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LONGITUDINAL BONE GROWTH THE NUTRITION OF THE EPIPHYSEAL CARTILAGES AND THE LOCAL BLOOD SUPPLY

An Experimental Study in the Rabbit

BY

HARALD BRODIN

EJNAR MUNKSGAARD
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Mr. L. James Brown

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INTRODUCTION

The desire to accelerate the healing of fractures has given rise to many experimental investigations. Despite this fairly wide interest, it appears that no reliable repair-promoting method has become available. An unavoidable difficulty in the comparison of the rate of repair of experimental fractures in a given series is that such fractures nearly always differ from one another with regard to position, appearance, dislocation, injury to the soft parts and fixation, all factors which must be taken into account in the evaluation of the rate of healing. Another difficulty is the assessment of the degree of healing.

Mc Lean *et al.* (1951) stressed the histological similarity between the calcification of the healing fracture and "the endochondral growth in length of the long bones". It is known that the activity of the epiphyseal cartilage can be increased with consequent increased cell production and increased rate of longitudinal growth of the bone. This suggested to the writer that if it were possible to demonstrate some factor stimulating the longitudinal growth of a long bone, this factor might also be capable of accelerating the repair of a fracture.

With this in mind attempts were made to find suitable methods that might be able to disclose the factor or factors directly responsible for increased growth in length.

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Chapter I

GENERAL REMARKS ON THE ANATOMY, PHYSIOLOGY AND PATHOPHYSIOLOGY OF LONG BONES

A. Anatomy

The growing long bone has a proximal and a distal epiphysis (between epiphyseal cartilage and joint cavity), proximal and distal epiphyseal cartilage, a proximal and a distal metaphysis (between the epiphyseal cartilages and the shaft proper, *i. e.* the region in which the bone is approximately constant in diameter throughout its length, LACROIX, 1951) and diaphysis. The diaphysis is lined with periosteum, which is structurally continuous with the perichondrium clothing the non-articular surfaces of the cartilaginous ends.

The vascular anatomy of the tibia of the dog was studied by JOHNSON (1927) and of the femur+tibia of the rat by REICHEL (1947). It appears that the best investigations available of the anatomy of the vessels in long bones are those carried out by DE MARNEFFE (1951) on the tibia and the femur of the rabbit. He demonstrated a subperiosteal capillary network communicating with the peripheral capillaries in the corticalis. Roughly in the middle of the diaphysis a nutritional artery passes through the periosteum and the corticalis to enter the marrow cavity of the diaphysis in which it divides into a distal branch and a proximal branch. The smallest branches extend to either epiphyseal cartilage where they form a capillary network. These capillaries show bulges or dilatations believed to fill spaces arising on degeneration of cartilaginous cells near the metaphyses. DAHL (1936) and POLICARD (1941) expressed the opinion that the capillary bulges should be interpreted as ruptures of the vessels. Several small arteries enter the marrow cavity *via* the corticalis

of the metaphyses, and their small branches form part of the capillary network adjacent the epiphyseal cartilage. This capillary network empties into a central vein extending through almost the entire marrow cavity. This vein has several drains. The small ones pass through the corticalis together with the nutritional artery and the metaphyseal arteries, while the larger ones pass through the corticalis by themselves. In the femur of the rabbit two or three large veins leave the marrow cavity proximally-dorsally; in the tibia of the rabbit, one or two proximally-dorsally and one ventrally in the distal third of the diaphysis. Each epiphysis is entered by two or more arteries, which branch to form a capillary network in the marrow cavity there. Here the veins accompany the arteries (Fig. 1).

LAMAS *et al.* (1946) showed that the nutritional artery in the tibia of the dog has a thick wall with a strong muscularis, a thick intima and a small lumen. He suggested that the artery might, by means of a sphincter mechanism, regulate the flow of blood to the marrow cavity. DE MARNEFFE (1951) found no such thickness of the wall of the nutritional artery of the rat, guinea-pig or rabbit.

RUTISHAUSER (1952) demonstrated arteriovenous anastomoses in the marrow cavity in man. (DOAN described such anastomoses in the pigeon as early as 1922.)

The vessels are accompanied by a small nerve, which is practically always unmyelinated (DE MARNEFFE, 1951). In human beings (KELLGREN, 1939) and in rabbits (OLLIER, 1867) the marrow cavity is sensitive to pain.

The epiphyseal cartilage is built up of hyaline cartilaginous cells. Adjacent the epiphysis (reserve zone, proliferation zone) no definite arrangement of the cells can be detected. Here mitoses have been demonstrated (HARRIS, 1933; POLICARD, 1941; LACROIX, 1951). In the ground substance, which is relatively sparse, are transverse collagenous fibrils (POLICARD, 1941). Near the metaphysis the cartilaginous cells arrange themselves into columns ("groupes sériés" POLICARD, 1941) and increase in size as they approach the metaphysis. In the cells, which undergo vacuolisation, glycogen and fat have been demonstrated

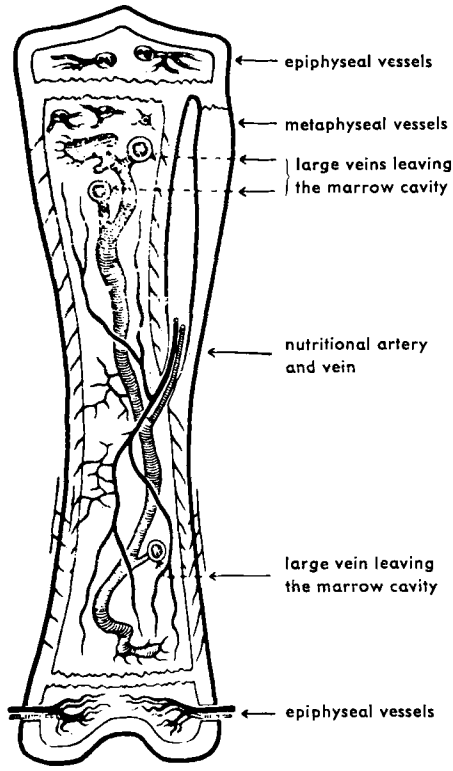

Fig. 1. Diagram of blood vessels in the tibia of the rabbit (From de Marneffe: Recherches morphologiques et expérimentales sur la vascularisation osseuse, Bruxelles, 1951).

(LACROIX, 1951). Here the ground substance is more abundant and its fibrils run in the longitudinal direction of the bone (POLICARD, 1941). At the level of the two to three cells nearest the metaphysis in every column the ground substance is completely or partly calcified (LACROIX, 1951) and shows newly formed bone on the surfaces facing the marrow cavity and the cartilage cells, although the calcification extends further into the cartilage than the newly formed bone (MC LEAN *et al.*, 1951). In the periphery of the epiphyseal cartilage in very young individuals the corticalis of the metaphysis extends up beyond

the line of ossification and appears later as a perichondrial ring of bone (LACROIX, 1951).

Vessels have been seen in epiphyseal cartilage (HARRIS, 1933), but they are described as rare. According to MAXIMOW & BLOOM (1948), cartilage is avascular. The cartilage nearest the vessels is eosinophilic, otherwise basophilic. Arteries begin to appear in the cartilage before its final ossification (ANSEROFF, 1934).

B. **Physiology**

Growth in length of long bones occurs from the epiphyseal cartilage (HALES, 1727, cited by LACROIX, 1951; DUHAMEL, 1739—1743, cited by LACROIX, 1951; GATEWOOD *et al.*, 1927; HARRIS, 1933; SILFVERSKIÖLD, 1934; BISGARD, 1936; CORTES-LLADO, 1936; BLOMQVIST *et al.*, 1943; VAHLQUIST, 1944). As to the growth mechanism, two theories have been advanced. According to the classical and widely accepted theory, of which LACROIX is also an adherent, the length of the bone is increased by a continuous proliferation of the cells of the epiphyseal cartilage. The other theory (POLICARD, 1941) claims that growth in length occurs periosteally, that the shaft of the growing diaphysis-metaphysis presses the epiphyses apart, and that the main purpose of the epiphyseal cartilages is to prevent the development of an empty space in the middle of the bone. This theory has been strongly assailed by LACROIX (1951).

The epiphyses grow from the joint cartilage (BOEREMA, 1941, cited by LACROIX, 1951, and SIEGLING, 1941).

The diaphyses of long bones increase in thickness by appositional growth subperiosteally. A corresponding adsorption occurs endostally. The metaphyses are formed endochondrially and are remodelled during growth (HARRIS, 1933, and LACROIX, 1951).

The marrow grows not only proximally, distally and circumferentially (HUGGINS *et al.*, 1938) but, according to experiments with thorium oxide on rabbits, also interstitially (LACROIX, 1951).

Growth of the periosteum is shown to be interstitial (LACROIX, 1951).

The circulation in the marrow cavity of the diaphysis is slow. Indigo carmine injected into the ext. iliac artery of the dog spreads to the paw of the animal within four seconds, but requires one minute to be visible in the marrow cavity, where it is not more than faintly demonstrable, and even then only for a short time (LAMAS *et al.*, 1946).

The growth hormone of the hypophysis and the hormones from the thyroid and sex glands stimulate growth of the epiphyseal cartilage (LICHTWITZ *et al.*, 1951).

As to the nutrition of the hyaline cartilage, two theories have been presented. According to one of them, the less recent and defended by REITZ (1868) and ARNOLD (1878), for example, the cartilage contains paths for the supply of nutrition and for the removal of waste products. According to the more recent theory accepted by SCHAFFER (1930), COWDRY (1934), PETERSEN (1935), BACSICH *et al.* (1945), MAXIMOW & BLOOM (1948), HAM (1953) and FAWCETT (1954) the cartilaginous cells are nourished and drained by diffusion. SCHAFFER (1930), KESTLER (1947), MAXIMOW & BLOOM (1948), HAM (1953) and FAWCETT (1954) claim that the vascular region from which such diffusion comes is the perichondrial, while ARNOLD (1878) expressed the opinion that nutrition comes from both the perichondrium and the marrow cavity.

LICHTWITZ *et al.* (1951) studied the effect of various hormones on the epiphyseal cartilage. They found the most distinct effect on the cells nearest the metaphysis, but no effect on those nearest the epiphysis. They therefore concluded that the nutritional pathways arose in the metaphysis. They denied the possibility of any communication with the epiphysis. They did not discuss whether they believed the nutrition to occur by diffusion or *via* paths in the cartilage.

The consumption of oxygen by hyaline cartilage is low, and the aerobic glycolysis is slightly less than the anaerobic (DICKENS *et al.*, 1936).

C. Pathophysiology

I. Clinical observations

a) *Decreased and increased activity.* — It is known that the growth in length of a paretic limb is slower than that of a normal limb. Such inhibition is accompanied by a relatively greater inhibition of appositional growth (ARMSTRONG, 1946). In contrast to the inhibition of growth of a paretic limb, increased exercise within normal physiological limits of a normal limb produces a slight increase in the growth in length (INGELMARK, 1943).

b) *Fractures.* — According to BERGENFELDT (1933), fractures involving the epiphyseal cartilage fairly seldom influence the rate of growth in length of the bone. Only in fourteen of two hundred and seventy-eight patients did he observe such an inhibition of growth. However, other authors have found a higher frequency. Thus IRELAND (1933) reported the inhibition in six out of sixteen cases, and COMPERE (1935) observed it in eighteen out of nineteen cases of slipped epiphysis.

BERGENFELDT (1933) found the use of metal pins through epiphyseal cartilages in the fixation of fractures in children not to inhibit growth in length of the bone with certainty.

Fractures of the diaphysis in children do not infrequently cause some increase of growth in length of the fractured bone (TRUESDELL, 1921; BURDICK *et al.*, 1923; DAVID, 1924; LEVANDER, 1929; BLOMQUIST *et al.*, 1943; HEDBERG, 1947). TRUETA (1950) likewise found an increase in growth in length of a bone after fracture of the diaphysis, but only when the marrow cavity had been completely occluded by callus formation.

c) *Inflammatory conditions.* — In an osteomyelitis series with involvement of eighty-five legs WILSON *et al.* (1936) observed inhibition of growth in eighteen, in which the process was located in the metaphyses, increase in eighteen in which the process was in the diaphyses, and normal growth in length in the remaining forty-nine. In an osteomyelitis material TRUETA (1950) observed that when the process was located in the diaphysis, it was accompanied by an increase in growth in length

of the bone if the marrow cavity was occluded anywhere by the process, but that if the epiphyseal cartilage was involved, growth in length was inhibited. Of forty-eight children with tuberculosis of the knee, increased growth in length of the leg was found in thirty-three and decreased growth in three (BERGMANN, 1933). PHEMISTER (1936) described a case of osteomyelitis in which an abscess broke through the epiphyseal cartilage without disturbing growth.

HELFERICH (1887) reported a case of chronic skin lesion of one of the tibial tuberosities of a sixteen-year old girl in whom the lesion had caused an elongation of the leg by three centimeters in the course of 6 years. No affection of the bone was seen at operation. Extirpation of a subperiosteal cyst in the tibia after diagnostic excision of a tumour (ostitis fibrosa general.) in a six-year old child was followed by increased growth of six centimetres during the subsequent four years and a half (SALMON *et al.*, 1951).

d) *Disturbances of the local circulation.* — Arteriovenous anastomoses cause increased growth (HORTON, 1932; LERICHE, 1949) as do varices in children (SERVELLE, 1948), angioma+varices (BRÜCKE, 1938; SERVELLE, 1945) and prolonged stasis (HELFERICH, 1887).

e) *Growth stimulating procedures.* — In seven children with a shortening of one leg PEASE (1952) inserted screws of ivory or metal (vitallium, stainless steel, vanadium, brass) into the proximal tibial epiphysis and/or distal femoral epiphysis. Increased growth was observed in all, though it was sometimes only slight. Ivory was found to be the most suitable material. WILSON (1951) used the same method, but with screws of two metals, of which copper and constantan were found most suitable. Application of the method in two cases was followed by a relative increase of seven millimetres in the length of the tibia of one of the children: in the other the method produced no increase.

JANES *et al.* (1952) described a six-year old child with a shortening of one of the legs after poliomyelitis. Establishment of an arteriovenous shunt in one of the groins was followed by a

relative increase in the growth in length of the leg by 0.6 cm in 10 months.

HARRIS *et al.* (1936) found that sympathectomy carried out to increase the circulation in the legs of children with sequelae from poliomyelitis caused an increase of the growth in length of the bones on the operated side in half of the cases. FAHEY (1936) found the operation to produce no such effect on children with normal legs.

FERGUSON (1933), who studied increased growth in length of long bones of children after fractures, expressed the view that it is the injury to the marrow cavity that is mainly responsible for any such increase. In the treatment of a child with a shortening of one of the legs he bored a hole through the corticalis, *via* which he destroyed the marrow cavity at that level. The operation was followed by a slight increase in longitudinal growth.

BERTRAND *et al.* (1948) and PEASE (1952) found that longitudinal growth of the tibia could be stimulated by loosening the periosteum of the bone.

In children slight stimulation of growth in length can be produced by short wave therapy (BERTRAND *et al.*, 1948).

f) *Growth inhibiting operations.* — Inhibition of growth in human beings has been described after epiphyseodesis (PHEMISTER, 1933), after stapling, *i. e.* fixation of the metaphysis to the epiphysis by means of staples (BLOUNT *et al.*, 1949; BLOUNT *et al.*, 1952) and after boring a hole diametrically through the epiphysis and another through the metaphysis, through both of which a strong wire was passed and knotted (HAAS, 1945).

II. Experimental observations

a) *Disuse.* — As far back as 1867 OLLIER showed that division of the sciatic nerve of the rabbit was followed by a decrease in growth in length of the leg.

b) *Mechanical injury.* — Fracture of the diaphysis of kittens (BISGARD, 1936) and of rabbits (LEVANDER, 1929; COMPERE *et al.*, 1937) is followed by an increase in growth of the fractured bone.

IMBERT (1951) found that the drilling of small holes through the epiphyseal cartilage *via* the corticalis and the marrow cavity does not produce any change in the growth in length, but that the boring of large holes and scraping out of the epiphyseal cartilage inhibits growth.

KÜHNE (1952) found that on incision of the epiphyseal cartilage from the perichondrial surface perpendicular to the longitudinal axis of the bone of the rabbit the injury did not disturb the normal ossification of the cartilage between the incision and the metaphysis and that within ten to twelve days the cartilage assumed its ordinary appearance. According to GELBKE *et al.* (1953), who used rabbits and dogs, only major lesions — produced with chisel or saw — of the epiphyseal cartilage inhibits growth. When the epiphyseal cartilage of the rabbit was removed and replaced, growth was considerably inhibited, even when fragments of adjacent bone were removed together with the cartilage. Two to three months after the operation ossification no longer occurred (SOUSA, 1937). RING (1953) carried out similar experiments and found that ossification was not essentially impaired by autotransplantation as long as the cartilage healed firm.

According to DAHL (1936) and CHAPCHAL *et al.* (1948), roentgen irradiation cannot produce increased growth in length. If given in large doses, it destroys the cartilaginous cells and thereby inhibits growth. In addition, it can severely damage the vessels in the marrow cavity (DAHL, 1936).

Judging by experiments on puppies by HERRICK *et al.* (1951), ultrasonics in relatively large doses are very destructive to growing bone.

c) *Inflammatory irritation.* — The application of various substances near the epiphyseal cartilage has been tried in an attempt to stimulate growth. BOHLMAN (1929) tried 22 different substances including lead, asphalt, and vaccine of staphylococcus aureus, but without any demonstrable effect. PAVLIK (1928) tried steel, iron, tin foil, lipiodol, iodine tincture, zinc sulphate, likewise wihout success. KISHIKAWA (1936) found the application of staphylococcus albus or of turpentine to stimulate

growth. WU *et al.* (1937) found cotton, gauze, paper, wood and iron and HERNDON *et al.* (1953) copper wire to promote growth. CHAPCHAL *et al.* (1948) found the insertion of an ivory peg into the epiphysis and/or metaphysis to stimulate growth in six out of nine animals treated in this way.

d) *Circulatory disturbances.* — JANES *et al.* (1950), who used puppies, found artificial arteriovenous fistula in the iliac region usually to cause a slight increase in the growth of the femur and tibia, but occasionally even a marked inhibition. In four of the animals they measured the temperature in the marrow cavity of the femur and found it to be higher on the operated side.

HELFERICH (1887), WU *et al.* (1937) and DICKINSON (1953) did not find artificial stasis to stimulate growth in the rabbit and the dog. KISHIKAWA (1936), however, found ligature of the femoral vein of the rabbit and stasis produced by a rubber bandage for a period of up to eight weeks, to stimulate growth. DICKINSON (1953), who used oscillometry, found stasis of the leg to stimulate growth in four of eight puppies, to decrease it in one and to have no effect on the remaining three. BERGMANN (1933) found ligature of the femoral vein to stimulate growth in two of four rabbits. SERVELLE (1948) reported that ligature of the popliteal vein or femoral vein or the internal or external saphenous vein to produce an increase of two to eight millimetres in growth of the tibia of puppies in twelve to eighteen months. Ligation of both femoral artery and vein or of iliac artery of the kitten (BISGARD, 1936) and of the femoral vein only, of the rabbit (WU *et al.*, 1937) had no demonstrable effect on growth in length of the leg.

Destruction of a transverse layer of the marrow of the tibia of the rabbit *via* a hole drilled through the corticalis produced no demonstrable effect on growth (WU *et al.*, 1937). BERTRAND *et al.* (1948) carried out a corresponding operation on guinea-pigs and applied turpentine, chloroform and formol into the marrow cavity, but the complications (deaths, spontaneous fractures) were so frequent that it was not possible to assess the stimulating effect, if any, of such procedures. COMPERE *et al.*

(1937) found destruction of the marrow cavity in six rabbits to produce a slight growth stimulating effect.

e) *Growth stimulating operation of the periosteum.* — In his systematic investigation of bone OLLIER (1867) found freeing of the periosteum of the diaphysis of the tibia of the rabbit to cause an increase of up to five millimetres in the length of the tibia in three months. His finding has since been confirmed on the rabbit by WU *et al.* (1937), VOUTEY (1948) and LACROIX (1951) and on guinea-pigs by BERTRAND *et al.* (1948). If the perichondrium is loosened together with the periosteum, it will inhibit growth (SOUSA, 1938). Scraping of the soft parts outside the periosteum and perichondrium have no demonstrable effect on growth (SOUSA, 1938). If the epiphysis is freed from periosteum and joint cartilage, or if the "epiphyseal cartilage plate on the side nearest the joint" is destroyed, closure of the epiphyseal cartilage is hastened (GATEWOOD *et al.*, 1927).

D. Theories on increased growth in length of long bones

The increase in the growth in length of the bone after surgical intervention has been ascribed to hyperemia (LEVANDER, 1929; BERGMANN, 1933; KISHIKAWA, 1936; COMPERE *et al.*, 1937) abnormal circulation, probably hyperemia (BISGARD, 1936), increased epiphyseal blood flow (FRIEDENBERG, 1953), increased metaphyseal blood flow (TRUETA, 1950), stasis (SERVELLE, 1948), increased flow of blood (HELFERICH, 1887; HARRIS *et al.*, 1936), irritation of the epiphyseal cartilage (OLLIER, 1867), disturbance of the circulation in the marrow cavity (FERGUSON, 1933), electrolytic reactions (WILSON, 1951), and liberation of a growth stimulating substance called osteogenin (LACROIX, 1951).

That increased flow of blood (arterial hyperemia) might be the cause of increased growth in length is supported by LEVANDER's (1929) angiograms of rabbits in which the intra-osseous arterial system was distinctly greater in the fractured leg than in the other. After experimental stasis and sympathectomy the temperature of the skin was found to be increased, for a short

period after stasis (BIER, 1906) and for a long period after sympathectomy indicating an increased flow of blood to the extremity.

Judging by the experiments carried out by ZINN *et al.* (1941), who injected carbon particles into the rat, sympathectomy is followed by hyperemia of the leg with the exception of the marrow cavity. They found a smaller amount of carbon particles in the marrow cavity of the bone of operated rats than in the controls. They explained this difference by the assumption that dilatation of the vessels in the soft parts is accompanied by a smaller flow of blood through the bone marrow.

Arteriovenous anastomosis causes a peripheral stasis (LERICHE, 1949). In SERVELLE's case of thrombosis in a child and in cases of anomalies, stasis also seems to have been present.

The other theories referred to above appear to be less well founded and will therefore not be discussed here.

Chapter II

THE PURPOSES OF THE INVESTIGATION

It is known that certain types of local stimulation can increase the rate of growth of a long bone. It appears that the most effective method for producing such acceleration in the rabbit is to loosen the periosteum from the diaphysis and from the metaphysis. It is, however, not known how long such acceleration persists, nor is it known by how much each epiphyseal cartilage contributes to growth in length (growth pattern) under normal conditions or after such a growth-stimulating operation.

Growth in length of the long bones occurs from the epiphyseal cartilages. Changed longitudinal growth might be correlated with changed nutrition of the epiphyseal cartilage. This changed nutrition might in turn be correlated with changes in the blood circulation round the borders of the epiphyseal cartilages.

These considerations prompted the present investigation, the purposes of which were thus:

1. To form an opinion of the growth pattern of a long bone normally and after local stimulation.

2. To clear up from where (bone marrow or perichondrium) epiphyseal cartilage can take up substances from the blood.

3. To find out whether any change occurs in the circulation in the bone marrow or in the perichondrial surface of the epiphyseal cartilage after a growth-stimulating operation, and, if so, how long it persists.

Chapter III

GROWTH IN LENGTH OF THE TIBIA OF THE RABBIT

When planning the investigation it was realised that many animals would be necessary, and that the animals should preferably be fairly large, in order to facilitate operative orientation. The use of larger animals would also permit qualitatively better roentgenograms. The animals should also preferably grow quickly, in order to keep the duration of the experiments within reasonable limits. In view of these requirements it was decided to use rabbits.

The bone selected for the experiments was the tibia. The ventral and medial aspects of this bone are covered by only a thin layer of soft tissue: this facilitates the operative procedure. Another advantage of the tibia is that it can be placed in practically one and the same position for roentgen examinations of the animals on different occasions during growth.

Of methods available for stimulating growth in length, that described by OLLIER (1867) appeared the most reliable. According to this method, the periosteal sheath is loosened. The procedure is both simple and quick. Two to three days after the operation the animals ran about on all fours as usual: the loss of functional use of the operated leg was thus relatively short. Unlike earlier investigators using this method, *e. g.* WU *et al.* (1937), the loosened periosteal sheath was not sutured after being freed, it being considered unwise to introduce a foreign body into the operative field unnecessarily. The inclusion of such a foreign body might prove irritating and blur the results.

Operative technique. — With the exception of a dorsal strip, all the hair was removed from the distal part of either thigh down to the ankle. The animals were anesthetized with ether,

and an incision about 2 cm. long was drawn from the region of the tuberositas down along the ventral ridge of the tibia. The incision was carried through the periosteum medial to the lateral musculature. After making a small transverse incision from the periosteal wound, a sharp 3 mm. wide periosteum elevator was introduced between the periosteum and the bone. The periosteum was loosened from the proximal half of the diaphysis and from the proximal metaphysis up to just below the proximal epiphyseal cartilage. The periphery of the epiphyseal cartilage could be spared because its ventro-medial and ventro-lateral part could be discerned through the thin, soft parts, and because its posterior aspect is protected by a flange-like projection of the bone. The arteria nutritia was spared by refraining from elevating the periosteum adjacent its foramen nutritium. Notes were made of any hemorrhage from the dorsal aspect of the tibia.

In order to measure growth from either epiphyseal cartilage, a radiopaque indicator was fixed to the ventral ridge of each tibia. In one leg it was fastened through the distal end of the above mentioned incision. In the other leg a small incision was made roughly in the middle of the lower-leg, care being taken to loosen as little of the soft parts as possible. A fine oblique hole was drilled through the corticalis, care being taken to avoid the marrow cavity. A stainless steel wire (indicator) with a knot in one end was threaded through the hole (Fig. 2). The knot was drawn as close as possible to the corticalis, after which the other end of the wire was bent so that it lay flat against the surface of the bone. It might be assumed that any irritation by the indicator was roughly the same for both legs.

Only the skin was sutured.

Roentgen examination. — Roentgenograms (Fig. 2) of the legs were taken immediately after the operation. The animal was mounted on a frame (Figs. 3, 4) with the hind legs stretched backwards. The knees were pressed against the roentgen film by means of a belt drawn tight over the posterior of the animal, and the ankles were held in position by means of a clamp arrangement (Fig. 2). Pairs of films were wrapped in paper. In

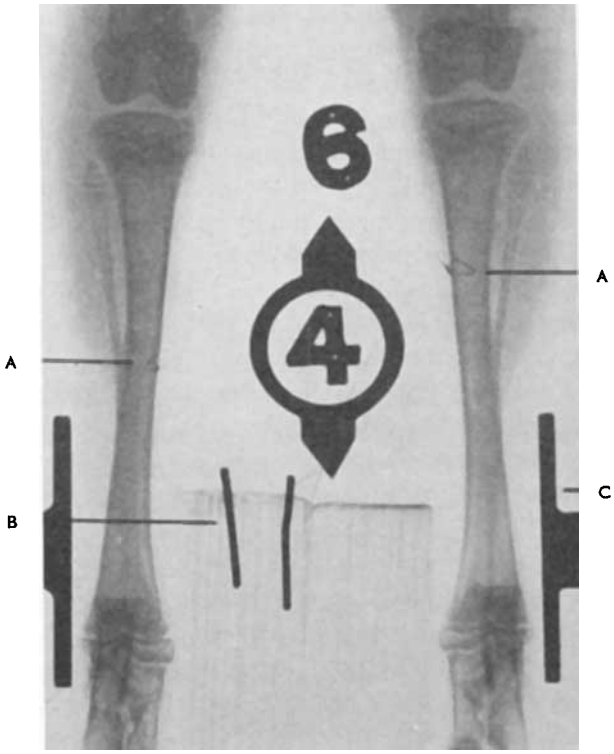

Fig. 2. Roentgenogram of the tibiae. The indicators in the corticalis (A) are reference points for the evaluation of growth from the two epiphyseal cartilages. Metal indicators (B) are in the wooden peg (H in Fig. 3) and indicate the left side. The metal plates (C) fix the distal ends of the tibiae to the wooden peg.

order to check any subsequent contraction or expansion of the films, a standard length of metal was mounted on the paper wrapper. A metal number (No. of animal) was also fastened to the paper.

The focus-film distance used was 2 metres, the exposure time 0.62 sec., kV 55—60 and the diaphragm the smallest possible. No grids or intensifying screens were used. As a rule, only frontal films were taken. The examination was repeated once a week or once every other week.

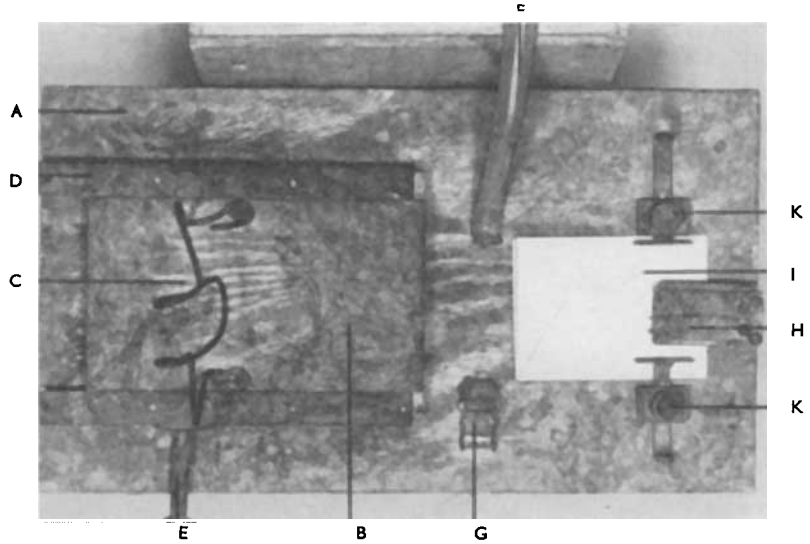

Fig. 3. Frame for mounting the rabbit for taking films of the tibiae. The frame consists of a wooden board (A) provided with a smaller movable wooden board (B) to which is fastened a 2 mm. copper wire (C) bent to form a holder for the rabbit's neck. The holder is 7 cm. high and its width can be adjusted as desired. At the top the wire is bent to form two eyes, through which a metal pin is passed over the head of the animal. The smaller wooden board runs between a pair of metal strips (D) and can be fastened in position by a screw (E). Close to the small board is a band (F), which by means of a lock (G) can be fixed over the back of the rabbit. One end of the large board is provided with a small wooden peg (H). The peg is about 2 mm. above the large plate. The roentgen film is inserted in this space (in the photograph the film is substituted by a piece of white paper (I). A metal plate (K) can be adjusted relative to the pieces of wood (H). The longitudinal edges of these are provided with a groove, under which the medial malleolus is pressed in by the plate (K).

Measurements of the lengths of the tibiae (Fig. 5). — It was not always possible to place the legs in exactly the same position for roentgen examination. Any difference in the rotational position of the leg would result in false measurement of the distance between the proximalmost and distalmost part (always the malleoli) of the tibia, because the malleolus, and sometimes also the tibial spine, did not lie in the longitudinal axis of the

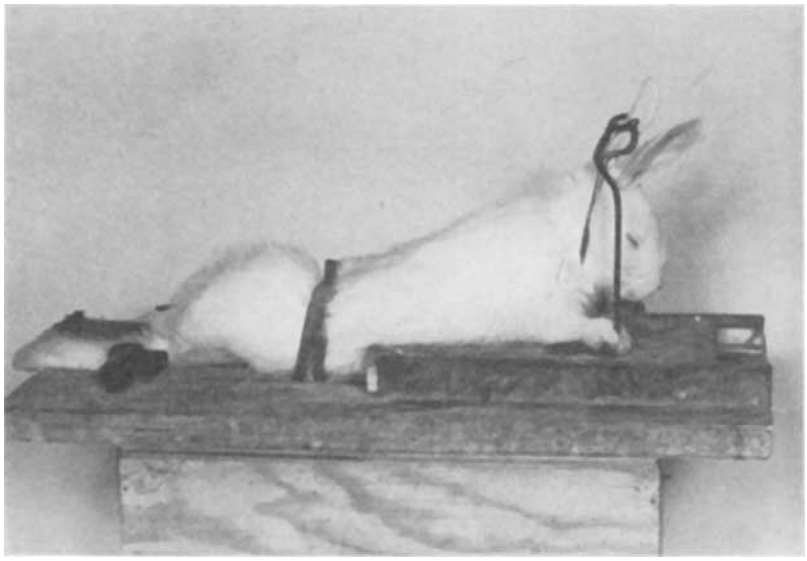

Fig. 4. The animal mounted in the frame shown in Fig. 3.

leg. Therefore, the longitudinal axis of every leg was determined and marked on the film. On the film a perpendicular was drawn from the axis to the tip of the eminentia intercondyloidea and to the tip of the medial malleolus, which usually appeared more distinctly than the lateral malleolus, and of any indicators. The distance between these points on the axis was measured with a slide caliper provided with a pair of translucent tips to facilitate adjustment (according to BARCLAY, 1951). The caliper was graduated in 0.1 mm.

The accuracy of the method for determining the difference between the lengths of the legs was checked in the following way. The distance between the tibial spine and the tip of the lateral malleolus of tibia preparations of eighteen rabbits was measured twice with a slide caliper. The differences in the lengths of the tibiae of each animal was recorded. Duplicate roentgen films were taken of these tibiae. The differences between the length of the right and left legs, as seen in the roent-

gen films, was measured and noted. For four pairs of legs the difference found by one method proved to differ by 0.2 mm. from that found by the other, the corresponding difference for seven other pairs was 0.1 mm. As to the remaining seven pairs, no difference was demonstrable between the results found by these two methods. Statistical analysis of the measurements made on the roentgen films with the aid of Barclay's slide caliper showed a statistical variation of ± 0.3 mm. $(=2.5\ \sigma)$ round the differences in leg-length, as determined with the ordinary slide caliper. The measurements of the prepared tibiae with the ordinary slide caliper were taken as the more correct. The method cannot be regarded as giving exact values. The

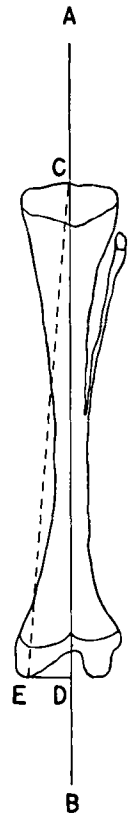

Fig. 5. Measurement of the length of the tibia on the roentgen film.
A—B: longitudinal axis
C: projection of the proximalmost point of the tibia onto the axis
D: projection of the distalmost point (E) of the medial malleolus onto the longitudinal axis.
Rotation of the bone a few degrees about its longitudinal axis will result in a change of the distance E—D and thereby of the distance C—E, but not in the distance C—D. The distance C—D was taken as the length of the bone.

points between which the measurements were made are situated on cartilaginous surfaces, and it cannot be excluded that the thickness of the cartilage at the site of measurement may be greater on the left side than on the right or *vice versa*. In two cases the measurements noted on the second occasion were 0.1 mm. smaller than the first. This was probably due to compression of the cartilage on the first occasion.

The lengths measured on the roentgen films are probably less exact. The projection of the proximalmost and distalmost parts onto the longitudinal axis constitutes a source of error, as does the size of the points made on the line. The size of the grains of the film with consequent diffuseness can hardly have any influence on the results of the measurements. No signs of stretching or shrinkage of the films were observed.

With but few exceptions the indicators in the bones produced no irritation of the tissues, and were surrounded by newly formed bone.

RESULTS

A. Normal growth in length

Four rabbits submitted to the growth stimulating operation (see above) of one of the legs were allowed to live for 140, 160, 160 and 160 days and the growth from the proximal and distal epiphyseal cartilage of the unoperated leg was measured. These measurements may be taken as a measure of the normal rate of growth. It is hardly likely that the minor operation for the insertion of an indicator in the corticalis of the diaphysis can have had any appreciable effect on growth. It was observed that growth from the proximal end was more active than from the distal, the quotient between the proximal and distal growth increasing from 1.27 to 1.40 from 1.03 to 1.43, from 1.16 to 1.57 and from 1.14 to 1.46, respectively. Only in the last of these animals did the distal epiphyseal cartilage appear to be closed at the time of the last measurement (Figs. 6 and 7). The preceding quotient in this case was 1.37.

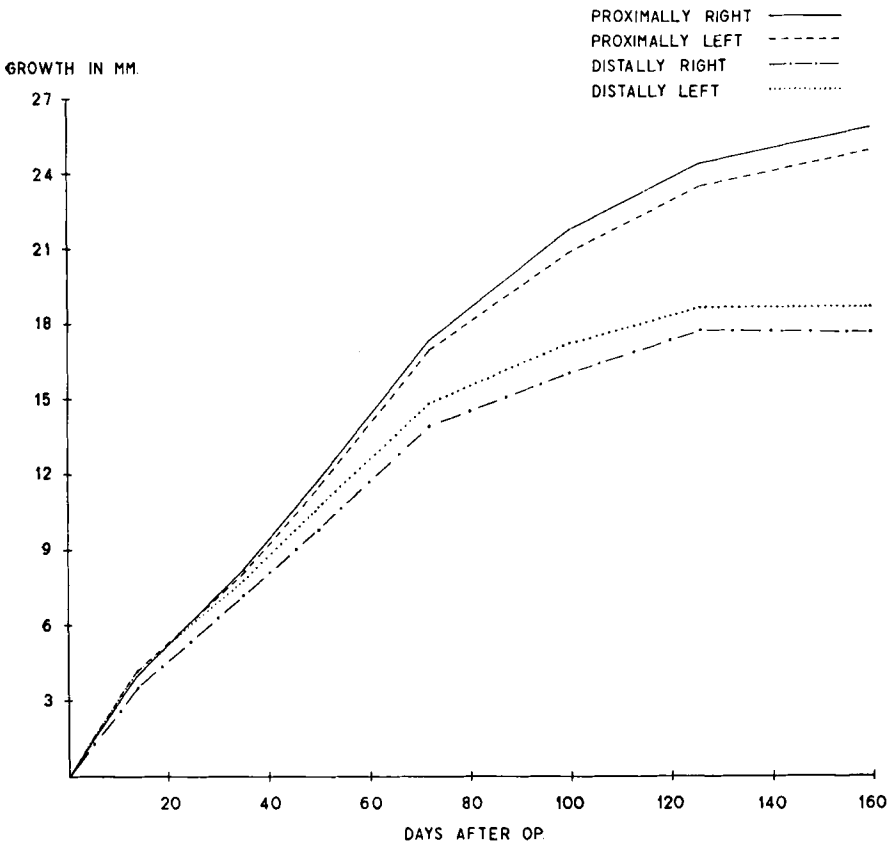

Fig. 6. Curve showing the growth from the proximal and distal epiphyseal cartilages of the tibia of the rabbit. The left leg has been operated on. The proximal part of the right tibia grew quicker, and the distal part slower, than on the left side.

B. **Growth after operation (loosening of periosteum)**[1]

a) *Proximal epiphyseal cartilage* (Fig. 8, lower curve). — The measurements of the lengths of the operated legs showed that during the first week after operation there was a slight increase

[1] Increase and decrease in rate of growth are to be understood as relative to the rate of growth from the corresponding epiphyseal cartilage on the control side.

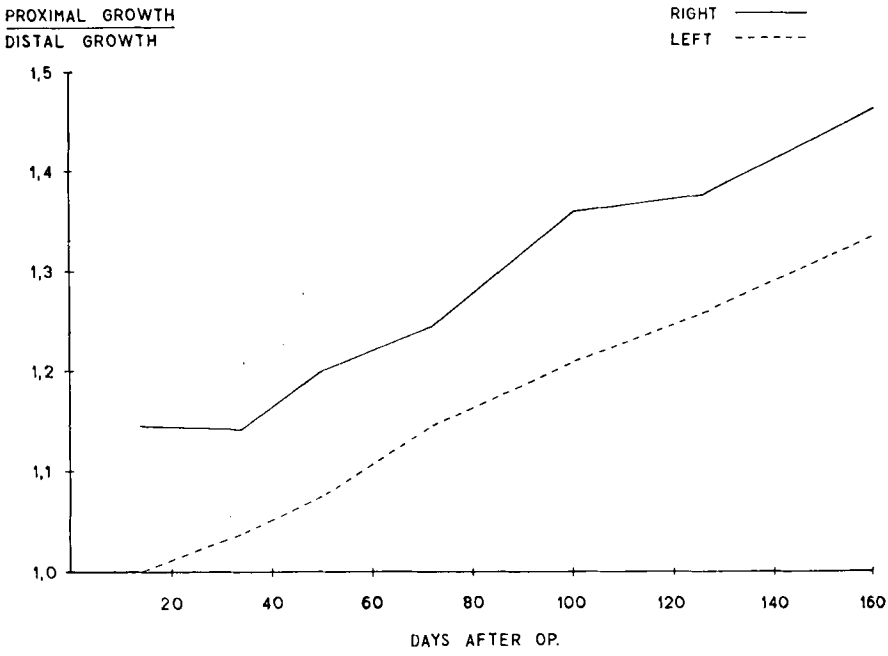

Fig. 7. Curve for the quotient between the proximal and distal growth. The quotient was lower for the operated leg (left). Same animal as in Fig. 6.

in the rate of growth of 0.1 mm.—0.5 mm. in twenty-five of the forty-four animals. In these forty-four animals the average increase was 0.12 mm. During the following week the rate of growth decreased by 0.08 mm., calculated as the average for forty-three animals. Of these, the rate of growth after the operation was lower or equal to that before in twenty-four. It is apparent that, with the exception of the first week, the operation was followed by a successive decrease in the rate of growth from the proximal epiphyseal cartilage. (The points plotted in Fig. 8 represent averages of values recorded for 44, 43, 29, 22, 23, 23, 10, 8 and 8 animals.) In four cases measurements were made up to twenty-three weeks after the operation. The greatest decrease (1.0 mm.) was measured in one animal ten weeks after operation.

b) *Distal epiphyseal cartilage* (Fig. 8, upper curve). — On the operated side measurements one week after the operation

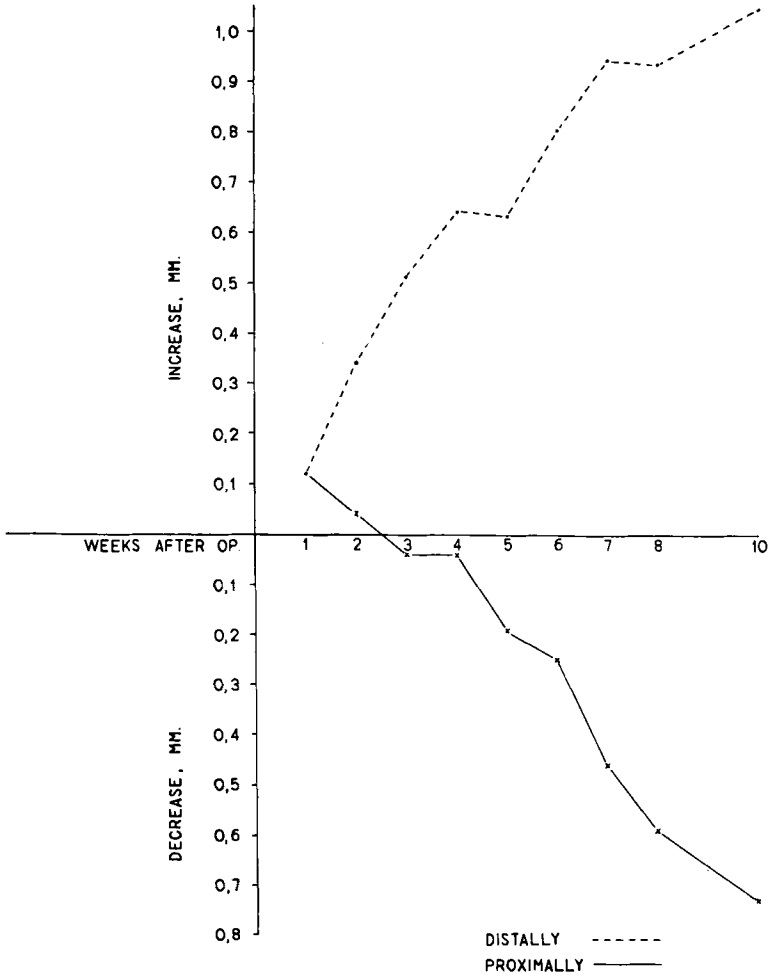

Fig. 8. Average difference between the operated leg and the unoperated leg regarding the growth from the proximal and the distal epiphyseal cartilages. (See text for number of animals.)

showed an average increase of 0.12 mm. in forty-four animals. In twenty-five an increase of 0.1—0.9 mm. was noted, and in ten a decrease of 0.1—0.3 mm. and in one a decrease of 0.6 mm. Two weeks after operation a further average increase of 0.22 mm. was noted for forty-three animals, of which, however,

three had shown a decrease of 0.1—0.3 mm. since the operation. In the upper curve in Fig. 8 every point represents an average for the same animals as the corresponding point in the lower curve. Generally speaking, a successive increase was noted in the growth from the distal epiphyseal cartilage after operation. One of the animals showed an increase of as much as 1.5 mm. seven weeks after operation, another 1.6 mm. sixteen weeks after operation.

The amount of bleeding from the dorsal aspect of the tibia did not appear to have any influence on the changes in growth in length.

COMMENTS

The intervention on the tibia was followed by prolonged increased activity of the epiphyseal cartilage most remote from the operative field, while the growth from the less remote cartilage was inhibited for the same length of time. The stimulation of growth appeared to be greatest during the first four weeks after the operation, after which it did not cease completely. The inhibition was most marked one to eight weeks after the operation and it did not cease within the duration of the experiment either (up to twenty-three weeks).

The slight unevenness of the curves is due to the fact that the animals were not examined every week and that the changes in the rate of growth varied from one animal to another.

It does not sound unreasonable to suppose that the decreased activity of the proximal epiphyseal cartilage might have been due to injury of metaphyseal vessels and large veins from the marrow and of the cartilage or perichondrium at operation. But even then it will hardly explain why the inhibition did not appear until the second week after operation. One might also imagine that this delayed inhibition was due to a growth stimulating effect of the operative trauma *per se* with consequent hyperemia and release of debris from injured soft tissue.

TO RECAPITULATE, normally the longitudinal growth of the tibia occurs to a relatively increasing extent from the proximal epiphyseal cartilage.

After a growth stimulating operation (loosening of the periosteum from the proximal half of the diaphysis and from the proximal metaphysis) the growth pattern was the same as normally. However, the longitudinal growth from the distal epiphyseal cartilage was accelerated, and from one week after the operation it was inhibited from the proximal epiphyseal cartilage. These changes were demonstrable up to at least 160 days after the operation.

Chapter IV

SUPPLY OF NUTRITION TO THE EPIPHYSEAL CARTILAGE

One of the purposes considered in the planning of the investigation of the circulation was to find out which vascular region (in the metaphysis, epiphysis or perichondrium) showed the best communication with the epiphyseal cartilage. This required a charting of the intracartilaginous paths of nutrition. It was thought that this point might be cleared up by studying which side of the epiphyseal cartilage was entered by identifiable substances administered intravenously. A search of the literature failed to reveal reports of any such attempts.

1) *Vital staining.* — According to McCLUNG JONES (1950) vital staining of the cartilage is not possible. This was confirmed in the present study as far as trypan blue and carmine are concerned.

2) *Autoradiography.* — In the search for a suitable tracer substance that could be administered intravenously and afterwards be demonstrable, even in low concentration, in the epiphyseal cartilage, it was perhaps only natural to consider radioactive isotopes. Both $P^{32}O_4^{--}$ and I^{131-} were tried. Attempts were made to demonstrate isotopes and their distribution in section from freeze dried epiphyseal cartilage by means of autoradiography (GROSS *et al.*, 1951, and DONIACH *et al.*, 1953). The films showed dark spots at the sites of small bone fragments, but not at the site of the cartilage. This may be explained by uptake of $P^{32}O_4^{--}$ by bone tissue and of I^{131-} by thyroid tissue. In view of the reports in the literature that nutritional substances diffuse through the cartilage, it was thought that the isotopes would require one to three hours to reach the cartilagenous tissue. This period was, however, afterwards (pages 36—43) found to be far too long. Autoradiography thus failed to give the information desired.

3) *Fluorescence studies.* — Experiments using the following method gave some information on the paths of nutrition to the epiphyseal cartilage. The fluorescence of 3,4-benzpyrene has

been studied by several investigators (readers interested in a review of the literature are referred to NORDÉN, 1953), and that of sodium 3-oxypyrene-5, 8, 10-trisulphonate by STRUGGER (1949), who demonstrated intercellular spaces in plants. The substance has apparently not been used in animal experiments. Even in low concentration both of the above-mentioned substances emit a readily recognised fluorescence when excited by ultraviolet light.

Material. — Fourteen rabbits four to six weeks old.

3,4-benzpyrene (synthesized by Professor H. Lund, Aarhus) dissolved in Tween 20 [1] in varying concentrations (0.5—2.5 per cent). The solution was mixed with 0.9 per cent sodium chloride (0.5—2.5 ml. per 10 ml.).

Sodium 3-oxypyrene-5, 8, 10-trisulphonate, 5 per cent solution in 0.9 per cent sodium chloride. The pH of the solution was 5.3. The solution showed a bright yellow-green fluorescence.

The younger rabbits, *i. e.,* those about four weeks old, could not tolerate the benzpyrene-Tween-water solution, and all six died within a few minutes of the injection. When the amount of Tween in proportion to the amount of benzpyrene was small, the benzpyrene was precipitated as crystals. These crystals became lodged in the lung capillaries.

The older rabbits, *i. e.,* those about six weeks old, tolerated the benzpyrene solution fairly well.

The injection of oxypyrene did not produce any demonstrable effect on the general condition of the animals.

Method. — The fluorescence solution was injected into an ear vein: After a varying interval the animal was killed by a blow against the back of the head. The distal epiphyseal cartilage of the radius or ulna was removed as soon as possible, frozen and cut into 10 μ thick sections which were mounted in liquid paraffin.

Fluorescence technique. — A Reichert Lux U V fluorescence microscope with a Philora 500 W super high pressure mercury

[1] Polyoxyethylene sorbitan monolaureate, supplied by Atlas Powder Company, Delaware, U. S. A.

lamp was used. The benzpyrene fluorescence was judged with the naked eye as well as with the aid of a Hilger spectrometer with a photomultiplier (R C A 1P 21). The same microscope was used in experiments with sodium 3-oxypyrene-5, 8, 10-trisulphonate. In these cases the fluorescence could be photographed, but not in experiments with benzpyrene, on account of a relatively weak fluorescence and the photo-sensitivity of benzpyrene not permitting prolonged exposure.

RESULTS

A. *In experiments using benzpyrene.* — Ten animals were used. Animals that died or were killed within one hour of the injection showed fairly strong fluorescence in all organs, *e. g.* brain, lungs, gastric mucosa, liver and kidneys. In the marrow cavity of the long bones and in the centre of the epiphysis fairly pronounced benzpyrene fluorescence was noted around the trabeculae. The perichondrium adjacent the epiphyseal cartilage also showed distinct fluorescence.

Within ten minutes of the injection the cartilaginous cells nearest the metaphysis showed slight benzpyrene fluorescence fading with increasing distance from the marrow cavity. Fluorescent cartilaginous cells were also seen in a zone adjacent the epiphysis; such cells were also seen near the perichondrium, but there they appeared to be limited to a region narrower than that near the epiphysis or metaphysis. Around vessels passing through the cartilage was bright fluorescence, which rapidly decreased in intensity with increasing distance from the vessel. Nowhere else in the inner portion of the epiphyseal cartilage was any fluorescence seen.

The difference in benzpyrene fluorescence between the inner and the outer parts of the epiphyseal cartilage could not be ascribed to the capacity of the central region to take up benzpyrene being lower than that of the periphery, because sections, fluorochromed in a benzpyrene solution showed benzpyrene fluorescence of approximately equal intensity in all of the cartilaginous cells.

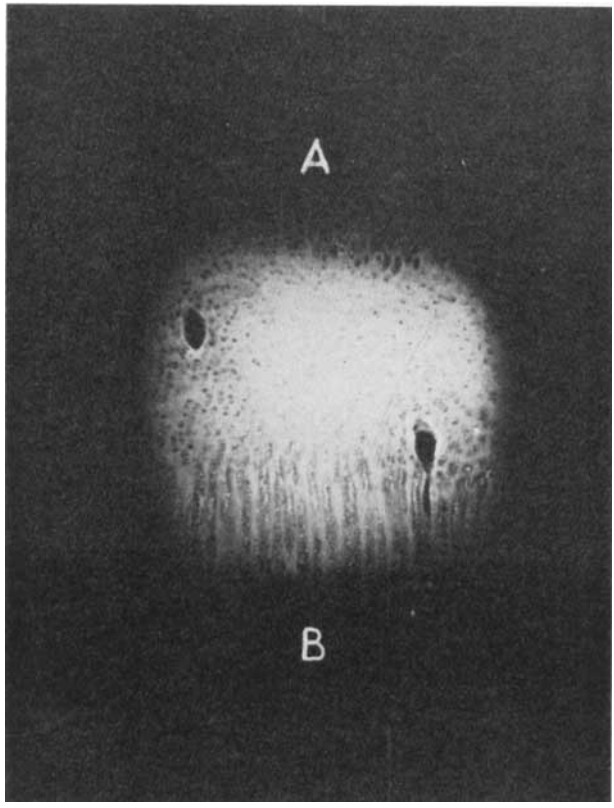

Fig. 9. Epiphyseal cartilage 10 minutes after intravenous injection of 100 mg. sodium 3-oxypyrene-5 8, 10-trisulphonate. The fluorescence is roughly equally strong in the entire preparation. The vessel walls, but not their surroundings, show increased fluorescence. A: epiphysis; B: metaphysis.

In no instance did the intercellular substance of vitally stained preparations show benzpyrene fluorescence. No spontaneous fluorescence was seen in untreated cartilaginous sections.

Parenthetically it might be mentioned that in the fluorochromed sections the cartilaginous cells showed one or two distinct, cytoplasmic spots with a considerably stronger fluores-

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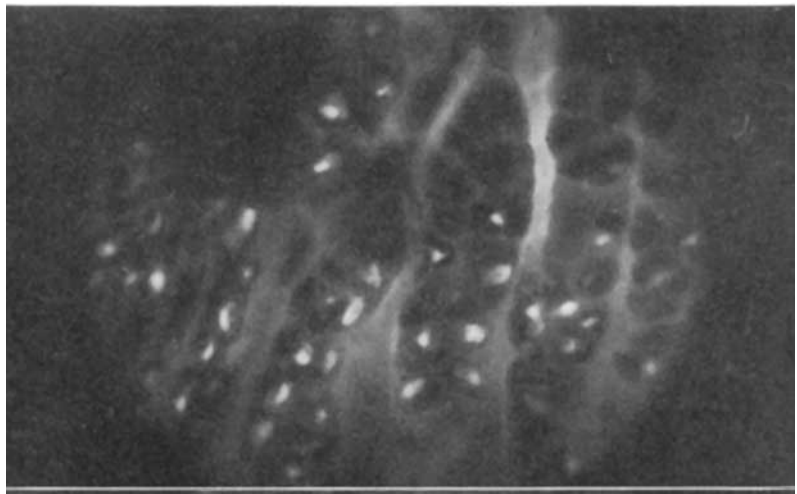

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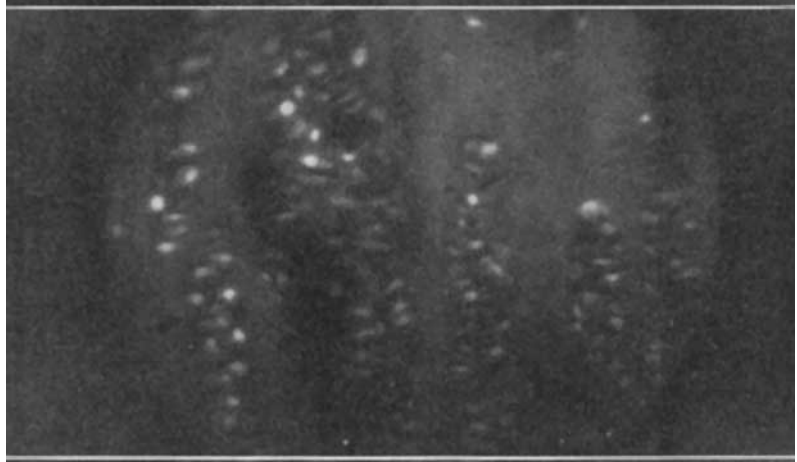

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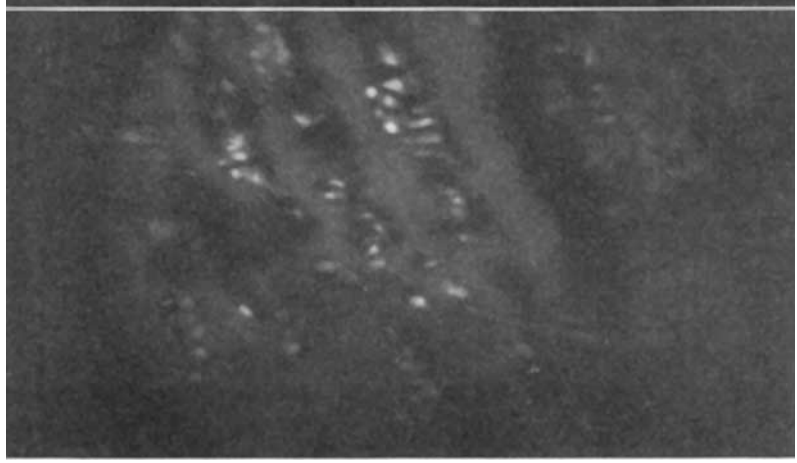
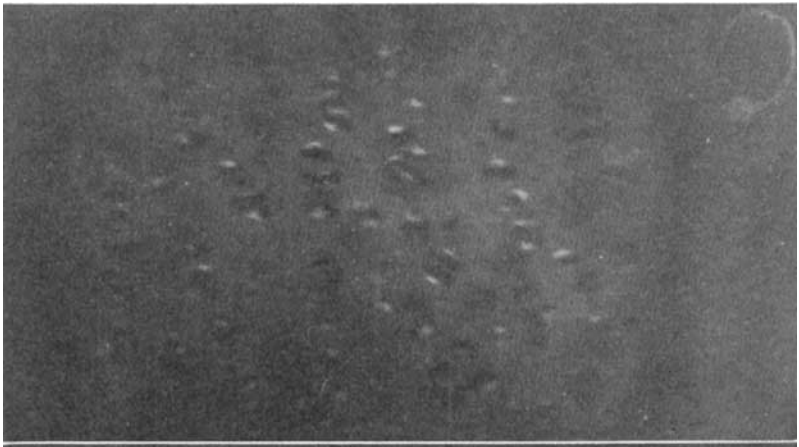


Figs. 10—15. Epiphyseal cartilage 3 1/2 minutes after intravenous injection of 50 mg. sodium 3-oxypyrene-5, 8, 10-trisulphonate. Nos. 10—15 show confluent cartilaginous regions extending from the metaphysis to the epi-



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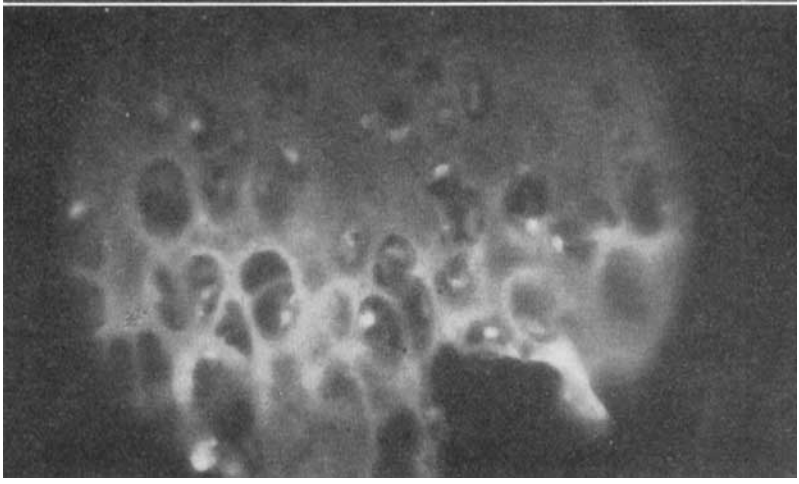

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physis. No. 10 illustrates a region immediately in the neighbourhood of the metaphysis. No. 15 from the border of the epiphysis. The fluorescence decreases slowly with increasing distance from the metaphysis and increases rapidly near the epiphysis.

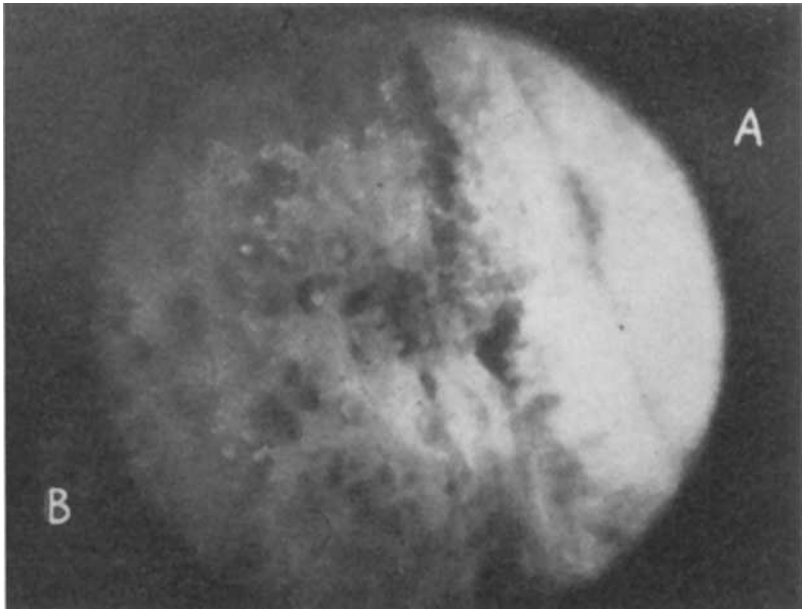

Fig. 16. Same preparation as in Figs. 10—15. The perichondrium (A) is strongly fluorescent, while the adjacent cartilage (B) is slightly fluorescent.

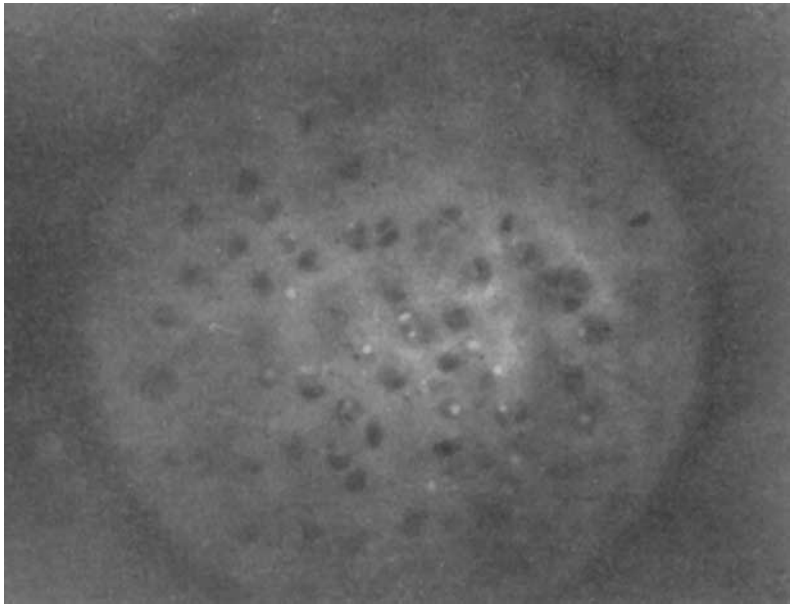

Fig. 17. Same preparation as in Figs. 10—16. One field of vision located further into the cartilage than in Fig. 16. Slight but distinct fluorescence.

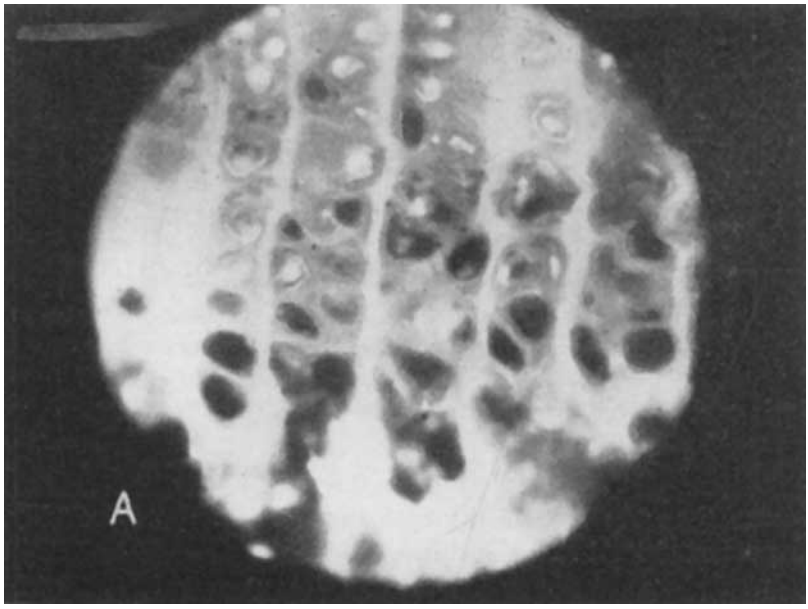

Fig. 18. Epiphyseal cartilage 30 seconds after injection of 100 mg. sodium 3-oxypyrene-5, 8, 10-trisulphonate. Border of the metaphysis. Some of the bony tissue shows particularly strong fluorescence. A: metaphysis.

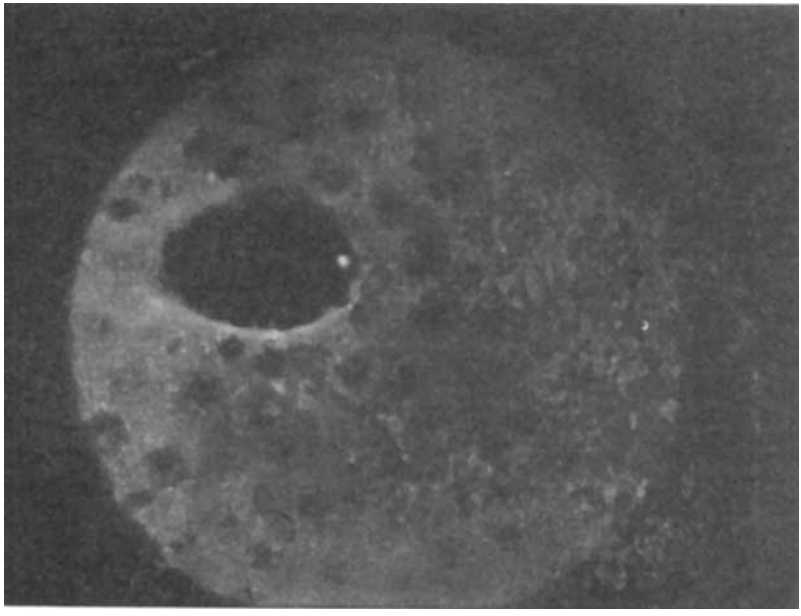

Fig. 19. Same preparation as in Fig. 18, roughly in the middle of the epiphyseal cartilage. Oblique section through a vessel, whose wall is strongly fluorescent. Very little fluorescence in the surroundings of the vessel.

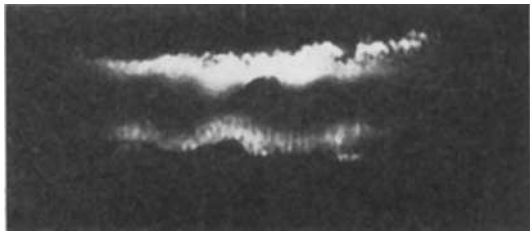

Fig. 20. Epiphyseal cartilage placed for 30 sec. in 0.3 per cent solution of sodium 3-oxypyrene-5, 8, 10-trisulphonate, rinsed in water, frozen and cut for microscopic examination. The substance entered the cartilage from the epiphyseal (uppermost in the picture) as well as from the metaphyseal borders.

cence than the rest of the cytoplasm. These spots probably represented lipid-rich structures.

B. *In experiments using sodium 3-oxypyrene-5, 8, 10-trisulphonate* (Figs. 9—20). — Four rabbits were used. None of them showed any untoward reaction to the intravenous injection containing 50 or 100 mg. of the fluorescent substance. The animals were killed half a minute, one minute, three minutes and ten minutes after the injection. In the animal killed after ten minutes the fluorescent substance had spread uniformly throughout the epiphyseal cartilage (Fig. 9), while in the one killed three and a half minutes after injection (Figs. 10—17), the fluorescence was much stronger in the periphery of the cartilage than in the centre, and it appeared to be strongest in the region nearest the marrow cavity of the metaphysis. The fluorescent zone was approximately just as narrow at the perichondrial surface as at the border of the epiphysis. The same picture was seen in the animals killed one minute and half a minute (Fig. 18) after the injection. Only faint fluorescence appeared round vessels in the cartilage (Fig. 19). The preparations showed a weak but distinct postvital diffusion of fluorescence, which was much less in the sections mounted in paraffin than in those mounted in glycerol. The osseous tissue regularly showed strong fluorescence.

In a fifth animal an epiphyseal cartilage was removed and

placed for 30 seconds in 0.30 per cent oxypyrene. It was then rinsed in water, frozen and sectioned. Photography shows (Fig. 20) that also *in vitro* the oxypyrene soon penetrates into the epiphyseal cartilage.

COMMENTS

3,4-benzpyrene is insoluble in water, sodium 3-oxypyrene-5, 8, 10-trisulphonate is readily soluble and acidic, both substances were demonstrated in the same parts of the epiphyseal cartilage examined soon after the intravenous administration of the fluorescent substance. This suggests that the spread of the substances in the epiphyseal cartilage is not due to their electrolytic dissociation. They can hardly have entered the cartilage by ordinary diffusion only, because within as short a time as 30 seconds the fluorescent substances, though in low concentration, could be detected in the inner portion of he epiphyseal cartilage.

It therefore appears most likely that the substance spread *via* some sort of minute canals in the cartilage. Judging by the spread of the fluorochrome, these canals communicate mainly with the spongiosa in the marrow cavity of the metaphysis in a direction parallel to the cartilaginous columns. The perichondrium readily takes up the fluorochrome, but it appears that only a small portion of it passes further to the epiphyseal cartilage.

TO RECAPITULATE, fluorescent substances administered intravenously entered the epiphyseal cartilages mainly from the metaphyseal side, where the chief nutritional pathways are supposed to arise. The substances also entered from the perichondrial and epiphyseal borders, but to a less extent.

Chapter V

STUDIES IN THE CIRCULATION OF THE BLOOD IN THE LOWER HIND LEG OF THE RABBIT

Since the epiphyseal cartilage is nourished mainly from the metaphyseal side, it is the circulation in the metaphysis that is of greatest interest in the investigation of the correlation between changes in longitudinal growth and changes in circulation following growth stimulating operation. However, the epiphyseal cartilage is also nourished to a certain extent *via* the surfaces facing the perichondrium. Therefore such an investigation should be extended to include the study of the circulation in that region, especially as it cannot be excluded that changes there can influence the flow of blood to the marrow cavity *via* the arteria nutritia and the small metaphyseal arteries.

In the present investigation of the local circulation the following methods were used: A. temperature recordings (skin and marrow cavity), B. determination, after the growth stimulating operation, of the amount of blood in the region of the epiphyseal cartilage relative to the amount in a corresponding region of the contralateral control leg. Plethysmography was not considered suitable for these studies. Its degree of accuracy would surely be low because of the difficulty in applying the apparatus to the short thick conical thigh of the rabbit. C. clearance of isotopes from the marrow cavity, D. determination in the metaphyses of the amount of intravenously injected substances (fluorochromes, Na_2CrO_4) reaching the metaphyses.

A. Temperature recordings

I. Skin

MONTGOMERY *et al.* (1941) and FELDER *et al.* (1954) studied the flow of blood through the fingers and the toes, respect-

ively, of apparently healthy persons with a plethysmograph and at the same time measured the temperature of the skin. They reported that once the temperature of the skin had become "relatively constant", a positive correlation was demonstrable between the flow of blood through the fingers and toes and the temperature of the skin. These findings suggested to the writer that the cutaneous temperature of the lower leg of the rabbit might be used as a measure of the flow of blood through that part.

Apparatus. — 1) Temperature indicator, type T.E.3, supplied by Electro-laboratoriet, Copenhagen. A special applicator for rabbit experiments was manufactured by the suppliers. The apparatus works on the thermocouple principle.

2) A frame for mounting the experimental animal (Fig. 21).

Method. — Both hind legs of the animal were depilated, from the distal portion of the thigh down to the tarsus, care being taken to keep the depilated areas as equal as possible. In the areas under consideration the hair was first cut short and the remainder removed by a depilatory consisting of one part of barium sulphide and 5 parts of talcum and made up to a creamy consistency by stirring with water. A day or so later the animals were operated on. No temperature recordings were made until any irritation of the skin had passed off. In those cases in which the temperature was measured one week or more after depilation, it was often necessary to treat the skin again with the mixture. Irritation of the skin was fairly common, especially after repeated treatment with the depilatory.

The temperatures were measured with the animals held on the back by means of slings of thin rubber tubing tied round the forelegs. Adjustable, rubber-lined clamps gripped the hair under the foot soles. Care was taken to adjust the position of the knees and ankles on either side in as corresponding positions as possible. Variation in the position of the knee joint had no definite influence on the skin temperature over the ventral and medial aspect of the proximal tibia. On the other hand, small differences in the position of the ankle were sufficient to bring about relatively large changes in the temperature of the skin

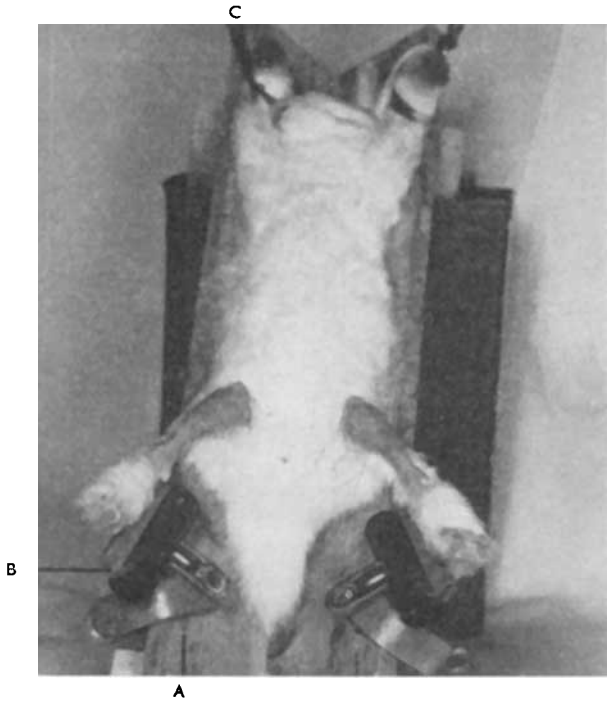

Fig. 21. Rabbit mounted in the supine position for measurement of the skin temperature. A: woden frame. B: rubber lined metal clamps gripping the hair underneath the feet. C: rubber tubing for fixing the front legs in position.

over the distal ventral part of the tibia. The temperature was lowest when the ankle was extended. Slight flexion of the joint was enough to cause a distinct rise in temperature. Owing to the looseness of the attachment of the skin, complete immobilisation of the foot was not possible by the method used: this should be borne in mind in the evaluation of the results.

In the present investigation it was decided to measure the temperature of the skin over the tuberositas tibiae and over the medio-ventral aspect of the proximal epiphyseal cartilage. In these areas the skin was separated from the periosteum and the perichondrium by a thin layer of loose connective tissue. These measuring sites were readily recognised. The tuberositas ap-

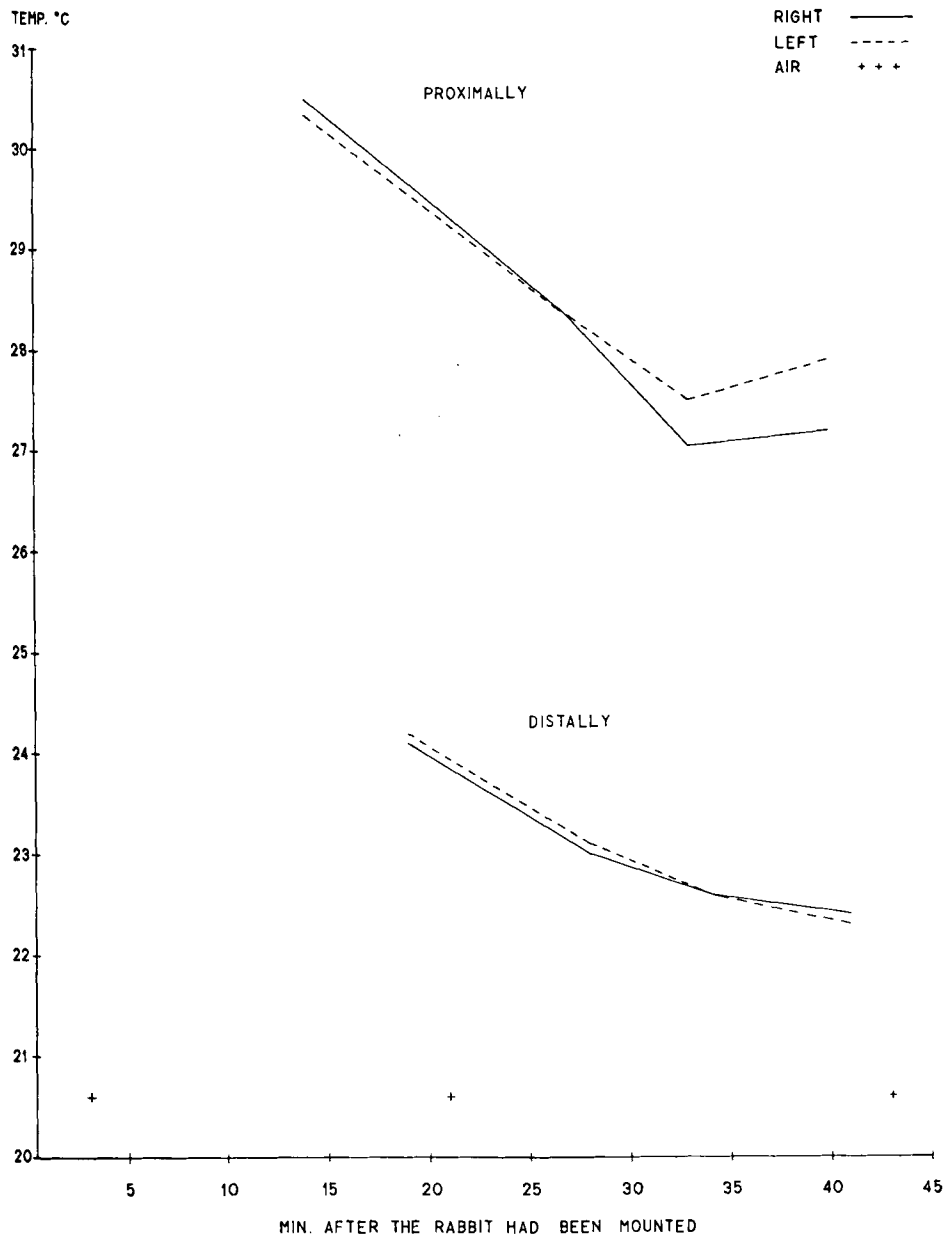

Fig. 22. Temperature of the skin of a rabbit, whose right leg was operated on 7 days before measurement. The values for the proximal ends represent means of 6 measurements; for the distal ends, of two measurements. Proximally, then, the control leg was slightly warmer than the operated leg. Distally there was no demonstrable difference.

peared distinctly and the vascular pattern medial to it provided useful landmarks. Triplicate measurements were made at each site. No measurements were made of the temperature over the dorsal or lateral parts of the epiphyseal cartilage because of the fairly thick layer of muscle under the skin there and because of orientation difficulties. Disto-ventrally the temperature was measured over the epiphyseal cartilage between two conspicuous tendons. Two determinations were made for each leg. Medially and laterally in this region it was difficult to apply the thermocouple satisfactorily against the skin because of the unevenness of the surface there. (As duplicate measurements were practically always identical, it was considered unnecessary to measure the temperature three times.)

The thermocouple was applied in the longitudinal direction of the leg. It was held against the skin with just enough pressure to cause a shallow impression. Slight variation in the application pressure did not cause any demonstrable differences in the results of the measurements.

The recordings were read with an accuracy of 0.1° C.

Care was taken to avoid any appreciable variation in the environmental temperature during the experiment. The animals were not anesthetized. Sometimes the animals were restless and then jerks were accompanied by sudden transient temperature peaks. No temperature recordings were made until the animal had again become quiet. Examples of typical curves are given in Fig. 22.

When the temperature varied by at most 0.3° C between two measurements made at an interval of two minutes, or by 0.6° C at an interval of five minutes, the temperature of the skin was said to be "relatively constant". The mean was calculated a) of all the temperature recordings made on one occasion, of the skin over the proximal part of the leg, b) of such means for two examinations, between which the temperature varied within the above limits. Every figure in Table I and every value in the curve in Fig. 23 thus represents the difference between the means of twelve single measurements of each leg.

Only animals showing no signs of local infection were used

TABLE I

Comparison between the temperature of the skin over the proximal part of the operated leg and of the control leg. About one week after operation the temperature of the control leg appeared to be higher than that of the operated leg, and to persist so for about two weeks. Every figure represents the difference between the mean of twelve measurements of each leg.

Days after op.	Experimental leg warmer	Control leg warmer
2	0.75, 0.90	
3	0.35, 0.80, 0.90	0.05
5	0.22	0.07, 0.85
7	0.07, 0.65, 1.00	0.05, 0.05, 0.05, 0.37, 0.40, 0.55, 0.60, 1.25
10	0.20	
18	0.12 0.12	0.05, 0.27, 0.30, 0.30 0.40
20		0.60
22		0.05, 0.15, 0.32
25		0.22
31	0.07	

for the temperature studies. A slight swelling of the operated area regularly persisted for a week after the operation. This was regarded as normal.

The difference found between the temperature over the proximal epiphyseal cartilage of the tibia of the left and right side respectively, of 5 controls were 0.08, 0.18, 0.0, 0.1 and 0.03° C and distally 0.0, 0.05, 0.05, 0.0 and 0.05° C. Thus the difference in temperature of the proximal areas was probably 0.2° C and less than 0.1° C for the distal areas. However, with reference to what was said above (pages 45—46) the error of the distal measurement in the individual cases may be larger.

RESULTS

During the first few days after the operation the temperature of the skin over the proximal part of the operated leg was higher than that over the corresponding area of the control leg (Fig. 23). As from one week after the operation the temperature of

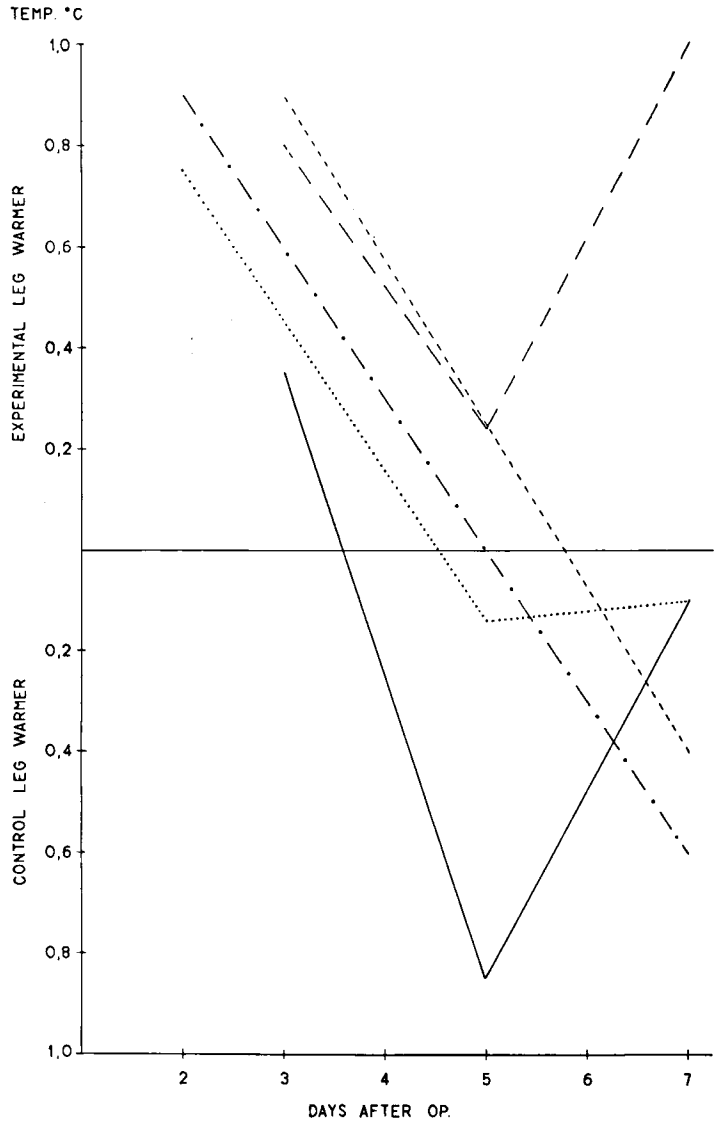

Fig. 23. Changes in the temperature of the skin over proximal part of the tibia in five rabbits the first week after operation. Every value represents the difference between the means of twelve measurements of each leg.

the operated leg was, as a rule, lower than that of the control leg (Table 1). The difference in temperature between the legs decreased with increasing interval after the operation.

One week after the operation the temperature of the distal part of the operated leg was lower than that of the control leg in one of three animals. Of four animals the temperature, as measured three weeks after the operation, was found to be lower for three. On no occasion did the temperature of the operated leg increase with certainty.

COMMENTS

Of the values given in Table I, several are within the range of error of the method (0.2° C). Even if these values also be taken into account, the table shows that the postoperative increase in temperature did not last more than about a week. Therefore, judging by the results obtained with the use of this method, any increase in the flow of blood through the region under consideration did not persist for more than about seven days. The experiments gave no information as to whether the flow inside the bone was also increased. Although the circulation in the bone marrow normally differs widely from that in the subcutis (LAMAS *et al.*, 1946), it was presumedly also damaged by the operation. However, the observations made in the study of the circulation in the marrow cavity (pages 68—82) suggested that the increase recorded in the temperature was ascribable to an increased flow of blood in the superficial vessels caused by the operative trauma.

II. Marrow

It was reasoned that any change in the flow of blood through the metaphyses following the growth stimulating operation would be demonstrable by temperature differences. If the flow of blood was increased, it would be reflected by an increase in the temperature of the operated leg as compared with that of the control leg. Therefore in an attempt to demonstrate any difference in the local circulation after the operation, the temperature of the marrow in the proximal and in the distal metaphyses was recorded.

JANES *et al.* (1950) measured the temperature of the marrow cavity in dogs by means of thermistores. They found differences between the temperature of the marrow cavity of the two femora after arteriovenous anastomosis in the groin on one side. They gave no details of the technique used. HUTT *et al.* (1952) measured the temperature of the marrow cavity of the femur and ulna of a number of patients immediately after the withdrawal of a medullary nail. They found the temperature in the cavity to decrease slightly in distal direction. HERRICK *et al.* (1949) and NELSON *et al.* (1950) compared the value of thermistores with that of thermocouples of copper-constantan. They found the former to be better and more sensitive for the measurement of differences. For the purpose of the present investigation the method using thermistores was considered less satisfactory because the instrument, being fragile, might be damaged during its introduction into the marrow cavity. In addition, its application would be more time-consuming, and it could hardly be applied simultaneously to both legs. It was therefore decided to use thermoelements, which are handier and stronger.

An apparatus for measuring temperatures was designed by P. Petersen (1955). This apparatus consists of two 0.42 mm. thick needle-shaped thermoelements of stainless steel-constantan coupled so that the difference in the electromotive force can be recorded. A multiflex galvanometer according to Lange, type M G 1, was used. Every mark on the scale corresponds to a difference of 0.03° C between the two elements.

Method. — The animals were anesthetized with ether and mounted on the frame in the same way as for measuring the temperature of the skin. After the apparatus had been adjusted to zero, an incision was made across the medial aspect of each leg immediately proximal to the medial malleolus. The periosteum was loosened over a small area immediately distal to the medial attachment of the ligament over the ventral part of the tendons of the leg. With an electric drill a hole measuring about 0.7 mm. in diameter was made through the corticalis of both tibiae. Thermoelements were introduced *via* both holes simultaneously, and the values were read as soon as the indicator of the galvanometer had become steady.

In the proximal part of the tibia the incision was placed medial to the tuberositas tibiae. Immediately ventral to the attachment of the pes anserinus the periosteum was loosened over a small area and the corticalis was perforated with the same small drill as above. The measurements were then made in the way described.

Material. — The material consisted of six rabbits ranging in weight from 800 g. to 2,000 g.

RESULTS

Relatively large differences were found in the temperature of various parts of the marrow cavity of one and the same leg, especially distally. It was observed that the temperature varied widely with the position of the needle in the marrow cavity. Thus the temperature in the ventral parts was lower than in the dorsal parts. This difference was anything up to 1.5° C. A small decrease in the temperature was noted when the tip of the needle was introduced through the proximal hole towards the diaphysis. As a rule, the drilling of the hole through the corticalis caused no hemorrhage. On introduction of the needle, however, bleeding from the hole was common. When such bleeding occurred the temperature recorded was usually relatively low. It was not possible to place the tip of the needle in exactly corresponding positions in both tibiae.

COMMENTS

The apparatus appeared to be very sensitive and to be able to measure the temperature with satisfactory accuracy. As it was observed in control rabbits that the temperature of the marrow cavity varied from one level to another, and as it was not possible to measure the temperature at exactly corresponding sites in both tibiae, results obtained by this method must be regarded as unreliable. Therefore these measurements could not be expected to permit any valid conclusions about the effect of the operation on the temperature in the marrow cavity. The difference between temperatures in various parts of a marrow cavity must be borne in mind in the evaluation of the results found by JANES *et al.* (1950) in their investigation of the temperature in the marrow cavity of the femur. It is, however, possible that the temperature in the marrow cavity of the femur does not vary so widely from one level to another as in that of the tibia, the femur being surrounded by much more musculature than the tibia.

TO RECAPITULATE, after a growth stimulating operation (loosening of the periosteum from the proximal half of the diaphysis and from the metaphysis) the temperature of the skin over the operated area was higher than that of the control leg during the first five days or so. During the subsequent two weeks it was somewhat lower than that of the control leg. In the region of the distal epiphyseal cartilage of the operated leg the skin temperature was never found to be increased. No cor-

relation was found between the changes in the temperature of the skin and the changes in longitudinal growth.

The temperature in the marrow cavity was found to vary from one level to another, but the accuracy of the temperature recordings was not sufficient to permit any valid conclusions.

B. Determination of the relative amount of blood in part of a limb

A search of the literature failed to reveal reports of any method available for the determination of the amount of blood in a part of an extremity in vivo. This together with the fact that stasis (=passive hyperemia=increased amount of blood) has been mentioned as a probable explanation for the increased growth in length of long bones, such a method was considered desirable. It was thought that such a method might be devised on the basis of the communications presented by GRAY *et al.* (1950) and ROWLANDS (1953). They tagged radioactive chromium,[1] Cr^{51} in the form of Na_2CrO_4 to erythrocytes, which take up the chromate. The chromate is believed to be tagged to the hemoglobin. This bond is stable for at least 1 day. The serum protein also takes up a certain portion of the chromate.

Experiments using 1μ C $Na_2Cr^{51}O_4$ in 3 ml. water showed that the number of counts registered per minute was not demonstrably changed by 3 mm. or 4 mm. aluminium filter. When a 2 mm. lead filter was used, the number of counts per minute decreased to about half the number.

Principle. — *In vitro* tagged red blood cells were injected intravenously. The radioactivity over corresponding parts of the legs was measured simultaneously. It is supposed that the injected blood cells spread in equal amounts from the aorta blood to both of the iliac arteries and to both legs. The counts give a measure of the amount of blood in the one measuring area in relation to that in the other (control leg).

Apparatus. — 1) Two scintillation counters made at the De-

[1] Half-life 26.5 days, γ-radiation 330 keV.

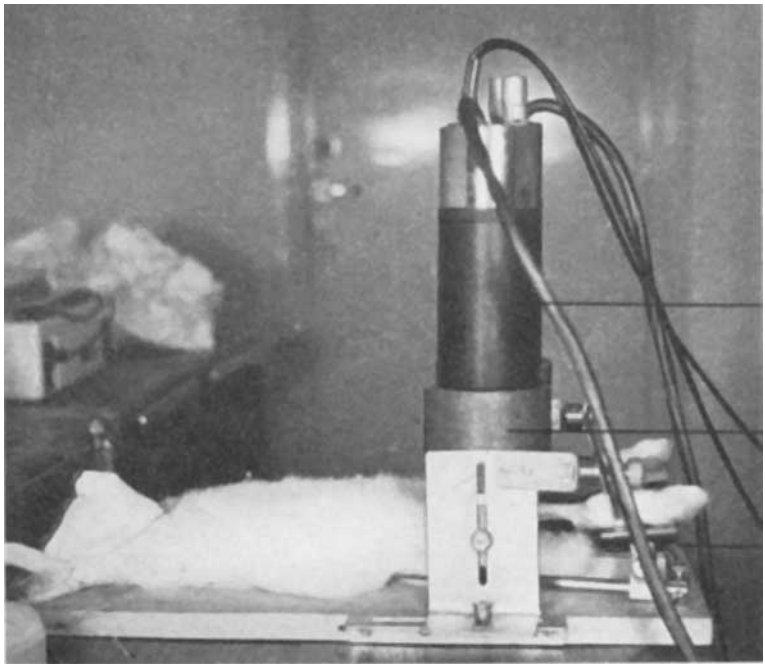

Fig. 24. Anesthetized rabbit mounted in the supine position and held by rubber-lined clamps (A) gripping the hair of the soles of the feet, and by thin rubber tubing bound over the forelegs. A photocell+crystal (B) in a collimator (C) has been placed over each knee.

partment of Physics, University of Lund, provided with cylindrical crystals of sodium iodide measuring 35 mm. in diameter and 20 mm. in height. 2) A frame (Fig. 24) for mounting the experimental animal. The frame was provided with adjustable lead collimators (Fig. 25) for the scintillation counters.

Method. — The hearts of animals not belonging to the experimental series were punctured and up to 45 ml. of blood was collected from each animal. The blood, which was mixed in the syringe with sodium citrate, was centrifuged. The erythrocytes were washed three times with physiologic saline after which 6 parts of physiologic saline were added to 4 parts of blood cells. About 30μ C $Na_2Cr^{51}O_4$ was then added to 20 ml. of the cell sus-

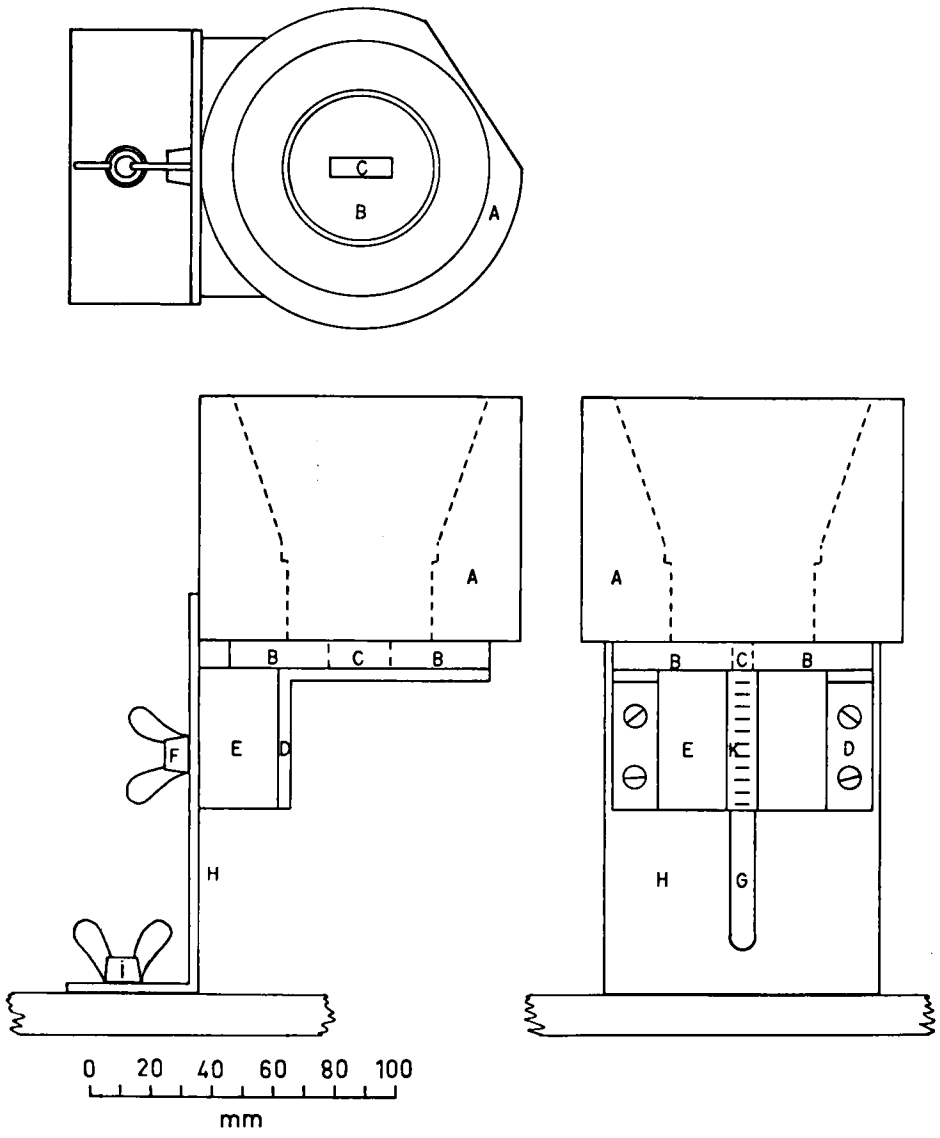

Fig. 25. Sketch of collimator and its attachment. The measures are apparent from the scale. The collimator consists of a lead cylinder with a beveled edge. The central parts of the cylinder are turned partly conically and partly cylindrically so that the photocell and crystal housing fit in as closely as possible. The collimator rests on a lead plate (B) with a rectangular hole (C). The plate B is supported by two iron metal angles (D) fixed to a wooden block (E). By means of a screw (F), which can be shifted along a guide (G) in a metal angle (H) the height of the collimator can be adjusted. By means of another screw (I) and a groove in the same metal angle the collimator can be adjusted horizontally and in various angles of rotation. A scale (K) is fixed to the wooden block (E).

pension. Three hours later the erythrocytes were again washed with abundant physiologic saline and again suspended as before. The radioactivity of the last rinse was too small to be measured with certainty.

The experimental animals were anesthetized with ether and mounted in the frame (Fig. 24) in the same way as for measuring the temperature. The temperature over the operative field and the corresponding area on the contralateral leg were checked until it had become steady. Up to 30 ml. of the cell suspension was injected into an ear vein. Both knees of the animal were carefully placed under the rectangular opening in the collimators (Figs. 26—27). The tuberositas tibiae was visible at the distal edge of the opening in the diaphragm. The epiphyseal cartilage and its immediate surroundings were thus exactly under the opening. The collimators were lowered until they were as close as possible to the knee. A 1-cm. thick lead plate was placed between the legs of the rabbit. After an interval of at least 15 minutes after the injection the counters were placed in the collimators and measurement was started. Measurements were made during repeated 2-minute periods. The detectors changed sides between consecutive measurements, so that every detector was used for both legs. Corresponding measurements were made over the distal epiphyseal cartilage and then the malleoli were used as landmarks. It was possible to make at least two to four measurements of the region of the proximal epiphyseal cartilage. As the experiments were fairly prolonged, respiratory trouble sometimes occurred, and then only one to three measurements could be made of the distal end of the tibia.

Both legs were examined simultaneously for activity, preliminary experiments having shown that when one leg was measured at a time the experiment was so prolonged that many of the animals died, besides which the measurements then showed a fairly wide variation. This wide range of variation might be ascribed to circulatory disturbances due to manipulation of the animal and changes in the depth of the narcosis during the experiment. These sources of error were circumvented by measuring both sides at the same time.

The counters were switched on in good time. The discrimi-

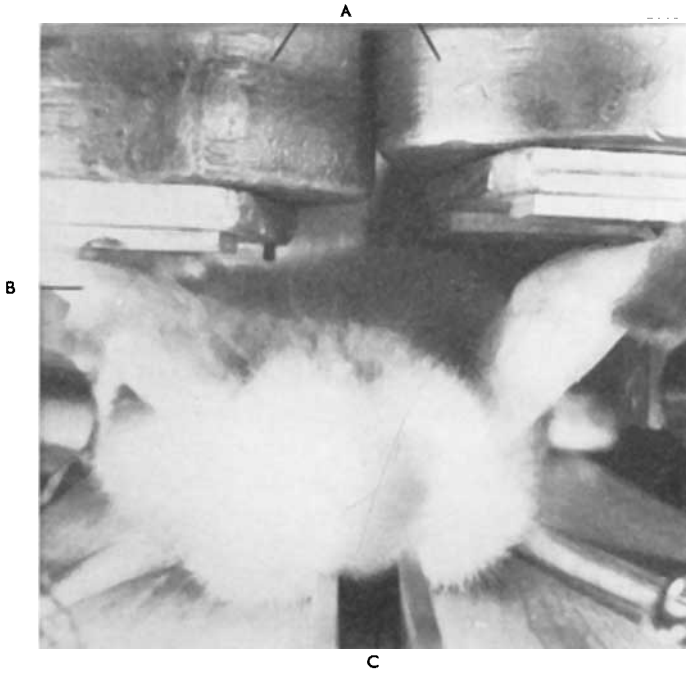

Fig. 26. Under the two collimators (A) are the two legs of the rabbit (B). A metal gutter (C) is provided for a 10 mm. lead plate screen. By cutting a segment from each collimator, the diaphragms can be brought together close enough to be centered over the knees of the abducted legs of even small rabbits.

nators were adjusted until the difference between a Cr^{51} source +background and the background was the greatest possible. The two counters were fed with high voltage from one and the same unit.

If the animal moved during the experiment, the measurement was interrupted (two animals) or the measurement was interrupted and resumed about 15 minutes later after readjustment of the legs (nine animals). For some of the animals that died in preliminary series differences were seen between the values recorded before death and those noted afterwards. Of the animals originally included in the present investigation one died during measurement and three before.

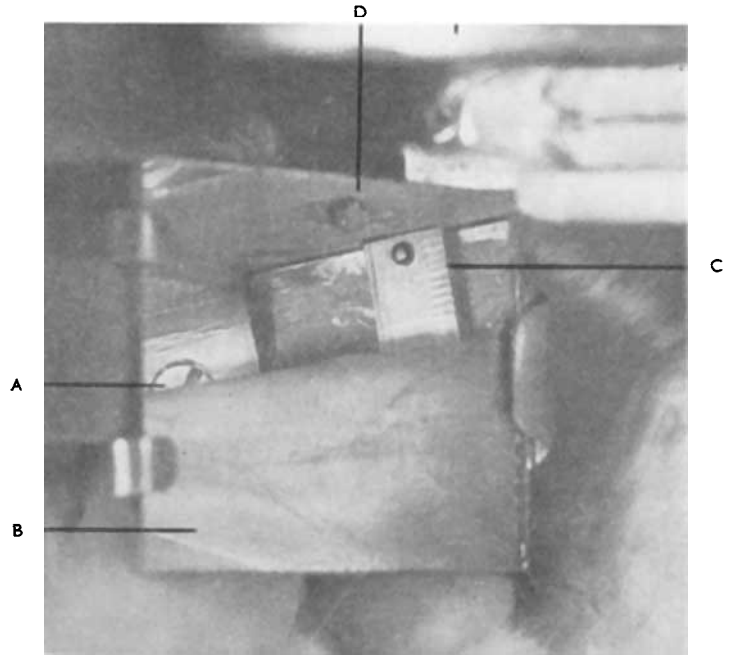

Fig. 27. Under one of the collimators is a mirror (A). This reflects one (B) of the rabbit's legs, the scale (C) and the hole in the lead plate (D) of the collimator.

The investigation included five control animals, five animals operated on one week before the experiment, seven operated on two weeks an a half before the experiment and one animal operated on four weeks before the experiment.

The values recorded were analysed. In the evaluation of the values recorded the analysis of variance was used. The calculations of the difference between the proximal measuring areas of those animals operated one week before the experiment are given in Tables II and III.

The other calculations for this group and the calculations for the other groups are not given. The procedure used was one and the same throughout. The accuracy of the difference found between the right leg and the left leg was calculated for every

TABLE II

Amount of blood in operative field one week after operation.
Primary values: number of counts per 2 minutes. Right leg operated. The figures in the top row for every animal indicate the values found by one of the recording apparatuses, the lower row, those found with the other apparatus. Total mean-value, m = 1434.

Rabbit No.	Proximally right x				Σx		n	m	
2	1599	1464	1503		4566	8815	6	1522	1469
	1460	1404	1385		4249			1416	
3	1604	1612	1548		4764	10593	7	1588	1513
	1443	1441	1416	1529	5829			1457	
4	1451	1547	1596		4594	8836	6	1531	1473
	1373	1424	1445		4242			1414	
5	1317	1270	1284	1329	5200	6596	5	1300	1319
	1396				1396			1396	
6	1739	1701	1577	1656	6673	13054	8	1668	1632
	1636	1595	1586	1564	6381			1595	
							32		1497

Proximally left x				Σx		n	m		Proximally right + left ΣΣx	n	ΣΣx^2
1325	1328	1363		4016	8153	6	1339	1359	16968	12	24.062.846
1392	1389	1356		4137			1379				
1436	1409	1399		4244	9783	7	1415	1398	20376	14	29.760.802
1330	1492	1337	1380	5539			1385				
1332	1291	1408		4031	7976	6	1344	1330	16812	12	23.667.144
1251	1307	1387		3945			1315				
1201	1102	1126	1361	4790	6001	5	1198	1200	12597	10	15.954.625
1211				1211			1211				
1473	1556	1497	1488	6014	11969	8	1504	1497	25023	16	39.277.059
1644	1459	1470	1382	5955			1489				
						32		1371	91776	64	132.722.476

Symbols in tables II and III.

x = observed values m = arithmetic mean

n = number of observations f = degrees of freedom

S_M^2 = variation of m_i round m (=mean-values for individual animals round total mean).

S_I^2 = variation of x round m_i (=of observed values round mean-values for individual animals).

S_m^2 = variation of m_{ij} round m_i (=of mean-values for legs round mean-values for individual animals).

S_i^2 = variation of x round m_{ij} (=of observed values round mean-values for legs).

TABLE III

The figures are deduced from table II.

	f.	Sums of squares	Quotient
Between class means (animals)	4	598.241	$S_M^2 = 149.560$
Within classes (animals)	59	517.451	$S_i^2 = 8.770$
Between subclass means (legs)	5	253.994	$S_m^2 = 50.799$
Within subclasses (legs)	54	263.457	$S_I^2 = 4.879$
Total	63	1.115.692	$S_T = 17.709$

Legends to table III.

Group: animals	Subgroup: legs
$\Sigma\Sigma\Sigma x = 91.776$	$\Sigma\Sigma\Sigma x^2 = 132.722.476$
$n = 64$	$\Sigma n_i m_i^2 = 132.205.025$
$m = 1434$	$\Sigma\Sigma n_{ij} m_{ij}^2 = 132.459.019$
$\Sigma\Sigma\Sigma x^2 - nm^2 = 1.115.692$	$nm^2 = 131.606.784$
$\Sigma n_i m_i^2 - nm^2 = 598.241$	
$\Sigma\Sigma n_{ij} m_{ij}^2 - \Sigma n_i m_i^2 = 253.994$	

$\frac{S_M^2}{S_i^2} = 30.65$, significant ($P < 0.001$) indicates variations not ascribable to chance.

$\frac{S_m^2}{S_i^2} = 10.41$, significant ($P < 0.001$) indicates that the difference found between the legs is a true difference.

$\frac{S_M^2}{S_m^2} = 2.94$, not significant ($0.05 < P < 0.2$) indicates that the difference between the animals can be ascribed to differences between the legs.

animal. These procedures are essentially the same as those described above.

RESULTS

In one of the control animals a difference was found between the proximal ends of left and right legs. No explanation can be offered for this discrepancy. As the difference was not realised until after statistical treatment of the data, the leg could not be examined *post mortem*. No difference between the legs of the other four animals in this group or between the distal measuring areas of the first mentioned animal could be demonstrated.

All of the animals operated on one week before the experiment showed a definite increase in the amount of blood round the proximal epiphyseal cartilage in relation to the corresponding area on the unoperated control leg. When the group was taken as a whole, the increase in the amount of blood round the distal epiphyseal cartilage (four animals) on the operated side was statistically significant. In two of the animals the difference between either leg was definite, in the other two it was less certain.

In the group operated on two weeks and a half before the examination, the amount of blood in the proximal measuring area was found to be greater on the operated side ($0.001 < P < < 0.01$). Of the seven animals in this group, only two showed a significant difference between the left and right legs. The other five showed no difference. In none of the seven animals was any difference found between the distal measuring areas on either side.

The animal that was operated on 4 weeks before the examination showed no difference between the left and right proximal measurements or between the left and right distal measurements.

Proximally the temperature of the skin of one leg never differed by more than 0.5° C from that of the other, and the

mean difference between the temperatures of the two legs was 0.1° C for each group. No correlation was demonstrable between the amount of blood in the leg and the skin temperature.

COMMENTS

Microscopic examination of blood samples from some ten animals showed no clumping of erythrocytes that had been washed, tagged and re-washed. In four cases such blood cells were added to serum from the host, and in no instance was agglutination observed. As MARCUSSEN (1941) had injected erythrocytes into the rabbit without any consideration to the blood groups and evidently without complications, cross testing was thought unnecessary.

As to those animals that died during the experiment, it was apparently not the injection of erythrocytes, but the narcosis, that was responsible for the fatal issue. Should small agglutinates appear intravitally, *i. e.* agglutinates not large enough to be life-threatening, there is no reason to suppose that they should occur with greater frequency in one leg than in the other.

The concentration of the injected erythrocytes decreased only slowly in the peripheral blood. During the first two to three hours after the injection no definite decrease could be found in five animals on examination of blood from an ear vein. The first specimens were collected within half an hour of the first injection of erythrocytes, the last 120, 120, 125, 140 and 180 minutes after the injection. After some twenty-four hours the activity of the peripheral blood was found to have decreased by at least half. All measurements were made within two hours of the injection.

In the statistical analysis the variations in the background activity and the small differences between the recording apparatuses did not make themselves felt. After every measurement each counter+scaler changed sides so that any difference between the counters was compensated for. These changes and the relatively large number of observations in every animal

were regarded as compensating the influence of non-systematic variations on the results of measurement.

In view of the uncertain results of measurement on the animals operated on two weeks and a half before the experiment and the result of the measurements on the animal that had been operated on four weeks before the experiment it was not thought necessary to extend the series to include animals operated on at a still longer period before measurement. The experiments suggested that the loosening of the periosteum resulted in an increased amount of blood in the operated area, as compared with a corresponding area in the unoperated leg. This difference was statistically significant one week after operation, and in two of seven animals it could also be demonstrated with certainty as long as two and a half weeks after operation.

TO RECAPITULATE, after a growth stimulating operation (loosening of the periosteum from the proximal half of the diaphysis and from the metaphysis of the tibia) the amount of blood in the operated area was found to be increased for anything up to two and a half weeks. In the region of the distal epiphyseal cartilage the amount of blood was found to be increased one week after operation. No correlation could be demonstrated between the amount of blood in the operated leg and the change in longitudinal growth.

C. Clearance of I^{131} from the marrow cavity of the tibia

The circulation (arterial supply, venous and lymphatic drainage) of a given tissue part can be assessed by determining the clearance of a diffusible substance from the region under consideration (KETY 1949, cited by MCGIRR 1952). According to this method, a known amount of tracer substance is deposited into the tissue, and its subsequent decrease in amount there is measured at certain intervals. In practice use is made of an isotope such as Na^{24} or I^{131}, which is injected into the tissue to be studied. The adsorption of the isotope is then followed with the aid of a suitable detector. The relationship between the quantity of the isotope and the time can, according to MCGIRR (1952), be calculated by the formula

$$K = \frac{\log C_1 - \log C_2}{0.4343\ (T_2 - T_1)}$$

where C_1 and C_2 are net counts per minute at time T_1 and T_2, and K the clearance constant which indicates the gradient of the adsorption curve. Another way of assessing the adsorption is to calculate the half-time, *i. e.* the time necessary for the activity measured to decrease by half.

McGirr (1952) studied the clearance of Na^{24} from muscles, subcutis and cutis. He found the application of an arterial tourniquet to inhibit clearance and muscular activity or vasodilatation by the administration of histamine to increase it.

A fairly thorough search of the literature failed to reveal any reports on the clearance from the bone marrow. However, the method was thought worth while trying in the investigation of any difference in the circulation in the marrow cavity of the tibia of the rabbit after the growth stimulating operation (pages 22—23).

Material. — The same apparatus was used as for determining the amount of blood with the aid of marked erythrocytes (page 55). As isotope use was made of I^{131}, which has the advantage over Na^{24} that practically all of it is taken up by the thyroid gland after adsorption from the site of injection. In this way the results are not blurred by the return of circulating isotopes to the point of measurement. This advantage is particularly valuable when it is desired to make a series of determinations on one and the same animal, *i. e.* a procedure requiring a fairly large dose of the isotope.

Four control animals were used. On one of them only one determination was made (proximal metaphysis of the tibia) because of technical difficulties. On each of the remainder four determinations were made (both metaphyses of both tibiae).

Method. — The animals were anesthetized with ether. The metaphyseal region to be studied was exposed by means of a small medial incision where the soft parts are thin. The purpose was to check that the fluid injected did not flow back through the hole in the corticalis. The incision of the soft tissues minimised contamination of the extramedullary tissue by the tip of the needle on its withdrawal. Sometimes a small drop of isotope solution was seen over the hole in the corticalis on withdrawal of the needle. It was wiped off with filter paper. Contamination with isotope outside of the bone will cause a source of error. It will increase the number of impulses per minute during the experiment, and at the end of the experiment the curve will not drop to the level it would have in the absence of such contamination.

A preliminary roentgenographic experiment with the injection of 0.15 ml. water soluble contrast medium into the proximal metaphysis

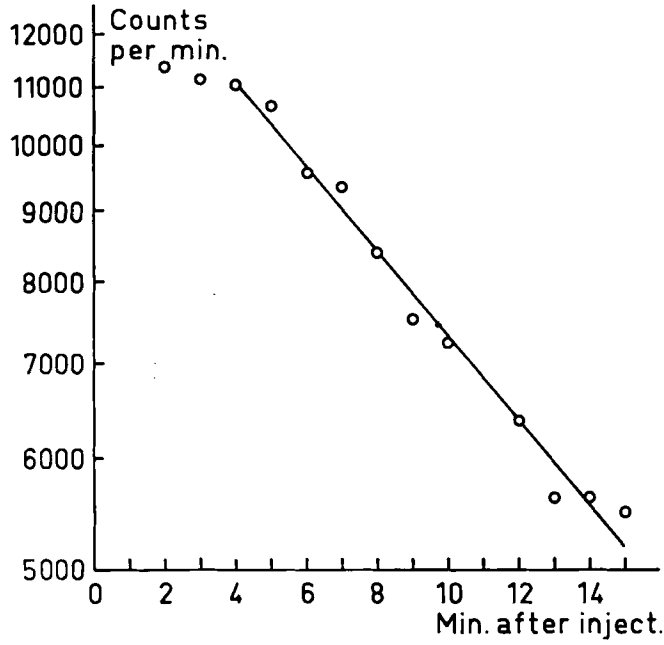

Fig. 28. Example of the results of investigation of the clearance of I^{131} from the marrow cavity of the proximal part of one tibia of the rabbit. The slow drop in the curve for 2—4 minutes after injection of the isotope was also noted for the curves of other animals.

of the tibia gave a fairly good filling of the marrow cavity of the metaphysis. An amount of 0.1—0.15 ml. was therefore considered suitable for the isotope solution which was diluted so as to contain 20μ C per 0.1 ml. The solution was injected with an ordinary hypodermic needle and, with the exception of one case, in which a bone fragment had occluded the lumen of the needle, no appreciable resistance was offered to the injection. After injection of the solution into the marrow cavity of the metaphysis, the scintillation counter was placed over the site of injection (Figs. 24—27) and counting was started as soon as possible. The counter was allowed to run continously and the results were read every half minute.

Results. — Of the thirteen experiments made, four failed completely. In one case the amount of isotope solution had probably been insufficient owing to occlusion of the tip of the injection needle. In the other three the amount of solution injected was apparently sufficient, but

the measurable activity was extremely low and could not be determined with certainty. As to the remaining nine determinations, the activity was found to vary within a fairly wide range. For instance, the activity measured in one animal was only one tenth of that measured in another animal. This irregularity was probably not due to leakage from the site of injection or to unequal amounts of isotope solution injected. The most likely explanation for these differences is that in some cases the solution was injected into a large vein, whence the isotope was soon carried off. Measurement could not be started until at least one minute after the injection, as a rule not until after one minute and a half.

Fig. 28 shows a curve for the proximal metaphysis of the left tibia. The plateau at the top of the curve was found for all of the experiments in which the determinations could be evaluated. The rest of the curve was practically linear.

COMMENTS

The method does not seem to be suitable for comparing the circulation in the marrow cavity of the tibial metaphysis on one side with that on the other. First of all the actual injection of the isotope solution involves a source of error, in that a varying proportion of it is carried off fairly quickly, probably because it is deposited in one of the large veins in the marrow cavity. The consequent decrease in the measurable activity might render the results unsatisfactory. Furthermore, the possibility cannot be excluded that the introduction of the needle into the marrow cavity can injure a blood vessel with consequent change in the adsorption of the isotope. It cannot be excluded that the injection per se might have caused compression of intact vessels, varying in degree from one animal to another. In some cases a portion of the isotope might spread to parts of the marrow not covered by the counter. Comparison between the gradients of the different curves would also present considerable difficulties. If the activity were high enough, it would, of course, have been best to judge clearance by the half-time, but this was not possible in the present investigation.

Judging by the above observations, then, the method used was too crude for the purposes of the present investigation.

TO RECAPITULATE, the clearance of isotopes from the marrow cavity gave no information about the local circulation.

D. Determination of the blood supply of the tibia by means of intravenously deposited substances

The circulation in the marrow cavity of the tibia is not readily accessible to investigation. The purpose of the experiments described below was to form an opinion of the supply of blood to the marrow cavity, or, to be precise, of the quantity of blood supplied within a short period to the metaphyseal and epiphyseal portions of a bone with changed longitudinal growth as compared with that supplied to a corresponding portion of the normally growing bone on the other side.

Principle of the methods. — It is assumed that a substance administered intravenously and passing the lungs to the aorta is homogeneously mixed by the time it leaves the aorta, so that the iliac arteries receive equal amounts of the substance administered. Every part of the bone with intact circulation receives part of the substance. If the circulation is impaired, the amount supplied per unit of time will be smaller, but if the supply of blood is increased, the tissues will receive a larger amount of the substance per unit of time. The experimental period must be kept short, otherwise the tissues will be saturated with the substance and then any difference in blood supply will not be detected.

I. Sodium chromate

In these experiments it was decided first to try *sodium chromate*. This substance is apparently non-toxic and can be demonstrated even in small quantities by means of colorimetry (SANDELL, 1944).

Material: 1 per cent solution of Na_2CrO_4 and 0.25 per cent solution of diphenylcarbazide in alcohol.

Beckman B spectrophotometer.

Method. — The animal was anesthetized with ether and mounted in the supine position for five to ten minutes. Of the chromate solution, 2 ml. was injected into an ear vein and fifteen to thirty seconds later the hind quarters were cut off by means of a guillotine-like knife. The tibiae were freed from the soft parts and sawed off transversely at corresponding levels. The pieces of bone thus obtained were ashed at 900° C in an electric oven for up to four hours and a half. During this process the epiphyses loosened. Thus four pieces were obtained from

each bone. The corresponding parts of the right and left legs were dissolved in equally large amounts of 1 N HCl. To each of the specimens was added 0.25—0.5 ml. of diphenylcarbazide. This coloured the sample red violet and the extinction of the colour was determined in a spectrophotometer at a wavelength of 5400 Å. The extinction was found to be unchanged at pH 0.35—0.70 for anything from 5 to 230 minutes after the addition of diphenylcarbazide.

Results. — The values (quotient right/left) obtained for the control animals were anything up to 750 per cent (average of 42 pairs: 146 per cent). Nine determinations were below 110 per cent and eight were above 150 per cent.

COMMENTS

The solution of the ashed bones in hydrochloric acid was sometimes cloudy, no matter whether the bones had been heated for one hour and a half, three hours or four hours. The cloudiness had an extinction of at most 0.036 and might thus imply a considerable source of error. The cloudiness did not disappear on dilution of the solution with 0.2 N HCl. On the other hand, the cloudiness disappeared when the sample was heated, but reappeared with the cooling of the specimen. No explanation can be offered for the nature of this cloudiness.

The cloudiness could not explain the wide variation of the measurements. Specimens were therefore watched while being heated in a crucible by means of a Bunsen burner. Fairly striking eruptions from the marrow cavity were observed. Drops of boiling fat sputtered far out into the surroundings.

A third source of error and perhaps the most important was that drops of blood oozed out of foramina in the corticalis on removal of the soft parts. This also occurred when the bone was not cleaned until several days after the death of the animal. Intravenous injection of thrombin sometimes appeared to depress this leakage, but provided no guarantee. One and the same dose of thrombin killed some animals immediately, but had no demonstrable effect on another. Removal of the soft parts with a cautery knife did not prevent the leakage either. Attempts to fix the leg in alcohol and formalin before the soft parts were removed only resulted in all of the chromate running out into the fixation fluid.

Any reduction of Na_2CrO_4 to Na_2CrO_3 would surely be the same for both bones and thereby not involve a source of error on comparison.

The sources of error could thus not be controlled and therefore the method was considered unacceptable for the present investigation.

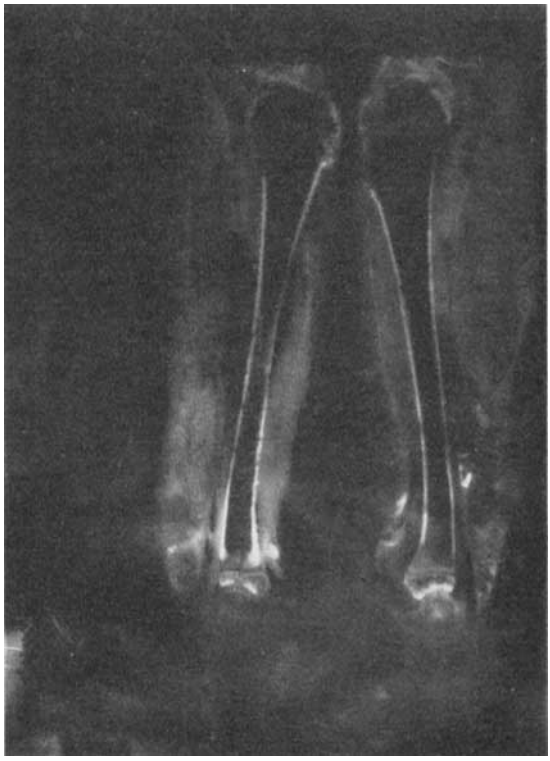

Fig. 29. Tibiae of a rabbit, that received 100 mg. sodium 3-oxypyrene-5, 8, 10-trisulphonate 3 days before the investigation. The corticalis is still fluorescent, but not the metaphyseal regions.

II. Fluorescent substances

In another experimental series the spread of a fluorescent substance in the marrow cavity and the epiphysis was studied.

In the investigation of the nutrition of the epiphyseal cartilage it was found that sodium 3-oxypyrene-5, 8, 10-trisulphonate had a great affinity for bone tissue. Three days after the injection the corticalis of the tibia was fluorescent (Fig. 29). The substance appeared to be suitable, because it could be assumed that it would be taken up by the bone tissue.

Another fluorochrome, thioflavine-S (methyl dehydrothio-p-toluidine-sulphonate) was used by SCHLEGEL (1950) in the investigation of blood vessels and lymphatics in the mesenterium of the guinea-pig and in the uterus of the Rhesus monkey. The substance is water soluble, and in the quantities necessary for such an investigation it was non-toxic. The substance was used in 4 per cent solution for the investigation of the kidneys of the rabbit (SCHLEGEL *et al,* 1950). ALGIRE *et al.* (1950) demonstrated a thrombic effect of thioflavine-S in experiments on mice, in which they used a 4 per cent solution. They reported that in solution the substance was blue fluorescent, but that the crystals were yellow fluorescent. When an artery containing thioflavine-S was exposed to ultraviolet light, marked vasoconstriction was observed. MOSES *et al* (1951) found that the monomeric form had a bluish fluorescence. They dissolved thioflavine-S in distilled water in the proportion 1 : 5 at room temperature and obtained a 16 per cent solution of polymers. This fraction of thioflavine-S was called vasoflavine. This substance has a "definite affinity for the walls of the blood vessels", according to MOSES *et al.* (1952).

This last mentioned property of vasoflavine suggested that the substance might be suitable in the investigation of the circulation of the bone marrow.

Material. — Sodium 3-oxypyrene-5, 8, 10-trisulphonate in 5 per cent solution in physiologic saline.

Vasoflavine in 4 per cent solution in physiologic saline, prepared according to MOSES *et al.* (1951).

Trimex ultraviolet lamp (AB TRIMEX, Malmö).

Cold room with a temperature of minus 10° C to minus 15° C.

Photometer (type Chalonge & Laffineur) with a slit of about 0.01 mm.

Model experiment. — With the source of ultraviolet light, microscope, photocell and Hilger spectrometer used in the investigation of the paths of nutrition of the epiphyseal cartilage, the fluoresence of thin layers (0.01 mm.) of a 4 per cent solution of vasoflavine showed a distinct maximum at 5700 Å and another maximum, though less distinct, at 4650 Å. A 2 per cent solution showed two maxima at 5700 Å and 4650 Å respectively. The 1 per cent solution showed only one maximum, at 4550 Å. Further dilution of the vasoflavine resulted in a marked decrease of the fluorescence. The investigation showed that the

degree of polymerisation of vasoflavine changes on dilution of the substance.

The same experiment was performed with *sodium 3-oxypyrene-5, 8, 10-trisulphonate.* Solutions in concentrations of 0.005 per cent to 0.63 per cent showed a distinct maximum at 5100 Å.

Seven solutions of various concentrations were frozen and the ice surface was planed flat. The specimens were photographed in ultraviolet light in the cold room with an exposure time of 5, 10, 15, 20, 40 and 80 seconds. The photographic density was determined. The gradation curve was linear from the density value of 0.13 to at least 1.70.

Method. — The animals were anesthetized with ether and mounted in the supine position for about 10 minutes, after which 100 mg. oxypyrene or 80 mg. vasoflavine was injected into an ear vein. About 20 seconds later the hind quarters were cut off by means of a guillotine-like knife, and placed immediately into a freezing mixture consisting of alcohol and solid carbon dioxide with a temperature of about minus 80° C. In the cold room the legs were taken out of the freezing mixture and the thighs sawn off. With a plane the medial side of both legs was removed including a necessary part of the femur and tarsus. Planing was continued until the veins through the posterior of the tibia could be seen and, as a rule, the tibial tuberosity. The legs were held with the flat surfaces uppermost against two plane and horizontal parallel bars. The planed surfaces were thus parallel. The source of light was placed about 50 cm. from the distal end of the bones. The central ray of the beam fell between the two preparations at an angle of 60 degrees to the longitudinal axis of the bones. Thus corresponding parts of the legs were equally illuminated. The specimens were photographed in the cold room with a yellow-green filter (Figs. 30—32). The quantity of fluorochrome in the marrow cavity varied somewhat from one animal to another because of small differences in the time interval between the injection and the removal of the hind quarters.

The intensity of fluorescence (blackening) near the epiphyseal

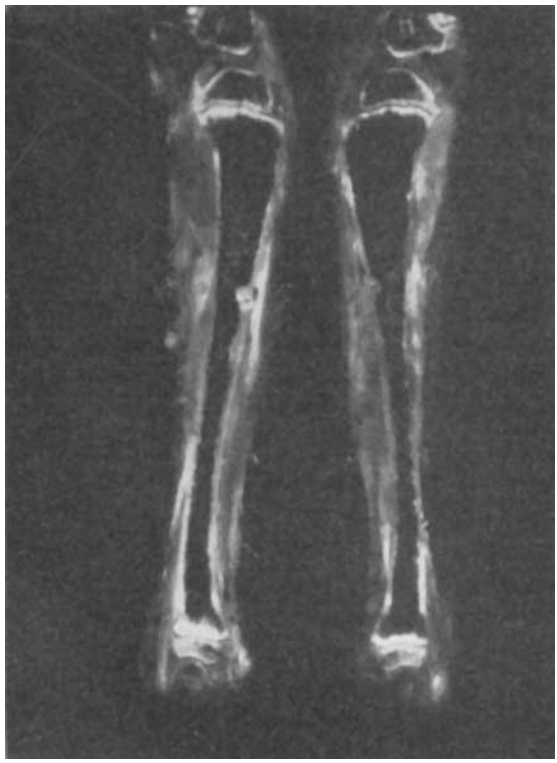

Fig. 30. Tibiae after the injection of vasoflavine, freezing and planing. The substance did not enter the epiphyseal cartilages. Uneven fluorescence close to the right proximal epiphyseal cartilage.

cartilages was measured on the film in a photometer along a line across the epiphyseal cartilage and roughly parallel to the longitudinal axis of the bone. It crossed the blackening in the metaphysis and, as a rule, in the epiphysis. Fig. 33 illustrates such a curve. As a rule the epiphyseal cartilage had taken up a fair amount of oxypyrene, but not so much as that of the adjacent bone. Therefore at the peak the curve shows a short abrupt indentation at the site of the epiphyseal cartilage. As a rule 3 measurements (1—4 measurements) were made at different sites over each epiphyseal region.

Vasoflavine did not appear to enter the epiphyseal cartilage.

The greater the photographic density the higher was the peak. When the blackening was maximal the curve reached the level of the zero line.

In the photometric curves the photographic density was determined according to the formula $S = \log \frac{I_0}{I}$ (see ORNSTEIN, MOLL & BURGER, 1932). S indicates the photographic density, I_0 is the intensity of the light through the unexposed part of the film, I the intensity of the light through the blackened part of the film. Only values of S above 0.2 of all curves of the regions measured of both sides were used. The quotient right/left was determined for proximal epiphyseal cartilaginous regions and for the regions over the distal epiphyseal cartilages. The width of the fluorescent zone always included the metaphyseal and the epiphyseal fluorescence adjacent the epiphyseal cartilage. It was not considered possible to measure the width of two such closely located bands separately, because it must be assumed that the photometric curve for one of the sections would be influenced by that for the other in the region where they meet.

The width of the peaks of the curves indicating the width of the fluorescent zone were determined in the following way. At a relatively equal distance from the top of the curves to be compared (one half and one third of the total height) horizontal lines were drawn. The width of the peak was measured along these lines. The mean of these two values was taken as a measure of the width of the fluorescent zone. For the comparison the mean of the values of the zonewidth for each metaphyseal-

Figs. 31 and 32. Tibiae after injection of sodium 3-oxypyrene-5, 8, 10-trisulphonate, freezing and planing. The epiphyseal cartilages, the border of the corticalis and subchondral bone are somewhat fluorescent. The right bone had been operated on 2 weeks before the study. Proximally (Fig. 31) the metaphyseal region of the right, operated bone was less fluorescent than that of the left, control bone. The dots are reference marks for photometry. Distally (Fig. 32) the fluorescence round the right (operated leg) epiphyseal cartilage was more intense than round the left.

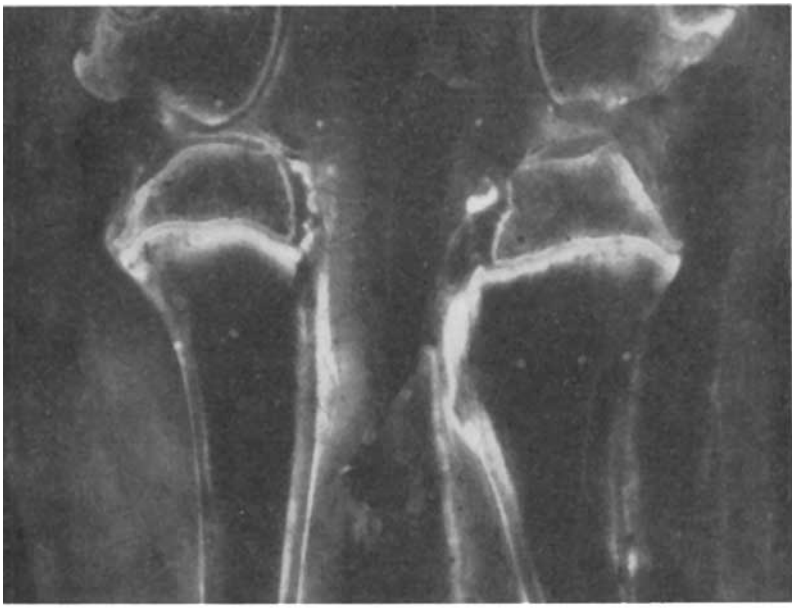

Fig. 31

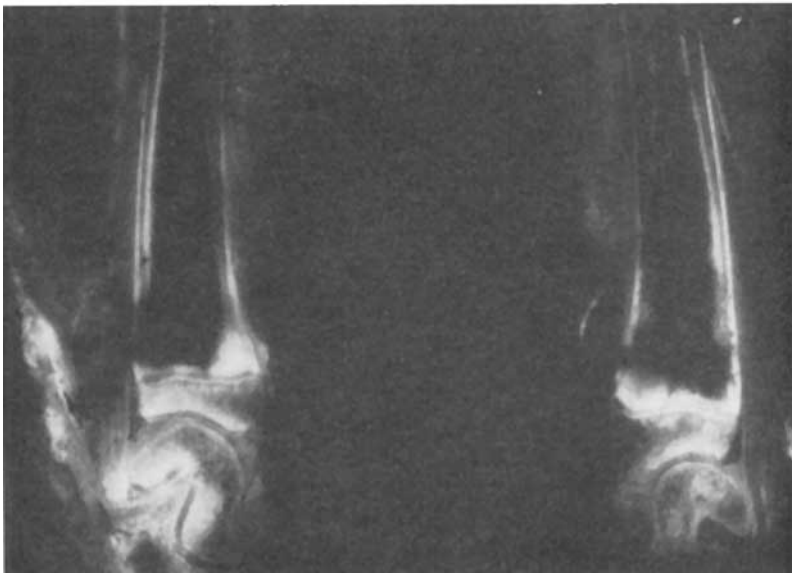

Fig. 32

epiphyseal region was calculated and the quotient right/left determined for each animal. When the fluorescence of the endosteum and periosteum was registered relatively close to the fluorescence in the vicinity of the epiphyseal cartilages it was necessary to measure the widths of the peaks at one fifth and one tenth of the total height. Widths of the peaks less than 10 mm. were measured with an accuracy of ± 0.1 mm.

Fig. 33. Photometric curve for the region of the proximal epiphyseal cartilage of a control animal. The peak A represents the fluorescence in the subchondral bone close to the knee joint, the peak B the fluorescence in and around the epiphyseal cartilage. The 8 mm. deep indentation near the top of the peak B represents the relatively weak fluorescence of the epiphyseal cartilage. The lowest point of the curve (equal on the sides of the base of the peak B) represents those parts of the film not blackened by fluorescent light.

The product of photographic density and width of the peak of the photometric curve was calculated for proximal and distal epiphyseal regions in each group. It gives a rough relative measure of the amount of fluorescence. The quotient right/left between the products of corresponding areas was determined.

RESULTS

Only values obtained with sodium 3-oxypyrene-5, 8, 10-trisulphonate could be used. In experiments using vasoflavine the fluorescent area close to the epiphyseal cartilage often showed defects, probably due to small thrombi. The two components measured (the fluorescence intensity and width of the

fluorescent zone) are given as the quotient between the right and left legs with the mean error of the mean. The values for the photographic density in the region of epiphyseal cartilages are given in Table IV and Figs. 34 and 35, the widths of photometric curves in Table V and Figs. 34 and 35. The importance of each component separately could not be determined. The values for the control animals showed that normally the quotient between the two components does not differ with certainty from 1.

TABLE IV

Photographic density, measured with photometer, as quotient right/left. C=controls. In the other groups the right leg was operated on.

Days after op.	Proximally	Number	Distally	Number
C	0.986 ± 0.037	6	1.021 ± 0.055	2
1	1.169 ± 0.002	2	1.145 ± 0.174	2
7	1.107 ± 0.039	6	1.268 ± 0.121	5
14	1.107 ± 0.063	4	0.996 ± 0.016	2
28	1.194 ± 0.072	4	1.216 ± 0.038	3
42	0.967 ± 0.047	7	1.224 ± 0.078	5

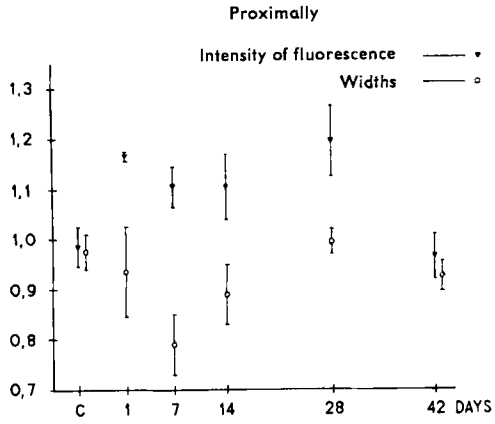

Fig. 34. Results of photometric measurements of the intensity of fluorescence and of the widths of the fluorescent zone round the *proximal* epiphyseal cartilages, given as quotient right/left. C=controls. In all of the experimental animals the right leg was operated on. Days=days after operation. For exact quotients and numbers of animals, see Tables IV and V.

As to *the regions of the proximal epiphyseal cartilages* in the experimental animals, it is remarkable that the values for each of the two components during the first four weeks after operation are situated on either side of the line for the quotient 1.

TABLE V

Width of photometric curves as quotient right/left.
C = controls. In the other groups the right leg was operated on.

Days after op.	Proximally	Number	Distally	Number
C	0.974 ± 0.039	6	1.100 ± 0.235	2
1	0.933 ± 0.091	2	1.448 ± 0.373	2
7	0.789 ± 0.058	6	1.280 ± 0.208	5
14	0.892 ± 0.057	4	1.257 ± 0.405	2
28	0.994 ± 0.026	4	0.993 ± 0.164	3
42	0.924 ± 0.029	7	1.120 ± 0.059	5

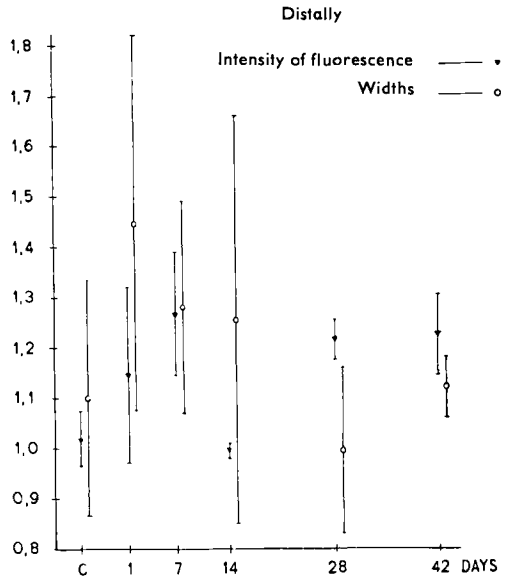

Fig. 35. Results of photometric measurements of the intensity of fluorescence and of the widths of the fluorescent zone round the *distal* epiphyseal cartilages, given as quotient right/left. C = controls. In all of the experimental animals the right leg was operated on. Days = days after operation.

For exact quotients and numbers of animals, see Tables IV and V.

By six weeks after operation the two values approached one another and then lay practically under the line for the quotient 1 and thereby suggest that the blood supply was then probably less round the right proximal epiphyseal cartilage, which showed poorer activity than the left (control). The values for the amount of fluorescence (Table VI and Fig. 36) was less in the operated leg seven days and forty-two days after operation and greater twenty-eight days after operation.

The blood supply to *the regions of the distal epiphyseal cartilages* showed a distinct increase during the first week after operation. The intensity and the width of the fluorescence, judged separately, did not show any definite change in the circulation, one, fourteen, and twenty-eight days after operation. However, seven and forty-two days after operation the flow of blood seemed to be increased. The product of the two components shows values above 1.0 in all operated groups (Table VI and Fig. 36).

The average growth from the proximal epiphyseal cartilage in millimetres during the last week[1] for groups seven, fourteen, twenty eight and forty-two days were —0.05 (six animals), +0.07 (four animals), —0.05 (four animals), —0.1 (seven animals). The corresponding figures for the distal epiphyseal cartilage were +0.28 (five animals), —0.2 (two animals), —0.07 (three animals), +0.06 (five animals). In view of what was pointed out in an earlier section concerning the error of the measurement of growth in length, the figures do not permit any valid conclusions concerning a correlation between the growth in length and the circulation. The data obtained from the study of the circulation should rather be correlated with the composite curves for growth in length after the operation (Fig. 8), the basis of which includes the animals used for circulation studies.

The *proximal* values of the forty-second day after operation are probably more informative than the others, because the

[1] +indicates that the right leg grew more than the left, — the opposite relationship.

TABLE VI

Amount of fluorescence expressed as the quotient right/left.
C = controls. In the other groups the right leg was operated on.

Days after op.	Proximally	Number	Distally	Number
C	0.960 ± 0.053	6	1.123 ± 0.250	2
1	1.091 ± 0.106	2	1.658 ± 0.485	2
7	0.873 ± 0.071	6	1.623 ± 0.306	5
14	0.987 ± 0.085	4	1.252 ± 0.405	2
28	1.186 ± 0.078	4	1.207 ± 0.203	3
42	0.894 ± 0.052	7	1.371 ± 0.113	5

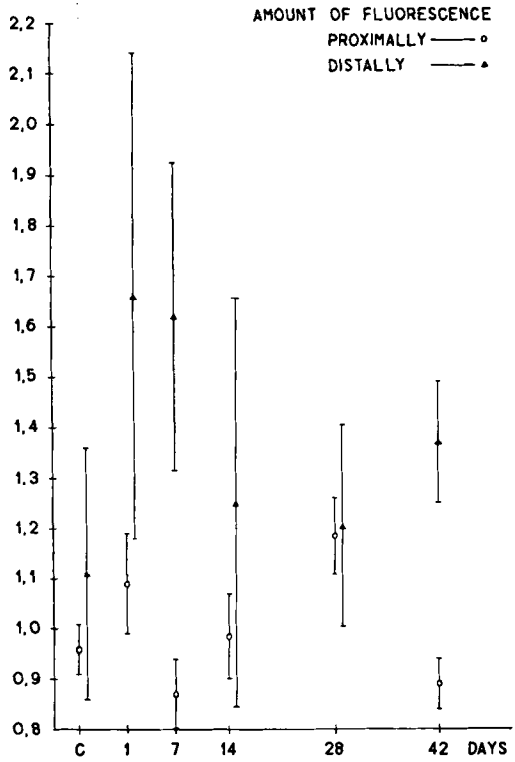

Fig. 36. Relative amount of fluorescence (products of the values recorded in Figs. 34 and 35), given as quotients right/left. Days = days after operation. The exact values are given in Table VI. C = controls. In all of the experimental animals the right leg was operated on.

postoperative changes might not have disappeared (see temperature recordings of the skin) in the earlier groups. On the forty-second day all the values for the proximal region lay below the unit-line and probably indicated a decreased blood supply at the time when the growth curve was distinctly on the decline. *Distally* the change in flow of blood was more marked than proximally (increased flow of blood) in all groups and the growth curve was on the incline.

COMMENTS

The method described requires that the flow of blood to the two legs is interrupted at a favourable moment, *i. e.*, when the fluorochrome has just reached the region under consideration. This time is, of course, somewhat earlier for the vessels round the proximal epiphyseal cartilage than round the distal epiphyseal cartilage. Owing to the difficulty in assessing exactly when sufficient fluorochrome has reached the environments of the distal epiphyseal cartilage, ten of the animals could not be used for the investigation of the circulation in the region of the distal epiphyseal cartilage.

Proximally, it was not difficult to obtain an approximately sagittal plane at the same level of the two legs to be compared. Distally, however, it was not possible to judge the depth of the planed surface with certainty. Therefore the data obtained for the region of the distal epiphyseal cartilage must be regarded as somewhat less reliable than those obtained for the proximal.

In no instance had the fluorochrome demonstrably passed the marrow out into the veins.

No explanation can be offered for the high intensity and the narrowness of the fluorescent zone proximally in the operated tibia (Tables IV and V, Fig. 34). It is, however, possible that the destruction of the metaphyseal vessels at the operation might be the cause .One might also be inclined to imagine a changed capacity of the newly formed bone to take up the fluorochrome, although this possibility sounds less likely, because the fluorescent zone under consideration was very intense and narrow

even one day after the operation, by when any change in the uptake of the fluorochrome due to bone new formation could hardly have made itself felt.

TO RECAPITULATE, the amount of intravenously administered substances could not be determined quantitatively with certainty in ashed bones.

In experiments using a fluorochrome, sodium 3-oxypyrene-5, 8, 10-trisulphonate, deposited intravenously, it was found that the increased longitudinal growth was correlated with increased blood supply to the metaphyseal and epiphyseal regions of the "stimulated" epiphyseal cartilage. Certain evidence produced suggested that retarded longitudinal growth is correlated with a decreased blood supply to the region of the "inhibited" epiphyseal cartilage.

DISCUSSION

The experiments described gave clear cut information on the growth pattern after the growth stimulating operation and on the vascular region from which the epiphyseal cartilage takes up tracer substances presented to it by the blood stream. On the other hand, the investigation permitted no definite conclusion as to any correlation between changes in the blood circulation and increase in growth in length.

Of the methods tried, determination of the temperature in the marrow cavity, colorimetric determination of intravenously deposited sodium chromate and clearance of isotope (I^{131}) from the marrow cavity were found to be of no value for the purposes of the investigation.

The other methods, measurement of the skin temperature, determination of the total amount of blood around the epiphyseal cartilages and the determination of the amount of intravenously injected fluorochrome that reaches the vascular network were informative. The purpose of these methods was to elucidate the following factors: superficial circulation, relative amount of blood in a segment of the extremity and the rate of blood supply to the marrow cavity nearest the epiphyseal cartilage. The first two methods showed changes during the first two—three weeks after the operation. Therefore the factors measured appear not to influence growth in length, except possibly during the first two to three weeks after operation. Experiments with the third method showed somewhat low values for the supply of blood of the vicinity of the slowly growing proximal epiphyseal cartilage six weeks after the operation and relatively high values for the supply of blood of the vicinity of the rapidly growing distal epiphyseal cartilage in all the operated groups, the latest forty-two days after operation.

The investigation did not permit any definite conclusions as to the correlation between increased growth in length and blood supply. But it showed with a fair degree of probability that after a growth stimulating operation the blood supply around a rapidly growing epiphyseal cartilage is increased and that when growth in length is decreased, the blood supply round the actual epiphyseal cartilage is also decreased at least at six weeks after operation.

The literature on the cause of increased growth in length calls for a few remarks. No observations made in the present investigation argued for the opinion that stasis (passive hyperemia) is of any importance as a cause of increased longitudinal growth. The increased amount of blood demonstrated by Cr^{51}-labeled erythrocytes was of too short a duration to explain the continuous change in growth in length. Moreover, it is not certain whether passive hyperemia really was present. The increase in the temperature of the skin over the area under consideration when the increase in the amount of blood was most pronounced suggests that the hyperemia was rather of active nature. Neither did any observations made in the present investigation argue for active hyperemia being of any importance as a cause of increased growth in length. The investigation only showed that the flow of blood is probably increased close to a relatively rapidly growing epiphyseal cartilage and decreased close to a relatively slowly growing epiphyseal cartilage. No evidence was produced to indicate that the change in the circulation is the cause of the change in the rate of growth. It is true that the growth stimulating operation damaged the small metaphyseal vessels in the proximal part of the tibia and the drainage of the marrow cavity and that circulatory disturbances in the marrow cavity should reasonably be expected.

The opinion has, however, been expressed that a growth stimulating substance, osteogenin (LACROIX, 1951), is released from the subperiosteal tissue, when the periosteum is loosened. It is supposed to exert a growth stimulating effect directly on the cartilage cells. The power of the supposed osteogenin to exert its effect for at least five months after the loosening of the

periosteum appears less likely. Moreover, it seems strange that the substance released from the proximal part of the tibia should stimulate the distal end, while the growth of the nearer epiphyseal cartilage, which should reasonably be more readily stimulated, should be inhibited.

Even though the experiments argue against the occurrence of the hypothetic osteogenin, it cannot be concluded that the changes in circulation are the cause of the change in the rate of longitudinal growth. Before it is possible to clear up any such causal relationship, it would be necessary to produce a persistent disturbance in circulation without damaging the bone, and then study the growth pattern of the bone and determine the amount of blood supplied to the surroundings of the epiphyseal cartilages.

SUMMARY

The following observations of interest were made in an investigation of a) the effect of periosteal loosening on the longitudinal bone growth, b) the nutrition of the epiphyseal cartilages and c) the local blood supply of the regions of the epiphyseal cartilages of the tibia of the rabbit.

1. Loosening of the periosteum of the proximal half of tibia by the method described by OLLIER in 1867 was followed by increased growth in length from the distal epiphyseal cartilage and decreased growth from the proximal epiphyseal cartilage. These changes in rate of growth persisted throughout the period covered by the present investigation (160 days).

2. Intravenously injected fluorescent substances (water insoluble benzpyrene and water soluble sodium 3-oxypyrene-5, 8, 10-trisulphonate) entered the epiphyseal cartilage mainly *via* the bone marrow and then to a much greater extent from the metaphyseal side than from the epiphyseal.

3. Measurement of the temperature in the marrow cavity of the tibia, the clearance of isotopes from the marrow cavity of the tibia and colorimetric determination of the amount of intravenously injected sodium chromate taken up by the tibia proved valueless in the investigation of any correlation between disturbances of the local circulation and changed growth in length of the bone.

4. The first few days after operation the temperature of the skin over the operated area was higher than that over a corresponding area of the control leg, but one week after the operation it was lower, after which it gradually increased to reach that of the control side within a few weeks.

5. Judging by the radioactivity measured over corresponding parts of both legs after the intravenous injection of erythro-

cytes tagged with $Cr^{51}O_4$, the amount of blood in the operative field was greater than that in a corresponding region of the control leg for anything up to two and a half weeks after operation.

6. Photometric determination of the fluorescence of frozen, exposed marrow cavity of the tibia of rabbits that had received an intravenous injection of sodium 3-oxypyrene-5, 8, 10-trisulphonate suggested a certain correlation between the rate of growth in length of the bone and the local blood supply six weeks after operation. Distally, the flow of blood and the rate of growth were increased from the day after operation.

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Harald Brodin

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