 16	Right	6.3	0.1	6.0	6.5	64.3	3.5	0.1	3.3	3.6	35.7
	Left	6.6	0.2	6.2	7.0	68.4	3.0	0.1	2.7	3.4	31.6

The development of length discrepancy has been analysed above on the basis of total longitudinal growth of the bone. In order to illustrate the growth phenomenon, Table 12 shows the growth from each epiphyseal cartilage at a certain age, in both absolute and percentual terms.

It will be seen from Table 12 that normal growth, represented by the growth of the left tibia, occurred between the ages of 6 and 8 weeks in 53.7 per cent of the cases from the proximal and in 46.3 per cent from the distal epiphyseal cartilage. At 8—12 weeks normal growth occurred in 60.2 per cent from the proximal epiphysis and in 39.8 per cent from the distal epiphysis. The normal growth at 12—16 weeks occurred in 68.4 per cent from the proximal and in 31.6 per cent from the distal epiphyseal cartilage. The growth from the proximal epiphysis was thus more pronounced, and the difference is accentuated by the fact that the distal epiphyseal cartilage closed earlier than the proximal.

To obtain a clearer picture of the distribution of growth between the proximal and distal epiphyseal cartilage, the mean growth quotient proximal growth/distal growth was calculated for the animals of series D. Fig. 17 shows graphically the changing of this quotient and the confidence limits for the change for both the right and the left tibia.

Development of mean growth quotient* in series D .

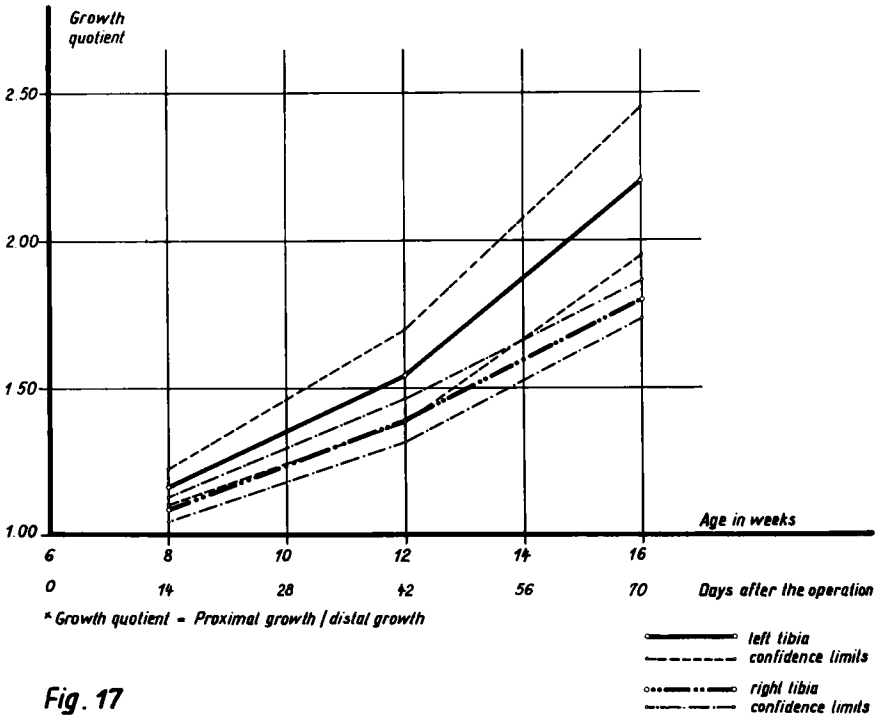


Fig. 17

The growth quotient of the operated leg was significantly smaller than that of the control limb owing to the increased activity of the distal epiphyseal cartilage.

Table 13 shows the distribution of the length discrepancy originating between the right and the left tibia relative to the proximal and distal epiphyses.

Table 13. Length discrepancy in mm due to proximal and distal epiphyseal cartilage in series D (8 rabbits)

Age intervals	Proximal			Distal			
	Mean	Standard error	Confidence limits	Mean	Standard error	Confidence limits	
6 — 8	0.1	0.1	-0.2 0.3	0.4	0.1	0.2	0.5
6 — 12	0.1	0.1	-0.2 0.2	0.8	0.2	0.3	1.4
6 — 16	-0.3	0.1	-0.6 0.1	1.3	0.3	0.7	1.9

Length discrepancy due to proximal and distal epiphyseal cartilage.

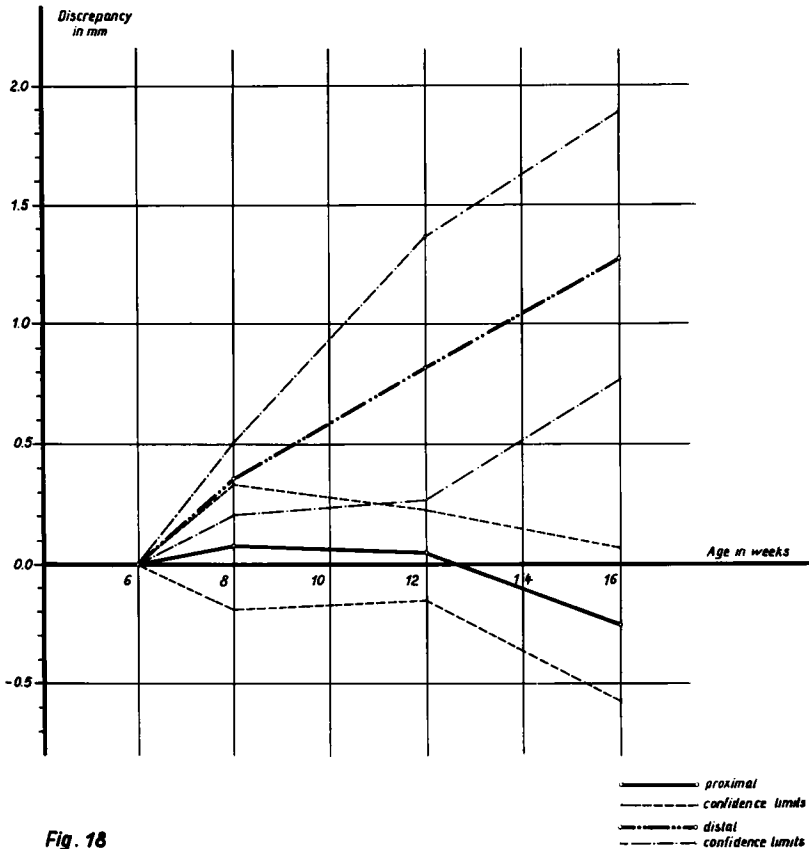


Fig. 18

Table 13 and Fig. 18 reveal that when the periosteal detachment was performed as described above growth stimulation occurred princi-

pally from the distal epiphyseal cartilage. After 7 postoperative weeks the proximal epiphyseal cartilage also caused some growth stimulation, but then the proximal epiphyseal cartilage began to retard growth. The effect of the proximal epiphyseal cartilage on growth stimulation was not statistically significant during the test, as the confidence limits show.

To find out whether the growth stimulation from the distal epiphyseal cartilage differed statistically significantly from growth stimulation from the proximal epiphyseal cartilage, the confidence limits for the difference were calculated.

Table 14. **Difference between discrepancy due to distal and proximal epiphyseal cartilage**

Age intervals	$D \pm s_D$	C i
6 ... 8	0.3 ± 0.1	0.0 0.6 v)
6 ... 12	0.8 ± 0.2	0.2 1.3 v)
6 ... 16	1.5 ± 0.3	0.9 2.2 v)

The stimulation of longitudinal bone growth was considerably greater from the distal than from the proximal epiphyseal cartilage at all ages.

To summarise, it may be said that the longitudinal growth of rabbit tibia occurs for the greatest part from the proximal epiphyseal cartilage, and that the difference becomes even more distinct when the animal approaches adulthood. Although longitudinal growth occurs mainly from the proximal epiphyseal cartilage, the postoperative positive discrepancy in longitudinal growth derives from the activity of the distal epiphyseal cartilage.

Roentgenological changes

Irrespective of the operation performed, all the rabbits developed a soft-tissue swelling in the operative area. It was demonstrable roentgenologically 2—4 weeks postoperatively. A week after the operation all the groups showed thickening of the periosteum and increased calcification in the region of the periosteal incision. The processes disappeared in about a month. There were, however, other changes in some groups.

More pronounced roentgenological changes were seen only when skin had been used as the implantation material. A semi-translucent lesion usually developed in the skin groups in c. 2 weeks at the site of implantation in the operated tibia. Within a week this lesion became more demarcated and took on the appearance of a cyst-like formation, the periosteum rose and the bone became eroded at the site. The cyst then went on growing for some 18 weeks, spherical in shape and pushing towards the centre of the bone (Figs. 19, 20, 24, 25, 33, 34 and 35) or spreading under the periosteum to encircle the diaphysis like a belt and eroding it only superficially (Figs. 31 and 32). The semi-translucent lesion was sometimes multilocular with the different parts of the transplant growing in different ways (Figs. 22 and 23). Enlargement towards the periphery occurred in control group A₄ in which skin was implanted in the medullary cavity. All but one of the cases with no roentgenologically demonstrable cyst formation showed later, after the animal was sacrificed, that the skin graft had slipped from beneath the periosteum into the surrounding soft tissues. The exception was rabbit No. 33 in which the graft had been implanted in the bone marrow cavity; at autopsy, at the age of 1 year, not a trace of the implant could be found.

The cyst was encircled by a dense calcified zone which gave it distinct margins. The bone thickened near the cyst and the cortex also thickened. As the cyst often raised the periosteal line it frequently broke and a bowl-shaped depression originated in the bone, as can be seen in Fig. 33. If a cyst formed, it appeared within 4 weeks of the operation. The only exception to this rule was rabbit No. 39, in the marrow control group of series A. In this case no roentgenographical changes could be seen right up to the age of 20 weeks, i.e. 18 weeks postoperatively, but the roentgenographs taken 5 weeks later revealed a distinct cyst. The same rabbit also developed around the site of the cyst a pronounced callus at the age of 42 weeks. The surrounding normal bony tissue disappeared from around the cyst and was replaced by loose, irregular

newly-formed bone. The bone diameter in this case was 3 times its original size near the cyst.

Similar vigorous resorption of bone and formation of new bone occurred in 2 other rabbits in which the skin graft was inserted under the periosteum, i.e. rabbit No. 5 (Group A₁) and rabbit No. 21 (Group A₁). Figs. 26, 27 and 28 are roentgenograms of rabbit No. 21. In rabbit No. 5 the reaction set in 8 weeks and in the other rabbit 12 weeks post-operatively.

As the roentgenograms of the rabbits were conventional antero-posterior pictures and only in isolated cases other projections, it was generally impossible to assess whether the medullary cavity was completely blocked. Not even in the skin graft groups did a cyst form in all the rabbits. If a cyst appeared it grew gradually as the rabbit approached the age of 24 weeks but then remained more or less unchanged. The margins of the cyst became less clearly defined owing to the decrease in the calcium density of the encircling bone and to possible degenerative changes in the cyst. Two rabbits, Nos. 103 and 104, (Group C₁) showed distinct cysts 10 weeks postoperatively, but in the roentgenogram taken 22 weeks later the cyst had disappeared completely in one case and in the other was seen only as a streak-like radiolucent line parallel to the longitudinal axis of the bone. The table 15 shows the incidence of roentgenologically demonstrable cyst in the different series.

Table 15. The occurrence of roentgenological changes in operated rabbits

Series	Group	Total No. of rabbits	Cyst was found roentgenologically	
			No. of rabbits	Per cent
A	A ₁	21	20	96
	A ₄	12	11	92
B	B ₁	21	6	29
C	C ₁	17	12	71
D		8	8	100

In Group A₁ 96 per cent of the cases showed a cyst. The frequency was roughly the same (92 per cent) in control groups A₄ in which the skin graft was implanted in the medullary cavity. The percentage of cyst formations was low (29 per cent) in group B₁, but higher in group C₁ (71 per cent). A cyst formed in all the animals of series D.



Fig. 19. Both tibiae of rabbit No. 91 (Group C₁) at 12 weeks of age exposed for the length measurement. The right tibia is 0.5 mm longer than the left tibia. A clearly demarcated cyst can be seen in the operated right tibia.



Fig. 20. The same lesion as in Fig. 19, but at 24 weeks of age. The right tibia is 0.7 mm shorter than the left. The cyst has become larger and its boundaries more sharply defined.



Fig. 21. The same lesion as in Figs. 19 and 20, but at 32 weeks of age. The right tibia is 0.5 mm shorter than the left. The cyst is no longer so clearly demarcated as before.



Fig. 22. Antero-posterior projection. The lesion in the right tibia of rabbit No. 3. (Group A₁) at 20 weeks of age. The right tibia is 2.8 mm longer than the left. A multilocular cyst. The cyst raises the periosteum. The corticalis has become thicker. Figs. 42 and 43 are microphotos of the same lesion.

Fig. 23. Diagonal projection.



Fig. 24. Antero-posterior projection. The lesion of rabbit No. 4 (Group A₁) at 20 weeks of age. The right tibia is 2.3 mm longer than the left. Ellipsoid-like cyst raising the periosteum. Fig. 46 is a microphoto of the same lesion.

Fig. 25. Diagonal projection.

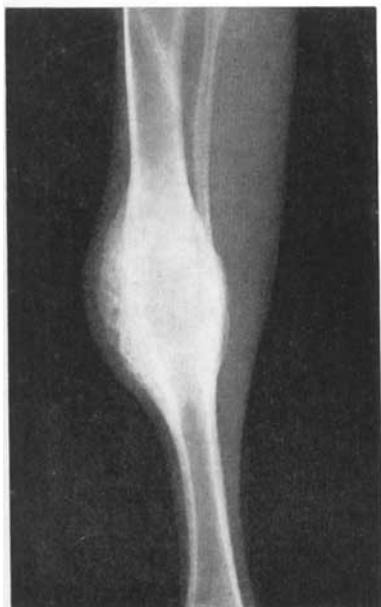


Fig. 26. Antero-posterior projection. The lesion in the right tibia of rabbit No. 21 (Group A₁) at 24 weeks of age. The right tibia is 6.3 mm longer than the left. The normal corticalis has disappeared around the cyst and is replaced by cancellous new bone.

Fig. 27. Diagonal projection.



Fig. 28. The same lesion as in Figs. 26 and 27, but at 53 weeks of age. The right tibia is 6.4 mm longer than the left. The boundaries of the cyst cannot be seen as clearly as at 24 weeks.



Fig. 29. The lesion in the right tibia of rabbit No. 48 (Group A₃) at 24 weeks of age. The right tibia is 0.3 mm shorter than the left. There is a roundish semi-translucent area with small calcified spots in the middle. The bone has become thicker around the cyst. The diameter of the bone at the height of the lesion is 8.6 mm.



Fig. 30. The same lesion as in Fig. 29, but at 53 weeks of age. The right tibia is 0.9 mm shorter than the left. The semi-translucent area in the bone has almost disappeared but the calcified spots are still clearly visible. The diameter of the bone here is 7.7 mm. Fig. 53 is a microphoto of the lesion.



Fig. 31. Antero-posterior projection. The lesion in the right tibia of rabbit No. 10 (Group A₁) at 52 weeks of age. The right tibia is 0.6 mm longer than the left. An epidermal cyst belt-like around the diaphysis, growing under the periosteum.



Fig. 32. Side projection.



Fig. 33. The lesion in the right tibia of rabbit No. 8 (Group A) at 22 weeks of age. The tibia is 1.7 mm longer than its control. A well demarcated cyst in the diaphysis. The periosteum is raised and has ruptured to form a bowl-like depression.



Fig. 34. A planigram of the same lesion as in Fig. 33. The boundaries are clear. Cortical thickening around the cyst.



Fig. 35. Roentgenogram of the same lesion as in Fig. 33, but $\times 1.4$ and taken by planigraph. The boundaries are clearly visible.

Macroscopic changes

The majority of the rabbits in which the skin graft was implanted under the periosteum displayed at necropsy when the rabbits were 24 weeks old a pale elevation with crater-like edges in the operated tibia. The light area was softer than the surrounding bone. Its content was a white tallow-like mass with remnants of hairs. In some cases the contents included clear fluid. It was possible to empty the content of the bone cyst. The skin graft had generally dropped under the periosteum and its location was slightly more distal from the original site of implantation. In several cases the elevation could be palpated from above the skin. In some cases the skin graft had slipped into the surrounding muscular tissue from under the periosteum, obviously between the sutures or through the possible ruptures originating on detachment of the periosteum. Typical of these cases were the rabbits (Table 2) which even roentgenologically revealed no permanent changes. The epidermal cyst was the size of the nail of the little finger and lay in the surrounding muscular tissue. In bony tissue the cyst had grown to c. 2—3 times its size in the muscular tissue.

The medullary cavity group generally revealed a cyst of corresponding nature, again originating when the skin graft was under the periosteum. There was one case, rabbit No. 33, in which not a trace of the graft implanted into the marrow cavity could be demonstrated at necropsy.

When subcutaneous tissue was used as the implant it was resorbed during the period of observation and not a trace of it could be found at necropsy. These cases gave a finding similar to that in the animals in which periosteal detachment only was performed. Their crural fascia was thicker than usual and more cicatrised, but no major reaction was observed. None of the animals displayed signs of infection.

Histological changes

Local changes

The epidermis of the skin transplant formed a cyst which was lined by squamous epithelium and contained a necrotic mass with remains of keratinous lamellae and hair. There were moreover smaller cysts in the environment in the form of hair follicles.

If the cyst was associated with the bone its epithelium was shallow, sometimes atrophic, and in some cases it disappeared and the cyst was lined only by scar tissue. Under the initially thick epithelium the granulation tissue layer proliferated as the epithelium thinned. The degeneration of the epithelium progressed until it disintegrated completely. Scar tissue developing from the granulation tissue often grew into the cyst. If the cyst was adjacent to soft tissues the epithelium was higher, rich in pigment and there were hair follicles and sebaceous glands in association with it (Fig. 49). Sequestra were often observed inside the cyst (Figs. 42, 43).

Especially when implanted under the periosteum, the graft developed a cyst which grew expansively towards the centre of the bone (Fig. 40). The bone anterior to the growing cyst was resorbed and new bone was formed in places. The encircling tissue generally contained numerous multinuclear giant cells. They were foreign body giant cells in the connective tissue (Fig. 41) and osteoclasts in the lacunae along the edge of the bone (Fig. 45). The bone adjacent to the cyst showed degeneration manifest as less regular bone lamella structure. There was not a single case of cartilaginous tissue in the cysts or around them. It was possible to distinguish histologically an epithelial cyst growing in a belt-like manner (Fig. 52). The compact cortical bone surrounding the cyst was sometimes resorbed and replaced by loose newly-formed bone. Fig. 50 shows an example of this development.

Newly-formed bone trabeculae (Fig. 44), an indication of increased osteogenesis, were established especially around a graft implanted in the medullary cavity. The graft implanted in the medullary cavity disintegrated in one case, it produced no cyst and all that remained was an ossified cicatricial focus of indeterminate shape containing remains of hairs and epidermis (Fig. 53). In another rabbit, No. 33, no trace of a cyst could be found after a year.

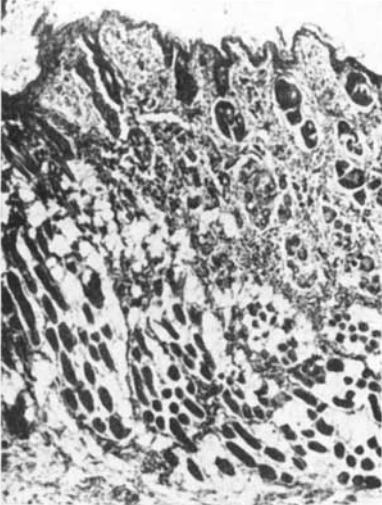


Fig. 36. A sample of whole-thickness skin from a 2-week old rabbit. The epidermis is thin. Large amount of hair follicles extending to the subcutaneous fat layer.
 Enlargement $\times 40$. H.- van Gieson

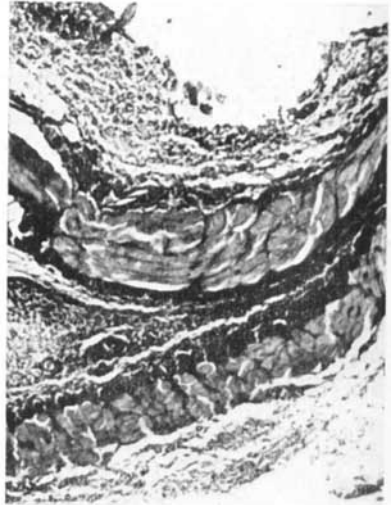


Fig. 37. A sample of subcutaneous tissue from a 2-week old rabbit. Rabbit subcutaneous tissue also has striated muscle tissue.
 Enlargement $\times 40$. H.- van Gieson



Fig. 38. A microphoto of the lesion in the right tibia of rabbit No. 13 (Group A₁) at 24 weeks of age. The right tibia is 0.5 mm longer than the left. In one part an isolated cyst is preserved, everywhere else the scar tissue has grown between the masses of horny lamellae. Bone trabeculae around the cyst.
 Enlargement $\times 20$. H.- van Gieson



Fig. 39. A detail of Fig. 52. There are multinuclear giant cells in the connective tissue.
 Enlargement $\times 120$. H.- van Gieson

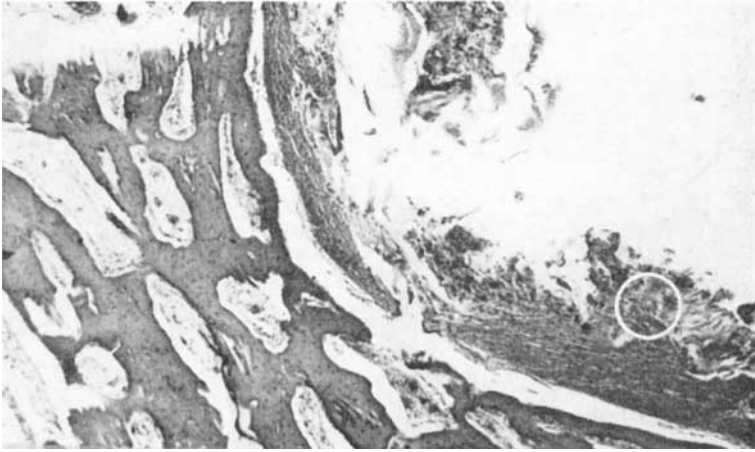


Fig. 40. The lesion in the right tibia of rabbit No. 98 (Group C) at 24 weeks of age. The right tibia is 2.3 mm shorter than the left. A cancellous bone layer isolates the cyst from the bone marrow cavity. The epithelium of the cyst wall is wholly degenerated and is replaced by scar tissue. In the middle of the cyst are remnants of horny lamellae and hair follicles. Enlargement $\times 40$. H.- van Gieson



Fig. 41. A detail of Fig. 40. A foreign body giant cell near the disintegrating remnants of hairs. Enlargement $\times 890$. H.- van Gieson



Fig. 42. A microphoto of the lesion in the right tibia of rabbit No. 3 (Group A) at 25 weeks age. The right tibia is 3.1 mm longer than the left. On the concave side is a thin epithelial lining. Figs. 22 and 23 give X-ray pictures of the same lesion.
Enlargement $\times 40$. H.- van Gieson



Fig. 43. A detail of Fig. 38. There is scar tissue under the epithelium. In the cyst there is a bone sequester surrounded by horny lamellae.
Enlargement $\times 160$. H.- van Gieson

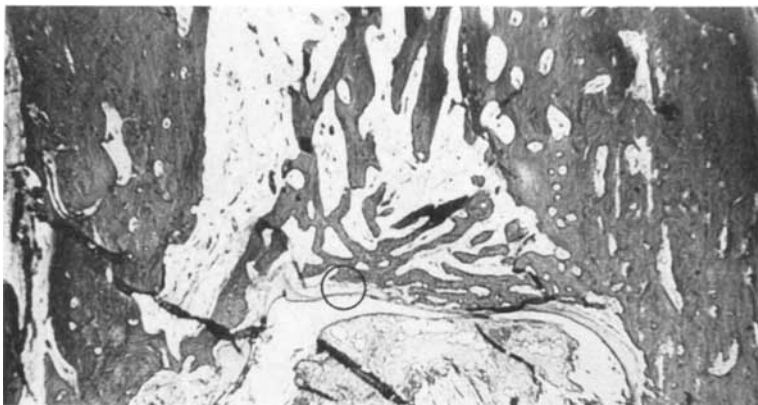


Fig. 44. The lesion in the right tibia of rabbit No. 38 (Group A₄) at 12 weeks of age. The right tibia is 0.4 mm longer than the left. Around the cyst are bone trabeculae, a sign of increased osteogenesis. In the cyst, remnants of hair follicles and horny lamellae and sequesters.
Enlargement $\times 35$. H.- van Gieson

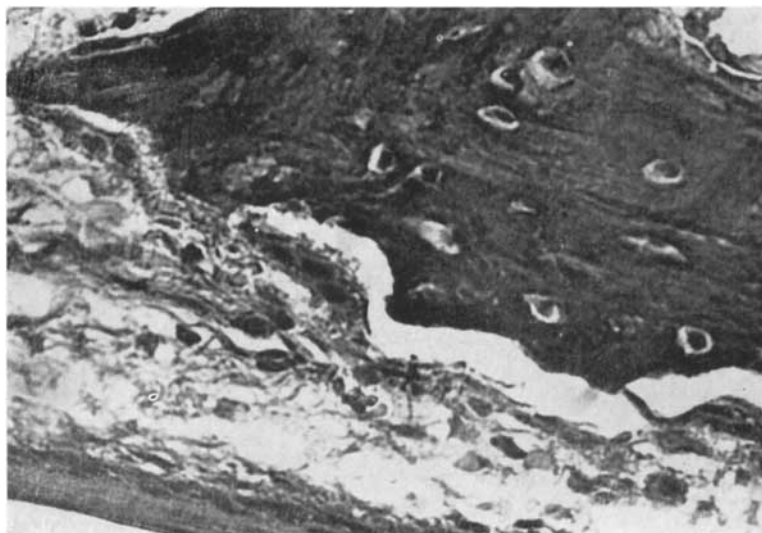


Fig. 45. A detail of Fig. 44. Cyst wall in the left lower corner. Along the bone edge osteoclasts in Howship's lacunae.
Enlargement $\times 490$. H.- van Gieson

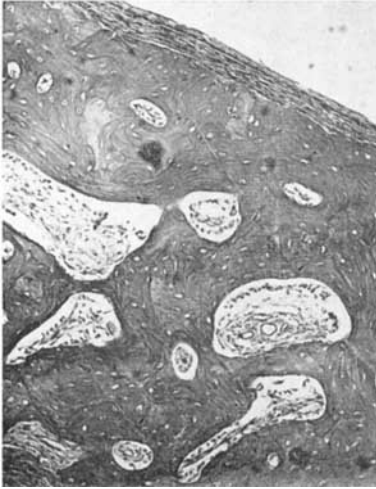


Fig. 46. A microphoto of the lesion in the right tibia of rabbit No. 4 (Group A₁) at 25 weeks of age. The right tibia is 2.2 mm longer than the left. Wall of epidermal cyst at the top. Layer of thin, coarse fibrillar compressed hyaline scar tissue separates the atrophic epithelium from the bone. The bone is more cancellous than normally. Figs. 24 and 25 are X-ray pictures of the same lesion.

Enlargement $\times 20$. H.- van Gieson



Fig. 47. A microphoto of the lesion in the right tibia of rabbit No. 90 (Group C₁) at 24 weeks of age. The right tibia is 4.0 mm shorter than the left. Under the epithelium is a thick layer of scar tissue. There is slight degeneration of the bone.

Enlargement $\times 20$. H.- van Gieson



Fig. 48. A microphoto of the lesion in the right tibia of rabbit No. 11 (Group A₁) at 7 weeks age. The right tibia is 0.6 mm longer than the left. The epithelium does not line the cyst completely. It is partly lined by granulation tissue.

Enlargement $\times 40$. H.- van Gieson



Fig. 49. A microphoto of the lesion of the right leg of rabbit No. 64 (Group B₁) at 24 weeks of age. An epidermal cyst in a muscle. The epithelium is well preserved, there are hairs and sebaceous glands. Especially the stratum spinosum is hyperplastic.

Enlargement $\times 130$. H. - van Gieson

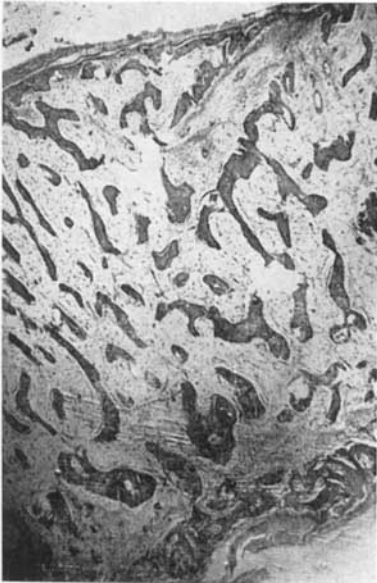


Fig. 50. The lesion in the right tibia of rabbit No. 5. (Group A₁) at 20 weeks of age. The right tibia was 3.0 mm. longer than the left tibia at 17 weeks. A strong reaction has occurred in the bone. The whole corticalis is replaced by loose cancellous bone.

Enlargement $\times 20$.

H.- van Gieson

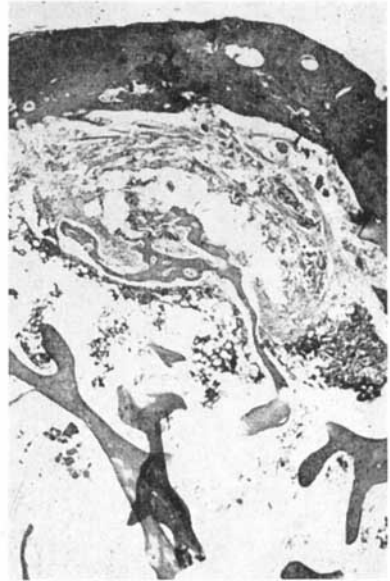


Fig. 51. The lesion in the right tibia of rabbit No. 91 (Group C₁) at 32 weeks of age. The right tibia is 0.5 mm shorter than the left. Only remnants of hairs and horny lamellae are left of the epithelial lining of the cyst. Between the remnants are scar tissue and bony trabeculae.

Enlargement $\times 20$.

H.- van Gieson

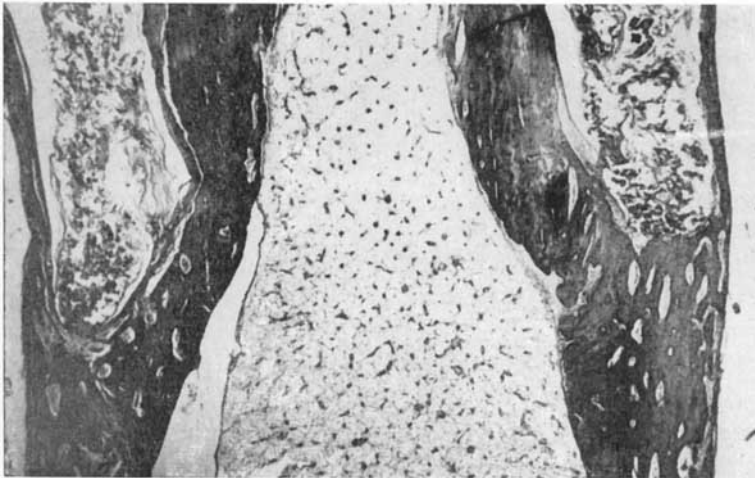


Fig. 52. A microphoto of the lesion in the right tibia of rabbit No. 7 (Group A₁) at 10 weeks of age. The right tibia is 1.1 mm longer than the left. The epidermal cyst is growing under the periosteum. The bone marrow cavity is narrowed. The diameter of the bone is scarcely increased at all.

Enlargement $\times 25$.

H.- van Gieson



Fig. 53. A microphoto of the lesion in the right tibia of rabbit No. 48 (Group A₃) at 53 weeks of age. The right tibia is 0.8 mm shorter than the left. In the middle of bone marrow cavity is a group of bone trabeculae, between which there are scar-like connective tissue and remnants of horny lamellae. Enlargement $\times 20$. H.- van Gieson

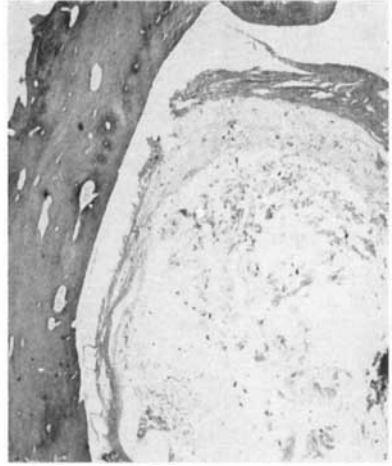


Fig. 54. The lesion in the right tibia of rabbit No. 51 (Group B₁) at 52 weeks of age. The right tibia is 0.6 mm shorter than the left. The epithelium lining the cyst has disappeared almost completely. The scar tissue is growing into the cyst. Enlargement $\times 20$. H.- van Gieson

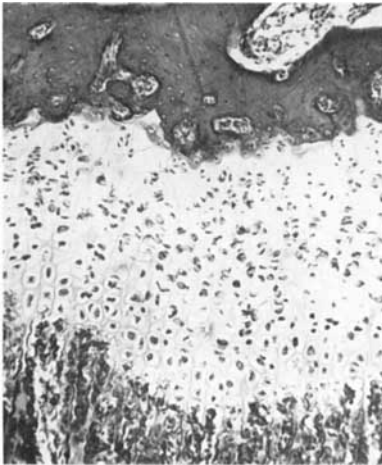


Fig. 55. The proximal epiphyseal cartilage of the right tibia of rabbit No. 107 (Group C₂) at 24 weeks of age. The right tibia is 1.5 mm shorter than the left. This cartilage was disturbed by extending the detachment of the diaphyseal periosteum beyond the epiphyseal line at 2 weeks of age. The arrangement of the cartilage cells is disturbed in all zones. The cells of the proliferating zone are hypertrophied and the border between this zone and that of maturing cells has become unclear. Enlargement $\times 60$. H.- van Gieson



Fig. 56. The proximal epiphyseal cartilage of the left tibia of rabbit No. 107 (Group C₂) represents a normal epiphyseal cartilage at 24 weeks of age. The form of the cartilage columns is regular. Enlargement $\times 60$. H.- van Gieson

The piece of fat implanted under the periosteum was resorbed rapidly and no sign of it was demonstrable 6 months later. Nor was any appreciable change observed in the bone preparations in the cases in which the periosteum had been detached half a year earlier.

There was not a single case in which the irritation was found to have caused malignancy.

Histological study of the epiphyseal cartilage

The epiphyseal cartilage is the centre of the growth process. The problem here was to establish whether stimulation of the epiphyseal cartilage affects its morphology.

Epiphyseal cartilage consists of rows of cartilage cells arranged parallel to the longitudinal axis of the bone. Various investigators have divided the cartilage into zones according to the degree of maturity of the cartilage cells. Mention may be made of MÜLLER (1858), HAM (1957) and TRUETA (1957) in this connection. The present author adopted the zonal division of Ham for the present study: the zone of resting cartilage, the zone of proliferating young cartilage, the zone of maturing cartilage and the zone of calcified cartilage. No striking changes were established in the present study between the stimulated epiphyseal cartilages and the corresponding controls in series D. In series D the degree of stimulation achieved was measured between the proximal and distal epiphyseal cartilages. The arrangement of the cartilage cells remained unchanged. But pronounced changes were demonstrated in series C in which the periosteal detachment was prolonged to the area of the perichondrium lining the epiphyseal cartilage, a measure which provoked a definite slowing-down of longitudinal growth. The arrangement of the cartilage cells was irregular, the parallel organisation had disappeared (Fig. 55). The margins of the cellular zones were increasingly difficult to distinguish and the epiphyseal bone plate often tended to herniate from the side of the epiphysis. No true bony bridges were observed at the age of 24 weeks. In the next observation period, 30 weeks of age, the epiphyseal cartilage had disappeared completely in all the proximal epiphyseal cartilages examined.

Changes in the height of epiphyseal cartilage have been mentioned from time to time. It was measured in the present investigation by the histoquantitative method developed by UOTILA and KANNAS (1952). The principle of the application was to project a section of the epiphyseal cartilage on white paper and draw on it the boundaries of the zones

of the epiphyseal cartilage. The width of the zones was measured later from the paper. It was apparent from the outset that the boundary between the zones of resting cartilage and proliferating cartilage as well as the final limit of the calcified cells could not be distinguished with sufficient certainty. Hence the resting and proliferating cartilage zones were measured as one zone and the maturing cartilage zone as another.

The changes in the stimulated epiphyses were studied in the proximal and distal epiphyseal cartilages of series D. The height of the cartilage and these two zones, called in the following the zones of proliferating cells and hypertrophic cells, were measured in the direction of the cell columns from fixed points of the epiphyseal cartilage. The samples were taken when the animals were 16 weeks of age. Table 16 shows the height of each zone. The measurements were made on a total of 8 rabbits. The mean of 6 measurements was recorded. The figures are given in centimetres, measured from the enlarged projection, as are also all other figures for epiphyseal cartilage measurements.

Table 16. **The calculated heights for the epiphyseal cartilages in series D**

	Zone of proliferating cells		Zone of hypertrophic cells		Height of the total cartilage	
	Mean	Standard error	Mean	Standard error	Mean	Standard error
Right proximal	4.45	0.38	1.84	0.13	6.29	0.43
Right distal	3.21	0.62	1.19	0.24	4.40	0.80
Left proximal	4.73	0.47	1.95	0.26	6.68	0.68
Left distal	3.51	0.52	1.34	0.15	4.85	0.65
Sum of proximal epiphyseal cartilages	9.19	0.67	3.79	0.33	12.98	0.88
Sum of distal epiphyseal cartilages. . .	6.71	1.05	2.53	0.30	9.24	1.32

The mean heights of both epiphyseal cartilages of the operated right tibia and the left tibia were calculated separately for Table 16. The sum of the figures of proximal and distal epiphyseal cartilages was also calculated. The table also gives the standard error for all these heights.

Table 17 gives a comparison of the results in Table 16. It shows the difference of the means compared, their standard error and the 95 per cent confidence limits for the difference. Number of degrees of freedom $f = 14$.

Table 17. The effect of stimulation on the epiphyseal cartilage as indicated by comparing the height of the whole epiphyseal cartilages and their zone heights

	Zone of proliferating cells				Zone of hypertrophic cells				Height of the whole cartilage			
	Difference	S _D	95 %	Ci	Difference	S _D	95 %	Ci	Difference	S _D	95 %	Ci
lp-rp	0.28	0.21	-0.18	0.73	0.11	0.10	-0.10	0.33	0.39	0.28	-0.22	1.00
ld-rd	0.30	0.29	-0.32	0.92	0.15	0.10	-0.07	0.36	0.45	0.36	-0.33	1.23
p-d	2.48	0.44	1.52	3.42	1.26	0.16	0.92	1.60	3.74	0.56	2.54	4.94

The symbols used in Table 17:

lp - rp = left proximal less right proximal

ld - rd = left distal less right distal

p - d = sum of proximal epiphyseal cartilages less the sum of distal epiphyseal cartilages

S_D = standard error

Ci = confidence limits

It will be seen from Table 17 that there is no significant difference in height between the stimulated and the control epiphyseal cartilages. This applies to both proximal and the distal epiphyseal cartilages. Similarly, no difference in height was established between the proliferating and the hypertrophic cell zones. On the other hand, taking the sum of the heights of the proximal and distal epiphyseal cartilages of both bones and of the heights of their components, a statistically significant difference is elicited. The height of the proximal epiphyseal cartilage is greater than that of the distal. The same applies to the different zones of the two cartilages.

Discussion

The results indicate that the size of the periosteal detachment had a distinct effect on the stimulation of longitudinal growth. A small detachment on the boundary between the metaphysis and the diaphysis caused no growth stimulation. A mild degree of stimulation in the operated tibia was produced by detaching the proximal half of the tibial diaphysis as far as the metaphysis. This growth, however, levelled off in the course of the observation period. When the detachment of the periosteum of the proximal half of the diaphysis was extended to the area of the epiphysis, i.e. the perichondrium of the epiphyseal cartilage was detached at the same time, a definite retardation of longitudinal bone growth between the ages of 12 and 24 weeks regularly resulted.

Other workers such as OLLIER (1867), WU and MILTNER (1937) and LACROIX (1951) produced a greater stimulation of growth than the present author by detaching the tibial periosteum, and the resulting increase in growth was permanent. WU and MILTNER performed varying degrees of periosteal detachment. The smallest growth stimulation in their series was in the group in which the periosteum was detached only on the anterior of the tibia. Modest growth stimulation was provoked by BRODIN (1955) by detaching the periosteum from the proximal half of the diaphysis and metaphysis. The present author followed this technique in series A and D.

Seeking an explanation for the small growth stimulation achieved by periosteal detachment in the present work, the first factor that comes to mind is the age at which the operation is performed. OLLIER established that the age at surgery affects the degree of stimulation. LANGENSKIÖLD (1957) produced only small growth stimulation when he operated on rabbits aged 14–36 days, roughly the same age at which the operation was performed by the present author. BRODIN did not mention the age of his animals, but WU and MILTNER performed their operations on animals aged 5–8 weeks. This age is probably the most suitable for surgery since the most distinct length

discrepancies provoked by stimulation are those reported by WU and MILTNER. The present investigation was not concerned specifically with the influence of age on stimulation. Some of the animals were operated on at the average age of 3 weeks, some (series D) at 6 weeks, and no significant difference could be established from the results of the first 11 postoperative weeks when it was possible to make comparative studies. Another reason for the conflicting results obtained by different workers may be their attitude to the nutrient artery. Not all authors have mentioned whether they touched it in their technique, but TRUETA (1953) regarded the ligation of the nutrient artery as an important stimulation-inducing factor while BROOKES (1957) succeeded in retarding bone growth by simple ligation of the nutrient artery. WU and MILTNER (1937) destroyed the nutrient artery in detaching the periosteum. The present author, applying the method of detachment introduced by BRODIN (1955), left the nutrient artery intact. FREJKA and FAIT (1957), who were careful not to cut the nutrient artery when they detached the periosteum of child patients, produced fairly considerable length increase after stimulation.

In series C, in which the detachment was extended to the area of the epiphyseal cartilage, retardation of longitudinal growth on the operated side was the rule at the age of 12 and 24 weeks. This observation concurs with the findings of GATEWOOD and MULLEN (1927): The central part of the epiphyseal cartilage has been reported to be less sensitive to trauma than its peripheral part. According to IMBERT (1951), only extensive curettage of the cartilage or comparable trauma affects bone growth. It is of course possible, in certain conditions, to pass nails and drill holes through the epiphyseal cartilage without disturbing growth, as has been ascertained by GREEN (1950) and CAMPBELL and his co-workers (1959). According to TRUETA (1957) it is through the epiphysis that the epiphyseal cartilage obtains the vasculature which affects growth. It is precisely these vessels that are obviously damaged in detachment of the perichondrium. TRUETA and BRODIN assumed in fact that possible growth stimulation is due to the increased circulation of blood in the epiphyseal cartilage. HUTCHISON and BURDEAUX (1954), again, believed that all growth stimulation surgery produces stasis in the circulation of the epiphyseal cartilage.

One control group of the present investigation in all the series compared animals in which subcutaneous tissue was inserted under the periosteum in lieu of a skin graft. The measure provoked a very slightly greater growth stimulation than simple periosteal detachment. It is thought

that the subcutaneous graft was resorbed so rapidly that it had no time to produce any appreciable effect on the bony tissue.

The formation of an epidermal cyst and other bone changes did not in themselves cause growth stimulation. Stimulation also requires extensive periosteal detachment. The formation of an epidermal cyst alone did not accelerate longitudinal growth.

In series D, in seeking to discover from which epiphyseal cartilage the growth stimulation derived, the acceleration was found to be due to the distal epiphyseal cartilage. This excludes the possibility that longitudinal growth was caused by lengthening the bone at the site of origin of the cyst since the mark placed in the bone was on the distal side of the cyst. Growth stimulation was thus due to the increased activity of epiphyseal cartilage and not to bone lengthening at the operation site. This agrees with the observations made by COMPERE and ADAMS (1937) who stated that the growth increase established after femoral fractures is not attributable to growth at the site of the fracture but obviously to the increased activity of the epiphyseal cartilage.

The general growth of the bone from different epiphyseal cartilages was followed. It is a problem that has been studied extensively before. In general, tibial growth has been found to occur from the proximal epiphyseal cartilage in 55 and from the distal in 45 per cent of cases. According to the author's own investigation, maximum growth occurred from the proximal epiphyseal cartilage and the growth quotient (proximal growth/distal growth) increased with the age of the animal. The growth quotient of the stimulated limb did not increase as sharply as that of the control limb. In BRODIN's investigations (1955) the graphs of the growth quotients of the unoperated tibia remained parallel and smaller for operated limb than the control. BRODIN was the only one to study from which epiphyseal cartilage accelerated longitudinal bone growth derived after stimulation surgery. The present author confirmed BRODIN's finding that stimulation occurred from the distal epiphyseal cartilage. He did not, however, establish a growth retardation from the proximal epiphyseal cartilage of corresponding size to that produced by BRODIN. BRODIN's investigations are not in fact fully comparable with the present study for the method employed here provoked greater stimulation than the technique used by BRODIN.

The present investigation showed that detachment of the diaphyseal periosteum is the basis of growth stimulation operations, confirming earlier findings to this effect by e.g. BERTRAND & TRILLAT (1948), KRAMER (1955) and VACIRCA & CANEPA (1956). Small metaphyseal

detachment did not stimulate growth. In no case may the detachment be extended to the area of epiphyseal cartilage since that will retard growth.

The roentgenological changes observed following periosteal detachment were soft-tissue swelling, thickening of the periosteum and increasingly dense calcification after about a week at the operation site. LACROIX (1951) observed corresponding changes. More pronounced changes were seen only when skin, too, was used as an implant. The epidermal cyst that developed usually appeared within 2 weeks of the operation as a small semi-translucent lesion in the roentgenogram. From this the cyst gradually grew for some 19 weeks, pushing towards the centre of the bone in spherical form or spreading under the periosteum to encircle the bone in a belt-like manner and eroding it only superficially. The semi-translucent lesion was sometimes multilocular with the different parts of the implant growing in different ways. The zone of dense calcification surrounding the cyst gave it distinct margins. The cortex was thickened near the cyst. Three rabbits showed a marked callus reaction near the cyst after the normal bony tissue around it had disappeared and been replaced by loose irregular newly-formed bone. The bone diameter could on occasion be 3 times its normal size.

In addition to the changes in the bone surrounding the epidermal cyst, changes without any sign of infection were also discovered. The bony tissue was more spongy, revealed signs of bone destruction and in places of new bone formation. The epidermal cyst often grew towards the medullary cavity, filling it. The author has found no similar reports of roentgenological and histological changes in cases of bone growth stimulation. LANGENSKIÖLD (1957), after stimulating longitudinal growth by inserting a plastic film under the periosteum, noted considerable callus formation in several cases and in some cases sequestration of the part of the diaphysis wrapped in the film, without manifest infection. On the other hand, there are several studies in the literature which report bony changes unconnected with longitudinal growth acceleration. BOHLMANN (1929) implanted various substances in rabbit metaphysis, producing considerable bony changes but no stimulation of longitudinal growth. Similar findings were reported by GUERRIERO (1927) after injection of staphylococcal and streptococcal suspension into dog metaphysis. TRUETA (1953) established stimulation of bone growth in his osteomyelitis material if the medullary cavity was blocked by bone. He accounted for the growth stimulation by suggesting that the periosteum had been stripped in these cases, with pus collecting readily

in osteomyelitis just under the periosteum. Narrowing of the medullary cavity occurred also in the present series and may have been a factor promoting stimulation.

The literature contains few and conflicting reports of stimulation-provoked changes in the morphology of the epiphyseal cartilage. ASLING ET AL. (1950) produced considerable growth stimulation in rat tibia by injecting growth hormone. The stimulation of growth occurred from the proximal epiphyseal cartilage, the height of which increased appreciably. After stimulating longitudinal growth in dog by means of periosteal detachment, VACIRCA and CANEPA (1956) established an increase in the height of the epiphyseal cartilage. KISHIKAWA (1936), on the other hand, found that the growth stimulation he had produced in different ways caused shallowing of the epiphyseal cartilage. HUTCHISON and BURDEAUX (1954) stimulated bone growth by stasis and found that the periosteum thickened and became still richer in cells. The vascular connective tissue between the bone trabeculae increased, but the cartilage height remained unchanged. Reports on the changes in the epiphyseal cartilage produced by retardation of bone growth are more numerous in the literature. SIFFERT (1956) and AMAKO and HONDA (1957) found a reduction in the height of the epiphyseal cartilage. The present author noted no difference 10 weeks postoperatively in the height of the epiphyseal cartilages of the stimulated bone and their zones of proliferating cells and hypertrophic cells. But there was a distinct difference between the proximal and distal epiphyseal cartilage and its components. The proximal values were regularly higher than the distal. The height of the epiphyseal cartilage was thus proportional to the growth from it in that the higher, i.e. proximal, epiphyseal cartilage showed greater growth than the distal.

Implantation of a skin graft under the periosteum after extensive detachment of the diaphyseal periosteum stimulates more than average bone growth compared with other growth stimulation operations. Interesting though it would have been to study the effect on longitudinal growth of the dermis alone placed like whole-thickness skin in the present investigation, it was impossible as the hair follicles in a fur-bearing animal like the rabbit extend deep into the subcutaneous tissue (see Fig. 36). As regards the possible clinical significance of the experiments, the animal material does not make a suitable basis of assessment since human skin differs considerably in structure from rabbit skin.

The epidermal cyst originating under the periosteum is interesting. No corresponding report has been found in the literature. Closest to

it, perhaps, come the epidermal cysts observed in the distal phalanges of the fingertips and assumed to originate from a piece of skin that has entered the bony tissue during the trauma which usually precedes these cysts. In the author's own experiments these cysts, demonstrable roentgenologically, histologically and sometimes also clinically, grew expansively inside the bone for half a year and then stopped growing. The cyst developed around itself a fibrocollagenous scar reaction with numerous foreign body giant cells. In the next 6 months the epithelium of the epidermal cyst gradually degenerated and fibrocollagenous tissue tended to replace the cyst. PEER and PADDOCK (1937), studying experimental epithelial cysts, found that the epidermis disappeared also from them. Epidermis transferred to tissues generally degenerates, and the process is faster the greater the functional strain on the transplant (e.g. KETTUNEN 1958, KIVILAAKSO 1955, WOLFE and DIAMOND 1956).

It is difficult to say what makes the skin graft form a cyst of this kind in the bony tissue. After invading the soft parts the transplant was transformed into a considerably smaller cyst. The conditions in growing bony tissue and especially under the periosteum obviously stimulate the growth of the cyst. The fact that the graft implanted under the periosteum tended to invade the surrounding soft tissues from under the periosteum as far as this was possible would seem to indicate that subperiosteal pressure may be greater than the environmental pressure. The role of this circumstance in the genesis of the cyst is hard to explain. After the epidermal tissues, deriving from the ectoderm, of the cyst gradually disappeared in the course of a year a cyst lined by fibrotic tissue finally originated. The ultimate result was thus somewhat reminiscent of fibrous osteitis. The resemblance was particularly striking roentgenologically. GESCHICHTER and COPELAND (1949) regarded osteitis fibrosa or simple bone cyst as a healing reaction, i.e. a protective measure against some invasion. The invasive irritant destroying the bone may have been an abscess, foreign body, trauma, sarcoma or giant cell tumour. The last-mentioned is the most common of these irritants. LICHTENSTEIN (1952), on the other hand, denies any relation at all between unicameral bone cyst and giant cell tumour. In the cases of DEETZ (1900) and GLIMM (1905) the cyst originated as the result of irritation by subperiosteal hematoma, but BLOODGOOD (1910) held that a cyst never originates for this reason. In the present investigation growing epidermal cyst was the irritant. An experimental bone cyst produced by implanting a skin graft under the periosteum of the bone might perhaps help to clarify the behaviour and properties of bone cysts.

Further study of the question is planned. One method that could be used is the injection of some virus suspension into the cyst formations.

It is worthy of note that when KETTUNEN (1958) performed experimental skin arthroplasties in the hip joint of cats, a procedure which involved neither subperiosteal application nor growing bone, no cyst formation resulted. This was due to the function of the joint and intra-articular pressure. It was the same with the cases of clinical arthroplasties followed-up by KALLIO (1958). They, too, displayed no epidermal cysts.

Summary and conclusions

The purpose of the present investigation was to establish the effect of subperiosteally implanted autogenous whole-thickness skin graft on the tibia of growing rabbits and on its longitudinal growth. The implant was free, without function. A total of 143 young rabbits was used for the tests. The animals in which subcutaneous tissue was similarly implanted or periosteal detachment alone was performed served as the controls. In some control animals the skin graft was implanted in the medullary cavity of the bone. In addition, which epiphyseal cartilage was responsible for the surgically achieved stimulation of longitudinal growth was studied. The tibial lengths were measured roentgenologically at intervals up to a certain age. The longest observation period was a year.

The stimulation experiments by implanting a skin graft subperiosteally led to formation of an epidermal cyst in the bone. If the periosteal detachment was extensive, from the area of the proximal metaphysis to half way along the diaphysis, the cyst originating from the piece of skin inserted between the metaphysis and the diaphysis caused a reaction which stimulated longitudinal bone growth considerably more than simple periosteal detachment. When this operation was performed on rabbits aged 2—4 weeks the maximal mean length discrepancy was 2.3 mm at the age of 17 weeks. From this maximum the length discrepancy fell slightly to 1.6 mm at 25 weeks and 1.4 mm at the age of 12 months. Cyst formation in the bone did not in itself cause stimulation of longitudinal bone growth. Stimulation required extensive periosteal detachment. Implantation of a piece of subcutaneous tissue subperiosteally produced slightly greater growth stimulation than simple detachment of the periosteum. The acceleration in bone growth obtained after simple periosteal detachment and after this plus implantation of subcutaneous tissue was minimal, about 0.5 mm, and transient. Periosteal detachment was established to be the basis of growth stimulation operations, but the detachment had to be sufficiently large and outside the area of the epiphyseal cartilage or retardation of growth ensued between 12 and 24 weeks. Although longitudinal growth of rabbit

tibia occurred with advancing age in an increasingly great degree from the proximal epiphyseal cartilage, the acceleration of growth produced by stimulation surgery occurred as an increase in the activity of the distal epiphyseal cartilage.

Young rabbits in which the skin graft remained under the periosteum always developed 2 weeks postoperatively a roentgenologically demonstrable epidermal cyst thrusting into the bony tissue. This cyst went on growing for 19 weeks, then remained roughly unchanged in size up to the age of one year which was the longest period of observation in the present work. During the last 6 months the cyst also became less clearly visible, obviously because of the decreasing density of calcification around and the degenerative changes in the cyst. A similar cyst formed from the graft implanted in the medullary cavity, with the exception of one case in which the implant disappeared completely. A subcutaneous graft implanted under the periosteum produced no changes in the bones. On histological examination the cyst was found to be an epidermal cyst lined by squamous epithelium and filled with remnants of horny lamellae and hair follicles and often clear fluid. A pronounced scarring reaction developed around the cyst. The epithelium gradually degenerated and was supplanted by fibrocollagenous scar tissue. The surrounding bony tissue showed signs both of bony resorption and of new bone formation. In the animals in which subcutaneous tissue was implanted under the periosteum not a trace of the tissue could be seen at the age of 24 weeks; it had become completely resorbed.

A histoquantitative method was used to determine in both the stimulated limb and the control limb the height of the proximal and distal epiphyseal cartilage and of the zones of proliferating and hypertrophic cells. For this purpose the samples were taken when the animals were 16 weeks of age. The stimulative operation was performed for these animals at 6 weeks. No statistically significant difference was established between them, but the height of the epiphyseal cartilage and of its different zones was proportional to the growth from it in that the most substantial bone growth occurred just from the higher epiphyseal cartilage.

The following conclusions may be drawn from the animal experiments:

1. Autogenous whole-thickness skin graft implanted subperiosteally in growing tibia resulted in the formation of an epidermal cyst in the bone. The cyst was demonstrable roentgenologically c. 2 weeks postoper-

atively. It grew expansively into the bony tissue for some 6 months, but remained unchanged in size for the next 6 months becoming, however, less clearly defined in outline. The cortex around the cyst often thickened. The cyst was histologically lined by squamous epithelium. After gradual degeneration the squamous epithelium was replaced by fibrocollagenous scar tissue. Malign degeneration was never present.

2. After detachment of the periosteum of the proximal diaphysis of the operated tibia the cyst caused in the bone a reaction which stimulated its longitudinal growth. The cyst forming in bone did not cause stimulation of growth unless accompanied by extensive periosteal detachment.

3. Growth stimulation was produced through an increase in the activity of the distal epiphyseal cartilage. No difference was established between the height of the epiphyseal cartilage and its different zones of the stimulated bone and the corresponding values of the epiphyseal cartilage of the control bone.

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