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Some aspects of longitudinal bone growth

AN EXPERIMENTAL STUDY OF

THE RABBIT TIBIA

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innerligt tack för värdefull
handledning, ständigt stöd
och vänlig uppmuntran under
tillkomsten av denna bok.

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Introduction

For many decades, the factors regulating the longitudinal bone growth have aroused interest. Alimentary and hormonal factors that influence the organism as a whole have been studied in detail. In orthopaedics, the main interest has been to determine the underlying cause of a changed growth rate in individual skeletal parts. These factors have been sought by experimentally accelerating in different ways the longitudinal bone growth. However, some problems are still unsolved, and certain obscurities need clarifying in more detail. Therefore the present work has been carried out with the object:

of producing an accelerated longitudinal growth in a bone (rabbit tibia) with the aid of a method that is non-traumatic as far as the bone is concerned;
of analysing the factors involved in such a growth acceleration;
of studying, at this accelerated growth, the intra-osseous blood flow.

Chapter 1 Accelerated bone growth

I. Earlier investigations

The possibility of influencing the longitudinal bone growth was investigated as early as 1867 by OLLIER, who observed the occurrence of this after periosteal stripping of the tibia in the growing rabbit. Since then, a series of growth stimulating methods have been tested experimentally. The experiences thus gained have been applied clinically. At various diseased conditions, a locally more rapid skeletal growth has also been noted.

A survey of the literature reveals one group of growth stimulating methods that involves direct traumatization of the investigated skeletal part, whereas another group leaves the bone intact, its effect being indirect.

A. STIMULATION OF LONGITUDINAL BONE GROWTH BY DIRECT MEASURES

1. **Röntgen**

The effect of ionizing radiation on the growing skeleton has been the object of several investigations. BAUNACH (1935) showed experimentally on rabbit that a low röntgen dose produced an initial and brief growth stimulation as an expression of an irritative effect. Larger doses were followed by growth retardation, the younger the experimental animal the more pronounced the effect. This accords well with the findings of other investigators (*inter al.* LANGENSKIÖLD & EDGREN 1949). Of more recent works can be mentioned that of PHILLIPS & KIMELDORF (1966), who studied in detail the retardation of skeletal growth in rat, taking into account dosage and age of the animal.

2. **Heat**

RICHARDS & STOFER (1959) reported stimulated longitudinal bone growth in experimental animals after increasing the skeletal temperature by the application of an electrically heated wire. A positive effect produced by increased heat was also obtained by DOYLE & SMART (1963), who used moderate doses of short-wave diathermy. WILSON & THOMSON earlier

(1939) used that method on a small group of patients suffering from unilateral paralytic growth retardation of the lower extremity. There was usually no further increase in the existing length discrepancy after the treatment. A slight growth stimulation was also noted clinically by BERTRAND & TRILLAT (1948).

According to WISE *et al.* (1949) the application of heat results in growth retardation in young rats. The amount of heat they mention, however, has been considered too large (DOYLE & SMART 1963, GRANBERRY & JANES 1963). The latter gave a moderate application to growing dogs, but failed to record any significant effect on longitudinal growth; nor could DE FOREST *et al.* (1953) do so with ultrasound, or RING & LEE (1958) with a carbon resistor in the distal ulnar epiphysis in rabbit. RING (1961) observed no effect from increased heat on the sequelae of poliomyelitis in children.

3. Trauma to the medullary cavity

KISHIKAWA (1936) noted that drilling into the tibial diaphysis in rabbit produced an increase in the longitudinal growth of the bone. HUTCHISON & BURDEAUX (1954) obtained some effect after drilling into the femoral metaphysis in dog.

FERGUSON (1933) obtained growth stimulation after drilling and curettage of the bone marrow in the tibial and fibular diaphysis in children.

Both COMPERE & ADAMS (1937) and WU & MILTNER (1937), on the other hand, failed, experimentally, to record any effect; HANSSON & WIBERG (1963) reported only insignificant increase in length after drilling in the tibial metaphysis of rabbit.

4. Application of various materials in the medullary cavity

Since VON LANGENBECK's (1869) positive experiment of inserting an ivory plug into the medullary cavity in experimental animals, a large number of experimental works have been carried out where, by the irritation of a foreign body in the vicinity of the growth plate, an accelerated growth has been attempted. (In some instances, two different metals have been implanted with the object of using also the electrolytical effect). A great variety of materials have been used. MEISENBACH (1910), KÖNIGSWIESER (1925), BERGMANN (1931), KISHIKAWA (1936), WILSON & PERCY (1956) are among those who, by this type of operation, could observe a growth stimulation. This was also observed by TRUETA (1953, 1958) with a somewhat modified method involving extensive destruction of the medullary cavity, which is then filled with foreign material.

Many investigators, *inter al.* TROUT (1915), BOHLMAN (1929), WU & MILTNER (1937), CHAPCHAL & ZELDENRUST (1948), HERNDON & SPENCER (1953), HAAS (1958) either recorded no effect or observed a retardation in the longitudinal bone growth after implantation of foreign materials.

In several of the mentioned experiments, infectious material was used. It is an old clinical experience that a limb with a local inflammation often shows signs of stimulated skeletal growth (LANGENBECK 1869, BERGMANN 1931, WILSON & MCKEEVER 1936, TRUETA 1953).

The experimental knowledge was used in 1952 by PEASE, who in 7 children with anisomelia applied different metals or ivory into the metaphyses at the knee joint: he noted growth stimulation in all patients. Effects of similar operations have also been reported by *inter al.* CARPENTER & DALTON (1956, 1963) and NORDENTOFT & GULDHAMMER (1964), but they emphasize that the gain obtained in longitudinal bone growth is so slight that in most instances it does not justify the operation. WILSON & PERCY (1956) could detect no effect whatever from this procedure.

5. Periosteal stripping

OLLIER (1867) loosened most of the periosteum from the diaphysis in growing rabbit tibia and three months later observed an increase in length of 2—5 mm. Several investigators, (WU & MILTNER 1937, LACROIX 1947, BERTRAND & TRILLAT 1948, BRODIN 1955, ELO 1960, and SOLÁ *et al.* 1963) later, using the same method, noted a significant increase in the longitudinal bone growth; BRODIN was the first to show in more detail that periosteal stripping from the proximal rabbit tibia is followed by decreased activity of the proximal growth plate and increased of the distal. The latter effect predominates; therefore the net result is a slight increase in the growth rate of the tibia as a whole.

A modified method has been tried by placing foreign matter or organic tissue between the periosteum and the cortex (LANGENSKIÖLD 1957, ELO 1960) but SOLÁ *et al.* (1963) consider this method to be inferior to stripping alone.

A second stripping procedure does not appear to increase the stimulating effect (SOLÁ *et al.* 1963) and if the periosteum as well as the perichondrium is removed, growth retardation occurs (HAAS 1917, SOUSA PEREIRA 1938, ELO 1960).

The clinical value of the method has been appraised by *inter al.* BERTRAND & TRILLAT (1948), ZANOLI (1949), PEASE (1952), TAILLARD (1959), and SOLÁ *et al.* (1963). Periosteal stripping in children suffering from growth retardation of a lower extremity thus produces a moderate growth stimulation.

6. Fracture

Experimental diaphyseal fractures (BISGARD 1936, COMPERE & ADAMS 1937, GREVILLE & JANES 1957) are followed by stimulated growth of the fractured bone. Both BISGARD (1936) and COMPERE & ADAMS (1937) think that the stimulation is local in character and does not refer to the other bones in the extremity involved. This is contradicted by WRAY & GOODMAN (1961), who found that a fracture through the tibial diaphysis in rat causes, during the immediately following period, growth retardation of the femur both in the fractured and the non-fractured extremity. A period then ensues when both femurs show increased growth, but where the growth of the femur of the fractured extremity is significantly greater than that of the corresponding bone on the contralateral side. Earlier, LEVANDER (1929), after fracturing the tibial diaphysis in rabbit, observed a temporarily increased growth of the femur in the same extremity.

Several investigators have noted that diaphyseal fractures in children are often followed by an increased longitudinal growth of the fractured bone (TRUESDELL 1921, BURDICK & SIRIS 1923, COLE 1925, LEVANDER 1929, BISGARD 1936, AITKEN *et al.* 1939, HEDBERG 1945, BERTRAND & TRILLAT 1948, TRUETA 1953, EMNÉUS & WIBERG 1958, GULDHAMMER 1963, *inter al.*). Sometimes an increased growth originates also in other bones in the extremity (COLE 1925, BERTRAND & TRILLAT 1948, GOFF 1960).

The general opinion is that the accelerated growth after fracture is not due to an increased amount of bone tissue at the place of the fracture, but is a result of increased metaphysial activity. BISGARD (1936) illustrated this in his experiments and WRAY & GOODMAN (1961), besides COLE (1925) and LEVANDER (1929), have also shown that the stimulus was not solely localized to the fractured bone.

In the above survey, investigations of experimental or clinical nature, where two or more methods were combined to obtain growth stimulation, have been omitted.

The causal relationship

The following theories have been suggested concerning the mechanism underlying the growth stimulating methods reported above:

Röntgen. The growth increase observed here has been interpreted as an expression of an irritative effect due to the ionizing radiation.

Heat. By increasing the temperature in the tissues, heat has been thought to produce a diffuse hyperaemia or in some cases a more local one. RICHARDS

& STOFER (1959) describe both an arterial and a venous hyperaemia, which they found in injection studies and which they then thought stimulated the growth plate.

Trauma to the medullary cavity, as well as the *application of various materials* into it, produces a greater or lesser damage to intra-osseous vessels. The growth stimulating effect according to FERGUSON (1933) is to be found in interruption of the metaphysial blood flow; HUTCHISON & BURDEAUX (1954) look for the explanation in the venous stasis that occurs; TRUETA (1953) and CARPENTER & DALTON (1956) think that, by blocking periosteal and diaphysial vessels, the arterial blood is forced to a greater extent than usual through the metaphysial vessel channels, resulting in a stimulation of the longitudinal bone growth. HANSSON & WIBERG (1963), however, call attention to the hyperaemia that accompanies a healing process and that, by way of the metaphysial vessels, can act as a stimulus.

Application of various materials into the medullary cavity also includes the introduction of what is usually a foreign body and sometimes of infectious material. This creates a condition of irritation with varying degrees of necrosis that induces a hyperaemia. The hyperaemia acts also on the growth plate and stimulates longitudinal bone growth (LANGENBECK 1869, KÖNIGSWIESER 1925, KISHIKAWA 1936, CHAPCHAL & ZELDENRUST 1948, PEASE 1952).

It is the irritation element at *periosteal stripping* that acts as a stimulus according to earlier investigators (*inter al.* OLLIER 1867). LACROIX (1947) thinks that the procedure has a direct effect upon the growth plate by liberating a growth stimulating substance; most investigators, however, consider that the changed rate of growth is due to disturbed circulation caused by the damages to the periosteal-osseous vascular connexions (SOUSA PEREIRA 1938, BRODIN 1955). The effect, according to TAILLARD (1959), is a growth stimulating hyperaemia in the bone, most pronounced in the epiphysial region (SOLÁ *et al.* 1963). The latter suppose that the operation, through damage to the vascular connexions to the diaphysis, increases the blood flow in the vessels between periosteum and epiphysis. ZUCMAN (1960), by means of an injection method, showed that a loosening of the periosteum was followed by an extensive dilatation of the periosteal vessels; earlier, BRODIN (1955), with the aid of isotope-labelled erythrocytes and a fluorescence method, thought he could detect an increased blood flow to the epiphysial-metaphysial regions.

The mechanism underlying the growth stimulation at *fracture* of long bones is generally thought to lie on the vascular plane. Some authors believe that

the fracture causes, *inter alia*, irritation of the periosteum resulting in an increased blood flow, which can effect and thereby stimulate the growth plate (LEVANDER 1929, BISGARD 1936, BERTRAND & TRILLAT 1948). HEDBERG (1945) considers the hyperaemia to be related to the degree of the callus formation, whereas TRUETA (1953) thinks that the essential is a blocking of the medullary vascular channels with an activation of the periosteal circulation (CAVADIAS & TRUETA 1965) whereby the blood is, to an increased extent, forced into metaphysial vessels.

WRAY & SPENCER (1960) could establish a pronounced increase of the arterial inflow in the limb, after fracture of a long bone in dog; but because a stimulated longitudinal bone growth could also be noted on the contralateral side, a generally acting factor, possibly an increased liberation of growth hormone, could be important here (WRAY & GOODMAN 1961).

B. STIMULATION OF LONGITUDINAL BONE GROWTH BY INDIRECT MEASURES

1. Disturbed innervation

As is well known, a disturbed innervation in a growing person can be reflected in the structure and development of the skeleton, including a disturbed longitudinal bone growth.

Many investigations have been made into the skeletal innervation, and nerve ends have been found in compact bone as well as in bone marrow. (SHERMAN 1963 surveys the earlier literature.)

CURCIO (1898) stated that he had experimental evidence that a trophic centre of the skeleton was situated in the spinal cord. Many of his contemporaries had the same belief about the tropism relationship between the nervous system and the skeleton. (For review, see CASSIRER 1912.)

The trophic relationship was later adopted by HURRELL (1938), who observed in cat unmyelinated nerve endings in the matrix next to the bone cells. He thought that "they may be the sensory and effector endings required for a reflex control of bone growth and maintenance."

MILLER & KASAHARA's (1963) work can be mentioned among more recent investigations. They applied methylene-blue immersion technique and investigated long bones from *homo*, rabbit, and rat. No principal species difference was found. Numerous myelinated and unmyelinated nerve fibres pass through the nutritional foramen and ramify in the bone marrow and endosteum in the diaphysis. Similar thin nerve fibres pass through the cortex by way of the many foramina found in the epiphysial-metaphysial region. Myelinated nerve fibres can be seen winding around bone trabeculae and ramifying under the inner surface of the joint cartilage. The

authors think that these fibres are sensory and can have something to do with the positional sense. Unmyelinated nerve fibres are found mostly around vascular structures. These have also been noted by SHERMAN (1963), who presumes that they belong to the autonomic nervous system and are concerned with the regulation of the blood flow. Little evidence supports the idea that the skeletal bones get fibres from anterior-horn cells of the spinal cord (GILLESPIE 1954).

The large number of experimental works that exist concerning the effect of denervation upon bone growth can probably be related to the marked changes recorded clinically in the growing skeleton at injuries of the nervous system and the thereby connected therapeutic problems that arise at, for instance, the treatment of the sequelae of poliomyelitis.

The results of experiments must be judged against the background of how the denervation is carried out. TROUPP (1961) has partly touched on this. Anatomically, the same result of nerve root sectioning as of peripheral nerve sectioning cannot be expected. Sectioning of the posterior root thus produces a sensory denervation. Sectioning of the anterior root, besides motor denervation, injures preganglionic sympathetic nerve fibres—if the root involved lies cranially to the lowest level of the preganglionic outflow to the sympathetic trunk—and also causes an interruption to a number of postganglionic sympathetic fibres, as shown in recent investigations by DAHLSTRÖM & FUXE (1965). They demonstrated that noradrenalin-containing nerve fibres of supraspinal origin leave the spinal cord through the anterior roots and bypass the ganglion cells of the sympathetic trunk without synaptic contact. Finally, the result of peripheral nerve sectioning is an interruption of sensory, motor, and postganglionic sympathetic nerve fibres.

Sensory nerve injury

GREY & CARR (1915) and GILLESPIE (1954) failed to affect the bone growth by cutting sensory roots (GREY & CARR, however, say nothing about the age of the experimental animals). RING (1961) also failed with peripheral sensory denervation (section of the sural nerve in rabbit). TROUPP (1961), however, observed, after sectioning posterior roots in rabbit, that the femur on the denervated side showed marked growth retardation, but the tibia did not. The same experiment was repeated with the denervated extremity protected by the abdominal skin. The growth retardation of the femur was then considerably less, and in some cases the longitudinal growth of the tibia was accelerated.

Motor nerve injury

GREY & CARR in 1915 made a selective denervation by sectioning anterior roots. GILLESPIE (1954) did the same experiment in young cats. However,

these investigators could observe no effect upon the longitudinal bone growth. According to TROUPP (1961) various degrees of anterior root lesions in rabbit causes growth retardation of the denervated bones in relation to the extent of the denervation. Sectioning anterior roots in young dogs resulted in a significant increase in longitudinal growth of the tibial diaphysis on the denervated side (RING 1961) but the author could observe no effect from the same operation in kittens.

Injury of sympathetic nervous system; pharmacological sympathetic blockade

The effect of sympathectomy on the longitudinal bone growth was studied by, *inter al.*, KISHIKAWA (1936), who obtained moderate growth stimulation in the lower extremity after lumbar sympathectomy in puppy. GULLICKSON *et al.* (1951) achieved the same result in a similar experimental animal; they noted also a growth retardation after prolonged stimulation of the lumbar sympathetic trunk. GOETZ *et al.* (1955) sympathectomized young rabbits and observed increased growth of the foot skeleton.

Most experimental works, however, present no evidence that sympathectomy stimulates growth (CANNON *et al.* 1929, BACQ 1930, BERGMANN 1931, BISGARD 1931, HARRIS & MCDONALD 1936, RING 1961, TROUPP 1961).

HARRIS & MCDONALD (1936) reported four patients with Hirschsprung's disease; all underwent unilateral lumbar sympathectomy. The lower extremity on the same side in all patients was longer than that on the other side. FAHEY (1936) studied two similar cases, but found no signs of stimulated skeletal growth. The lumbar sympathectomy and the pharmacological sympathetic blockade lessen the growth retardation after poliomyelitis, according to HARRIS & MCDONALD (1936), BARR *et al.* (1950), KOTTKE *et al.* (1958), whereas WHITE (1931), NORDENTOFT & GULDHAMMER (1964) could not positively observe this favourable effect. STEWART (1937) studied the effect of the operation on bone growth in children with cerebral paresis, but noted only minor results.

Peripheral nerve injury

Many experimental works concern the effect upon bone growth of peripheral nerve sectioning. MILNE EDWARDS (1860) cut off the mandibular nerve and thereby produced hypertrophy of the mandible. OLLIER (1867) is the first to mention atrophic elongation. He noted several times in experimental animals how a denervated bone had diminished in width but had increased in length. NASSE (1880) and GHILLINI (1897) made similar observations. RING (1961) showed that peripheral nerve sectioning in rabbit produces a significant increase in the length of the tibial diaphysis.

Several investigators, *inter al.* BOREL (1922), BERGMANN (1931), SELYE & BAJUSZ (1958), HĚŘT (1959), are of the opinion that peripheral nerve sectioning has no effect whatsoever on the longitudinal bone growth. Others (HOWELL 1917, ALLISON & BROOKS 1921, ARMSTRONG 1946, GILLESPIE 1954, *inter al.*), however, noted bone atrophy with varying degrees of growth retardation.

Growth retardation has been found clinically after peripheral nerve injury in children (VON LANGENBECK 1869, FISCHER 1871, COMBES *et al.* 1960, *inter al.*).

Poliomyelitis

The sequelae of paralytic poliomyelitis have been studied in detail, and important findings have here been made concerning the influence of the nervous system upon the growing skeleton. Some disagreement exists about whether the condition, apart from the damage to the anterior-horn cells and to sensory structures (MOLDAVER 1944), also results in injury to the sympathetic innervation. Most investigators (*inter al.* COLLINS *et al.* 1947, SMITH *et al.* 1949, STENPORT 1951, MCPHERSON & KESSEL 1955, GOETZ *et al.* 1955, HAGELSTAM 1956, KOTTKE *et al.* 1958, FANCONI 1959, SPENCER *et al.* 1960) believe that the autonomous nervous system is always affected at poliomyelitis. GOETZ *et al.* (1955) emphasize that poliomyelitis is a disease of the gray matter, and that there is no reason why cells responsible for the preganglionic sympathetic outflow should be exclusively spared; indeed, lesions have been found in those cells (SABIN 1942, MOLDAVER 1944). HORÁNYI-HECHST (1935), however, found them to be completely intact in most cases; SHARRARD (1959) asserts that those cells in particular are capable of resisting poliomyelitis virus and are affected only at very extensive damages. RING (1961) thinks that no permanent damage of the sympathetic nervous system occurs, whereas TELFORD & STOPFORD (1933), GASK & ROSS (1937), and TROTT *et al.* (1958) find no signs whatever that the autonomous system is affected at poliomyelitis.

The effect of poliomyelitis on the longitudinal bone growth has been discussed exhaustively. SEELIGMÜLLER (1879) was able to note an initial growth acceleration in a paretic extremity. This is rather the rule during the first year of the disease (GREEN & ANDERSON 1955). This phenomenon was also observed by LERIQUE (1956) and RATLIFF (1959). RING & WARD (1958) found that the immediate effect of the nerve injury at poliomyelitis is an increased longitudinal growth of the affected bones. This occurs during the first year of the disease and can also remain during the second year. Thereafter, the longitudinal growth of the paralysed limb is retarded, and the length discrepancy will increase throughout the entire remaining growth period with a shorter paretic extremity as the net result.

The retarding effect of the disease on the skeletal growth has been investigated by many, *inter al.* HUMPHRY (1862), STINCHFIELD *et al.* (1949), GULLICKSON *et al.* (1950), GREEN & ANDERSON (1955), RING (1957, 1958 a), RING & WARD (1958), RATLIFF (1959), LINDHOLM (1961).

The causal relationship

The analysis of experimental works as well as of clinical observations concerning innervation and skeletal growth is complicated in so far as many factors must in this connexion be considered; namely sensory, somato-motor, and autonomous. This has not always been taken into account. The changes observable regarding the longitudinal bone growth after *sensory denervation* has been interpreted as secondary to the increased traumatization resulting from reduced sensibility (RING 1961, TROUPP 1961).

Sectioning of anterior root, injury to peripheral nerve, or affection by poliomyelitis virus in all instances result in, *inter alia*, interference with *the motor innervation*. According to SEELIGMÜLLER (1879) disturbance of the trophic relationship is also included; this could lie behind a growth acceleration. LANGENBECK (1869) and FANCONI (1959), however, would like in that way to explain a decreased growth activity.

Many investigators have called attention to the stresses and strains that the long bones in a normally functioning extremity are subjected to. Thus, OLLIER (1867) seeks the explanation of the atrophic elongation in the decreased pressure and muscle tension, an opinion that was later shared by, *inter al.* SEELIGMÜLLER (1879), NASSE (1880), GHILLINI (1897). RING & WARD (1958) and RING (1961) interpret a reduced or abolished pressure and tension on the skeleton as at least one contributory cause of such an elongation.

Mechanical factors in the form of normal stress and strain are regarded as essential for normal skeletal growth, and a change in these has been considered as contributing cause of the growth retardation observed after motor nerve damage (HARRIS & MCDONALD 1936, GULLICKSON *et al.* 1950, PEASE 1952).

Several authors (*inter al.* STINCHFIELD *et al.* 1949, GULLICKSON *et al.* 1950, RING 1957, 1961) treat particularly the relation between the extent of growth retardation and the degree of paresis from the standpoint of changed conditions in pressure and tension. (See further the section on mechanical factors.)

A motor denervation produces marked changes in the circulation in an extremity, and the relation between muscle activity and skeletal blood flow has been emphasized (OLLIER 1867, BLAIR 1938, MORGAN 1959). In this respect bone and cartilage with surrounding musculature are regarded

as one functional unit (GEISER 1957, BROOKES 1958, RING 1961, TRUETA 1961, 1964). The bone elongation at polio, in accordance with this, has been ascribed to a shunting of blood from the muscles to the skeleton (FANCONI 1959, TROUPP 1961), the skeleton getting a relatively larger share of the extremity's blood supply because of the reduced metabolic demand by the paretic musculature (RING & WARD 1958, RING 1961). On the other hand, a reduced circulation, secondary to defective muscle activity in a paretic extremity, has been indicated as the chief cause of the observed growth retardation (HARRIS & MCDONALD 1936, GULLICKSON *et al.* 1950, PEASE 1952, RING 1957, 1961). TELFORD & STOPFORD (1933), maintain that the primary factor here is a reduced venous backflow to which GASK & ROSS (1937) ascribe the greatest importance; others, however, lay greater emphasis on the reduced arterial inflow (GULLICKSON *et al.* 1950, PEASE 1952, RING 1957, 1961). A disuse of an extremity since childhood, caused by an innervation disorder, can reveal itself as an organic change in the vascular system in the form of an arterial infantilism (GOETZ *et al.* 1955, MCPHERSON & KESSEL 1955). (The relation between muscle activity and blood circulation are further discussed in the section on immobilization.)

As mentioned, sectioning of motor roots as well as peripheral nerves results in injury to the *symphathetic nervous system*; many consider this to be the case at poliomyelitis too. The changes in the bone growth demonstrated in these instances or after sympathectomy have been thought to be due to changed vasomotion and thereby changed blood circulation.

The accelerated skeletal growth that could be established experimentally and clinically after sympathectomy is explained by a reduced vasoconstriction resulting in increased blood flow and thus stimulation of the growth plates (KISHIKAWA 1936, HARRIS & MCDONALD 1936, GULLICKSON *et al.* 1951, GOETZ *et al.* 1955). Measurements of blood flow indicate (HERRICK *et al.* 1932, THEIS 1933, COLLINS *et al.* 1947, DETERLING & ESSEX 1949, SAKO *et al.* 1959, FOLSE 1965) an increase of the total flow of a limb, but it has been questioned (WILKINS & EICHNA 1941, STEIN *et al.* 1948) whether this increase is wholly or partly to be referred to the skin. PAPPENHEIMER & MAES (1942) state that the flow increase concerns the musculature too; this is also reported by LAMBERT (1957, 1960), SCHÖNBACH (1964), and VAN DE BERG *et al.* (1964).

FELL described in 1949 how injury to the sympathetic innervation can produce hyperaemia in the skeleton; LOWENSTEIN *et al.* (1958) as well as TROTMAN & KELLY (1963) point to an increased blood flow in dog tibia after lumbar sympathectomy. Bone clearance of radiostrontium (SHIM *et al.* 1966) indicates a significantly increased blood flow in talus and calcaneus

in rabbit 1 to 2 weeks after sectioning the sciatic nerve. The author attributes the change entirely to interruption of the sympathetic nerve supply of the bone. Analogous to this, DRINKER & DRINKER (1916), PETRAKIS (1954), HERZIG & ROOT (1959), WEISS & ROOT (1959), CUMMING (1962), AZUMA (1964), SHIM (1966) also interpret their findings as a reduction of the intra-osseous blood flow after sympathetic stimulation or administration of sympathomimetics.

The mentioned results are contradicted by the slight or completely absent stimulation of the skeletal growth as reported in other experimental and clinical works. Many investigations, both symptomatologic (PALOSCHI & LYNN 1964) and those concerning the haemodynamic after sympathectomy, are in total agreement with this. SHAW *et al.* (1964) give an account of a 10-year material of sympathectomies and find so slight an increase in muscle circulation that it can be considered of no practical significance, especially as no change after exercise could be detected. BEACONSFIELD (1954) and MYERS & IRVINE (1966) report similar results. BARCROFT & SWAN (1953) could, it is true, by plethysmography measure initially in *homo* an increased muscle blood flow after sympathectomy, but a return to normal level occurred after a few hours or days. LAMBERT (1957, 1960) and SCHÖNBACH (1964) emphasized also the short duration of the circulation increase experimentally. SCHÖNBACH shows also that the vascular tonus post sympathectomiam after a varying time not only returns to the original level, but even takes a position above the normal.

The return of tonus in the sympathectomized vessels according to MONRO (1959) can best be explained by "reorganization of preganglionic fibres by collateral sprouting" whereby functional connexion with intact ganglion cells is established.

Another explanation has been sought in sensibilization of the vascular musculature of a circulating vaso-constrictor substance (*inter al.* LECOMPTE 1941). This effect has later proved to be due to circulating noradrenalin (MACMILLAN *et al.* 1962).

The angiographic picture is quite unchanged after sympathectomy in rabbit (HULTH & OLERUD 1960). CUTHBERTSON *et al.* (1964 a), VALDERRAMA & TRUETA (1965), and SHAW (1965), who recorded blood flow change with the aid of intra-osseous pressure measurement, could find no signs of autonomous influence on the skeletal blood vessels.

Finally, it should also be mentioned that ZINN & GRIFFITH (1941) refer to reduced flow (dye injection) in rat tibia after sympathectomy; this they interpret as a result of a relatively larger dilatation of the soft tissue vessels. BRAUNSTEINER & GRABNER (1958) at flow measurements in the sternal medulla in *homo* (thermocouple) found that sympathomimetics produced an increase of the blood flow.

It has been postulated that an important factor in the origin of muscle atrophy is a disturbance of the autonomous nervous system, which secondarily can be thought to affect the skeletal growth. Among recent works referring to this problem, can be mentioned that of HELANDER (1960). He showed in rabbit that a sympathectomy does not affect the histological picture of the skeletal muscle; he emphasizes at the same time, however, that the sympathetic tone in rabbit is weak and that the results are therefore not with certainty reproducible in other species.

Concerning poliomyelitis, several authors (see above) have referred to—besides other nervous disfunctions—a simultaneous injury to the sympathetic nervous system; the retarded longitudinal bone growth, a result of reduced circulation, has to a varying extent been attributed to a disturbed sympathetic innervation (OGILVIE 1932, GULLICKSON *et al.* 1950, GOETZ *et al.* 1955, RING 1957, KOTTKE *et al.* 1958). This also manifests itself in other vascular phenomena such as coldness, cyanosis, swelling, clamminess of the involved limb; the explanation has been sought in an abnormal reaction to cold in the form of pronounced angiospasm (TELFORD & STOPFORD 1933, HARRIS & MCDONALD 1936, COLLINS *et al.* 1947, SMITH *et al.* 1949, STENPORT 1951, KOTTKE & STILLWELL 1951). The disease is believed to destroy internuncial neurons, situated close to the sympathetic neurons in the intermediolateral column and normally inhibiting these neurons. The result of the reduced or lost inhibition is hyperactive responses to afferent stimuli, among which is cold. This changed sympathetic activity concerns in the main the vessels in the skin (WILKINS & EICHNA 1941); others (TROTT *et al.* 1958, KOTTKE *et al.* 1958) state that also deeper-lying vascular structures are similarly affected. Thereby the blood flow to the skeleton is also influenced, resulting in a disturbed longitudinal bone growth. A pharmacological sympathetic blockade increases the blood flow measured at the ankle (COLLINS *et al.* 1947) and can to some extent reduce a growth retardation (KOTTKE *et al.* 1958).

2. Disturbed circulation

By eliminating to a varying extent the arterial flow to a long bone, it was possible to observe experimentally different degrees of avascular necrosis, delayed healing of fractures, and reduced growth potential. (For literature survey, see TROUPP 1961, TRUETA & CAVADIAS 1964.) A growth stimulation has in some cases been obtained. It has then been a question of direct traumata to the vascular system as a result of an operation of the type earlier reported (drilling, stripping, etc.).

Increase of arterial flow

Experimental attempts to increase the arterial flow to a long bone by direct surgical diversion of an arterial channel into bone have been made in recent years (WOODHOUSE 1963, DICKERSON & DUTHIE 1963, BOYD & AULT 1965, DICKERSON 1966). The artery, with maintained blood flow, has been implanted under conditions as physiological as possible. Long-term experiments were involved; the acute traumatizing effect could therefore be eliminated. When the method was applied to growing animals, an obvious increase of the growth in length was obtained compared with the control side and with a group of sham operated animals (DICKERSON 1966).

Arterio-venous fistula

More than a century ago, BROCA (1856) *inter al.* noted an accelerated growth of both femur and tibia in a child with an arterio-venous aneurysm situated immediately distal to the inguinal ligament. Several similar reports have since been published: FRANZ (1905), HORTON (1932), HARRIS & MCDONALD (1936), BIRNSTINGL (1962) *inter al.* These clinical observations were followed by a number of experimental works. JANES & MUSGROVE (1950), KELLY *et al.* (1959), DOERR & JANES (1959), VANDERHOEFT *et al.* (1963), KECK & KELLY (1965) created in puppies iliac or femoral arterio-venous fistulas, and observed an increased growth in length of the bones in the lower extremity.

The method was introduced clinically in 1952 by JANES & ELKINS and has been applied to children with length discrepancies in the lower extremities, resulting in growth acceleration (HIERTONN 1957, 1961, COOLEY *et al.* 1960, JANES & JENNINGS 1961).

Venous stasis

One interpretation of the reason for the increased growth in length at an arterio-venous fistula is venous stasis. Obstructing the venous return has been attempted with the object of accelerating the bone growth. BIER (1906) mentions in his monography "Hyperaemie als Heilmittel" that a venous stasis of an extremity is reflected deep into the medullary cavities of the bones and frequently gives rise to an increase both in length and in thickness of the bones. Experimental venous ligatures in the lower extremity in young rabbits resulted in an increased longitudinal growth of the skeletal parts involved (BOREL 1922). The experiment was later reproduced by BERGMANN (1931) KISHIKAWA (1936), SERVELLE (1948). HUTCHISON & BURDEAUX (1954) put tourniquets on the legs of puppies and obtained increased longitudinal bone growth; PEARSE & MORTON (1930) observed accelerated fracture healing at simultaneously created venous stasis. COLT &

IGER (1963) experimented on young dogs by stenosing, in varying degrees, the femoral vein. Both femur and tibia showed accelerated growth of moderate, and in some instances minimum, extent.

However, the literature gives examples also of experiments where the venous stasis was ineffectual (GREY & CARR 1915, DICKINSON 1953, KECK & KELLY 1965).

HELFERICH (1887) described several instances of one shorter lower extremity in children where a venous stasis, produced externally, resulted in an increased longitudinal growth in length. SCHÜLLER (1889) obtained a similar effect in two children with poliomyelitis. The pure venous stasis has been very little used as therapeutic aid during the present century.

Clinical conditions, including extensive varicose changes in the lower extremities since childhood, however, have been reported, often with marked increased skeletal growth in the limb concerned. Sometimes a simultaneous occurrence of haemangioma in the skin has completed the triad that composes Klippel-Trenaunay's syndrome (KLIPPEL & TRENAUNAY 1900, BOREL 1922, SERVELLE 1948, FOSTER & KIRTLEY 1959).

The causal relationship

The accelerated skeletal growth after the *implantation of artery* in a long bone of a growing animal has been explained by the stimulating effect an increased arterial blood flow exerts upon a growth plate. It has thus been possible already after three weeks to observe a rich amount of anastomoses between the implanted vessel and the intra-osseous arterial system (WOODHOUSE 1963).

An increased arterial flow to the skeleton has been mentioned as background to the growth stimulation at an *arterio-venous fistula* (JANES & MUSGROVE 1950, DOERR & JANES 1959, HIERTONN 1961) sometimes in the form of an arterial backflow in the veins (HOLMAN 1955). The venous stasis is a contributing factor here (FOSTER & KIRTLEY 1959, HIERTONN 1961), however, many (BIER 1906, HUTCHISON & BURDEAUX 1954, STEIN *et al.* 1958, HILLMAN *et al.* 1959, BIRNSTINGL 1962, COLT & IGER 1963) attach the utmost importance to it. VANDERHOEFT & KELLY (1964) and KECK & KELLY (1965) speak also of a venous congestion, meaning a "cascade-like recycling of mixed arterial and venous blood", an "active venous congestion", and consider this to underlie the stimulating effect.

Experimental measurements of the blood flow indicate that this, in the extremity as a whole, is reduced distal to a fistula, at least at first (COHEN *et al.* 1948, ROBERTSON *et al.* 1950, HENRIE *et al.* 1959). It gradually increases and can at times exceed the normal level (LEWIS 1940). Similar conditions concern the blood flow in individual skeletal parts distal of a

fistula. At first a decrease (PAUPORTE *et al.* 1958), thereafter an increase; however, not up to the normal level (WEINMAN *et al.* 1964, KECK & KELLY 1965).

It has been possible by angiography to establish a considerable development of both arterial and venous collaterals as well as a tendency to arborisation of periosteal and intra-osseous small vessels (ROBERTSON *et al.* 1950, KELLY *et al.* 1959, HIERTONN 1961, VANDERHOEFT *et al.* 1963, WEINMAN *et al.* 1964, VANDERHOEFT & KELLY 1964).

Venous stasis increases the blood flow to the growth plate (KISHIKAWA 1936) and this creates increased protein concentration and more favourable oxygen conditions (HUTCHISON & BURDEAUX 1954), making possible a stimulation of growth. Intra-osseous blood flow measurements are contradictory concerning the haemodynamic conditions. MCPHERSON *et al.* (1961), SHAW (1963, 1964), report increased flow, VALDERRAMA & TRUETA (1965) note the same change, although only initially. HERZIG & ROOT (1959) cannot measure any changes; WHITE & STEIN (1965), on the contrary, assert a reduction to half the normal flow.

3. Changed static and dynamic conditions

Pressure and tension

The classical HUETER-VOLKMANN law was formulated in 1862. This states that a pressure on a long bone applied longitudinally inhibits growth, whereas a reduction of such a pressure or a tension accelerates it (ARKIN & KATZ 1956 point out that DELPECH as early as 1829 observed this phenomenon). OLLIER (1867), *inter al.*, was of the same opinion; other views, however, were soon expressed in this matter. WOLFF (1892) most eagerly opposed HUETER-VOLKMANN's theory. He maintained that both an increased pressure and an increased tension stimulate bone growth.

The longitudinally exerted force that an active growth plate develops is great. For *homo*, about 400 kg (BLOUNT & ZEIER 1952); for calf, about 150 kg (STROBINO *et al.* 1956). The longitudinal growth can be completely stopped providing a sufficiently powerful pressure is applied to the epiphysial growth plate. MÜLLER (1928) and HAAS (1945) showed this experimentally with the aid of a wire loop around the growth plate: experiments that were repeated by, *inter al.* GELBKE (1951), SIFFERT (1956), SIJBRANDIJ (1963). SIJBRANDIJ states that a pressure of about 3 kg is needed to stop the longitudinal growth at the proximal tibial epiphysis in rabbit. The clinical application with the aid of metal staples was introduced by BLOUNT & CLARK (1949).

MÜLLER (1924), in animal experiments, increased the loading on the

ulna by partial resection of the radius and could detect a reduction of the growth in length of the ulna. Increased pressure was observed histologically by BRAGARD (1932) to impede the maturation of the cartilaginous cells in the growth plate, and by TRUETA & TRIAS (1961) to obstruct the normal progression of the metaphysial vascular loops.

STROBINO *et al.* (1956) state that the prevention of growth at increased pressure obeys an all-or-none law, but this is contradicted by others, for instance, ARKIN & KATZ (1956) and SIJBRANDIJ (1963), who emphasize that the inhibition varies according to the applied pressure, and also that the epiphysial growth plate can resume normal function providing the pressure has not been too prolonged (HAAS 1945, BLOUNT & CLARK 1949, GELBKE 1951, BLOUNT & ZEIER 1952, TRUETA & TRIAS 1961). Actually, very slight force can modify the growth in skeletal length, according to APPLETON (1934) and ARKIN & KATZ (1956). Thus, the normal force of gravity and the stresses that result from ordinary muscle activity have a retarding effect upon the longitudinal bone growth; however, this is accelerated when those factors are eliminated. OLLIER (1867) called attention to the fact that the amputation stump in the lower extremity of children often shows accelerated skeletal growth, and he reports an elongation of humerus in rabbit after experimental resection of radius. MÜLLER (1924) observed in experiments in rats, where one hind leg was placed beneath the abdominal skin, an elongation of the bones involved; this, he attributed to the elimination of normal pressure and tension. Similar results were reported by ARKIN & KATZ (1956) at plastering experiments on rabbits. BRASHEAR (1963) noticed an elongation of femur in rat after knee disarticulation. Division of the tendons around the ankle produced a significant increase in the length of rabbit tibia (RING 1961).

HAAS (1917), however, perceived scarcely any effect from plastering one hind leg in dog; similar findings were reported by BERGMANN (1931) at experimental luxations or division of tendons. Both these authors state, as did RING (1957, 1958 b) and SELYE & BAJUSZ (1958) later, that the normal growth in length occurs independent of mechanical elements.

The next stage in the experimental work was to expose a growing bone to tension. The reports here, however, are few. VON LANGENBECK (1869) thought that the fibular lengthening, seen when the tibia in the same extremity increased in length because of inflammatory irritation, was due to a purely mechanical tension. SMITH & CUNNINGHAM (1957), applying distracting forces to the epiphyses of calves, could measure accelerated bone growth, whereas GELBKE (1951) who exposed apophyses (tuberositas tibiae, olecranon, in dog) to tension, failed to do this. The histological picture, on the contrary, agreed more with that found in a growth plate exposed to increased pressure. HAAS (1958) obtained similar results.

Immobilization

Whereas the mentioned investigators attribute the changed growth rate that can be seen after exarticulation, luxation, plastering, &c., to the influence of the purely mechanical elements on the cells of the growth plate, another group of authors have assigned greater importance to the immobilization, i.e. the reduced or eliminated muscle activity resulting from such measures (RING 1961, *inter al.*). No differentiation of the underlying causal factors has thus been made, but different forms of motor nerve injuries, *inter alia*, polio (see these sections), have also been included here. The retarding effect upon the longitudinal growth has attracted most interest (OLLIER 1867, ALLISON & BROOKS 1921, GILL 1944, GILLESPIE 1954, GEISER 1957, HULTH & WESTERBORN 1963, LANDRY & FLEISCH 1964, AVDYUNICHEVA 1964).

The causal relationship

The investigators who studied the bone growth at *changed pressure and tension conditions* have sought the explanation of the obtained effects exclusively in changed mechanical demands on the growth zones of bone. As appears from the foregoing, however, the experimental methods are here to a great extent identical with those used at various immobilization experiments. Thus, different theories regarding the mechanism behind growth changes, secondary to such measures, have been combined in the next section.

Immobilization. The relation between muscle atrophy and growth retardation at disuse has been indicated (ARMSTRONG 1946, STINCHFIELD *et al.* 1949, GULLICKSON *et al.* 1950, RING 1957, 1961, RATLIFF 1959, KHARMOSH & SAVILLE 1965). GULLICKSON *et al.* (1950) maintain that skeletal growth is related more to the activity of the extremity than to the absolute muscular strength. Variations of this below the contraction level seem to have little importance, at least for the growth retardation (RING 1957).

As already mentioned, many regard bone and cartilage with surrounding musculature as a functional unit, where a normal muscular activity regulates the formation as well as the destruction of the bone component by way of the vascular system. GILL's (1944) observation that a long-term plastering of an extremity in children can lead to premature ossification of the epiphysial cartilaginous plates involved led GEISER (1957) to try to verify this experimentally. Plastering one hind leg or partially excising the Achilles tendon in a growing rabbit resulted in retarded growth in length, caused by premature ossification of growth plates, and pronounced osteoporosis. GEISER suggests, as did TRUETA (1964) and VALDERRAMA & TRUETA (1965)

later with more emphasis, that the balance in the osteogenetic function is upset by disturbance of the muscular pump activity on the venous backflow from the skeleton, whereby the bone destruction will predominate and ossification of the cartilaginous growth plate will be accelerated. Faradic stimulation of the plastered musculature considerably inhibited the mentioned effect of the immobilization, and the removal of the plaster was followed by a partial reconstruction of the skeletal part involved. However, GEISER & TRUETA earlier (1958), referring to identical experiments, maintained that "the mechanical pressure forces produced by muscle action to counteract gravity seem to be a most important factor for the maintenance of bone structure."

HEANEY (1962) stated that severe paralytic immobilization in an early phase shows signs of normal or increased bone formation rate and increased bone resorption rate. A second phase replaces this with normal or subnormal activity in both respects. LANDRY & FLEISH (1964) and KHARMOSH & SAVILLE (1965) have published experimental results that also indicate a higher activity ratio in an immobilized skeletal part during the first 2 to 4 weeks and a subnormal one in a later phase. The bone resorption, however, is predominant.

Changes in the vascular component in the bone-muscle complex, besides disturbed haemodynamic conditions here at immobilization, are illustrated in various ways.

Direct blood flow measurements indicate that an immobilization of an extremity leads to an increased arterial blood flow in it (IMIG *et al.* 1953), at least initially (KEMP *et al.* 1947). HULTH & OLERUD (1960) noticed, by angiography, signs of increased blood flow together with a dilatation of the large vascular trunks with a tendency to increased tortuosity; microangiographic investigation disclosed hypervascularization of muscle fasciae and of the connective tissue in the distal part of the extremity (HULTH & OLERUD 1961). The venous blood flow in a plaster-bandaged limb is considered to be increased in more than half of the cases (SCHRÖDER & SEYFARTH 1960).

GEISER (1957) and GEISER & TRUETA (1958) establish with the aid of injection methods that, at immobilization, a hypervascularization of the skeletal part involved occurs in conjunction with bone destruction, but that bone formation too is accompanied by increased vascular activity. Investigations of intramedullary blood disclose changes in its acid-base status (HULTH & SEMB 1966, SEMB 1966 a) as well as increased values of HbO_2 and pO_2 (SEMB 1966 b) after about 10 days immobilization, which is interpreted as signs of increased blood flow here. Blood flow measurement with the aid of plasma clearance of Sr^{85} by bone also indicates increased intra-osseous blood flow, but not until after about two weeks of immobilization (SEMB 1966 c).

HULTH & WESTERBORN (1963), however, judging by reduced uptake of S^{35} in the epiphysial cartilaginous plate, suppose that the nutritive circulation in the metaphysis is reduced during the first days of an immobilization, and SHIM (1966), using bone clearance of radiostrontium, notes a reduced flow in tibia and calcaneus in rabbit after two weeks of plaster immobilization. Prolonged immobilization, however, leads to a relative increase in the blood flow.

SCHEDEL (1958), using oscillography, found in *homo* a circulation less than normal in a plastered extremity and angiographic investigation disclosed a narrowing of arteries and veins. HULTÉN's (1951) investigations also indicated a reduced flow, but mainly in the skin vessels.

C. GENERAL SURVEY OF ACCELERATED BONE GROWTH

For clarity, a schematic list is given of examples found in the literature relating to accelerated bone growth and of theories advanced concerning the underlying mechanism.

A. Stimulation of longitudinal bone growth by direct measures

Röntgen BAUNACH (1935)

Heat WILSON & THOMSON (1939) BERTRAND & TRILLAT (1948) RICHARDS & STOFER (1959) DOYLE & SMART (1963)

Trauma to the medullary cavity FERGUSON (1933) KISHIKAWA (1936) HUTCHISON & BURDEAUX (1954)

Application of various materials in the medullary cavity VON LANGENBECK (1869) MEISENBACH (1910) KÖNIGSWIESER (1925) BERGMANN (1931) KISHIKAWA (1936) PEASE (1952) TRUETA (1953, 1958) CARPENTER & DALTON (1956) WILSON & PERCY (1956) NORDENTOFT & GULDHAMMER (1964)

Periosteal stripping OLLIER (1867) WU & MILTNER (1937) LACROIX (1947) BERTRAND & TRILLAT (1948) ZANOLI (1949) PEASE (1952) BRODIN (1955) LANGENSKIÖLD (1957) TAILLARD (1959) ELO (1960) SOLÁ *et al.* (1963)

Fracture TRUESDELL (1921) BURDICK & SIRIS (1923) COLE (1925) LEVANDER (1929) BISGARD (1936) COMPERE & ADAMS (1937) AITKEN *et al.* (1939) HEDBERG (1945) BERTRAND & TRILLAT (1948) TRUETA (1953) GREVILLE & JANES (1957) EMNÉUS & WIBERG 1958) GOFF (1960) WRAY & GOODMAN (1961) GULDHAMMER (1963)

The stimulated longitudinal bone growth is considered to be due to:

Liberation of growth stimulating substance at periosteal stripping (Lacroix 1947, Taillard 1959) fracture (Wray & Goodman 1961)

Changed circulatory conditions through:

a) *interruption of the metaphysial blood flow* at trauma to the medullary cavity (Ferguson 1933)
b) *hyperaemia* at heat (Richards & Stofer 1959), trauma to and application of various materials in the medullary cavity (Von Langenbeck 1869, Königswieser 1925, Kishikawa 1936, Chapchal & Zeldenrust 1948, Pease 1952, Hansson & Wiberg 1963) fracture (Levander 1929, Bisgard 1936, Hedberg 1945, Bertrand & Trillat 1948)
c) *increased circulation in epiphysial and metaphysial vessels* at trauma to and application of various materials in the medullary cavity (Trueta 1953, Carpenter & Dalton 1956) periosteal stripping (Brodin 1955, Solá *et al.* 1963) fracture (Trueta 1953, Cavadias & Trueta 1965)
d) *venous stasis* at trauma to and application of various materials in the medullary cavity (Hutchison & Burdeaux 1954)

B. Stimulation of longitudinal bone growth by indirect measures

Disturbed innervation

a) motor nerve injury Ring (1961)
b) injury of sympathetic nervous system; pharmacological sympathetic blockade Harris & McDonald (1936) Kishikawa (1936) Barr *et al.* (1950) Gullickson *et al.* (1951) Goetz *et al.* (1955) Kottke *et al.* (1958)
c) sectioning of peripheral nerve Milne Edwards (1860) Ollier (1867) Nasse (1880) Ghillini (1898) Ring (1961)
d) poliomyelitis Seeligmüller (1879) Green & Anderson (1955) Lerique (1956) Ring & Ward (1958) Ratliff (1959)

Disturbed circulation

a) increase of the arterial circulation Dickerson (1966)
b) arterio-venous fistula Broca (1856) Franz (1905) Horton (1932) Harris & McDonald (1936) Janes & Musgrove (1950) Hiertonn (1957, 1961) Doerr & Janes (1959) Kelly *et al.* (1959) Cooley *et al.* (1960) Janes & Jennings (1961) Birnstingl (1962) Vanderhoeft *et al.* (1963) Keck & Kelly (1965)

c) venous stasis HELFERICH (1887) SCHÜLLER (1889) KLIPPEL & TRENAUNAY (1900) BOREL (1922) PEARSE & MORTON (1930) BERGMANN (1931) KISHIKAWA (1936) SERVELLE (1948) HUTCHISON & BURDEAUX (1954) FOSTER & KIRTLEY (1959) COLT & IGER (1963)

Changed static and dynamic conditions

a) reduced pressure OLLIER (1867) MÜLLER (1924) ARKIN & KATZ (1956) RING (1961) BRASHEAR (1963)
b) tension VON LANGENBECK (1869) SMITH & CUNNINGHAM (1957)

The stimulated longitudinal growth is considered to be due to:

Trophic disturbance at injury to the motor innervation (SEELIGMÜLLER 1879)

Reduced pressure on the growth plate at injury to the motor innervation (OLLIER 1867, SEELIGMÜLLER 1879, NASSE 1880, GHILLINI 1897, RING & WARD 1958, RING 1961) at elimination of normal pressure and tension forces and normal muscle activity (OLLIER 1867, MÜLLER 1924, ARKIN & KATZ 1956, RING 1961, BRASHEAR 1963) at tension (VON LANGENBECK 1869, SMITH & CUNNINGHAM 1957)

Increased blood supply to the growth plate through:

a) *shunting of blood* from musculature to skeleton at defective motor innervation (RING & WARD 1958, FANCONI 1959, RING 1961, TROUPP 1961)
b) *arterial hyperaemia* at diversion of an arterial channel into bone (WOODHOUSE 1963) at arterio-venous fistula (JANES & MUSGROVE 1950, HOLMAN 1955, DOERR & JANES 1959, HIERTONN 1961)
c) *vasodilatation* at sympathectomy of pharmacological sympathetic blockade (HARRIS & MCDONALD 1936, KISHIKAWA 1936, GULLICKSON *et al.* 1951, GOETZ *et al.* 1955)
d) *venous stasis* at arterio-venous fistula (BIER 1906, HUTCHISON & BURDEAUX 1954, STEIN *et al.* 1958, FOSTER & KIRTLEY 1959, HILLMAN *et al.* 1959, HIERTONN 1961, BIRNSTINGL 1962, COLT & IGER 1963, VANDERHOEFT & KELLY 1964, KECK & KELLY 1965) venous occlusion (KISHIKAWA 1936, HUTCHISON & BURDEAUX 1954)

D. COMMENTS

An explanation of the sometimes extremely controversial results obtained experimentally in an effort to accelerate the growth in skeletal length can be sought in various methodological errors.

Little notice has usually been paid to the age of the experimental animals and the growth potential associated with it. In many instances, all that has been mentioned is that it was a "young" or a "growing" animal (Table 1). Moreover, some authors express the recorded difference in length in per cent of the length of the whole skeletal part, whereby comparisons with results from experimental animals of other age or other species cannot be made. RING (1961) mentions only the length of the diaphysis, making comparisons with measurements of the entire skeletal part impossible. Several investigators have measured the whole extremity or a part of it (e.g. the foot skeleton). This complicates comparisons with results concerning individual skeletal parts; moreover, the elasticity of a joint could influence the accuracy of the measurements.

The precision of the measuring method is fundamentally important. Several investigators fail to mention their procedure. Except for caliper measurements, the röntgenologic method is the most used. This is sometimes combined with radiopaque markers in the diaphysis, by which the contribution of the individual growth plates to the recorded length difference is determined. Few authors have discussed the size of the error in measurement with this method. LANGENSKIÖLD (1957) gives it as 1 mm, RING (1961) as 0.5 mm, BRODIN (1955) as 0.3 mm. BRODIN, however, bases this margin of error on comparisons with length measurements made directly on the bone with a slide caliper, which, as he himself points out, does not give exact values. ELO (1960) used a modified method, according to Brodin, and estimates the error at 0.1 mm. Length measurements with the aid of calipers are also affected by errors in measurement, which most authors make no comment on. GREVILLE & JANES (1957) are among those who estimated it at 1 mm; the corresponding dimension is given by RING & LEE (1958) as 0.5 mm. RING (1961) considers the figure to be lower, but fails to specify it more closely.

The size of the measurement error must then be seen in relation to the noted effects of various experimental procedures (Table 1).

A contributing cause of the varying results of growth stimulating operations can be the highly varied observation periods. With few exceptions, the investigators in the surveyed literature have made the measurements at the earliest two weeks after the operation, and often several weeks or months after. If the stimulating effect appears at an early stage in the experimental period and is later followed by a period of retarded growth analogous to the findings made concerning the sequelae of poliomyelitis (see above), the time point for the observation can be of essential importance.

Table 1. Experimental attempts to stimulate the longitudinal bone growth. Unless otherwise stated, the experimental procedure was directed towards the investigated skeletal part. When change in growth is expressed in per cent, the comparison has been made with the contralateral side.

Author	Year	Animal (number)	Age	Procedure	Investigated bone	Measuring method	Effect (mm)	Obs. time
BAUNACH	1935	rabbit (20)	young	rtg	femur	rtg	+ 2	1—6 months
PHILLIPS & KIMELDORF	1966	rat (120)	„	„ (whole-body dose)	tibia	„	neg.	2 months
WISE *et. al.*	1949	rat (40)	young	heat	femur	rtg	0 or neg.	2—36 weeks
DE FOREST *et. al.*	1953	rabbit (29)	3—8 weeks	„	tibia	caliper	− 3—26	2—18 weeks
DE FOREST *et. al.*	1953	dog (9)	4—8 months	„	„	„	− 3—24	24—29 weeks
RING & LEE	1958	rabbit (15)	2—3 months	„	ulna	„	0 or neg.	4—31 days
RICHARDS & STOFER	1959	rat (17)	25 days	„	femur	caliper?	+ 6 %	30—40 days
RICHARDS & STOFER	1959	dog (5)	6—8 weeks	„	„	„	+ 2.2 %	30—40 days
DOYLE & SMART	1963	rat (14)	21 days	„	„	caliper	+ 0.2—0.8	50 days
DOYLE & SMART	1963	rat (14)	21 days	„	tibia	„	− 0.2—+ 2.1	50 days
GRANBERRY & JANES	1963	dog (7)	4—5 months	„	femur	rtg	+ 0.15	63 days
GRANBERRY & JANES	1963	dog (7)	4—5 months	„	tibia	„	+ 0.1	63 days
KISHIKAWA	1936	rabbit (?)	young	trauma to medullary cavity (tibia)	hind leg	?	+ 1.77 %	4 weeks
COMPERE & ADAMS	1937	„ (5)	3 weeks	trauma to medullary cavity (femur)	femur + tibia	caliper	0	18—77 days
WU & MILTNER	1937	„ (6)	5—8 weeks	trauma to medullary cavity	tibia	rtg	insignificant	3—6 months
HUTCHISON & BURDEAUX	1954	dog (10)	3 months	„	femur	caliper	+ 0.8 %	16—140 days
HANSSON & WIBERG	1963	rabbit (27)	30 days	„	tibia	„	+ 0.5 (insign.)	5—64 days
LANGENBECK	1869	dog (1)	8 weeks	application of different material in medullary cavity	femur	?	+ 5	3 months
LANGENBECK	1869	„	8 weeks	„	tibia	?	+ 5	3 months
TROUT	1915	rabbit (10)	4—6 weeks	„	„	?	0 or neg.	6 months

Meisenbach	1910	„ (29)	6 weeks	„	„	rtg	pos.	2—12 weeks
Bohlman	1929	guinea-pig (92)	young	„	femur	caliper?	0 or neg.	3 months
Bergmann	1931	dog (2)	6 weeks	„	„	?	— 1	1—4 months
Bergmann	1931	„	6 weeks	„	tibia	?	± 1	1—4 months
Bergmann	1931	rabbit (7)	young	„	femur	?	± 1	6—9 weeks
Bergmann	1931	„	„	„	tibia	?	— 2—+ 1	6—9 weeks
Kishikawa	1936	„ (?)	„	„ (femur + tibia)	hind leg	?	+ 5.07 %	6—16 weeks
Wu & Miltner	1937	„ (10)	5—8 weeks	application of different material in medullary cavity	tibia	rtg	insignificant	1—3 months
Chapchal & Zeldenrust	1948	„ (14)	6—12 weeks	„	femur	„	— 3.5—+ 4	3 months
Herndon & Spencer	1953	„ (10)	3—4 weeks	„	„	caliper	varied	6 months
Wilson & Percy	1956	dog (46)	10—14 weeks	„	tibia	rtg	+ 1—5	1—4 months
Haas	1958	rabbit (6)	growing	„	radius	?	0	?
Ollier	1867	rabbit (3)	young?	periosteal stripping	tibia	?	+ 2—5	3 months
Wu & Miltner	1937	„ (18)	5—8 weeks	„	„	rtg	significant	20 days—5 months
Sousa Pereira	1938	„ (2)	6—8 weeks	„ „	ulna	„	neg.	41 days—6 1/2 months
Lacroix	1947	„ (8)	35 days	„	tibia	„	+ 1—2.3	2—8 months
Bertrand & Trillat	1948	guinea-pig (15)	young		„	?	0—17 %	3 months
Brodin	1955	rabbit (44)	„	„	„	rtg	+ 0.1—0.5[1] + 0.1—0.9[2]	1 week
Langenskiöld	1957	„ (7)	14—36 days	„	„	„	+ 1—3	1—8 weeks
Elo	1960	„ (14)	2—6 weeks	„	„	„	— 2—+ 0.7	3—53 weeks
Solá *et. al.*	1963	dog (37)	3—4 months	„	femur + tibia	caliper	+ 1.6	8—9 months
Levander	1929	rabbit (13)	2—4 months	fracture (tibia)	femur	rtg	+ 5	1—9 months
Bisgard	1936	goat (23)	3 months	„	tibia	„	0—+ 5.5	4—44 weeks

Author	Year	Animal (number)	Age	Procedure	Investigated bone	Measuring method	Effect (mm)	Obs. time
Compere & Adams	1937	rabbit (4)	7 weeks	„	„	„	pos. (insignificant)	5 weeks
Greville & Janes	1957	dog (20)	3—4 months	„	femur	caliper	+ 2.4—14	6—9 months
Wray & Goodman	1961	rat (144)	young	„ (tibia)	„	„	+ 5	3—48 days
Grey & Carr	1915	dog (1)	?	posterior root sectioning	tibia	rtg	0	2 1/2 months
Gillespie	1954	cat (10)	young	„ „ „	femur + tibia	„	0 or neg.	2 months
Ring	1961	rabbit (31)	„	peripheral sensory denervation	tibia	caliper	0	2—16 weeks
Troupp	1961	„ (14)	13—17 days	posterior root sectioning	femur	„	− 6—21 %	1—12 weeks
Troupp	1961	„	13—17 days	„ „ „	tibia	„	0	1—12 weeks
Grey & Carr	1915	dog (1)	?	anterior root sectioning	tibia	rtg	0	4—10 weeks
Gillespie	1954	cat (10)	young	„ „ „	femur + tibia	„	0 or neg.	2 months
Ring	1961	„ (16)	„	„ „ „	tibia	caliper	0—+ 1	3—6 weeks
Ring	1961	dog (21)	„	„ „ „	„	„	0—+ 4	3 months
Troupp	1961	rabbit (19)	13—17 days	„ „ „	femur	„	− 5 %	1—12 weeks
Troupp	1961	„	13—17 days	„ „ „	tibia	„	− 2.5 %	1—12 weeks
Bacq	1930	rat (9)	young	sympathectomy	hind leg	?	0	3 months
Bergmann	1931	dog (3)	„	„	tibia	?	0	1 month
Bisgard	1931	goat (4)	„	„	„	rtg	0	2—6 months
Harris & McDonald	1936	cat (10)	„	„	hind leg	?	0	?
Harris & McDonald	1936	dog (12)	„	„	„	?	0	?
Kishikawa	1936	„ (?)	„	„	„	?	0	?
Gullickson *et. al.*	1951	dog (6)	„	„	„	?	+ 6.5	?
Goetz *et. al.*	1955	rabbit (?)	„	„	hind paw	rtg	+ 4 %	14—80 days
Ring	1961	dog (11)	„	„	tibia	caliper	0—+ 1	3 months
Troupp	1961	rabbit (16)	13—17 days	„	„	„	0	4 weeks
Troupp	1961	„	13—17 days	„	femur	„	0	4 weeks

Milne Edwards	1860	dog (1)	8 months	peripheral nerve sectioning	mandibula	?	pos.	5 weeks
Ollier	1867	cat (3)	3 days	„ „ „	tibia	?	+ 0.5	7 days
Nasse	1880	dog (7)	young	„ „ „	metatarsals	?	pos.	2—8 months
Ghillini	1897	rabbit (?)	2 months	„ „ „	hind leg	?	0	6 weeks
Howell	1917	dog (2)	4 weeks	„ „ „	fore leg	rtg	− 5.5	19 weeks
Allison & Brooks	1921	„ (13)	young	„ „ „	humerus	rtg	neg.	10—200 days
Bergmann	1931	„ (3)	7 weeks	„ „ „	hind leg	?	0	2—10 weeks
Armstrong	1946	rat (8)	27 days	„ „ „	humerus	rtg	− 0.5	1—15 weeks
Armstrong	1946	„	27 days	„ „ „	radius	„	− 1.7	1—15 weeks
Gillespie	1954	„ (16)	young	„ „ „	femur + tibia	„	0	4 weeks
Selye & Bajusz	1958	„ (10)	„	„ „ „	tibia	caliper	0	30 days
Landry & Fleisch	1964	„ (16)	„	„ „ „	femur + tibia	?	0	12 days—5 months
Dickerson	1966	dog (16)	3—4 months	implantation of artery	femur	rtg	0—+ 2	14—120 days
Janes & Musgrove	1950	dog (10)	6 months	a-v fistula (iliac)	femur	?	pos. (90 %)	3—15 months
Janes & Musgrove	1950	„	6 months	„ „ „	tibia	?	pos. (60 %)	3—15 months
Doerr & Janes	1959	„ (15)	4 months	„ „ (femoral)	femur + tibia	?	+ 1—6	14 months
Kelly *et. al.*	1959	„ (12)	3—4 months	„ „ (iliac or femoral)	femur	?	+ 1—5	9—15 months
Kelly *et. al.*	1959	„	3—4 months	„ „ „ „ „	tibia	?	+ 2.5—4	9—15 months
Vanderhoeft *et. al.*	1963	„ (7)	3—4 months	„ „ (femoral)	femur + tibia	?	+ 5	6—18 months
Keck & Kelly	1965	„ (9)	4—6 weeks	„ „ „	femur + tibia	rtg	pos.	6 months
Grey & Carr	1915	dog (1)	?	venous stasis (lig. subclavian vein)	fore leg	rtg	0	2 months
Borel	1922	rabbit (3)	5 weeks	venous stasis (lig. femoral vein)	femur	?	+ 1	3—4 months
Borel	1922	„	5 weeks	„	tibia	?	+ 1—4	3—4 months
Kishikawa	1936	„ (?)	young	„	hind leg	?	+ 1.77 %	4—8 weeks
Servelle	1948	dog (7)	1 month	„	tibia	?	+ 2—8	12—18 months
Dickinson	1953	„ (8)	6 weeks	venous stasis (lig. ext. iliac vein)	hind leg	rtg	0	3—4 months

Author	Year	Animal (number)	Age	Procedure	Investigated bone	Measuring method	Effect (mm)	Obs. time
Hutchison & Burdeaux	1954	dog (11)	3 months	venous stasis (tourniquet)	radius	caliper	+ 1.04 %	18—132 days
Hutchison & Burdeaux	1954	„	3 months	„ „ „	femur	„	+ 0.52 %	25—75 days
Hutchison & Burdeaux	1954	„	3 months	„ „ „	tibia	„	+ 1.45 %	25—75 days
Colt & Iger	1963	„ (10)	young	venous stasis (stenosis of femoral vein)	femur	?	0—+ 2	8—10 months
Colt & Iger	1963	„	„	„	tibia	?	0—+ 1.5	8—10 months
Keck & Kelly	1965	„ (25)	2 1/2—6 months	venous stasis (lig. inf. vena cava + right femoral vein)	femur + tibia	rtg	0	6 months
Müller	1924	dog (3)	young	pressure (resection of radius)	ulna	rtg	neg.	10—70 days
Müller	1928	„ (?)	„	„	femur	„	− 12	2 months
Müller	1928	„	„	„	tibia	„	− 11	2 months
Haas	1945	„ (7)	growing	„	radius	„	− 10	55 days
Gelbke	1951	„ (?)	„	„	femur	„	− 15	19 weeks
Gelbke	1951	dog (?)	growing	tension	apophyses	rtg	0	19 weeks
Smith & Cunningham	1957	calf (7)	„	„	prox. tibia	„	+ 3	30 days
Haas	1958	rabbit (?)	„	„	femur	?	0	?
Haas	1958	„	„	„	tibia	?	0	?
Haas	1917	dog (5)	5—7 weeks	plaster	metacarpals	?	0—+ 1	12—42 days
Allison & Brooks	1921	„ (4)	young	„	humerus	rtg	neg.	10—200 days
Arkin & Katz	1956	rabbit (15)	6—8 weeks	„	tibia	„	+ 3	3—6 weeks
Hulth & Westerborn	1963	„ (34)	young	„	radius	„	− 1	2 hours—20 days
Bergmann	1931	dog (3)	5 weeks	muscle + tendon sectioning (knee)	tibia	?	− 2	2—16 weeks
Ring	1961	rabbit (17)	young	tendon sectioning (ankle)	tibia	caliper	+ 3	3—6 weeks

AVDYUNICHEVA	1964	dog (25)	„	muscle or tendon sectioning	?	?	neg.	?
AVDYUNICHEVA	1964	cat (15)	„	„ „ „ „	?	?	neg.	?
AVDYUNICHEVA	1964	rat (120)	„	„ „ „ „	?	?	neg.	?
OLLIER	1867	rabbit (1)	young	resection of radius	humerus	?	+ 4	5 months
BERGMANN	1931	dog (2)	7 weeks	luxation (hip)	tibia	?	− 1—0	3—5 weeks
BERGMANN	1931	rabbit (2)	8 weeks	„ „	„	?	0	2—5 weeks
BRASHEAR	1963	rat (57)	young	„ (knee)	femur	caliper	− 0.4—+ 0.8	4—92 days
MÜLLER	1924	rat (?)	young	limb beneath abdominal skin	radius-ulna; tibia	rtg	+ 1—2.5 „	1 1/2 months

[1] from the proximal growth plate
[2] from the distal growth plate

As mentioned earlier, big differences of opinion exist concerning the effect of sympathectomy on the longitudinal bone growth. Decisive for the experimental result is, naturally, to what extent the sympathectomy has been completed in the investigated region. Whereas several authors (BERGMANN 1931, KISHIKAWA 1936, GULLICKSON *et al.* 1951, RING 1961, TROUPP 1961) have completely omitted to check the extent of the sympathetic denervation, BACQ (1930), after resection of the lumbosacral sympathetic trunk in rat, carried out post-mortal examination of the operation field and omitted those instances where the intended ganglionectomy had not been complete. However, the rat has a large number of so-called intermediate ganglia distributed in the lower thoracic, lumbar, and upper sacral region (WRETE 1941). These intermediate ganglia are not found in a definite anatomical localization, but sometimes along the ramus communicans, sometimes at the paravertebral ganglion, and sometimes at the spinal nerve. Their preganglionic and postganglionic connexions can vary, and it is possible that a resection of the sympathetic trunk can leave them completely intact, resulting in an incomplete sympathectomy (WRETE 1941, MONRO 1959). Furthermore, skin temperature measurements (BISGARD 1931) sometimes after pre-cooling (GOETZ *et al.* 1955) have been used as a test of sympathetic denervation, as has pilo-erection after exposure to cold (CANNON *et al.* 1929). These methods, however, seem to provide scanty information concerning the degree of sympathetic denervation.

Finally, all results of the various investigations must also be appraised with reference to the size of the examined experimental groups (Table 1).

II. Own investigations

A. PURPOSE OF THE INVESTIGATIONS

The aim was to try to accelerate the longitudinal growth of the tibia in growing rabbit. The author thought it of importance to use here a growth stimulating method whose effect on the bone growth could to as great an extent as possible be analysed concerning the active factors involved. From this standpoint, an indirectly acting stimulation method, one that avoided direct trauma to the bone, was thought most suitable.

Previous investigations of growth disturbances in the skeleton after various nerve injuries indicate a relation with nervous functions; it has also been noted that the bones involved usually show increased growth in the initial phase after poliomyelitis in children. This gave the author the idea of attempting to influence the bone growth by experimental nerve injury—avoiding at the same time local effect on the bone by the experimental

measure—and to record changed growth conditions during the immediate post-operative period.

At first, the effect on the bone growth by sectioning peripheral nerves was studied. As pointed out earlier, this means an interruption of motor, sensory, and postganglionic sympathetic nerve fibres. With the object of trying to analyse the effect on the growth rate of these various components, the author carried out in later experiments a selective motor nerve injury by sectioning anterior roots, a sensory nerve injury by sectioning posterior roots, and an interruption of the sympathetic innervation by sympathetic ganglionectomy.

It has previously been discussed whether injury to motor nerve fibres, besides a disturbed neuromuscular function, also disturbs possible neurotrophic influence on the skeleton. With the object of producing defective muscular function with retained innervation and thereby avoiding this trophic factor, a resection of a part of the Achilles tendon was next carried out.

Because the trauma after the various experimental measures proved in general to have primarily a retarding effect on the skeletal growth, the observations first began on the third post-operative day when this effect seemed to have subsided.

B. MATERIAL—GENERAL

The material consisted of white rabbits aged—with few exceptions—6 weeks ± 2 days. The litter usually lived with the mother animal. At the experiments, neither the weight of the animals, the litter, nor the sex were taken into account (see statistical analysis). Every animal that at ocular examination showed the slightest symptom of illness was excluded from the material. All animals were raised on rabbit pellets and water ad libitum.

C. MEASURING METHOD FOR LONGITUDINAL BONE GROWTH; HISTOTECHNICAL METHOD

In the following experiments, the endochondral growth from the proximal growth plate of the diaphysis towards the metaphysis in the growing rabbit tibia was used as indicator of the longitudinal growth of this bone. The author employed the measuring method described by HULTH & OLERUD (1962), HANSSON (1964); this consists of intravital labelling of cartilage and bone tissue with the aid of tetracycline. Oxytetracycline (Terramycin, Pfizer) was administered intravenously in a dose of 1 mg/kg body weight. With this dosage for rabbit, no toxic effects on cartilage or bone tissue could be observed (HANSSON 1967).

Oxytetracycline was given to the experimental animals in two intravenous injections with a 24-hour interval. The animals were killed by a blow on the neck 10 minutes after the second injection. The proximal parts of the two tibiae were dissected out and fixed in absolute alcohol for 24 hours. The articular surfaces, as well as the cortical bone, with the exception of the cortical layer on the dorsal aspect, were removed from the preparation with a dental saw; the preparation was then embedded in paraffin in the usual way. Resting on the dorsal surfaces of the tibial condyles, it was then mounted on a wooden block in a sliding microtome. A small lateral part of one condyle was cut away sagittally with a knife. The thus produced sectional area was deparaffined and stained superficially with haematoxylin, after which the bone trabeculae in the metaphysis could easily be studied with the aid of a dissection microscope. By levelling the trabeculae parallel to the sectioning plane of the microtome, an oblique cutting of the mentioned trabeculae in their longitudinal extension was avoided. To prevent breaks in the section at the conventional stretching procedure, it was caught on tape according to the RYDBERG (1955) method. The sections, 60 μ thick, were mounted adhering to the tape in Entellan (Merck, Darmstadt), containing xylene (1 : 10) to dissolve the paraffin in the sections. This mounting material, similar to ordinary commercial tape, does not autofluoresce.

The sections were investigated in a Zeiss fluorescence microscope. The UV-light from a high-pressure mercury lamp (Osram, HBO 200) was passed through a Schott UG 1 filter, and the light emitted from the section was filtered through a Schott GG 9 filter.

At the microscopic examination, two fluorescent bands of oxytetracycline could be observed on the metaphysial side of the growth plate. They indicate the endochondral longitudinal growth here during the interval between the two injections, i.e. 24 hours (Fig. 1). The distance between the diaphyseal frontiers of the two lines was measured with a measuring ocular. In order to reduce the possible errors, measurements were made only in those parts of the section where the fluorescent lines formed a right angle to unbroken bone trabeculae. Thus, 15 measurements were made in different sections from one and the same preparation, and the arithmetic mean obtained was used at the later calculation.

Further, the 15 observations from 24 animals were used in a variance component model for estimating the precision of the measuring method. The calculations indicate that the arithmetic mean ± 5 μ is a 95 % confidence interval.

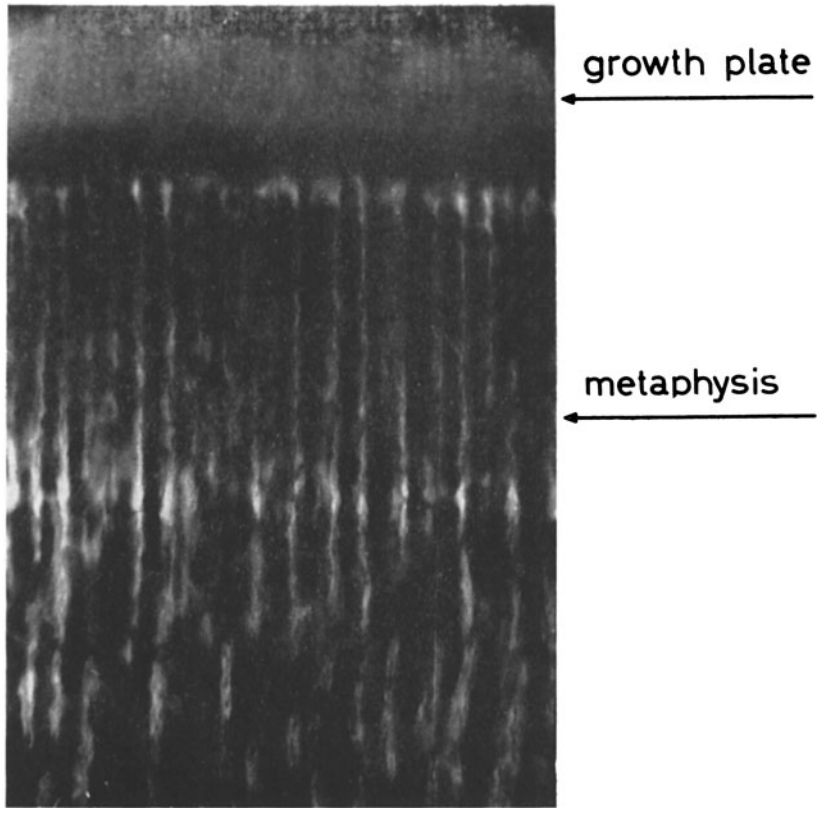

Fig. 1. Fluorescent microphotograph of the proximal tibial metaphysis in growing rabbit. The animal was given 2 injections of oxytetracycline with a 24-hour interval. It was killed 10 minutes after the second injection. The oxytetracycline appears as two fluorescent transversal bands located in the metaphysis. The distance between the diaphysial frontiers of the bands represents the longitudinal growth from the cartilaginous plate towards the metaphysis during the interval between the two injections. Magnification: 79×

D. METHOD FOR CONTROL OF SYMPATHETIC DENERVATION

As indicated above (p. 36) previous experimental works concerning the effect of sympathectomy on bone growth can be criticised as far as the lack of control of the extent of denervation is concerned. The use of a method that reveals an incomplete denervation must be regarded as an absolute necessity. Based on this, the author, after experimental injury to sympathetic nerve fibres, has appraised the degree of denervation by noting the disappearance of noradrenalin from the autonomic groundplexus of the intramedullary blood vessels (Euler & Purkhold 1951).

Noradrenalin, identified as the transmitter substance in the peripheral adrenergic system, and known to occur throughout the entire neuron but stored in especially high concentration in the nerve terminals (see EULER 1946, 1961), can be studied at the cellular level with FALCK & HILLARP's fluorescence method for demonstration of certain biogenic monoamines (FALCK 1962, FALCK *et al.* 1962, CORRODI & HILLARP 1963, 1964). A detailed methodological description has been given by FALCK & OWMAN (1965).

After postganglionic sympathectomy, the noradrenalin disappears owing to a massive leakage from the adrenergic nerves (EULER & PURKHOLD 1951), a process that begins in rat 8—24 hours after axotomy and is thereafter completed in 1—2 hours (MALMFORS & SACHS 1965). The course, interpreted as a consequence of degenerative processes in the nerve fibres, seems to be somewhat slower in other species (KIRPEKAR *et al.* 1962). Despite the extremely high sensitivity of the fluorescence method for demonstrating noradrenalin (FALCK 1962), however, a small amount of the amine can be thought to remain undetected towards the end of the mentioned leakage. If such be the case, this non-visualizable quantity should have disappeared during the course of a further few hours.

After systematic error has been excluded and an adequate time margin allowed, it can thus be established that a total lack of fluorescent noradrenalin product in adrenergic nerves for 24 hours indicates a complete interruption of the functional connexion between the innervation apparatus and the receptors of the effector cells.

The technique used by the author consisted of opening with a dental saw the medullary cavities in the two tibiae immediately after the animal had been killed. With the aid of a dissection microscope, a number of arteries were dissected out from various parts of the two medullary cavities and placed on each side of an engraved marking line on one and the same slide. It was easy to distinguish the arteries from the extremely thin-walled veins. The procedure with one slide ensured that the vessels from the two bones later underwent exactly the same treatment; thereby, the fluorescence intensity was directly comparable.

Another slide covered the preparations, the two slides being pressed together in a small vice. Thus, the tissue pieces were spread into thin membranes. To avoid adhesion between the preparations and the covering slide, a thin plastic separating film (PARAFILM "M", American Can Comp.) was placed here beforehand. The slide with the thus treated blood vessels was dried in a dessicator over phosphorus pentoxide for 1 hour and then exposed to formaldehyde gas from paraformaldehyde ($+80^{\circ}$ C) for 1 hour. After clearing in xylene for 5 minutes, the preparations were mounted in

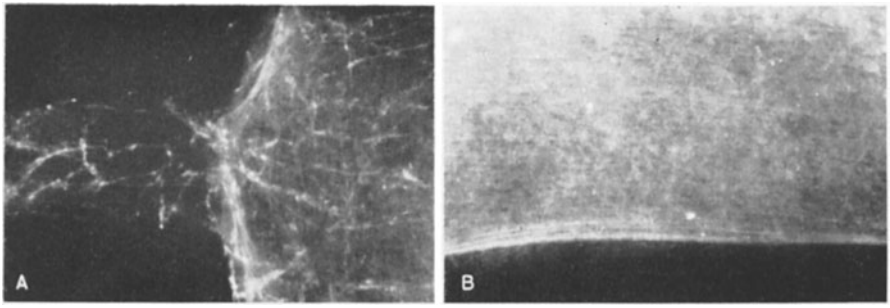

Fig. 2. Fluorescent microphotographs showing whole-mount preparations of tibial intra-medullary arteries treated with formaldehyde. Left-sided lumbosacral sympathectomy 48 hours earlier. The vessel from the control side shows a network of fluorescent terminal nerve fibres (A), whereas the vessel from the sypathectomized bone shows no specific fluorescence (B). Magnification: 165×

Entellan (Merck, Darmstadt). They could then be immediately examined in the fluorescence microscope. The source of light was a high-pressure mercury lamp and the UV-light passed through a Schott BG 12 filter. The light emitted by the preparation was filtered through a Schott OG 4 filter.

A great many previous investigations with the fluorescence method confirm the presumption that the green to yellow-green fluorescent network surrounding the vessels represents adrenergic nerve fibres running in a typical autonomic groundplexus. Fig. 2 shows intramedullary arteries from an animal that had undergone lumbosacral ganglionectomy 48 hours earlier (see page 58). The vessel from the sympathectomized bone shows no specific fluorescence whatsoever (Fig. 2 B), whereas a moderately tight network of fluorescent terminal nerve fibres with its typical varicosities appears on the vessel from the non-operated bone (Fig. 2 A). The width of the meshes, however, depends completely upon how much the vessel has been stretched at the compressing procedure and cannot be considered to reflect normal conditions. A cross-section of a similar freeze-dried vessel is shown in Fig. 3 (for details of the freeze-drying method, see FALCK & OWMAN 1965). The innervation apparatus appears as a superficial structure superimposed upon the media. However, a less complete picture of the innervation apparatus is obtained here compared with the whole-mount preparation.

E. STATISTICAL ANALYSIS

Within each experimental group reported here, there are for every investigation day six observations (six different animals) that indicate the noted

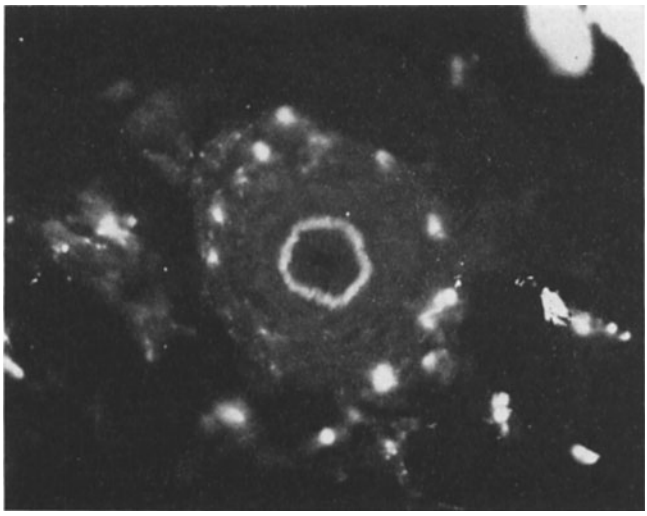

Fig. 3. Fluorescent microphotograph of a tibial intramedullary artery (freeze-dried) treated with formaldehyde. Cross-section (6 μ). The fluorescent innervation apparatus appears as a superficial structure superimposed upon the media. The internal elastic membrane of the artery shows unspecific auto-fluorescence. Magnification: 300×

growth difference between operated and non-operated side expressed in percentage of the non-operated control side. These observations are presumed to come from normal distributions, whose means are a measure of the effect of the experimental method.

The first observations were made in Group A during post-operative days 3, 6, 9, 12, 15, and 18. Six litters of six animals each were used here in the following manner (the animals belonging to one litter were randomly grouped):

Sub-groups	Litter number					
	1	2	3	4	5	6
A : 3	3 animals	3 animals				
A : 6	3 animals		3 animals			
A : 9		3 animals	3 animals			
A : 12				3 animals	3 animals	
A : 15				3 animals[1]		3 animals
A : 18					3 animals	3 animals

[1] One animal from another litter.

Application of t-test, as well as of Aspin-Welsh's test for A : 9, showed no significant difference between litters regarding the experimental effect. Moreover, because it appeared that no variance stability worth mentioning was gained by working in litters, the material was instead grouped in days.

To investigate whether any relation existed between the experimental effects and the original weight of the animals, or between the experimental effects and the relative increase in weight, corresponding correlation coefficients for the various subgroups within the entire Group A were calculated. These were:

Subgroups	Effect *contra* original weight	Effect *contra* rel. weight incr.
3	−0.67	+0.63
4	+0.80	+0.35
5	−0.64	+0.06
6	+0.44	+0.69
7	−0.57	−0.52
8	+0.19	−0.17
9	−0.25	−0.03
12	−0.51	+0.05
15	+0.46	−0.23
18	−0.38	+0.28

If normal distribution presumptions are made and two-sided tests of the hypotheses that the correlations are zero are carried out, no significant deviation is obtained from these hypotheses. Further, if the many sign changes are noted, there is on the basis of the material produced no reason to presume that the effects are to any extent due to the weight of the animals or to their weight changes.

In the continued analysis of the Groups A, B, C, D, and E, normal distributions with a common variance have been presumed. When the subgroups C : 9 and E : 18 show significantly less sample variances, they have been analysed separately. BARTLETT's M-test was performed on the variances of the other subgroups without any significant deviation from the presumption about common variance being observed. The pooled variance estimate is 12.16 with 130 degrees of freedom.

Of the subgroups within the sham-groups (A-, B-, C-, D-, and E-sham) B-sham : 9, D-sham : 6, and E-sham : 3, 9 have been analysed separately. BARTLETT's M-test on the remaining subgroups gives no significance and the pooled variance estimate is 1.76 with 40 degrees of freedom.

Here, the following code is used for significances:

* $1\ \% < P < 5\ \%$ (probably significant)

** $0.1\ \% < P < 1\ \%$ (significant)

*** $P < 0.1\ \%$ (highly significant)

F. EXPERIMENTS

1. Sectioning of peripheral nerves

With the exception of some proximal muscle groups, the skeletal musculature in the hind limbs of rabbit is innervated by the femoral and sciatic nerves, which issue with certain variations from nerve roots of L_5-L_6-L_7 and L_7-S_1-S_2, respectively (KRAUSE 1868, CRAIGIE 1948).

Besides sensory and motor components, such peripheral nerves contain, as mentioned, sympathetic postganglionic fibres.

The vascular nerve supply in the limbs follows two main patterns: a) a nerve supply, direct from the paravertebral sympathetic trunk, to the large vascular trunks in the proximal parts of the limb; b) a hereto connecting innervation to the more peripheral vascular ramifications through sympathetic fibres running in the spinal somatic nerves. Thus, the sympathetic nerves intended for structures distal to the knee are all found in the sciatic nerve (KRAMER & TODD 1914, WOOLLARD 1926, GASK & ROSS 1937, SHIM *et al.* 1966, ZIMMERMAN 1966).

Denervation (Group A)

The animals were anaesthetized for the operation with 6 % Mebumal-natrium (ACO), given intravenously in a dose of 1/2 ml/kg body weight, complemented with ether. After blunt dissection through covering musculature, the sciatic nerve was exposed on the right side where it emerges underneath the piriform muscle, sectioned, and a half-centimetre resected. The right femoral nerve was sectioned and resected similarly, just distal to the inguinal ligament. The skin was sutured with perlon.

During the post-operative course, the longitudinal bone growth was studied in the proximal parts of the tibiae with the aid of tetracycline labelling, as described above. The animals were killed at different times after the operation and were arranged in groups of six (see Table 2). Useful data were obtained from 60 animals.

In 9 different animals (distributed on the third, sixth, and ninth post-operative day) the extent of the postganglionic sympathetic injury caused by the nerve sectioning was examined with the aid of the fluorescence method according to FALCK & HILLARP.

Table 2. (Group A). Longitudinal growth in the proximal tibia in rabbit after peripheral nerve sectioning.

Days after operation	Animal	Non-operated side (μ)	Operated side (μ)	Difference op.— non-op. side (μ)	Difference in % of non-op. side
3	59/2	577.0	659.0	+ 82.0	+ 14.2
3	59/5	544.0	581.0	+ 37.0	+ 6.8
3	59/6	555.0	611.0	+ 56.0	+ 10.1
3	60/2	505.0	581.0	+ 76.0	+ 15.1
3	60/5	549.0	624.0	+ 75.0	+ 13.7
3	60/6	415.0	484.0	+ 69.0	+ 16.6
4	76/2	387.0	429.0	+ 42.0	+ 10.9
4	82/1	460.0	533.0	+ 73.0	+ 15.9
4	98/3	463.0	538.0	+ 75.0	+ 16.2
4	117/1	416.0	469.0	+ 53.0	+ 12.7
4	117/4	458.0	529.0	+ 71.0	+ 15.5
4	210/12	350.4	394.2	+ 43.8	+ 12.5
5	72/1	389.0	453.0	+ 64.0	+ 16.5
5	72/2	375.0	427.0	+ 52.0	+ 13.9
5	72/3	325.0	394.0	+ 69.0	+ 21.2
5	82/2	382.0	432.0	+ 50.0	+ 13.1
5	82/3	375.0	456.0	+ 81.0	+ 21.6
5	82/4	260.0	325.0	+ 65.0	+ 25.0
6	59/1	432.0	534.0	+ 102.0	+ 23.6
6	59/9	432.0	551.0	+ 119.0	+ 27.6
6	59/10	447.0	542.0	+ 95.0	+ 21.3
6	62/3	480.0	601.0	+ 121.0	+ 25.2
6	62/4	508.0	633.0	+ 125.0	+ 24.6
6	62/6	566.0	676.0	+ 110.0	+ 19.4
7	73/2	420.0	489.0	+ 69.0	+ 16.4
7	87/5	382.0	442.0	+ 60.0	+ 15.7
7	97/6	540.0	626.0	+ 86.0	+ 15.9
7	103/1	529.0	622.0	+ 93.0	+ 17.6
7	103/2	503.0	588.0	+ 85.0	+ 16.9
7	103/3	512.0	616.0	+ 104.0	+ 20.3
8	74/1	486.0	540.0	+ 54.0	+ 11.1
8	74/4	406.0	480.0	+ 74.0	+ 18.2
8	86/6	594.0	665.0	+ 71.0	+ 12.0
8	89/3	536.0	620.0	+ 84.0	+ 15.7
8	151/2	383.3	459.9	+ 76.6	+ 20.0
8	151/3	383.3	416.1	+ 32.8	+ 8.6
9	60/3	436.0	503.0	+ 67.0	+ 15.4
9	60/8	436.0	505.0	+ 69.0	+ 15.8
9	60/11	508.0	592.0	+ 84.0	+ 16.5
9	62/1	499.0	549.0	+ 50.0	+ 10.0

Days after operation	Animal	Non-operated side μ	Operated side μ	Difference op.— non-op. side μ	Difference in % of non-op. side
9	62/2	458.0	527.0	+ 69.0	+ 15.1
9	62/5	518.0	596.0	+ 78.0	+ 15.1
12	63/2	529.0	564.0	+ 35.0	+ 6.6
12	63/3	421.0	426.0	+ 5.0	+ 1.2
12	63/5	527.0	568.0	+ 41.0	+ 7.8
12	66/2	559.0	553.0	− 6.0	− 1.1
12	66/4	484.0	505.0	+ 21.0	+ 4.3
12	66/5	551.0	547.0	− 4.0	− 0.7
15	63/4	454.0	480.0	+ 26.0	+ 5.7
15	63/12	490.0	490.0	0.0	0.0
15	64/2	477.0	447.0	− 30.0	− 6.3
15	64/4	467.0	456.0	− 11.0	− 2.4
15	64/5	508.0	529.0	+ 21.0	+ 4.1
15	96/1	562.0	547.0	− 15.0	− 2.7
18	64/3	549.0	529.0	− 20.0	− 3.6
18	64/6	266.0	248.0	− 18.0	− 6.8
18	64/12	583.0	577.0	− 6.0	− 1.0
18	66/3	577.0	572.0	− 5.0	− 0.9
18	66/6	547.0	538.0	− 9.0	− 1.7
18	66/12	529.0	497.0	− 32.0	− 6.1

Sham-operation (Group A-sham)

These animals were anaesthetized in the same manner as those in the previous group. The same nerves were similarly exposed and freed from surrounding structures by careful dissection, but otherwise left intact. The wounds were sutured in a similar manner. The growth was studied after tetracycline labelling. Useful data were obtained from 18 animals (Table 3).

Results

After denervation, muscular control of the knee and ankle joint was lost. The affected limb, whose distal parts frequently showed a moderate oedema, dragged after the body, most often with knee and ankle joint extended. Ulcerations on the dorsal surface of the foot could easily be avoided by a soft layer on the floor of the cage (wood shavings). A gradually increased muscular atrophy of the denervated limb was noted.

The sham-operation restricted only moderately the movements of the operated extremity; this could be observed only during the first post-operative day. Thereafter, no disturbed function could be noted.

Table 3. (Group A-sham). Longitudinal growth in the proximal tibia in rabbit after sham-operation (peripheral nerve sectioning).

Days after operation	Animal	Non-operated side (μ)	Operated side (μ)	Difference op.— non-op. side (μ)	Difference in % of non-op. side
3	121/1	394.0	411.0	+ 17.0	+ 4.3
3	121/2	714.0	725.0	+ 11.0	+ 1.5
3	121/3	676.0	680.0	+ 4.0	+ 0.6
3	121/4	575.0	577.0	+ 2.0	+ 0.4
3	133/2	350.4	367.9	+ 17.5	+ 5.0
3	133/3	441.3	434.7	− 6.6	− 1.5
6	65/4	534.0	547.0	+ 13.0	+ 2.4
6	65/5	439.0	443.0	+ 4.0	+ 0.9
6	119/14	544.0	544.0	0.0	0.0
6	136/1	569.4	569.4	0.0	0.0
6	136/2	569.4	569.4	0.0	0.0
6	136/3	525.6	525.6	0.0	0.0
9	136/4	547.5	547.5	0.0	0.0
9	136/6	449.0	449.0	0.0	0.0
9	137/5	383.3	383.3	0.0	0.0
9	137/6	383.3	383.3	0.0	0.0
9	167/1	449.0	438.0	− 11.0	− 2.5
9	167/2	427.1	427.1	0.0	0.0

Table 4. (Group A). The effect of peripheral nerve sectioning on the longitudinal growth in the proximal tibial metaphysis in rabbit.

Days after operation	Observed length difference in % of the control side. Arithmetic mean ± 2.79	The effect ≠ 0 Significances (2-sided t-tests)
3	+ 12.8	***
4	+ 14.0	***
5	+ 18.6	***[1]
6	+ 23.6	***
7	+ 17.1	***[1]
8	+ 14.3	***
9	+ 14.6	***
12	+ 3.0	*
15	− 0.3	—
18	− 3.4	*

[1] The analysis shows that the effect during the 5th and the 7th day differs from the effect during the 6th day with a significance ** and ***, respectively, at one-sided t-tests, and the significance * and **, respectively, at two-sided t-tests.

Table 5. (Group A-sham). The effect of sham-operation (peripheral nerve sectioning) on the longitudinal growth in the proximal tibial metaphysis in rabbit.

Days after operation	Observed length difference in % of the control side. Arithmetic mean ± 1.09	The effect ≠ 0 Significances (2-sided t-tests)
3	+ 1.7	*
6	+ 0.6	—
9	− 0.4	—

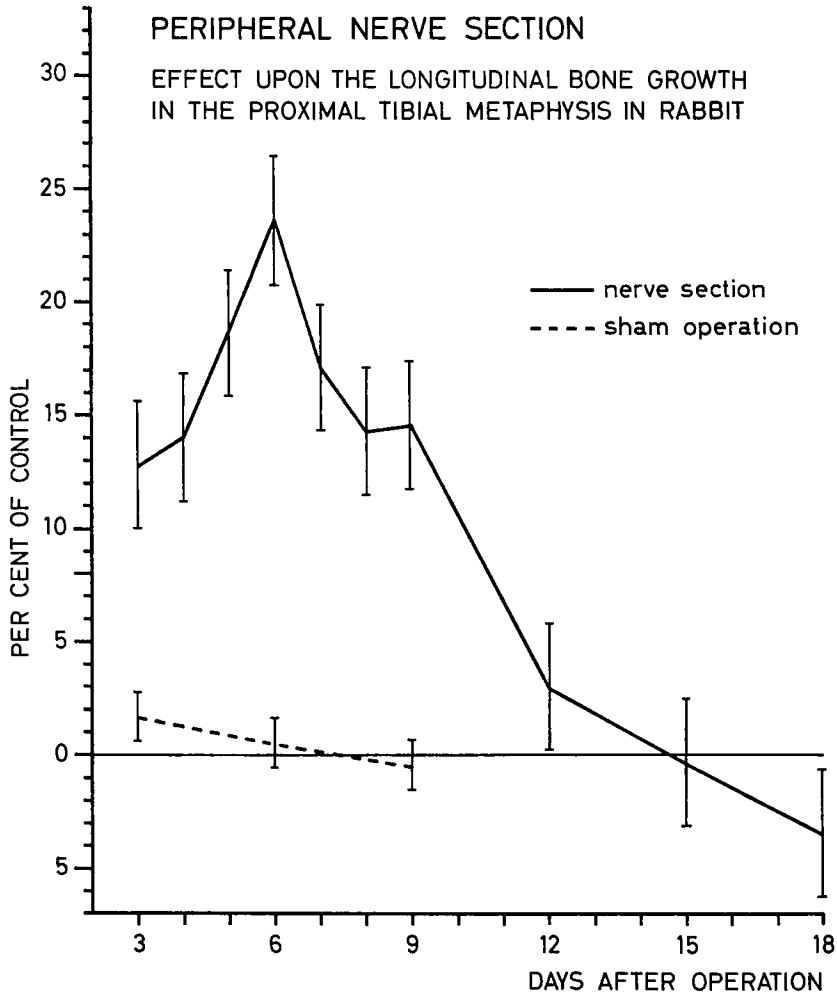

Fig. 4.

Tables 2 and 3 show the longitudinal growth in the investigated tibial region from non-operated (control) and from operated side. The difference between the two sides is expressed in percentage of the control side.

The arithmetic means of the percentage differences of each subgroup of six animals have been collated in Tables 4 and 5, and the degree of significance of the differences between operated and non-operated side has been reported. The results are shown graphically in Fig. 4.

Table 4 shows that the investigated tibial region, after ipsilateral sectioning of the femoral and sciatic nerves (Group A), displays a more rapid longitudinal growth (highly significant) than the corresponding region on the control side during a limited period. The accelerated skeletal growth increases post-operatively up to the sixth day, when it reaches its maximum. During the sixth day, the longitudinal growth on the operated side is more than 20 % bigger than on the control side (Fig. 5 A). After this, the accelerated growth decreases. Between the twelfth and the fifteenth day, there is no longer any side difference, and on the eighteenth day after the nerve sectioning, the longitudinal growth rate on the operated side is slower (probably significant) than on the non-operated control side.

The effects of the sham-operation (Group A-sham) were studied during the post-operative period when the effect in Group A is at its greatest, i.e. between the third and the ninth day (Table 5), (Fig. 5 B). The statistical analysis indicates highly significant differences between Group A and Group A-sham.[2]

Intramedullary blood vessels from the tibia on the denervated side show, in all examined instances, a total lack of fluorescent substance in the autonomic innervation apparatus in contrast to corresponding vessels from the non-operated side, which show a completely normal picture (Fig. 5 C).

2. Sectioning of three anterior nerve roots

In rabbit, the spinal nerves in the caudal parts of the vertebral canal are arranged in the usual fanshaped manner. Thus, the distance between the issuing of the nerve roots decreases in caudal direction. An opening of a certain size created by laminectomy therefore gives access to more roots in a more caudal part than in a more cranial. The anterior nerve roots

[2] Because a probably significant effect of the sham-operation appears during the 3rd post-operative day (A-sham : 3) a separate comparison is made here with the corresponding subgroup after nerve sectioning (A : 3). A t-test with ten degrees of freedom shows that the effect of A : 3 is greater than that of A-sham : 3 ($P < 0.1$ %). For the 6th and 9th day groups, the same conclusion is reached by establishing that the 99.95 % confidence intervals of A : 6 and A-sham : 6, as well as A : 9 and A-sham : 9, do not overlap.

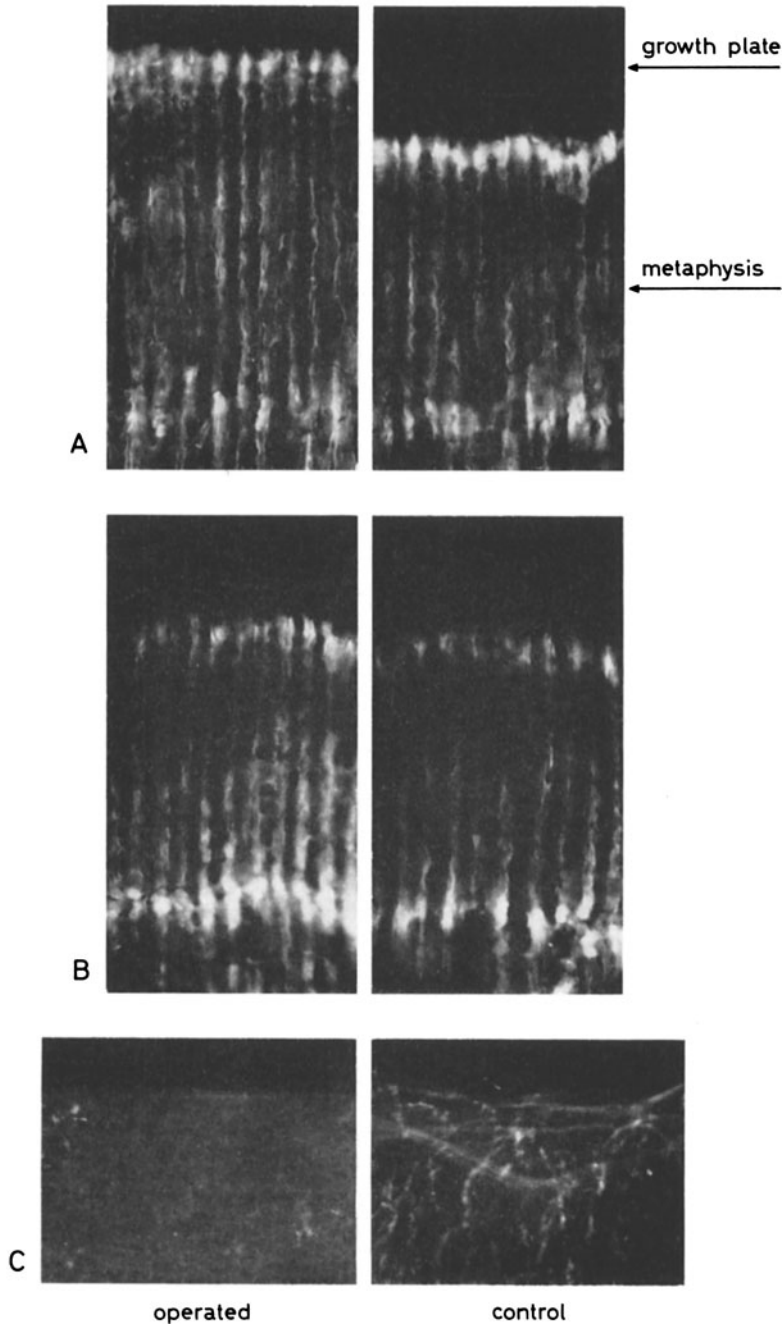

Fig. 5

L_5-L_6-L_7-S_1-S_2 must be sectioned so that the hind extremity in rabbit will to the greatest extent be motor denervated (KRAUSE 1868, CRAIGIE 1948). However, for this, an extensive exposure of the spinal cord is required; the author therefore chose to section only the L_7-S_1-S_2 roots, which for the mentioned topographical reason can be reached with a relatively much smaller operation. A sectioning of these three roots according to TROUPP (1961) results in a definite paresis, which renders the extremity unusable.

Denervation (Group B)

The animals here were anaesthetized in the same way as at the experiments with previous groups. A dorsal midline incision exposed the lumbar spine and adjacent parts of the sacrum; right sided hemilaminectomy of L_6 and L_7 and unroofing of the proximal part of the sacral canal were then performed. One of the biggest problems was the extensive bleeding that ensued. but this could to a large extent be checked by the hindquarters of the animal being raised from the base, thus accentuating the lordosis and reducing the pressure from the abdominal side (TROUPP 1965). In certain instances, Oxycel (Parke-Davis) had to be applied to the bleeding bony surfaces to ensure satisfactory haemostasis.

The three spinal nerves L_7-S_1-S_2 on the right side were lifted up by means of loose ligatures. After the dura had been opened, the anterior roots of the nerves were sectioned. The wound was sprayed with Polybacin (Astra) and then sutured in two layers with perlon.

The growth conditions in the proximal tibiae were studied post-operatively after tetracycline labelling. Useful data were obtained from 18 animals, arranged in groups of 6 (Table 6).

The noradrenalin content in the autonomic innervation apparatus of the intramedullary (tibiae) blood vessels was also examined in these 18 animals with the aid of the fluorescence method.

Fig. 5. Fluorescent microphotographs. Peripheral nerve section, 6th postoperative day. Oxytetracycline-labelling discloses the accelerated longitudinal growth in the proximal tibial metaphysis on operated side compared with control side after right-sided femoral and sciatic nerve sectioning (A). No such effect can be seen after corresponding sham operation (B). Whole-mount preparations of tibial intramedullary arteries, treated with formaldehyde, are depicted in C. The vessel from the operated side shows a total lack of specific fluorescence in contrast to the vessel from the control side, where a network of fluorescent terminal nerve fibres can be seen. Magnification: A, B 76×; C 190×

Table 6. (Group B). Longitudinal growth in the proximal tibia in rabbit after sectioning of three anterior nerve roots (L_7-S_1-S_2).

Days after operation	Animal	Non-operated side (μ)	Operated side (μ)	Difference op.— non-op. side (μ)	Difference in % of non-op. side
3	226/1	318.0	335.0	+ 17.0	+ 5.4
3	226/2	383.0	441.0	+ 58.0	+ 15.1
3	226/6	329.0	361.0	+ 32.0	+ 9.7
3	227/1	383.0	416.0	+ 33.0	+ 8.6
3	227/2	383.0	394.0	+ 11.0	+ 2.9
3	229/2	405.0	445.0	+ 40.0	+ 9.9
6	222/4	380.0	493.0	+ 113.0	+ 29.7
6	222/5	372.0	438.0	+ 66.0	+ 17.7
6	225/1	405.0	493.0	+ 88.0	+ 21.7
6	225/2	438.0	530.0	+ 92.0	+ 21.0
6	225/4	438.0	537.0	+ 99.0	+ 22.6
6	225/6	438.0	542.0	+ 104.0	+ 23.7
9	158/5	449.0	525.6	+ 76.6	+ 17.1
9	222/1	383.0	493.0	+ 110.0	+ 28.7
9	222/2	438.0	493.0	+ 55.0	+ 12.6
9	227/6	383.0	452.0	+ 69.0	+ 18.0
9	229/3	361.0	438.0	+ 77.0	+ 21.3
9	229/14	380.0	427.0	+ 47.0	+ 12.4

Sham-operation (Group B-sham)

With the animals under anaesthesia as mentioned, the vertebral canal was exposed and opened, as described above. The spinal nerves involved were lifted up and the dura was opened. This procedure was carried out with the utmost care in order to avoid trauma to the nerve fibres. The nerve roots were otherwise completely untouched, and the wound was sutured as in the previous group.

However, a number of animals post-operatively showed various degrees of paresis in the right hind leg; these were excluded from the material, which included only those where the motility in the hind extremities seemed of equal value.

The longitudinal bone growth in the proximal tibiae was observed post-operatively after tetracycline labelling in 18 animals (Table 7).

Results

Sectioning of the three anterior nerve roots caused a degree of paresis that prevented the animals from using the denervated limb. This was dragged

Table 7. (Group B-sham). Longitudinal growth in the proximal tibia in rabbit after sham-operation (sectioning of three nerve roots).

Days after operation	Animal	Non-operated side (μ)	Operated side (μ)	Difference op.— non-op. side (μ)	Difference in % of non-op. side
3	232/2	482.0	482.0	0.0	0.0
3	232/3	466.5	462.1	− 4.4	− 0.9
3	235/1	460.0	460.0	0.0	0.0
3	235/3	526.0	526.0	0.0	0.0
3	235/4	515.0	526.0	+ 11.0	+ 2.1
3	241/3	449.0	449.0	0.0	0.0
6	231/3	383.0	383.0	0.0	0.0
6	231/2	526.0	518.0	− 8.0	− 1.5
6	233/1	481.8	481.8	0.0	0.0
6	233/2	515.0	507.0	− 8.0	− 1.6
6	234/1	449.0	449.0	0.0	0.0
6	234/4	493.0	493.0	0.0	0.0
9	231/5	449.0	449.0	0.0	0.0
9	231/6	383.0	383.0	0.0	0.0
9	233/3	504.0	504.0	0.0	0.0
9	233/4	493.0	493.0	0.0	0.0
9	233/6	493.0	493.0	0.0	0.0
9	234/6	471.0	471.0	0.0	0.0

after the body in a manner similar to that after peripheral nerve sectioning and often showed an oedema distally. An increasing muscular atrophy was noted during the observation time.

The sham-operated animals showed the same degree of activity in both hind extremities.

The longitudinal growth in the proximal parts of the tibiae was studied

Table 8. (Group B). The effect of sectioning of three anterior nerve roots (L_7-S_1-S_2) on the longitudinal growth in the proximal tibial metaphysis in rabbit

Days after operation	Observed length difference in % of the control side. Arithmetic mean ± 2.79	The effect ≠ 0 Significances (2-sided t-tests)
3	+ 8.6	***[1]
6	+ 22.7	***
9	+ 18.4	***[1]

[1] The effect during the 3rd and the 9th day differs from the effect during the 6th day with the significance *** and *, respectively, at one-sided as well as at two-sided t-tests.

Table 9. (Group B-sham). The effect of sham-operation (sectioning of three nerve roots) on the longitudinal growth in the proximal tibial metaphysis in rabbit,

Days after operation	Observed length difference in % of the control side. Arithmetic mean ± 1.09	The effect ≠ 0 Significances (2-sided t-tests)
3	+ 0.2	—
6	− 0.5	—
9	0.0[1]	no analysis possible

[1] Mean value cannot be given.

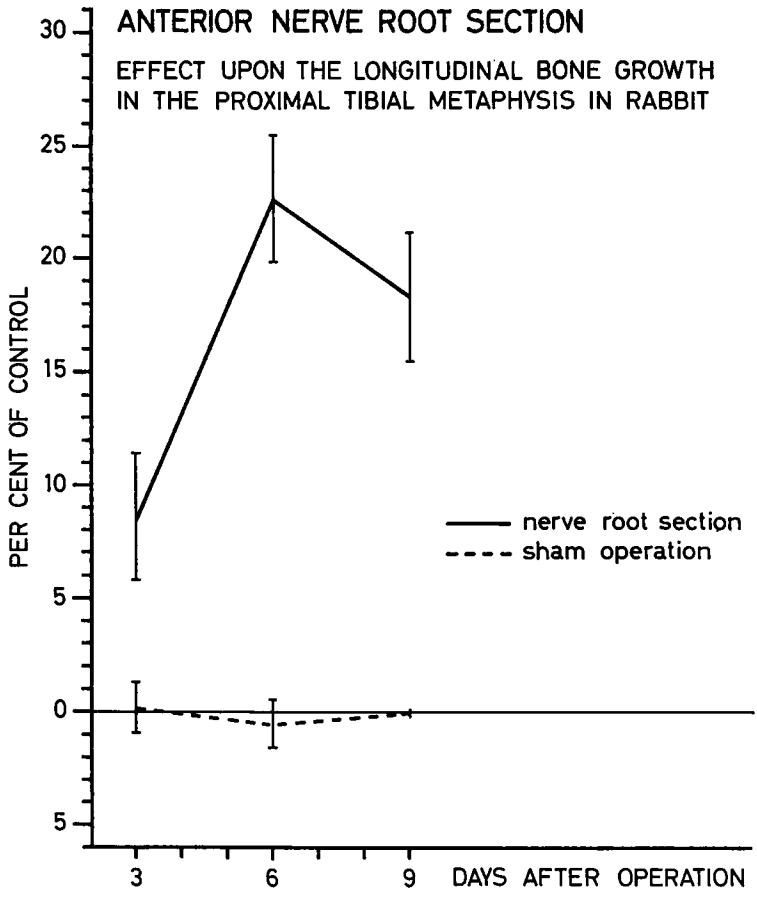

Fig. 6.

during the third, the sixth, and the ninth post-operative day, because the most marked changes in growth after peripheral nerve sectioning (Group A) were observed during this very period. Tables 6 and 7 show the differences in longitudinal growth in the two bones. The difference is given in percentage of the non-operated control side.

The arithmetic means of the percentage differences of each subgroup of 6 animals are collated in Tables 8 and 9, which report the degree of significance in the differences between operated and non-operated side. The results are presented graphically in Fig. 6.

Table 8 shows that the longitudinal growth in the proximal tibia after unilateral sectioning of the anterior nerve roots L_7-S_1-S_2 (Group B) is greater on the operated side than on the non-operated; this is highly significant. The most accelerated growth is demonstrated during the sixth post-operative day, when a difference of more than 20 % between the two sides is found. Highly significant and probably significant lower values, respectively, are noted during the third and the ninth days.

The effects of the sham-operation (Group B-sham) are quite insignificant (Table 9).

The statistical analysis shows that the effects in Group B are greater than those in Group B-sham with *** significance.[3]

Intramedullary blood vessels from all 18 animals in Group B show uniform noradrenalin fluorescence in both denervated and non-denervated tibia.

3. Sectioning of three posterior nerve roots

With the same motivation as in the previous section, the posterior nerve roots L_7-S_1-S_2 were chosen for sectioning.

Denervation (Group C)

The same technique for exposing the spinal nerves was used for this group as for Group B (see above) with the same kind of anaesthesia. After the dura had been opened, the mentioned posterior roots on the right side were sectioned proximal to the spinal ganglion. Spraying with Polybacin and suture were as above.

The longitudinal bone growth in the proximal tibial metaphyses was measured after tetracycline labelling.

The occurrence of fluorescent noradrenalin product in the autonomic groundplexus of the intramedullary (tibia) vessels was checked on both operated and non-operated side.

Useful data were obtained from 18 animals in this group (Table 10).

[3] The 99.95 % confidence intervals of the subgroups B : 3 and B-sham : 3, B : 6 and B-sham : 6, and B : 9 and B-sham : 9 do not overlap.

Table 10. (Group C). Longitudinal growth in the proximal tibia in rabbit after sectioning of three posterior nerve roots (L_7-S_1-S_2).

Days after operation	Animal	Non-operated side (μ)	Operated side (μ)	Difference op.— non-op. side (μ)	Difference in % of non-op. side
3	236/2	481.8	492.8	+ 11.0	+ 2.3
3	241/6	383.3	405.2	+ 21.9	+ 5.7
3	246/1	525.6	536.6	+ 11.0	+ 2.1
3	246/2	438.0	427.0	− 11.0	− 2.5
3	246/6	438.0	449.0	+ 11.0	+ 2.5
3	246/13	438.0	438.0	0.0	0.0
6	230/1	372.0	372.0	0.0	0.0
6	230/2	427.0	438.0	+ 11.0	+ 2.6
6	230/3	372.0	378.0	+ 6.0	+ 1.6
6	230/4	372.0	383.0	+ 11.0	+ 3.0
6	230/5	340.0	372.0	+ 32.0	+ 9.4
6	230/6	383.0	383.0	0.0	0.0
9	241/12	460.0	460.0	0.0	0.0
9	242/3	492.8	492.8	0.0	0.0
9	242/12	503.7	503.7	0.0	0.0
9	244/1	400.8	399.7	− 1.1	− 0.3
9	244/2	405.2	405.2	0.0	0.0
9	244/13	481.8	481.8	0.0	0.0

Sham-operation (Group B-sham)

Because this operation is identical with the sham-operation reported under 2: b, this animal group (Group B-sham, Table 7) was used as comparison material also for this experimental series.

Results

After unilateral sensory root sectioning, no positive side difference could be observed post-operatively regarding the motility in the hind limbs when the animals were allowed freedom of movement. However, when held up by the nape, the animals showed the same phenomenon as reported by TROUPP (1961): a tendency for the denervated extremity to sink downwards, possibly an expression of non-response by the positional sense. No positive muscular atrophy could be noted.

The differences in growth in length in the proximal tibiae in denervated (Group C) and in sham-operated (Group B-sham) animals were measured with the tetracycline method—shown in Tables 10 and 7, respectively. The observations were made during the third, sixth, and ninth post-operative

Table 11. (Group C). The effect of sectioning of three posterior nerve roots (L_7-S_1-S_2) on the longitudinal growth in the proximal tibial metaphysis in rabbit.

Days after operation	Observed length difference in % of the control side. Arithmetic mean	The effect ≠ 0 Significances (2-sided t-tests)
3	+ 1.7 ± 2.79	—
6	+ 2.8 ± 2.79	*
9	0.0 ± 0.13[1]	—

[1] Individual analysis. 5 degrees of freedom.

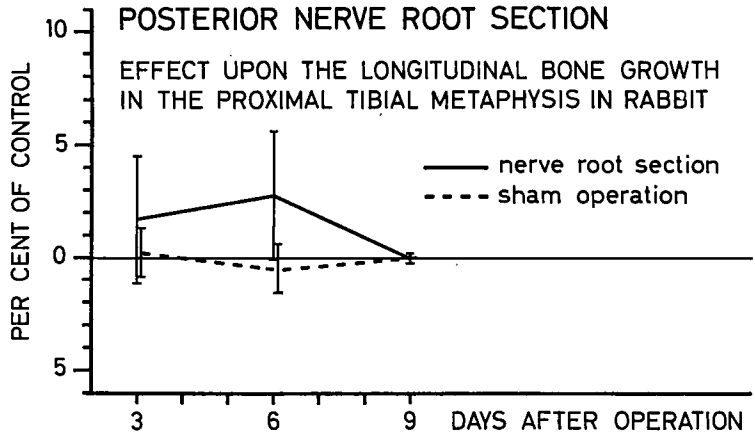

Fig. 7.

days, and as earlier, the differences are reported in percentage of non-operated control side.

The arithmetic means of each subgroup are shown in Tables 11 and 9, where the significance levels of the differences are also given. The results are presented graphically in Fig. 7.

Table 11 shows that the observed effect during the sixth post-operative day is on the verge of being non-significant.

At comparison of the subgroups in Group C and Group B-sham,[4] no significant difference could be established.

The tibial intramedullary blood vessels from the denervated as well as from the normal extremity show no differences in the amount of transmitter substance at fluorescence microscopy.

[4] The effects in Group B-sham are not significant.

4. Sympathectomy

The lumbosacral sympathetic trunks in rabbit are situated between the dorsal body wall and the abdominal aorta as well as between the dorsal body wall and the median sacral artery. The lumbar parts are situated close together and each contains seven ganglions. The ganglions on the two sides are separated only by the lumbar vessels. The sacral parts include four ganglions situated next to corresponding sacral foramina. Here, on the ventral surface of the sacrum, the ganglions of the two sides are well separated. The left sympathetic trunk is easier to reach (KRAUSE 1868, CRAIGIE 1948).

The author has found only one piece of information (GOETZ *et al.* 1955) regarding the postganglionic outflow from the sympathetic trunk to the lower limb in rabbit. These authors state that, because the lowest postganglionic outflow comes from the L_5 or the L_6 ganglion, the 4th—6th lumbar ganglions should be removed so that the postganglionic connexions to the lumbosacral plexus (L_4 to S_2) will be severed.

At preliminary experiments, attempts were made to verify this statement; left-sided resection of the 4th, 5th, and 6th sympathetic lumbar ganglion was performed on 6 animals (for technical details, see below).[5] Later investigation of tibial intramedullary vessels with the aid of the fluorescence method of FALCK & HILLARP showed in no instances signs of postganglionic denervation of these structures. Arteries from both operated and non-operated side showed a fluorescent network of similar appearance.

Left-sided ganglionectomies of varying extent were next carried out between the levels L_4 and S_2 on 11 animals. The results are reported in Table 12. They indicate that the lowest postganglionic outflow to the medullary cavity in the rabbit tibia occurs from the S_2 ganglion. To obtain complete postganglionic denervation of the medullary vessels, the ganglions between the L_5 and S_2 levels must be removed.

Sympathetic lumbosacral ganglionectomy (Group D)

Under the usual Mebumal-ether anaesthesia, the abdomen was opened by a midline incision. The abdominal viscera was moved over to the right side, and after the abdominal aorta and the inferior vena cava had also been carefully moved in the same direction, the sympathetic trunks could be exposed between the medial borders of the psoas muscles. The ganglions L_5-L_6-L_7-S_1-S_2 on the left side were removed, and after spraying with Polybacin, the abdomen was sutured in two layers with perlon.

[5] Here, as in the following, intervening parts of the sympathetic chain have also been removed.

Table 12. The occurrence of fluorescent noradrenalin product in tibial intramedullary arteries after sympathetic ganglionectomy of varying extent (+ indicates the presence and — the absence of fluorescence).

L_4	+
L_4—L_5	+
L_4—L_6	+
L_4—L_7	+
L_4—S_1	+
L_4—S_2	—
L_5—S_2	—
L_6—S_2	+[1]
L_7—S_2	—[1]
S_1—S_2	+
S_2	+

[1] The irregular localizations of intermediate ganglia in the lumbar region of rabbit (WRETE 1941) could cause the varying occurrence of fluorescence in these cases.

The growth conditions were studied post-operatively in the proximal tibiae after tetracycline labelling. Because earlier literature gives highly divergent information about the duration of a possible circulatory effect by the operation, the observations were extended over a period of 18 days (Table 13).

In all instances, the noradrenalin content was examined in the autonomic groundplexus of the tibial intramedullary arteries with the aid of the fluorescence method.

Useful data were obtained from 36 animals.

Sham-operation (Group D-sham)

With the animals anaesthetized as reported above, the abdomen was opened in a similar manner, the sympathetic trunks were exposed by careful dissection but otherwise left intact, and the abdomen was sutured after Polybacin spraying.

After tetracycline labelling, the longitudinal bone growth was studied in 18 animals (Table 14).

Results

No external differences between the hind limbs could be observed, and except for the first post-operative day, the animals in both experimental groups were normally agile.

The observed longitudinal growth in the proximal tibiae in both groups

Table 13. (Group D). Longitudinal growth in the proximal tibia in rabbit after sympathetic lumbosacral ganglionectomy (ganglion L_5-L_6-L_7-S_1-S_2).

Days after operation	Animal	Non-operated side (μ)	Operated side (μ)	Difference op.— non-op. side (μ)	Difference in % of non-op. side
3	177/1	317.6	295.7	− 21.9	− 6.9
3	177/5	293.5	277.0	− 16.5	− 5.6
3	216/1	328.5	328.5	0.0	0.0
3	216/2	328.5	328.5	0.0	0.0
3	216/3	350.4	350.4	0.0	0.0
3	220/1	391.0	394.0	+ 3.0	+ 0.8
6	182/1	372.3	372.3	0.0	0.0
6	214/2	427.0	438.0	+ 11.0	+ 2.6
6	214/3	438.0	438.0	0.0	0.0
6	215/14	526.0	493.0	− 33.0	− 6.3
6	215/15	504.0	482.0	− 22.0	− 4.4
6	216/4	441.0	449.0	+ 8.0	+ 1.8
9	193/4	401.9	434.7	+ 32.8	+ 8.2
9	215/3	482.0	482.0	0.0	0.0
9	215/4	468.0	449.0	− 19.0	− 4.1
9	215/5	457.0	449.0	− 8.0	− 1.8
9	221/1	493.0	493.0	0.0	0.0
9	221/2	449.0	438.0	− 11.0	− 2.5
12	183/4	405.2	405.2	0.0	0.0
12	196/12	503.7	503.7	0.0	0.0
12	200/2	441.3	449.0	+ 7.7	+ 1.8
12	213/4	485.0	471.0	− 14.0	− 2.9
12	213/5	450.0	471.0	− 21.0	− 4.7
12	218/5	383.0	383.0	0.0	0.0
15	185/6	339.5	339.5	0.0	0.0
15	185/12	416.1	410.6	− 5.5	− 1.3
15	213/6	449.0	452.0	+ 3.0	+ 0.7
15	213/12	438.0	438.0	0.0	0.0
15	219/4	449.0	449.0	0.0	0.0
15	219/5	438.0	449.0	+ 11.0	+ 2.5
18	186/12	438.0	434.7	− 3.3	− 0.8
18	220/3	438.0	449.0	+ 11.0	+ 2.5
18	220/6	438.0	438.0	0.0	0.0
18	223/2	438.0	441.0	+ 3.0	+ 0.7
18	223/4	438.0	442.0	+ 4.0	+ 0.9
18	223/5	464.0	460.0	− 4.0	− 0.9

Table 14. (Group D-sham). Longitudinal growth in the proximal tibia in rabbit after sham-operation (sympathetic lumbosacral ganglionectomy).

Days after operation	Animal	Non-operated side (μ)	Operated side (μ)	Difference op.— non-op. side (μ)	Difference in % of non-op. side
3	212/2	394.2	394.2	0.0	0.0
3	212/3	416.1	416.1	0.0	0.0
3	212/4	339.5	339.5	0.0	0.0
3	212/5	320.8	317.6	− 3.2	− 1.0
3	217/1	359.0	372.0	+ 13.0	+ 3.6
3	229/6	343.0	344.0	+ 1.0	+ 0.3
6	211/3	336.2	334.0	− 2.2	− 0.7
6	211/6	350.4	350.4	0.0	0.0
6	212/1	350.4	350.4	0.0	0.0
6	217/3	405.0	405.0	0.0	0.0
6	217/5	405.0	405.0	0.0	0.0
6	217/6	460.0	460.0	0.0	0.0
9	217/12	471.0	471.0	0.0	0.0
9	217/13	482.0	471.0	− 11.0	− 2.3
9	221/6	471.0	471.0	0.0	0.0
9	228/3	375.0	372.0	− 3.0	− 0.8
9	229/12	438.0	438.0	0.0	0.0
9	229/13	438.0	438.0	0.0	0.0

is reported in Tables 13 and 14, and as earlier, the differences are given in percentage of the non-operated control side (right).

Tables 15 and 16 show, besides the arithmetic means of the percentage differences of each subgroup, also the level of significance of the differences between operated and non-operated side. The results are presented graphically in Fig. 8.

Sympathetic lumbosacral ganglionectomy gives no significant effects (5 % level) during the first 18 post-operative days on longitudinal growth according to Table 15, nor does the corresponding sham-operation (Table 16).

The investigation of the tibial intramedullary arteries in Group D shows in all instances a total lack of fluorescence in the autonomic groundplexus of the arteries on the left ganglionectomized side, whereas corresponding vessels on the right (control) side show a completely normal picture (cf. Fig. 2 on page 41).

5. Immobilization with intact innervation

To produce partial inactivation without affecting the innervation apparatus, sectioning and resection (about 1 cm) of the Achilles tendon were

Table 15. (Group D). The effect of sympathetic lumbosacral ganglionectomy (L_5-L_6-L_7-S_1-S_2) on the longitudinal growth in the proximal tibial metaphysis in rabbit.

Days after operation	Observed length difference in % of the control side. Arithmetic mean ± 2.79	The effect ≠ 0 Significances (2-sided t-tests)
3	− 2.0	—
6	− 1.0	—
9	0.0	—
12	− 1.0	—
15	+ 0.3	—
18	+ 0.4	—

Table 16. (Group D-sham). The effect of sham-operation (sympathetic lumbosacral ganglionectomy) on the longitudinal growth in the proximal tibial metaphysis in rabbit.

Days after operation	Observed length difference in % of the control side. Arithmetic mean	The effect ≠ 0 Significances (2-sided t-tests)
3	+ 0.5 ± 1.09	—
6	− 0.1 ± 0.3[1]	—
9	− 0.5 ± 1.09	—

[1] Individual analysis. 5 degrees of freedom.

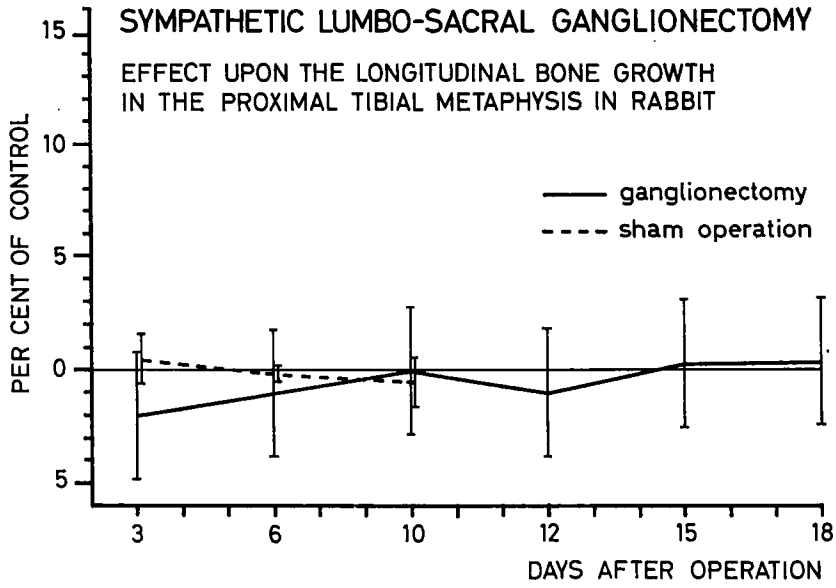

Fig. 8.

chosen. Merely sectioning of the tendon has been found to be insufficient because a good function was again established within one week (cf. GEISER & TRUETA 1958).

Partial resection of the Achilles tendon (Group E)

The animals were anaesthetized in the usual way with Mebumal-ether. After skin incision, the sheath round the triceps surae tendon on the right side was opened and about 1 cm of the tendon was resected just proximal to its insertion on calcaneus. The skin was sutured.

Because earlier investigations (GEISER & TRUETA 1958, LANDRY & FLEISCH 1964) have shown that, despite resection, scar healing of the tendon can occur with returning function of the gastrocnemius muscle after two or three weeks, the observation period for this experimental group was extended to 18 days.

The growth conditions in the proximal tibiae were studied at the appropriate post-operative period after tetracycline labelling in 36 animals (Table 17).

Sham-operation (Group E-sham)

This operation was performed in exactly the same way as the previous ones, except for the sectioning and the resection of the Achilles tendon, which was left untouched after being exposed by a 1 cm long incision in its sheath.

The skeletal growth in 18 animals thus treated was studied in the usual manner with the tetracycline method (Table 18).

Results

The tendon resection caused a considerable inactivation of the limb involved during the first ten to twelve post-operative days. The paw was kept dorsally flexed, and the hind leg was not used when the animal moved. No extension contracture of the ankle joint (HULTH & OLERUD 1960) could be observed. Towards the end of the second week, the animal usually began to use the tenotomized limb without, however, the mobility being altogether normalized during the eighteen days involved. Post-mortem examination showed in most instances a fibrous cicatrization between the two ends of the severed tendon, and also various degrees of muscular atrophy mainly in the gastrocnemius muscle.

No effect of the sham-operation could be observed after the first post-operative day.

Tables 17 and 18 show the longitudinal growth in the proximal tibial metaphyses of the two experimental groups and the differences are given

Table 17. (Group E). Longitudinal growth in the proximal tibia in rabbit after sectioning and partial resection of the Achilles tendon.

Days after operation	Animal	Non-operated side (μ)	Operated side (μ)	Difference op.— non-op. side (μ)	Difference in % of non-op. side
3	70/1	534.0	579.0	+ 45.0	+ 8.4
3	70/2	486.0	536.0	+ 50.0	+ 10.3
3	114/1	572.0	648.0	+ 76.0	+ 13.3
3	114/3	518.0	605.0	+ 87.0	+ 16.8
3	129/5	484.0	555.0	+ 71.0	+ 14.7
3	202/6	394.2	438.0	+ 43.8	+ 11.1
6	69/3	596.0	665.0	+ 69.0	+ 11.6
6	91/1	389.0	432.0	+ 43.0	+ 11.1
6	91/2	475.0	553.0	+ 78.0	+ 16.4
6	113/6	475.0	562.0	+ 87.0	+ 18.3
6	170/6	481.8	547.5	+ 65.7	+ 13.6
6	174/1	438.0	514.7	+ 76.7	+ 17.5
9	71/1	531.0	553.0	+ 22.0	+ 4.1
9	71/2	505.0	540.0	+ 35.0	+ 6.9
9	71/3	644.0	663.0	+ 19.0	+ 3.0
9	91/5	339.0	352.0	+ 13.0	+ 3.8
9	91/6	529.0	555.0	+ 26.0	+ 4.9
9	91/13	512.0	557.0	+ 45.0	+ 8.8
12	104/1	583.0	590.0	+ 7.0	+ 1.2
12	104/2	594.0	594.0	0.0	0.0
12	104/3	620.0	637.0	+ 17.0	+ 2.7
12	112/3	562.0	605.0	+ 43.0	+ 7.7
12	112/5	553.0	583.0	+ 30.0	+ 5.4
12	167/14	459.9	492.8	+ 32.9	+ 7.2
15	106/4	475.0	454.0	− 21.0	− 4.4
15	106/5	562.0	583.0	+ 21.0	+ 3.7
15	106/12	475.0	518.0	+ 43.0	+ 9.1
15	113/1	570.0	583.0	+ 13.0	+ 2.3
15	166/3	394.2	423.8	+ 29.6	+ 7.5
15	166/4	383.3	394.2	+ 10.9	+ 2.8
18	104/4	583.0	583.0	0.0	0.0
18	104/5	562.0	562.0	0.0	0.0
18	104/6	562.0	562.0	0.0	0.0
18	104/12	583.0	594.0	+ 11.0	+ 1.9
18	113/3	562.0	562.0	0.0	0.0
18	166/5	449.0	449.0	0.0	0.0

Table 18. (Group E-sham). Longitudinal growth in the proximal tibia in rabbit after sham-operation (sectioning and partial resection of the Achilles tendon).

Days after operation	Animal	Non-operated side (μ)	Operated side (μ)	Difference op.— non-op. side (μ)	Difference in % of non-op. side
3	138/5	383.3	383.3	0.0	0.0
3	142/4	383.3	383.3	0.0	0.0
3	142/5	383.3	383.3	0.0	0.0
3	148/2	339.5	339.5	0.0	0.0
3	202/12	459.9	462.1	+ 2.2	+ 0.5
3	206/1	449.0	449.0	0.0	0.0
6	142/1	427.1	427.1	0.0	0.0
6	142/2	416.1	416.1	0.0	0.0
6	142/3	459.9	459.9	0.0	0.0
6	169/5	514.7	503.7	− 11.0	− 2.1
6	169/6	470.9	468.7	− 2.2	− 0.5
6	169/12	533.3	536.6	+ 3.3	+ 0.6
9	167/3	438.0	438.0	0.0	0.0
9	167/4	394.2	392.0	− 2.2	− 0.6
9	168/2	449.0	449.0	0.0	0.0
9	169/2	492.8	492.8	0.0	0.0
9	169/3	492.8	492.8	0.0	0.0
9	170/2	479.6	480.7	+ 1.1	+ 0.2

in the same way as earlier in percentage of the non-operated control side.

The arithmetic means of the percentage growth differences in each subgroup are collated in Tables 19 and 20, which also show the levels of significance of the differences between operated and non-operated side.

The results are presented graphically in Fig. 9.

Table 19 (Group E) shows that the longitudinal growth in the proximal tibial metaphysis on the tenotomized side is (highly significant) more rapid during a limited post-operative period than the growth in the corresponding region on the control side. Maximum growth difference is observed during the sixth post-operative day with lower values (highly significant—two-sided t-test) during the third and the ninth day. An equalization of the differences occurs gradually. During the eighteenth post-operative day, the difference between operated and non-operated side does not differ significantly from zero.

The effects of the sham-operation (Group E-sham) are not significant (Table 20).

The statistical analysis indicates that the effects in Group E are significantly greater than those in Group E-sham.[6]

[6] The 99.9 % confidence intervals of the subgroups E : 3 and E-sham : 3, E : 6 and E-sham : 6, and E : 9 and E-sham : 9 do not overlap.

Table 19. (Group E). The effect of partial resection of the Achilles tendon on the longitudinal growth in the proximal tibial metaphysis in rabbit.

Days after operation	Observed length difference in % of the control side. Arithmetic mean	The effect $\neq 0$ Significances (2-sided t-tests)
3	$+ 12.4 \pm 2.79$	***[1]
6	$+ 14.8 \pm 2.79$	***
9	$+ 5.2 \pm 2.79$	***[1]
12	$+ 4.0 \pm 2.79$	**
15	$+ 3.5 \pm 2.79$	*
18	$+ 0.3 \pm 0.81$[2]	—

[1] The analysis shows that the effect during the 3rd and the 9th day differs from the effect during the 6th day with the significance * and ***, respectively, at the one-sided t-test, and the significance — and ***, respectively, at the two-sided t-test.
[2] Individual analysis. 5 degrees of freedom.

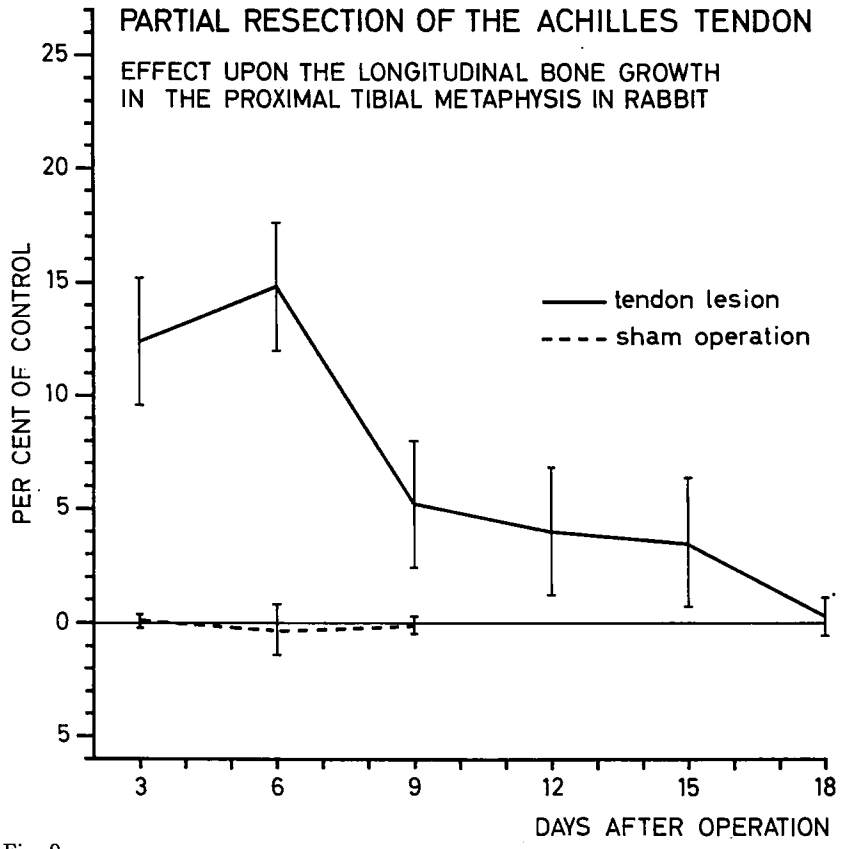

Fig. 9.

Table 20. (Group E-sham). The effect of sham-operation (partial resection of the Achilles tendon) on the longitudinal growth in the proximal tibial metaphysis in rabbit.

Days after operation	Observed length difference in % of the control side. Arithmetic mean	The effect $\neq 0$ Significances (2-sided t-tests)
3	$+ 0.1 \pm 0.22$[1]	—
6	$- 0.3 \pm 1.09$	—
9	$- 0.1 \pm 0.29$[1]	—

[1] Individual analysis. 5 degrees of freedom.

G. SUMMARY

The endochondral growth from the proximal growth plate of the diaphysis towards the metaphysis in growing rabbit tibia was studied with the aid of tetracycline labelling at disturbance of various nervous functions and at partial immobilization with intact innervation.

The results indicate that a *peripheral nerve sectioning* causes a brief (highly significant) initial acceleration of the longitudinal growth in the investigated region. Maximum effect is achieved during the sixth post-operative day; thereafter, the growth stimulation decreases, and during the eighteenth post-operative day, a retarded growth in length (probably significant) occurs on the operated side.

A *partial motor denervation* of the lower extremity by sectioning three anterior nerve roots also results in a changed growth rate. The maximum noted growth acceleration occurs at the same time point as after peripheral nerve sectioning, i.e. during the sixth post-operative day.

Sectioning of corresponding *posterior nerve roots* causes no positive change in the growth in skeletal length.

In the three mentioned experimental groups, the *vascular nerve supply* in the region involved was also studied. Tibial intramedullary arteries were thus investigated regarding the occurrence of fluorescent noradrenalin product in their adrenergic nerves. The results indicate that peripheral nerve sectioning also includes a postganglionic sympathetic denervation in the investigated skeletal region, whereas no such effect could be observed after motor as well as sensory root sectioning.

The *normal postganglionic sympathetic connexions* to the intramedullary arteries of the tibia were investigated with the aid of the same fluorescence method, and guided by these results, an animal group was subjected to *lumbosacral sympathectomy*. No significant effect on the longitudinal growth could be observed.

To judge by the investigations, partial immobilization by *sectioning and resection of the Achilles tendon* accelerates the longitudinal bone growth of the proximal tibia for a short period. During the sixth post-operative day, the effect of the operation reaches its maximum, as occurs after peripheral nerve sectioning and after motor root sectioning.

Chapter 2 Intra-osseous blood flow and its relation to accelerated skeletal growth

I. Earlier investigations

An increased blood flow to the growth zones of a long bone has for a long time been postulated as being one of the most essential factors for stimulating skeletal growth. The object of most growth stimulating procedures has therefore been to try to influence the haemodynamics in bone so as to increase the blood supply to the cartilaginous plates. As mentioned earlier, considerable uncertainty still prevails about the mechanism underlying the increased bone growth, and it has never been made clear whether, or in what manner, the intra-osseous circulation is changed in skeletal parts that have shown accelerated growth.

After a survey of the vascular anatomy of a long bone, a report follows of the various measurement methods applied to skeletal blood flow and the present concept of bone circulation according to skeletal blood flow measurements.

A. THE VASCULAR ANATOMY OF A LONG BONE

LANGER (1876) was the first to demonstrate the essential arterial inflows as well as their intra-osseous ramifications that supply a long bone. These investigations concerned femur and tibia in *homo*. Since then, numerous works have been published concerning the morphology of the intra-osseous vessels in various species.

Thus, the investigation in *homo* was carried out also by *inter al.* LEXER *et al.* (1904), TRUETA & HARRISON (1953), LAING (1953), SÜSSE (1955, 1956), HULTH (1956), STEINBACH *et al.* (1957), HARALDSSON (1959), TRUETA & MORGAN (1960), BROOKES *et al.* (1961); on calf and cow TILLING (1958); on dog, *inter al.*, DRINKER *et al.* (1922), MARNEFFE (1951), PETERSON *et al.* (1957), GAJÓ *et al.* (1963); on rabbit, *inter al.* DOAN (1922), MARNEFFE (1951), BROOKES & HARRISON (1957), BRÅNEMARK (1959), GÖTHMAN (1960), TRUETA & AMATO (1960), TRUETA & MORGAN (1960), ZUCHMAN (1960), LIŠKOVÁ & HERT (1961), GAJÓ *et al.* (1963), TRUETA & CAVADIAS (1964); on rat, *inter al.*, BROOKES (1958, 1965), IRVING (1964), ABDALLA & HARRISON (1966).

Various forms of injection technique, as well as angiography and vital microscopy have been used, and by experimentally eliminating one or more of the inflow channels, attempts have been made to establish the individual importance of these for the skeletal blood supply. Many of the investigations were made on rabbit tibia, but no fundamental differences appeared to exist between different long bones or between different species (TRUETA & MORGAN 1960).

When the reports of the various investigators are summarized, the following general main lines appear for the vascular pattern of a long bone:

Arteria nutritia: besides a main nutrient artery, there can occur a varying number of accessory nutrient arteries, which after entry into the medullary cavity mainly ramify in the periphery of the bone medulla, thus subcortically. During the course to the metaphyses, a rich amount of branches are given off, partly to the medullary tissue where a dense capillary network is formed by extensive anastomosis, partly also to the cortex. Disagreement prevails, however, concerning the vascularization of the cortical tissue of the diaphysis. Whereas many investigators state that this takes place both from medullary and from periosteal vessels, there are others who are of the opinion that periosteal vessels normally contribute with insignificant or no blood whatever to underlying bone.

In the metaphyses, the terminal nutritional ramifications anastomose with metaphysial arteries. Long, hairpin shaped, capillary loops supply the metaphysial side of the growth plate in the growing individual in such a manner that branches from arteria nutritia are responsible for the central 4/5 and metaphysial arteries for the remaining peripheral part.

Periosteal vessels: the periosteum, especially in a growing individual, is considerably vascularized. Thus, several large arteries are found running longitudinally along the bone. After extensive ramifying and anastomozing, they form a dense network of vessels that cover the periosteum. However, this network is also connected with intramuscular arteries. The periosteal network, particularly in the metaphysial regions, establishes a profuse amount of connexions with cortical vascular structures.

At both ends of a long bone, anastomoses of the coarse periosteal arteries occur in such a manner that two circular rings of vessels are formed, wherein branches from the larger soft part arteries around the joints are also included. The larger of the two rings of vessels are found level with the joint line, and branches from this condition the epiphysial arteries. The smaller is situated on the border between growth plate and metaphysis and contributes coarse vessels which, as metaphysial arteries, penetrate the cortex. From anastomoses between the two rings of vessels, the circumferential artery of the growth plate also emanates.

The epiphysial arteries find a way into the epiphysis immediately next to the capsular attachment, and after abundant ramifying and anastomosing, terminal ramifications contact the epiphysial side of the growth plate. No difference between central and peripheral parts, such as is found on the metaphysial side, has been observed here.

The metaphysial arteries divide, after passing through the cortex, into increasingly fine branches that anastomose with the ramifications from arteria nutritia and contribute to the blood supply of the growth plate in the manner mentioned.

Considerable disagreement has existed concerning the occurrence of vascular connexions between epiphysis and metaphysis through the cartilaginous growth plate. It seems that only exceptionally have these been observed. (When, however, the growth has ceased, vessels might cross the scar, thus establishing endosteal anastomoses between the two vascular beds.) Extensive extra-osseous connexions, however, exist via the described anastomosing ring formations of vessels. This "external" anastomosis is most obvious in the adult.

The *venous* blood in the marrow of a long bone collects in structures known as sinusoids. In the metaphysial regions, these have a vertical course which towards the middle of the shaft is increasingly transversal. The sinusoids in the diaphyseal medulla gradually drain into coarser vessels which, fan-shaped, flow together into a usually single central venous channel. A vena nutritia can be regarded as a branch of this structure.

The endosteal parts of the diaphyseal cortex drain their blood into medullary sinusoids or collection veins, whereas the external parts are drained through periosteal veins. There are also venous connexions that directly link together the medullary cavity with the outer surface of the bone. They are rather few in the middle of the shaft, but extremely profuse in the metaphysial regions.

Sinusoids of a similar type as in the medullary cavity of the diaphysis are found in the epiphyses. They are more irregular, however, in their distribution. The venous collecting vessels that sometimes flow together to a central channel drain their contents into veins chiefly corresponding to the arteries, but also often directly into large thin-walled vessels that pass the cortex establishing connexion with surrounding soft-part veins in a way similar to that in the metaphysial regions.

Only the larger extra-osseous veins are furnished with valves. These can often be found precisely at the exit through the cortex.

It can be said in conclusion that a long bone gets its arterial supply from

several directions, where arteria nutritia is only the largest inflow passage. Especially at the two ends of the bone, the relation with arterial structures in surrounding soft tissue is well developed. The venous outflow channels are more abundantly represented than the corresponding arterial structures. This concerns particularly the metaphysial regions in a growing bone, which appear as "a veritable sponge-work of canals, mainly occupied by veins" (MORGAN 1959). The intimate relation between the bone medullary vessels and the deep veins of the extremity has been commented on by many investigators.

B. MEASUREMENTS OF BLOOD FLOW AND HAEMODYNAMIC CHANGES IN BONE

The blood flow in bone belongs to those parts of the circulation physiology where many essential questions, in spite of intensive research, remain unanswered. The physical properties of skeletal tissue have here been the greatest obstacle. Another aggravating circumstance is the occurrence of multiple inflowing and outflowing vessels of which the veins are relatively much more abundant than the arteries and which, moreover, are largely of a very small calibre. Common methods for blood flow measurement could not therefore be applied. The methods used are the following:

1. Skeletal perfusion

One of the first attempts to register the blood flow in bone medulla was made by DRINKER & DRINKER (1916). The tibia from dog was perfused under constant pressure through the nutrient artery; the bone was otherwise completely isolated from the body. The velocity in the outflow from the bone *in toto* was measured by drop recorder. Under these experimental conditions, the authors stated that observed changes in the outflow were to be ascribed to changing vasomotor activities in the bone vessels. A fundamentally similar method was applied in later investigations by DRINKER *et al.* (1922).

CUMMING (1960, 1962) and CUMMING & NUTT (1962) cannulated the nutrient vein of rabbit femur. They mentioned that the outflow here measured by drop recorder represents the flow through the femoral medulla.

The shortcomings of these methods of measurement have been commented on by SHAW (1964). Besides the main nutritional vessels, no regard is paid to other inflow and outflow channels for the blood essential for the bone, i.e. epiphysial, metaphysial, and periosteal vessels, nor to the relation between skeletal blood flow and blood flow in surrounding musculature.

2. Venous occlusion plethysmography

EDHOLM *et al.* (1945) tried by this method to measure the blood flow in the humerus in *homo*. A plethysmograph was put over the distal third of the humerus. An arterial occlusion was applied between the plethysmograph and the proximal occlusive cuff on a level where the main nutrient arteries have an intra-osseous course whereby the occlusion would not affect the flow in these vessels. The proximal occlusive cuff prevented outflow through the main nutrient veins, and the blood that was collected in the soft parts underneath the plethysmograph was considered to represent the entire venous blood volume from the mentioned part of the bone.

This method was later criticised. Apart from the main nutrient arteries, it pays no regard to the other abundant arterial inflow that occurs or to the possibility of a venous outflow via the medullary cavity above the occlusion level (BRAUNSTEINER & GRABNER 1958, MCPHERSON *et al.* 1961, SHAW 1964).

3. Radio-isotopes

TUCKER (1950) was among the first to attempt to estimate the blood flow in bone with the aid of isotopes. He utilized *the bone-seeking property in* P^{32} and carried out studies in rabbit and then in *homo*. The isotope was injected intravenously and after about one hour measurements were made of the emitted beta-radiation from samples of bone. The demonstration of the isotope in the tissue was considered to indicate a functioning circulation, and the method was used for determining the viability in the femoral head. With the same object, the method was later applied by ARDEN & VEALL (1953) and BOYD *et al.* (1955) in animal experiments as well as in *homo*. These investigators, moreover, used a counting probe that made it possible to measure the radiation *in situ*.

A disadvantage of the method was that, at least at investigations in *homo*, only small tissue specimens could be obtained, and these were probably not representative for the whole skeletal region (TUCKER 1950). BOYD *et al.* (1955) for several reasons considered "the concentration of P^{32} in bone is not strictly a measure of circulation."

FREDERICKSON *et al.* (1955) were the first to utilize *the initial short term clearance of a bone-seeking isotope* (Ca^{45}) from blood as a measure of bone blood flow. The same method was used at later blood flow investigations with the aid of different isotopes: P^{32} (DE JONG *et al.* reported in COPP 1957), Ca^{47} (WEINMAN *et al.* 1963, 1964), Sr^{85} (WEINMAN *et al.* 1963, 1964, SHIM 1966, SHIM *et al.* 1966, SEMB 1966 c), F^{18} (VAN DYKE *et al.* 1965). These investigations were carried out in various experimental animals.

FREDRICKSON & co-workers presumed that all tracer atoms were removed

from the skeleton at one single circuit of the blood, and that the clearance value was a measure of the effective blood flow in bone. This has never been proved and has been the object of discussion. VAN DYKE *et al.* (1965) made their calculations on the same presumption, but did not rule out the possibility that the results for this reason could none the less be misleading. According to WEINMAN *et al.* (1963) the calcium and strontium radioisotopes are only incompletely eliminated from the blood of the skeleton; they state that the blood flow values indicate minimum blood flow in bone and should be interpreted as an expression of capillary flow.

Cr^{51}-labelled erythrocytes. Another method of utilizing radioactive isotopes for blood flow measurements was introduced by GRAY & STERLING (1950) and STERLING & GRAY (1950) who in *homo* calculated the circulating blood volume with the aid of Cr^{51}-labelled erythrocytes injected into the circulation. BRODIN (1955) measured the activity from such labelled cells in the tibiae in rabbit. The activity was considered to give a measure of the amount of blood in the investigated region so that a possible difference in blood amount in the two bones could be registered.

Quantitative measures of red cell volumes in various parts of femur and tibia in rat, based upon the dilution of chrome-labelled erythrocytes, have been established by BROOKES (1965).

A more dynamic picture of the blood flow in the skeleton is obtained by registration of the increasing activity in a bone during the initial period after systemic administration of chrome-labelled erythrocytes. WHITE *et al.* (1964), WHITE & STEIN (1965), and STEIN & WHITE (1966) studied rabbit tibia with this method, but SEMB (1966 c) considers the results unreliable because the method involves measurements made during a period of post-ischaemic hyperaemia.

A method based on a similar principle was devised by SAPIRSTEIN (1958). It is valid for many tissues that their content of intravenously administered *radioactive potassium-isotope* or *rubidium-isotope* during the first minutes after the injection is proportionate to the blood flow through the tissue. TROTMAN & KELLY (1963) used Rb^{86} in this way for semi-quantitative determination of skeletal blood flow in dog.

Radio-isotope depot clearance technique. This method was described in 1949 by KETY for the determination of the regional circulation in individual organs. If no significant loss of the depot material occurs via the lymphatic system, the method is believed to give a measure of the effective capillary circulation. The method was later applied for measuring skeletal blood flow with the J^{131}-isotope. PETRAKIS *et al.* (1953) applied the technique on human sternal medulla. The tibial and femoral medulla in rabbit (BRODIN 1955, BROWN-GRANT & CUMMING 1962) as well as the tibial medulla in dog (LOWENSTEIN *et al.* 1958) was investigated in a similar way.

4. Intra-osseous pressure measurement

A survey of the literature shows that LARSEN (1938) was the first to try to relate the intra-osseous pressure to the blood flow in bone. He was followed by many investigators who also used the intra-osseous pressure as indicator of the dynamics in the bone marrow circulation (BLOOMENTHAL *et al.* 1952, PETRAKIS 1954, SÜSSE 1956, STEIN *et al.* 1957, 1958, 1959, HERZIG & ROOT 1959, WEISS & ROOT 1959, DICKERSON & DUTHIE 1963, SHAW 1963, 1964, 1965, AZUMA 1964, CUTHBERTSON *et al.* 1964 a, 1964 b, TRUETA 1964, KECK & KELLY 1965, VALDERRAMA & TRUETA 1965, ARNOLDI & LINDERHOLM 1966). The measuring method is relatively simple; it could be used clinically too. The intra-osseous pressure conditions have been studied under varying circumstances in *homo*, dog, cat, and rabbit. It could throughout be established that the normal intra-osseous pressure is greater than the venous pressure. The average value of the pressure amounts to between one-third and one-fourth of the systemic blood pressure (LARSEN 1938, SHAW 1963, DICKERSON & DUTHIE 1963) but extreme individual variations have been observed (HERZIG & ROOT 1959, AZUMA 1964, CUTHBERTSON *et al.* 1964 a). At measurements in dog (212 bones) CUTHBERTSON *et al.* (1964 a) could demonstrate, moreover, considerable spontaneous pressure fluctuations in one and the same location.

PETRAKIS (1954) as well as ARNOLDI & LINDERHOLM (1966) considers the intra-osseous pressure to follow passively the pressure variations in the draining veins of the bone, whereas SÜSSE (1956) is of the opinion that it reflects the pressure in the venous sinusoids. Other investigators point out that the measurement results are obtained from an artificial blood pool and therefore rather correspond to a pressure situated somewhat between the arterial and the venous (BLOOMENTHAL *et al.* 1952, HERZIG & ROOT 1959, AZUMA 1964).

Uncertainty prevails concerning the extent that the measurement results reflect the intra-osseous blood flow. AZUMA (1964) in support of his perfusion studies in dog reports that a close relation exists between the velocity of the medullary blood flow and the intra-osseous pressure. VALDERRAMA & TRUETA (1965) consider the intra-osseous pressure to give some idea of the outflow of blood from the bone. BRAUNSTEINER & GRABNER (1958) and MCPHERSON *et al.* (1961) at blood flow measurements with thermoelectric method could observe obvious changes in bone blood flow without any appreciable simultaneous change in the intra-osseous pressure; therefore no consistent correlation between them seems to occur according to these authors. Most investigators establish merely that the measured pressure depends on arterial inflow and venous drainage.

5. Thermic methods

Attempts have been made to demonstrate changes in the local skeletal blood flow by registering *the intramedullary temperature* (JANES & MUSGROVE 1950, BRODIN 1955). Measured changes are difficult to interpret because not only blood flow changes but also changes in the local metabolism can be thought to lie behind them (JANES & MUSGROVE 1950). Considerable variations in temperature could also be registered from several points in one and the same medullary cavity (BRODIN 1955).

Another thermic principle introduced as early as 1902 by SHAKESPEARE is based on the fact that heat is transported away from a body warmer than its surroundings. It is considered that the heat transport in a tissue can be influenced by changes in its blood flow (SUZUKI & TUKAHARA 1963). However, no regard is paid here to tissue temperature variations, which can simulate a changed heat transport. This has been shown in experiments by *inter al.* JANSCÓ *et al.* (1965).

The method was further developed in *the heated thermocouple principle* by GIBBS (1933) and later modified by GRAYSON (1952) and HENSEL & RUEF (1954). It consists of one junction of a thermocouple being heated above the tissue temperature, while the reference junction is placed at an appropriate interjacent distance to prevent it being co-heated. Changes in surrounding tissue temperature do not influence the measure results because both junctions of the thermocouple are influenced to the same extent. Changes in the blood flow through the tissue, however, are revealed as change of the heat clearance from the heated junction of the thermocouple, that is to say, as an apparent change in thermal conductivity of surrounding tissue. A survey of the numerous investigations where this method is employed is given by GOLENHOFEN *et al.* (1963).

With the object of increasing the sensitivity in the measuring probe, some investigators have replaced the thermo-element with thermistors coupled in opposition in a Wheatstone bridge (FELIX & GROLL 1953, SHAW 1963, LAWN *et al.* 1964, *inter al.*).

The above-described temperature-compensated flow-velocity probe has been used extensively at blood flow investigations in different tissues. Some investigations involve skeletal blood flow. Among these can be mentioned measurements from human sternal medulla (GRAF & STEIN 1957, BRAUNSTEINER & GRABNER 1957, 1958) and from femoral medulla in cat (MCPHERSON *et al.* 1961, SHAW 1963, 1964, 1965).

There has been animated discussion as to what extent the measured heat clearance depends upon the flow velocity. Various reports contain contradictory information. GRAYSON (1952) thus found a linear relation between heat clearance and flow at perfusion experiments in rabbit and sheep kid-

ney, and sheep spleen. An approximative proportionality was observed by GRAF & ROSSEL (1958) at measurements of skeletal musculature (comparison with simultaneous drop recording), BETZ & HENSEL (1962) at perfusion of brain, BETZ *et al.* (1964) at experiments with kidney (comparison with volume determination of venous outflow and with inulin and PAH clearance). However, other investigators have only at low flow velocities found heat clearance proportionate to the flow through artificially perfused organs (LINZELL 1953, HENSEL & RUEF 1954, GRAF *et al.* 1957). BILL (1962) established also that this linear relation at low flow velocities is found only if the measuring probe is influenced by the flow in vessels that are both small in calibre (as upper limit, 180 μ is given for arteries and 450 μ for veins) and many in number. The importance of the calibre of the vessels here has also been pointed out by BETZ *et al.* (1966).

The measuring method must be regarded as purely qualitative (BILL 1962) but by suitable calibration with the measuring probe *in situ*, it can be converted to a semi-quantitative method (GOLENHOFEN *et al.* 1963) or purely quantitative (BETZ *et al.* 1966).

6. Vital microscopy

BRÅNEMARK (1959) studied the dynamics of skeletal blood flow by intravital microscopy of bone medulla in rabbit fibula. He could measure corpuscular flow velocity in the different vessel sections of the bone marrow as well as in capillaries in adjacent parts of diaphysial bone. This method is the only one that permits the determination of flow direction in the circulation in bone.

7. Non-radioactive blood-borne substances

ZINN & GRIFFITH (1941) injected *carbon particles* into the blood stream of rat. They thought there was some relation between the blood flow and the distribution of the particles in different tissues. Thus, they investigated tibiae histologically after unilateral sympathectomy and observed signs of qualitative difference in the blood flow of the two bones.

LAMAS *et al.* (1946) estimated the circulation in bone medulla in relation to other tissues by injecting *indigo carmine* into the external iliac artery in dog. They calculated the time required for the dye to become visible in skin, in a superficial wound on the paw, and in the bone medulla.

Fluorescent substance. The concentration in bone tissue of a fluorescent substance administered intravenously is measured with this method. In order for the method to reflect to some extent the blood flow, the measurement must be made before the tissue involved has had time to become

saturated with the fluorochrome (BRODIN 1955).

Radiopaque material. The fact that fluid injected into bone marrow leaves it very rapidly and appears in extra-osseous vein trunks has been utilized in various ways. Infusions of different fluids have been given in this manner, because intravenous injection has for technical reasons been difficult or impossible to carry out. Intra-osseous injection of radiopaque material, specially for venography, is used fairly extensively. (For survey, see HULTH 1956.) It has also been possible with this investigatory method to establish a varying drainage velocity of the contrast material from the medullary cavity in different diseases. JERGESEN (1956) and DICKERSON & DUTHIE (1963) determined the clearance velocity of intra-osseously deposited contrast material that would give a quantitative measure of the velocity of the venous outflow from the bone.

C. CONCEPT OF BONE CIRCULATION ACCORDING TO SKELETAL BLOOD FLOW MEASUREMENTS

Many measuring methods are, as can be seen from the above survey, purely qualitative, whereas others, with some reservations, give quantitative information.

High flow velocities could be established in bone tissues. FREDERICKSON *et al.* (1955) found them to be of similar size irrespective of the age of the animals (Ca^{45}-clearance in rat) but WEINMAN *et al.* (1963) observed a more rapid flow in young animals compared with adult (Ca^{47} and Sr^{85}-clearance in dog).

The velocity of the skeletal blood flow in dog is given by WEINMAN *et al.* (1963) as 5.6 ml/min/100 g tissue (adult animals), by SHIM (1966) as 10.2 ml; in both instances calculated on skeletal clearance of isotopes (Ca^{47}, Sr^{85}). Corresponding value for rabbit is 16 ml according to WHITE *et al.* (1964) (initial concentration of chrome-labelled erythrocytes), 9.6 ml according to SHIM (1966), but CUMMING (1962) reports the considerably higher value of 51 ml/min/100 g tissue according to results at venous effluent collection. FREDERICKSON *et al.* (1955) report a flow velocity of 10—30 ml/min/100 g tissue for rat (bone clearance of Ca^{45}). As comparison, it can be mentioned that the velocity of the blood flow in resting skeletal musculature is given as 3.5 ml/min/100 g tissue for *homo* (BARCROFT *et al.* 1952), as 3.4 ml/min/100 g tissue for dog (JONES & BERNE 1964).

The mentioned investigations concern whole skeletal parts. There is little information about the flow velocity in separate parts of a bone. According

to BRÅNEMARK (1959) (vital microscopy) the blood flows in the diaphyseal cortex at a higher velocity than in adjacent medullary parts. Investigations with the aid of chrome-labelled erythrocytes indicate that the arterial inflow is distributed in agreement with the degree of activity in different parts of the bone. In growing rat, this distribution between a metaphysis and a diaphyseal cortex is thus as 3 : 1 (BROOKES 1965). The blood flow to the ends of the bone seems to be significantly larger than to the shaft (bone clearance of Sr^{85}, SHIM 1966). SEMB (1966 c) notes a high velocity (25—30 ml/min/100 g tissue) in the distal radial metaphysis of dog (plasma clearance of Sr^{85} by bone), which according to the author can to some extent be explained by the extensive vascularization in this region. It is, moreover, well known that the venous outflow passages are specially abundantly represented in the ends of a bone, and the high clearance velocity of intra-osseously deposited radiopaque substance observed here indicates the rich flow in these channels (JERGESEN 1956, STEINBACK *et al.* 1957, *inter al.*).

At measurements of skeletal blood flow, it has throughout been possible to observe a reduction of the flow when the arterial inflow through occlusion of the main artery of the extremity or of the nutritional arteries of the bone is reduced. Various results, however, have been observed when the venous outflow from the bone was obstructed by occlusion of analogous venous structures. A decrease of the skeletal blood flow has been registered in some cases (PETRAKIS 1954, SÜSSE 1956, CUTHBERTSON *et al.* 1964 a, TRUETA 1964, VALDERRAMA & TRUETA 1965—intra-osseous pressure measurement, WHITE & STEIN 1965—chrome-labelled erythrocytes), at other investigations, increased blood flow has been observed as a consequence of the mentioned procedure (MCPHERSON *et al.* 1961, SHAW 1963, 1964—intra-osseous pressure measurements; thermo-electric methods).

DRINKER & DRINKER (1916) in their studies of skeletal blood flow reported a vasomotor activity in the intra-osseous vessels. A stimulation of the nerves involved as well as administration of sympathicomimetics resulted in a reduced bone blood flow. Other investigators have in a similar way interpreted observed changes in intra-osseous pressure after sympathetic stimulation as a constriction of medullary arterioles resulting in reduced flow (HERZIG & ROOT 1959, WEISS & ROOT 1959, AZUMA 1964). After sympathectomy, signs of increased skeletal blood flow have also been found by HERZIG & ROOT (1959) by means of intra-osseous pressure measurement; LOWENSTEIN *et al.* (1958) and TROTMAN & KELLY (1963) made the same observation, using radio-isotope methods.

On the other hand, investigations exist where the effect on the skeletal blood flow has completely failed to appear, both after sympathetic stimula-

tion (McPherson *et al.* 1961, Shaw 1963, 1964, Trueta 1964, Valderrama & Trueta 1965—measurement of intramedullary pressure, thermoelectric methods) as well as after sympathectomy (Cuthbertson *et al.* 1964 a—measurement of intramedullary pressure).

Several observations argue that the blood flow to a bone can be changed independent of the flow and pressure conditions in the systemic circulation. This has been mentioned by *inter al.* Petrakis (1954), Braunsteiner & Grabner (1958), Stein *et al.* (1958), McPherson *et al.* (1961), Shaw (1963, 1964), Cuthbertson *et al.* (1964 a), Stein & White (1966). Thus, most investigations indicate that adrenalin injected intravenously in a small dose exerts a locally constricting effect upon the skeletal vessels. The result of this is that the intra-osseous pressure will not follow the arterial systemic pressure and the intra-osseous blood flow will fail to reflect the flow conditions in the surrounding musculature. It has also been possible to establish that, after administration of various vaso-active substances, a marked delay occurs in the normalization of bone blood flow and intra-osseous pressure, compared with the systemic arterial changes (McPherson *et al.* 1961, Shaw 1963, Azuma 1964).

Because of the abundant vascular connexions that exist between a long bone and surrounding soft tissues, considerable efforts have been made to discover the haemodynamic changes in the bone at simultaneous muscular activity. Shaw (1965) is of the opinion that the intramedullary flow passively follows the varying flow in the muscles, whereby the individual muscle contraction increases the bone blood flow by squeezing blood via venous channels into the bone. According to that report, the net effect of repeated contractions is a reduction of the intra-osseous flow, probably as a result of the arterial inflow in the limb being distributed to a larger extent than earlier into muscular arteries because these on account of the muscular work are now dilated and offer less resistance. Trueta (1964) and Valderrama & Trueta (1965) insist that a somewhat different mechanism is involved. Their investigations of intra-osseous pressure changes indicate that the muscle contraction, after an initial short period with reversed flow direction and a venous backflow of blood into the bone, interrupts the venous outflow from the bone with an intra-osseous vascular engorgement resulting in reduced flow. The venous draining of the bone then takes place very rapidly when the contraction has ceased, whereby the relaxing muscles seem to some extent to exert a suction effect upon the intra-osseous vascular bed. Without interpreting their findings in more detail from a flow standpoint, Arnoldi & Linderholm (1966) have registered mainly analogous variations in calcaneous intra-osseous pressure in *homo* at muscle contraction.

D. BONE CIRCULATION STUDIES AT STIMULATED SKELETAL GROWTH

The investigation results in this respect have by no means been unambiguous. A review of these reports was given earlier in connexion with the survey of various growth stimulating methods; the reader is therefore referred to these sections.

II. Own investigations

A. PURPOSE OF THE INVESTIGATIONS

The various flow-measuring methods applied to skeletal blood flow have often involved a direct traumatization of the bone tissue as at perfusion studies, intra-osseous pressure measurements, and measurement made with the aid of thermo-electric methods. A characteristic of the two latter methods is that the measuring results are derived from an artificial blood pool. Common to all measuring methods described is the fact that they permit registration only for a limited number of hours. With some methods (isotope-clearance methods) the time is even shorter. A reliable dynamic picture has never been obtained of the total intra-osseous blood flow under varying experimental conditions. As mentioned earlier, it has never been completely explained how the intra-osseous circulation is possibly changed in skeletal parts that have shown accelerated growth.

The author's intention was to try to solve these problems by evolving a measuring method for continuous registration of skeletal blood flow, by registering with this method the flow at simultaneous muscular activity, and by following the circulatory events in the bone at simultaneous stimulation of the longitudinal growth.

Earlier investigation results have been extremely controversial concerning the effects of a sympathectomy both on bone growth and on blood flow; therefore a group of experimental animals have also been subjected to lumbosacral ganglionectomy with simultaneous continuous registration of the intra-osseous blood flow.

B. MEASURING METHOD

To be able to tackle the actual problems correctly, it was necessary that the method for measuring the skeletal blood flow should fulfil certain criteria. It should permit continuous registration from one and the same tissue part for a considerable time (several weeks). The measurement, moreover,

should—when necessary—be carried out in fully conscious animals, thereby avoiding an unphysiological effect by anaesthesia. At an examination of the available measuring methods, it was found that only the thermo-electric method met the mentioned requirements. This method allows a permanent implantation of the measuring probe, which was earlier done in experimental animals using various types of probes in heart muscle (BETZ & BENZING 1963) and in brain (BETZ & HENSEL 1962, BETZ *et al.* 1966). However, these types of measuring probes, which consist of thermocouples, are hardly suitable for implantation in bone tissue from the aspect of strength as well as of size. The principle of thermo-electric flow measurement can be found on page 76.

1. The measuring probe

At all work with electric measuring apparatus, the occurrence of artifacts, caused by the electronic equipment that comes after the transducer, is directly correlated to the size of the primary signal. Thus, it is of the utmost importance that the measuring probe has the greatest possible sensitivity. Therefore, the two junctions of the thermo-element were exchanged for two thermistors.

Thermistors (NTC-resistances) of type U23UD (Standard Telephones and Cables Ltd.) were used for the purpose. These are unmounted bead thermistors, each formed between two platinum alloy wires (2—3 cm long) where the glazed bead is approximately 400 μ in diameter. They therefore well fulfil the requirement of small dimension. In order to avoid errors caused by currents leaking to surrounding tissue, a low resistance value in the thermistor is to be preferred; the thermistor used has a resistance of 2 kΩ at 20° C. Its resistance *v.* temperature characteristics show a practically linear course (deviation about 2 % for each 10° C). The thermal time constant is equal to 0.8 seconds.

The thermistor bead is extremely brittle; its two platinum leads are even more so. They cannot be implanted in a tissue without protective covering, which is in any event necessary from an electrical insulation aspect. This covering, as well as the insulating coating on the wires, to which the thermistor is soldered, must be of a quality that combines indifference to the tissue with great strength. The end of the probe, where the thermistors are situated, must be able to withstand the rather harsh treatment unavoidable at the application into the bone tissue, and the wires leading away from it are subjected to considerable mechanical strain precisely where they issue from the bone and at the continued passage past joints. Various types of wire were tested. The one that best met the requirements was a

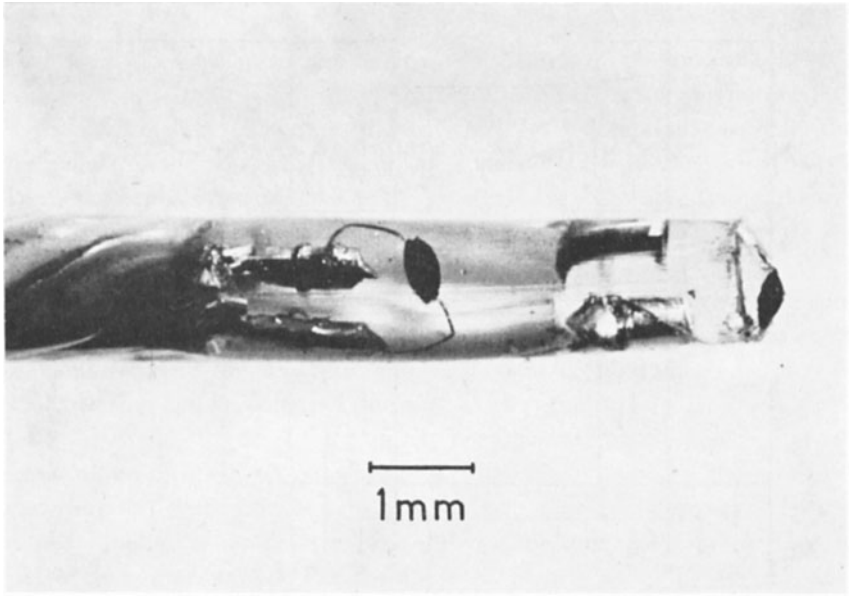

Fig. 10. End of measuring probe with the two thermistors embedded in Araldite.

teflon insulated 7-strand cable, 0.5—0.6 mm in diameter. Four of these were twisted firmly together and by simultaneous subjection to heat (approx. +300° C) the teflon coverings were forced to deform in such a way that the total diameter of the cable amounted to about 1 mm. At one end of the twisted cable, the two thermistors were soldered 3—4 mm apart.

The conventional manner of heating one thermistor is to utilize a separate heating coil. Similar heating is achieved at the use of thermo-elements. To restrict the dimension as far as possible, a different principle was followed here (see below). This meant that further components were not needed in the probe.

The terminal ends of the teflon insulated wires, together with attached thermistors, were bedded into ARALDITE D (CY 230) (Ciba),[1] a casting resin of epoxy-base, which because of its indifference to tissue has gained widespread use in medicine in connexion with chronic implantation. Fig. 10 shows the measuring end of a probe with one of the thermistors (the one that is later heated) superficially localized, thereby establishing good contact with surrounding medium.

[1] ARALDITE D (CY 230) is a solvent-free casting resin, which after the addition of LANCAST A (100:40 parts by weight) hardens (+80° C, 5—8 hours) to a product of good strength.

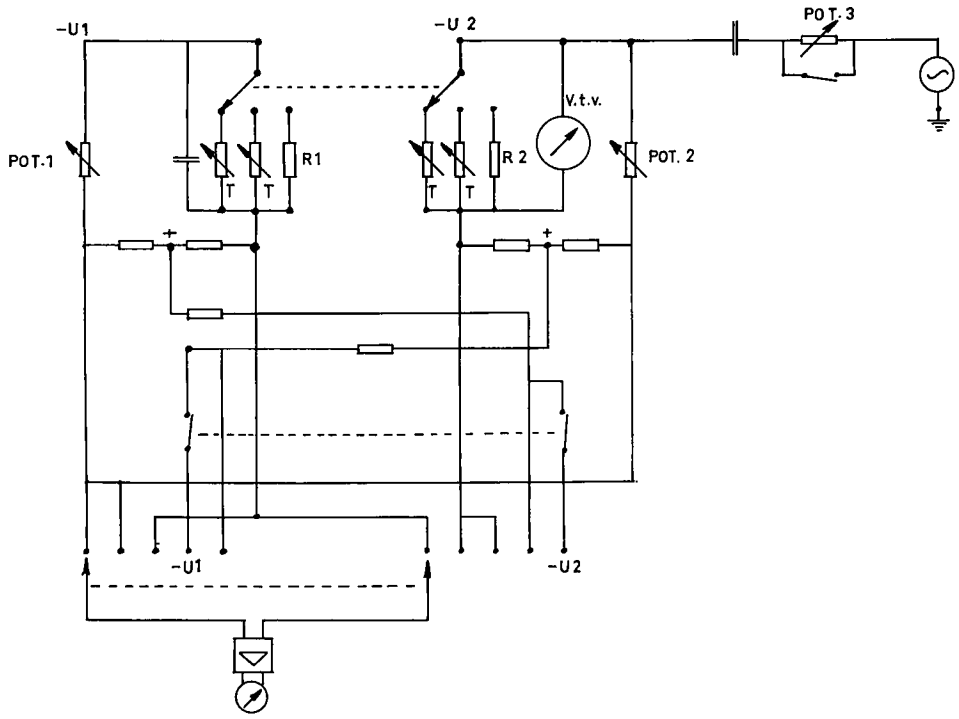

Fig. 11. Measuring equipment. *T*, thermistors, connectable in pairs, which for the purpose of voltage calibration are exchangeable for the fixed resistances *R1, R2. POT 1, 2, 3,* variable resistances each composed of a 10-revolution potentiometer (200 Ω) and fixed resistances with known values, connectable by steps. *U1, U2,* separate bridge-voltages. *V.t.v.,* vacuum tube voltmeter. A recording unit can be connected parallel with the measuring instrument (shown at the bottom of the wiring-diagram).

On the presumption that the two thermistors have identical temperature sensitivity, they can be connected into the opposing arms of a *Wheatstone* bridge, whereby changes in absolute temperatures do not upset the electric balance of the bridge, because the result of these changes is that the resistances in both thermistors change in the same direction and to the same extent. This presumes the use of "matched" pairs of thermistors, which are obtainable; but because a thermistor unit is easily damaged during mounting, many of these pairs can be expected to be destroyed. A more economic method of solving the problem is to use "unmatched" thermistors, which in pairs are made equivalent from the standpoint of temperature sensitivity. This can be effected by feeding them with currents of various size (see further, the section headed *Equalization of temperature sensitivity*). This, however, requires also two separate bridges.

2. **Bridge construction** (Fig. 11)

The thermistors (T) are put on identical points of the two *Wheatstone* bridges. With a double-throw switch they can be replaced by another pair of thermistors or by fixed resistances ($R1$, $R2$). The similarly polarized mid-points of the two bridges can be connected across a measuring instrument; the bridges can thus be balanced against each other. Imbalances of the same size and in the same direction in the two bridges will therefore not affect the instrument.

The applied bridge construction, moreover, allows the bridges to be connected separately with the measuring instrument, which can also in other positions measure the separate voltages ($U1$, $U2$), and these, because of their polarity, can be balanced one against the other.

Equalization of temperature sensitivity. The voltage across a thermistor is, according to Ohm's law, equal to the current times the resistance. In low voltage ranges, the temperature sensitivity in the type of thermistor used is, moreover, a direct function of the applied voltage.

Therefore, by changing the mutual relation between the voltages of the two thermistors to a required degree, the thermistors can be made to respond with equal resistance changes to a given temperature change, i.e. to show the same temperature sensitivity. This can be done in a practical way as follows:

Under identical conditions (the bridge voltages $U1$ and $U2$ are balanced against each other) the two thermistor resistances (the thermistors put in a thermostat-regulated water bath) are measured at two different temperatures (R_1, R_2 and R_1^1, R_2^1, respectively). Information concerning the sought relation between the two voltages $\frac{U_1}{U_2}$ is then obtained from the equation:

$$\frac{R_1 \times R_2^1}{R_1^1 \times R_2} = \frac{U_1}{U_2}$$

The relation is transferred to the voltage of the bridges as follows: By means of the double-throw switch, the thermistors are cut out and replaced by the fixed resistances $R1$ and $R2$ (Fig. 11). The potentiometers (Pot. 1, Pot. 2) in the other leg in each bridge are set so that their resistances are related to each other as U_1 is to U_2, according to the above formula. Thereby, in the bridges, imbalances result that go in the same direction but are of different size, whereby the sizes will relate to each other as U_1 to U_2 because they are functions of the resistances in the two potentiometers. This is reflected as an imbalance between the bridges, which can be read on the instrument. The size of the imbalance in one bridge can now be changed by altering the applied bridge voltage. (Here, the sensitivity of the bridge for

the imbalance is changed.) The bridge voltage is adjusted until the fault signal assumes the same size as the fault signal in the other bridge, which is noted on the measuring instrument as a resultant balance between the two bridges. The relation between the two voltages now established is directly correlated to the relation between the resistances of the potentiometers, which in their turn represent the sought relation $\frac{U_1}{U_2}$. The voltages adjusted to the bridges are, in other words, adjusted in such a way as to eliminate differences in the temperature sensitivity of the two thermistors. The relation between the voltages is easily read on the instrument by balancing them against each other. An instrumental value of this nature is thus characteristic of each thermistor pair. After correction according to this value, each thermistor pair was then tested in a thermostat-regulated water bath at two temperatures ($+38°$ and $+42°$ C) whereby it was established that the temperature sensitivity of the two thermistors was equalized.

As mentioned above, the linear relation between temperature sensitivity and applied voltage for the type of thermistor used prevails in the low voltage ranges (up to 0.5 volts). The author has used as maximum 0.25 volts D.C., i.e. this voltage was applied to the thermistor, which primarily showed least temperature sensitivity. At these low voltages the thermistor does not "feel" the flow, a condition that has been controlled in every single case.

3. Heating device

To restrict the size of the measuring probe as far as possible, the author has avoided using the conventional heating coil around one of the thermistors. This is, instead, heated with low frequency A.C. (20 kH_z). This has no effect on the direct current across the thermistor and thus not on its temperature sensitivity. An electronic filter prevented any effect on the other thermistor. The author has used in the thermistor an over-temperature of 4° C in relation to the temperature of the surrounding medium. Because the ARALDITE-covering shows a low coefficient of thermal conductivity (5.56×10^{-4} cal/cm · sec · °C) the surface temperature of the covering is considerably reduced. When measured, surface temperatures of about 2° C higher than the temperature of surrounding medium were noted. HENSEL & RUEF (1954) at measurements in muscle tissue found no signs of local vascular reaction at 3—4 degrees over-temperature, BETZ & HENSEL (1962) recommended the most suitable over-temperature at measurements in hypothalamus at 1—2 degrees. It must be added that these investigators used

measuring probes where the temperature fall to the surface is essentially less than occurs with the probes the author uses.

After the A.C. supply had been switched on, it took 3—4 minutes before stabilization of the temperature took place.

The voltage across the heated thermistor was recorded with a vacuum tube voltmeter (Fig. 11. V.t.v.). So that changes in the thermistor resistance will be proportionate to changes in heat clearance, it is necessary that the power developed in the thermistor be kept constant. But this power depends on the thermistor resistance, and to keep it constant, frequent measurements are necessary of both voltage and amperage, with adequate corrections of the latter. However, ROSENGREN (1961) has devised a method that eliminates these disadvantages. The thermistor resistance is measured, and the resistance in the heating circuit is equalized to it. (In the author's apparatus, this is done by a variable resistance [Fig. 11, Pot. 3] coupled in series with the A.C. source.) This makes it possible to keep the potential fall across the thermistor constant. A total deviation of the thermistor resistance of 4 %, which is a high value at flow measurements in bone, results in a maximum deviation in power of only 0.01 % according to ROSENGREN, a deviation that lacks relevance here.

4. Determination of heat clearance with the aid of thermistor probes

A body whose temperature is higher than that of the surrounding medium emits heat to it. The heat transport is determined by the heat conductivity of the medium. The heat conductivity displays an apparent increase if, at the same time, there occurs a convectional heat transport in the medium. It is these apparent changes in the heat conductivity of a tissue conditioned by variations in the blood flow through the tissue that are registered with the heated thermistor. The unheated thermistor merely eliminates the effect on the measuring unit by changes in the absolute temperature of the tissue.

For the determination of the heat clearance (apparent heat conductivity) in terms of heat conductivity with thermistors, the following applies:

$$\lambda = \frac{K \cdot P}{\vartheta}$$

λ is the heat conductivity with the dimension cal/cm · sec · °C, K is a constant conditioned by characteristics in the measuring probe, P is the developed effect in the heated thermistor, and ϑ is the temperature difference between the heated thermistor and the surrounding medium (the temperature in the unheated thermistor). This can also be expressed as follows:

$$\lambda = \frac{K \cdot U^2 \cdot k_t}{r \cdot \Delta R}$$

whereby U (read on the vacuum tube volt meter) indicates the voltage across the heated thermistor, which shows the resistance r (obtained as potentiometer value); k_t is the temperature sensitivity factor (resistance change/°C) of the heated thermistor (determined by calibration at two temperatures), and ΔR indicates the resistance difference between the heated and the unheated thermistor (obtained as potentiometer values). $\frac{U^2}{r}$ can be regarded as constant (see the section headed *Heating device*). This concerns also k_t. ΔR is variable. The size of K, which is characteristic for each measuring unit, is determined at stable temperature in a homogeneous medium with known thermal conductivity. The author has used 30 % gelatine at +37° C ($\lambda = 11.0 \times 10^{-4}$ cal/cm · sec · °C, GRAYSON 1952).

After various factors have been determined in the indicated manner, the following is applicable to each individual measuring unit:

$$\lambda = \frac{K_{Th}}{\Delta R}$$

ΔR is thus inversely proportionate to heat conductivity; its size can be determined with the aid of the measuring bridge potentiometer, and variations in it can be continuously registered on a recorder.

(Actually, the expression should be written:

$$\lambda = \frac{K_{Th}}{\Delta R} - a$$

where a indicates a λ-error conditioned by convectional heat transport via the wires of the measuring probe. This factor, however, is important only when the absolute value of heat conductivity is of interest, but causes no error when estimating relative changes in heat conductivity.)

Co-heating of the unheated thermistor can be a source of error. With the type of measuring probe used, this was determined at about 2 % or less, which can be considered of no practical importance (cf. GOLENHOFEN *et al.* 1963).

5. Application of measuring probes in bone

As mentioned earlier, the most reliable measuring results are obtained by this method from a tissue part with a large number of blood vessels of small and equal calibre. The most suitable placing of the probe in a long bone—in this case rabbit tibia—would be in the metaphysis. This, however,

is not practical for permanent implantation in a growing animal, because the probe, in that case, would after some days be situated in the diaphyseal marrow cavity in contact with vessels of extremely varying calibre. In order to get constant conditions, the probe must be placed in an epiphysis in a growing animal. For the present experiments, the proximal tibial epiphysis was chosen; in its more central parts, the vessels are predominantly small in calibre.

The measuring probes were applied as follows: the animals were anaesthetized with Mebumal-ether as earlier. After the hind leg had been extended, the skin was opened with a longitudinal incision (about 3 cm) over the thigh. The proximal part of the tibia could be brought forward in the opening of the skin by flexion of hip and knee joints. A thin guide wire was inserted from the lateral side by hand into the proximal tibial epiphysis straight through the layer of covering musculature. The guide wire was of a flexibility that allowed no penetration of joint surfaces or terminal bone plate to occur. An ordinary injection cannula, having the same external diameter as the end of the measuring probe, was passed over the protruding part of the guide wire with whose help it was inserted by hand into the epiphysis. After the guide wire and the cannula had been removed, the measuring part of the probe could be inserted under some resistance into this channel. This gave it a stable position, and no special fixation was necessary (Fig. 12).

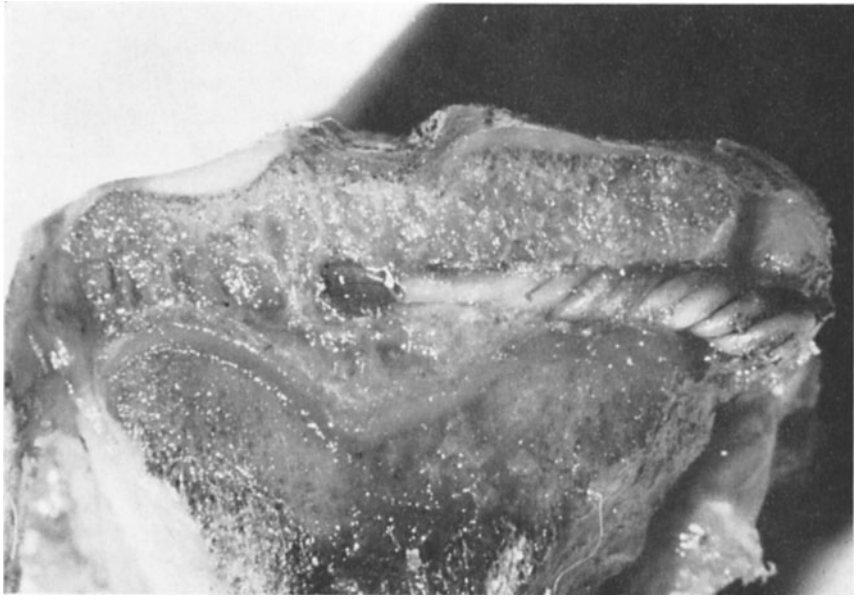

Fig. 12. Thermistor probe *in situ* in proximal tibial epiphysis.
Magnification: appr. 5×

At the end of the experimental period, the position of the probes in the epiphyses was checked by removing with a dental saw sufficient bone tissue to expose the intra-osseous parts of the probes. The restitution, around the probes, of the bone tissue that had been traumatized at the application was so pronounced that the probes, in most cases, could not be removed without their being damaged; therefore, they could not be used in more than one experimental animal.

A big problem was to prevent wear to the electric wires by the bony edge at the point where they issued from the epiphysis. The wires were therefore drawn some distance through the covering musculature and brought out at the tibial tuberosity. Here, they were fixed by suture through the attachment of the patellar tendon so that pulling on them would not affect the intra-osseous part of the probe.

The wires were then drawn up under the skin to the middle of the back, where they were connected to a contact device (strip connector, 221—647, Amphenol-Borg Electronics Corp.). In order to fix this and also to protect it from mechanical damage, it was mounted in a plexiglass-holder (Fig. 13). The base plate of this holder was attached under the skin by sutures in the back musculature. The skin on the leg and on the back were then sutured.

Every other day, for one week, the animals were postoperatively given penicillin (Penicillin-procain Novo vet.) in a dose of 100,000 I.U./kg body weight.

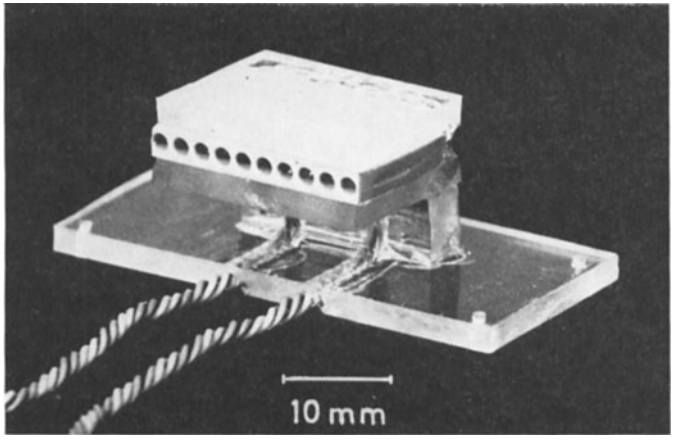

Fig. 13. Connecting-device. The base-plate is fastened subcutaneously to the back of the animal.

C. EXPERIMENTS

1. Preparatory measurements

At the experiments, the same breed of white rabbits were used as at the earlier experiments. As described, the thermistor probes were applied in the two proximal tibial epiphyses.

During the first post-operative days, heat clearance showed very large variations. Thereafter, these were reduced, and in the measuring values from each bone, a tendency to fluctuate around a level appeared. Variations from day to day, as well as during one and the same day, could be observed. Heat clearances from the two bones agreed more rarely; therefore the levels showed a parallel displacement. At the same time as a stabilization was achieved, which required 5—9 days, the tendency of the measuring unit to be influenced by blood flow changes increased too. This could be tested with the aid of digital compression of the femoral vessels. The course of the development was interpreted as the result of beginning healing. Now for the first time, the heat clearance could be thought to reflect circulatory events in the epiphysis. Before further experimental procedures were undertaken, the parallelity between the heat clearance values from the two sides was checked for at least three days, but usually longer. Because it was desirable for the sake of comparison that the planned measurements be performed on animals of the same age as those used at the earlier growth stimulation experiments, the probes must therefore be applied to the animals as early as four weeks of age.

It was also controlled, by digitally pulling the cables where they issued from the epiphyses, that the ends of the probes were securely fixed; i.e. no change of heat clearance ensued.

2. Suppressed blood flow; muscular activity

Various opinions have been expressed about what changes occur in the intra-osseous blood flow at changed outflow conditions in surrounding soft tissue. Associated with this are also the diverging ideas about the relation between skeletal flow and muscle activity.

Five animals about six weeks old were included in this experimental series. Thermistor probes had been implanted 10—14 days earlier. Stable conditions, from the standpoint of measuring values, therefore prevailed on the occasion of the investigation.

The usual Mebumal-ether anaesthesia was given. The femoral nerve and the femoral vessels on one side were exposed at the inguinal ligament, which was divided in order to have access to the vessels proximal to where the deep branches run off. Loose ligatures were laid here around the femoral

artery and the femoral vein. The femoral nerve was divided and its distal cut end was connected bipolarly to a square wave stimulator. The sciatic nerve on the same side was exposed at mid-thigh, the two main subdivisions of the nerve were divided, and here too the distal cut ends were connected in the same way to the stimulator. The distal ends of the nerves were stimulated to avoid central influence on the circulation in the leg. The parameters of the electric stimulation were: 6 V, 2 msec duration, 1/sec and 100/sec frequency.

On one animal, pharmacological sympathetic blockade was performed with the aid of bretylium in a dose of 20 mg/kg body weight given subcutaneously 4 hours earlier (BOURA & GREEN 1959).

Heat clearance from the proximal tibial epiphysis on the same side was, during the experiments, continuously registered on a pen-recorder (galvanometer-writer DS-1195, ABEM Comp.).

Results

Occlusion of the femoral artery (Fig. 14 A). A tightening of the loose ligature round the femoral artery was, after a brief delay, reflected as a lowering of heat clearance from the probe in the proximal tibial epiphysis. After the occlusion had been released, a return to the original level occurred quickly.

Occlusion of the femoral vein (Fig. 14 B). The ligature round this vessel caused a lowering of the heat clearance. The lowering was somewhat more slow than at corresponding arterial occlusion, but the return to the original level occurred in a similar way.

Isotonic contraction of the muscles (Fig. 14 C). Contractions at 1 second intervals caused a scarcely noticeable lowering of the heat clearance.

Isometric contraction of the muscles (Fig. 14 D). Heat clearance showed here an unmistakable lowering as long as the stimulation continued (1/sec). A more detailed study of the recordings discloses that the effect of the individual contraction is a lowering of the heat clearance (Fig. 14 E).

Tetanic stimulation of the muscles (Fig. 14 F). After an initial brief increase in heat clearance, it fell markedly and abruptly ceased when the stimulation was discontinued. A return to the original level quickly ensued.

If a similar stimulation was given after influence on the neuromuscular system in the leg, with repeated (8—10) brief, tetanic stimulations (5 secs interval) a different appearance of the curve was obtained (Fig. 14 G). The same initial increase of heat clearance of short duration could be observed,

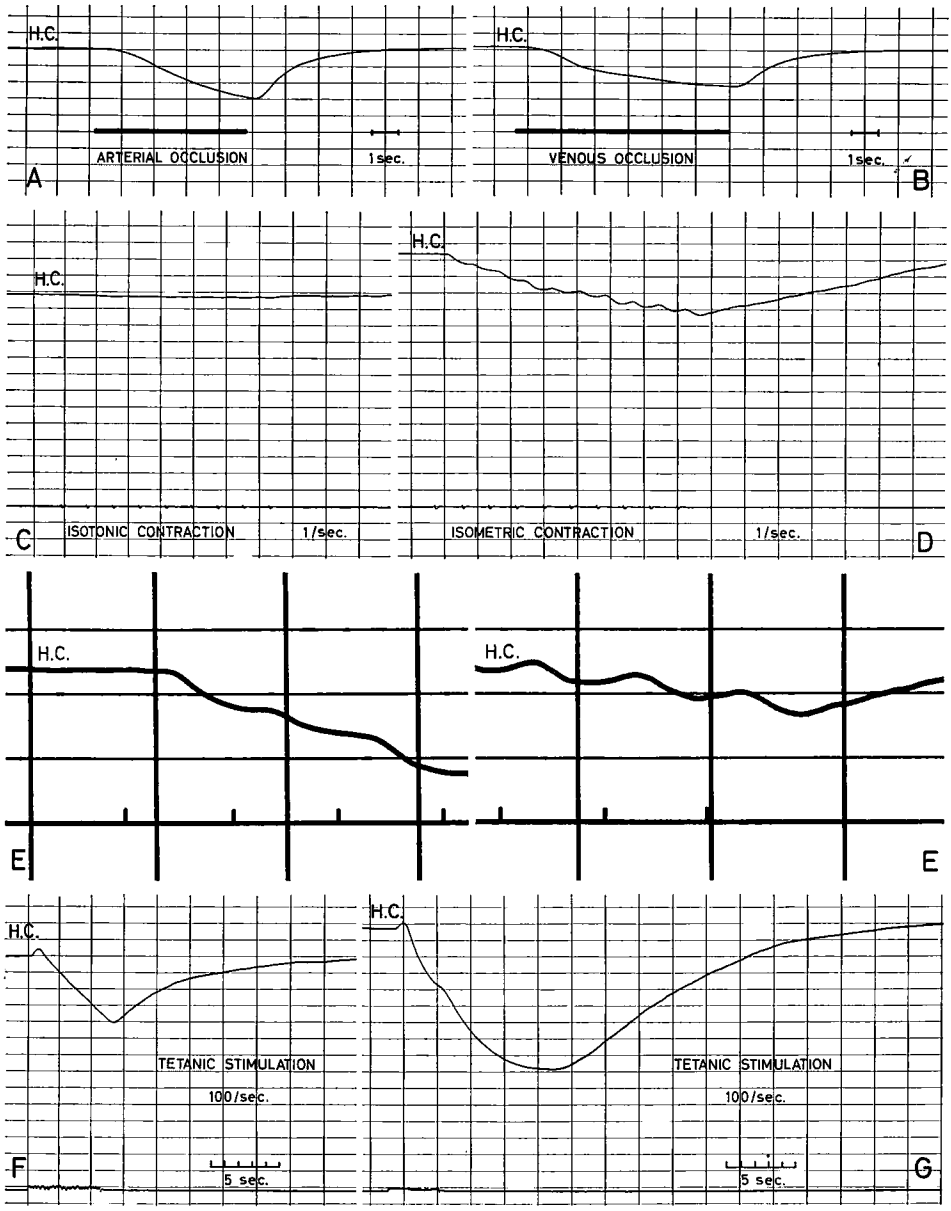

Fig. 14. Heat clearance (H.C.) recorded with the thermistor probe permanently implanted in the proximal tibial epiphysis. A and B are recordings from one animal; C, D and E from another, and F and G from a third. E denotes magnifications of parts from the recording D. Amplification of A—B lower than C—G.

as well as the lowering that ensued. After the stimulation had been discontinued—reflected as a slight deflection of the curve—heat clearance, however, continued to fall markedly. This was gradually succeeded by an increase towards the original level.

After a rest period of about 15 minutes, a renewed tetanic stimulation resulted in change of heat clearance similar to that seen in Fig. 14 F.

Results that in every respect were identical with those just described were also obtained after sympathetic blockade with bretylium.

3. Interference in the innervation

a. *Registration method*

Animals in which thermistor probes were implanted in both proximal tibial epiphyses were used for these experiments.

To standardize as far as possible the measuring conditions, the animals were placed in a box whose dimensions allowed them, without hindrance, to assume resting positions, but otherwise restricted their movements. At the measurements, the animals were also placed in half darkness, which further contributed to tranquilize them. During an adaptation period of 10 minutes, no measurements were made.

The measuring equipment did not permit simultaneous measurements from both bones. After the heat current was switched on, a further 3—4 minutes were required in order to stabilize the temperature conditions round the probes. During this period, smaller variations in heat clearance could perhaps occur. To eliminate any faulty side differences, the heat clearance was measured on control side and on operated side alternately—a total of three measurements on each side. The arithmetic mean of these three recordings indicated heat clearance on each side.

During a pre-operative period, it was first established that a parallelity existed in the fluctuations in the heat clearance values from the two bones. Exact agreement in these values could never be observed. The arithmetic mean of heat clearance during this period was calculated for each side. The difference between these means will be the basis for future calculations.

Based on the heat clearance from the control side at subsequent post-operative observations, corresponding heat clearance from the operated side could be predicted by the use of the mentioned mean difference. A difference on the operated side between the clearance value noted here and that predicted therefore illustrates a relative change of heat clearance on operated side compared with control side.

Knowledge of the resistance *v.* temperature characteristics of the ther-

mistors made it possible to calculate the intra-osseous temperature on both sides.

b. *Statistical analysis*

The statistical analysis of the calculated clearance values is based upon a variance analysis, mixed model. The construction of the model is seen in the following expression:

$x_{ij} = m + a_i + b_j + e_{ij}$, where

$i_{\text{peripheral nerve sectioning}} = 1, 2, \ldots, 17$ days (one day excluded because of missed observation)

$i_{\text{sympathectomy}} = 1, 2, 3, 4, 5, \ldots, 22$ time points, where 1—4 indicate ½—6 hours, 5—22 denote 1—18 days.

m = constant

a_i = constants that describe the effect of day/time point

b_j = stochastic variables that belong to the animals

e_{ij} = stochastic variables that correspond to measurement errors and interaction between animal and day/time point

It is presumed that the variables b_j and e_{ij} are independent and follow Gaussian distributions. In these distributions, the variance for b_j is the same for all j, and the variance for e_{ij} is the same for all i and j.

A comparison is also made between control sides and operated sides concerning the temperatures observed here. The method applied here is meant primarily to disclose larger differences between the two sides. Against this background, a variance analysis model—three-sided distribution—can be applied, with all effects that depend upon the animals as stochastic variables. With a further presumption of no interaction between animals and days, the comparisons can be based upon a variance analysis model of two-sided distribution where the day temperature on each side for every animal is added together. At the analysis, it is thus tested whether, on average, any difference exists between control sides and operated sides during the observed period.

The applications of the foregoing calculations are found below in each section.

c. *Peripheral nerve sectioning*

Except for two animals, the white rabbits used in this experimental series were aged 6 weeks ± 3 days; the two exceptions were 6 weeks + 4 days old. When they were about 4 weeks old, thermistor probes had been implanted in the proximal tibial epiphyses.

The animals were anaesthetized with 6 % Mebumal-natrium (ACO) (½ ml/kg body weight), complemented with ether. The right sciatic nerve was exposed at the piriform muscle, where it was divided and a half-cm resected. The right femoral nerve was exposed at the inguinal ligament and was divided and resected in a similar way. The skin was sutured with perlon.

During the next 18 post-operative days, heat clearance was recorded daily at the same time on both sides, as also was the intra-osseous temperature.

At the end of the experimental period, the position of the probes in the epiphyses was checked, and at macroscopic signs of inflammatory changes, the animal was excluded from the series.

Useful data were obtained from 10 animals.

Results — The effect of the experimental operation on heat clearance on the operated side is illustrated in Fig. 15. This shows graphically the difference between observed and predicted clearance value on the operated side of the ten animals. The same calculation method was later applied to the values from the pre-operative period. The results obtained thus indicate deviations in the clearance value of the thereafter operated side from the parallelity between the heat clearance of both sides. The operation day has been called day zero; heat clearance on this day represents a pre-operative value. Pre-operative days are indicated by a minus sign in front of the figure.

Fig. 15 shows that the calculated heat clearance in the ten animals has a marked tendency to increase during the immediately following post-operative period. The increase culminates around the sixth post-operative day; thereafter, it again falls towards its original level. Between the ninth and the thirteenth day, most observations tend to assume negative values, a tendency that is increasingly accentuated up to the eighteenth day.

The statistical analysis of the mentioned clearance values means that the hypothesis $a_1 = a_2 = \ldots = a_{17}$ is tested with the aid of variance analysis.

	Square sum	Degrees of freedom	F
Days	62447.024	16	41.85
Animals	8175.082	9	
Error	13429.918	144	
Total	84052.024	169	

The result is that the hypothesis can be rejected with $P < 0.1$ % (*** significance).

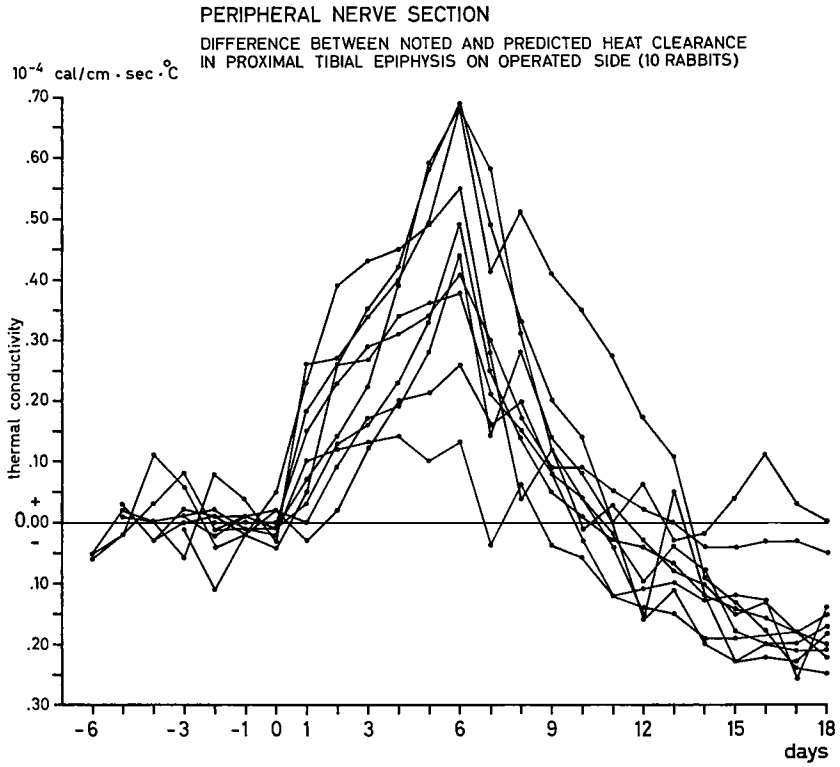

Fig. 15.

Separate tests of the hypotheses $a_5 = a_6$ and $a_6 = a_7$ result in their being rejected with * and *** significance, respectively (2-sided t-test).

The conclusion from the analysis is that the maximum for the observed effect of peripheral nerve division on heat clearance with high significance deviates from zero and occurs on the sixth post-operative day. The effect is more pronounced on the fifth day than on the seventh.

The intra-osseous temperature on the control side was also compared with that on the operated side during the experimental period. With the aid of variance analysis of the type shown above, the hypothesis that on average during the observation period there is no difference between control side and operated side was tested. The following results are shown in table on page 98.

According to the analysis, the hypothesis cannot be rejected. However, it can be thought that on one day, or on a few days, there is a significant difference despite the difference on average over the whole period not being significant. Day 6 is studied separately, because then there seems to be the

	Square sum	Degrees of freedom	F
Control-op.	14.0114	1	0.73
Animal	267.6382	9	
Error	172.6337	9	
Total	454.2833	19	

greatest difference. This, however, does not significantly differ from zero (2-sided t-test).

In conclusion, it can be said that with this analysis no significant difference in temperature between control side and operated side could be established after peripheral nerve sectioning.

d. *Sympathectomy*

The experimental animals in this group were aged 6 weeks $\pm$ 2 days. The thermistor probes had been implanted in the proximal tibial epiphyses about 14 days earlier.

Under normal Mebumal-ether anaesthesia, left-sided lumbosacral ganglionectomy according to the method described on p. 58 was performed. Heat clearance and intra-osseous temperature on both sides were registered post-operatively for eighteen days. Several previous investigations of the circulatory changes after sympathectomy indicate that these occur during a rather limited, early, post-operative period; therefore, clearance and temperature conditions were also studied during the hours immediately after the operation: ½, 1, 3, and 6 hours post-operatively.

In the same way as in the immediately preceding animal group, the position of the probes in the epiphyses was checked at the end of the experimental period. Those animals that showed infectious changes in the epiphyses were excluded here from the experimental series, too.

After the end of the experimental period, the noradrenalin content in the autonomous groundplexus of the tibial intramedullary arteries was also checked in all animals with the aid of the fluorescence method described on p. 39.

Useful data were obtained from 10 animals.

Results — The difference between observed and predicted clearance value in the proximal tibial epiphysis on operated sides represents, as in previous experiments, the effect of the experimental operation on heat clearance. The measurement values from the pre-operative period were treated in the same way as described above. The results are shown graphically in Fig. 16. Pre-operative observations are indicated by minus signs in front of the time figures, day zero is the operation day, but the observation is pre-operative.

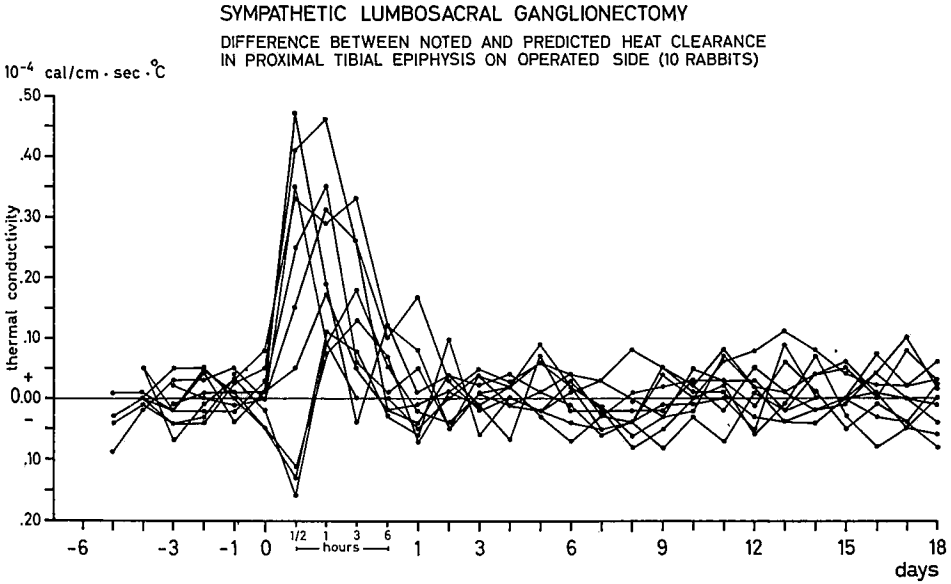

Fig. 16.

Heat clearance on the operated sides has a tendency to increase during the hours immediately after the ganglionectomy. The increase is shortlived, and the return to the original level usually occurs after six hours. During the following post-operative period (a total of 18 days) the clearance values are distributed round the same level as pre-operatively.

At the statistical analysis of the obtained clearance values, the hypothesis $a_1 = a_2 = \ldots = a_{22}$ was tested with the aid of variance analysis.

	Square sum	Degrees of freedom	F
Time point	7565.53	21	6.76
Animal	1340.66	9	
Error	10067.24	189	
Total	18973.43	219	

The hypothesis is rejected with $P < 0.1$ % (*** significance). However, the hypothesis $a_5 = a_6 = \ldots = a_{22}$ cannot be rejected. The hypothesis $a_2 = a_3$ is rejected with $P < 1$ % (** significance) by 2-sided t-test. (The observation a_1 was regarded as unreliable, because the animals at this time were usually still unconscious as a result of the anaesthesia.)

The analysis indicates that lumbosacral ganglionectomy has a significant effect on heat clearance on the operated side. The effect is maximum 1 hour post-operatively, and thereafter abates. From and including the first post-operative day, no effect can be observed from the experimental operation.

As in the previous experimental series, a comparison was made here between control sides and operated sides regarding the intra-osseous temperature during the experimental period. Models and methods were used analogous with those for corresponding calculations at peripheral nerve sectioning. With the aid of variance analysis, the hypothesis that on average during the observation period no difference exists between control sides and operated sides was thus tested. The following results were obtained:

	Square sum	Degrees of freedom	F
Control-op.	46.391	1	5.71
Animal	1186.891	9	
Error	73.097	9	
Total	1306.379	19	

The analysis shows that $1\ \% < P < 5\ \%$, i.e. there exists during the period on average a significant difference between control sides and operated sides in the available material. An individual study of the observations 1 hour post-operatively (2-sided t-test) shows that the difference at this point is not significant. Only when the entire series is examined, can it be established that a higher temperature exists on the operated sides.

The investigation of the tibial intramedullary arteries shows that there is a complete absence of fluorescence in the autonomous groundplexus of the arteries on the operated side, whereas corresponding vessels on the control side present a completely normal picture. This concerns all ten animals.

Data from the last two experimental series were also used for calculation of the mean values for heat clearance and intra-osseous temperature. The observations from the control sides on the eighteenth post-operative day were used here (20 animals). Heat clearance: $12.45 \pm 0.37 \times 10^{-4}$ cal/cm · sec · °C; intra-osseous temperature: 39.61 ± 0.23°C. In both instances, 95 % confidence interval.

D. SUMMARY

The thermo-electric method for estimating blood flow with temperature-compensated flow-velocity probe was further developed for continuous registration of intra-osseous blood flow with permanently applied probes.

Intra-osseous heat clearance shows an unmistakable lowering at *arterial* as well as at *venous occlusion.*

Heat clearance is hardly affected by *isotonic muscle contraction* but shows an obvious lowering at *isometric contraction* under otherwise similar experimental conditions.

Tetanic stimulation of surrounding musculature after an initial brief increase produces a marked lowering of heat clearance as long as the stimulation lasts. It thereafter quickly returns to its original level. A further lowering occurs after the stimulation has ceased, when the neuro-muscular system has previously been influenced by repeated brief tetanic stimulations.

The results referred to are not affected by previous sympathetic blockade with bretylium.

Peripheral nerve sectioning exerts a highly significant effect on heat clearance in the proximal tibial epiphysis. Maximum effect is obtained during the sixth post-operative day, whereafter it again decreases. The results indicate that after 9—13 post-operative days it is lower on the operated side than on the control side. This tendency continues up to the end of the observation period, 18 days after the denervation.

The nerve division seems to have no effect upon the intra-osseous temperature compared with the control side.

Lumbosacral ganglionectomy significantly influences heat clearance during the immediate post-operative hours. Maximum effect occurs 1 hour after the sympathectomy. Thereafter, there are no signs of effect from the experimental operation during the observation period involved (18 days).

After lumbosacral sympathectomy, the intra-osseous temperature is significantly higher on the operated side than on the control side.

Heat clearance in the proximal tibial epiphysis was $12.45 \pm 0.37 \times 10^{-4}$ cal/cm · sec · °C; the *intra-osseous temperature* registered in the same place was $+39.61^{\circ}$ C ± 0.23.

Summary and discussion

A survey of the literature that discusses various attempts to stimulate the skeletal longitudinal growth reveals a conspicuous divergence concerning the obtained results. Methodological errors are naturally of essential importance, and here an inadequacy in the recording of the changed growth rate, i.e. in the measuring methods, must be considered one of the most important factors.

Labelling with tetracycline in the skeleton—the method the author uses —provides excellent information about, *inter alia,* the endochondral growth from the growth plates (HULTH & OLERUD 1962, HANSSON 1964, 1967).

In the present investigation, the endochondral growth from the proximal growth plate of the diaphysis to the metaphysis in the growing rabbit tibia is taken as an indicator of the growth in skeletal length of this bone. No estimate of the contribution to the length of the bone from the distal growth plates or from the epiphyses in both ends of the bone has thus been made; nor have possible variations in the height of the growth plates been observed, which could also affect the total bone length. This would not necessarily be changed by accelerated growth in length in one section: the growth rate in other parts could at the same time be less than normal.

In view of the findings of other investigators, no regard was paid here to physiological anisomelia. According to GOFF (1960) and GULDHAMMER (1963) this in *homo* is insignificant. ARMSTRONG (1946) showed that the difference in weight of rat bone ash (front leg) is not significant; the radioactive uptake in right and left femurs of rabbit is equal (BOHR & SØRENSEN 1950). Growth studies, covering short periods with tetracycline labelling, revealed insignificant side differences that arose solely from the measuring methods (HANSSON 1967).

The investigations were carried out on 6-week old rabbits. At this age, more than 50 % of the growth potential remains in the tibia (HEIKEL 1960).

The absolute values of the growth in length of the proximal tibial metaphysis, tabulated for each experimental series, show great spreading also on the control sides. This was interpreted as an expression for a general effect —varying in degree—on the animals by the various operative interventions. Transperitoneally performed lumbosacral ganglionectomy, as well as open-

ing the vertebral canal and dividing spinal nerve roots, is naturally a major operation for a 6-week old rabbit. The absolute values on the control sides thus do not represent normal growth conditions, and all calculations of the effect of the various measures on the growth must therefore be based upon relative conditions. The mentioned general effect on the animals is expressed in a marked retarded and irregular growth during the first post-operative days, which made reliable measurements from this period impossible. For this reason, the first observations were not made until the third day.

The growth-stimulating effect of various methods was generally ascribed to a changed intra-osseous circulation. Such a change has been attempted by, *inter alia,* direct traumatization of the investigated bone (trauma to the medullary cavity—sometimes with the simultaneous application of material into it—and fracture). These conditions make it difficult to analyse to what extent the observed effect must be referred to abnormal intra-osseous flow conditions or to determine whether it is also conditioned by extra-osseous disturbances in the circulation. These can be supposed to appear secondarily to the disuse of the limb, which to varying extent results from the trauma. In these circumstances, the author chose peripheral nerve division as stimulating method, whereby the operative intervention leaves the bone completely intact.

The results indicate that *the peripheral nerve sectioning* causes a highly significant accelerated growth in length in the proximal tibial metaphysis. This is most pronounced during the sixth post-operative day. The findings agree with those of RING (1961) who, however, did not observe the diaphyseal length in rabbit tibia until after two weeks and thus obtained the net effect of the stimulation over that period.

There is also reason to compare the results with initial growth acceleration as observed in a paretic extremity in children with poliomyelitis (SEELIGMÜLLER 1879, GREEN & ANDERSON 1955, LERIQUE 1956, RING & WARD 1958, RATLIFF 1959). This acceleration is restricted mainly to the first year after the onset of the condition. Considering that it can here involve a somewhat different mechanism, it can be added that a year for *homo,* concerning skeletal ageing, corresponds to about 9 days for rabbit (HEIKEL 1960). This indicates, with some reservations, time parallelity between the growth acceleration at poliomyelitis and the acceleration that the author observed in rabbit after peripheral nerve division.

The noted accelerated growth in length of the operated side exceeds the growth of the control side by fully 20 %. It gradually reduces, and during the eighteenth observation day is significantly retarded on the operated side. The inclination of the growth curve indicates that the retardation during the immediately following period will be further pronounced. It is reason-

able to presume that the course of development is analogous with that of poliomyelitis concerning change in bone length, i.e. the initial growth acceleration gives way in a later phase to a marked and more prolonged growth retardation; the net effect of a peripheral nerve injury being retarded growth. This agrees completely with earlier observations (ALLISON & BROOKES 1921, ARMSTRONG 1946, GILLESPIE 1954, COMBES *et al.* 1960, *inter al.*) experimentally as well as clinically.

A sectioning of the femoral and the sciatic nerves causes, *inter alia,* a postganglionic sympathetic injury whose extent, as far as concerns rabbit tibia, the author has estimated with the fluorescence method (see below). The results point to a total sympathectomy of intra-osseous arteries and harmonize with the described distribution condition of sympathetic fibres to structures in the extremities (KRAMER & TODD 1914, WOOLARD 1926, WEISS & ROOT 1959, *inter al.*).

A peripheral nerve contains several components. To investigate which of these is of importance for the noted bone growth stimulation, a selective injury was done to the motor, the sensory, and the sympathetic component. These studies, however, have only included the period around the growth maximum, because the effect of the individual component can most safely be observed during this period.

Sectioning of the anterior roots belonging to the spinal nerves L_7-S_1-S_2 on one side causes a pronounced paresis in the corresponding hind limb. The denervation results in an accelerated growth in length of the proximal tibia on the same side (highly significant) which from the aspect of time agrees with the effect of peripheral nerve division. No quantitative comparison between these two effects can be made, because it can be presumed that the degree of motor denervation is not the same in the two experimental series. Further, there are other qualitative differences. RING (1961) observed also an accelerated skeletal growth in the tibia after motor root injury in dog, but could see no effect from the same intervention in cat. A reason for this can be that the root division in the former instance included more lumbal and sacral nerves than in the latter instance.

The accelerated growth reduces after maximum on the sixth day following the same pattern as at peripheral nerve sectioning.

The investigation of tibial intramedullary arteries showed no differences concerning fluorescent noradrenalin product (see below). However, sectioning of anterior roots causes an injury to preganglionic sympathetic fibres, but this does not affect the content of noradrenalin of corresponding postganglionic structures and therefore not the degree of fluorescence (FALCK 1962). Characteristic of the preganglionic fibres is also their over-

lapping pattern in the paravertebral trunks, whereby preganglionic fibres from intact levels can provide a preganglionic supply to ganglion cells that have been robbed of their original preganglionic synapses. The division of a smaller number of anterior roots thus will hardly affect the function of corresponding postganglionic structures (cf., MONRO 1959). New investigations (DAHLSTRÖM & FUXE 1965), however, point to the anterior roots also containing postganglionic sympathetic fibres. The disappearance of these at the applied method of root division is not reflected in the fluorescence pattern of the investigated vessels, probably for quantitative reasons.

Retarded skeletal growth has been noted after *sensory denervation*. This has been thought to be due to an increased traumatization through disappearance of normal protecting mechanisms (RING 1961, TROUPP 1961). The author did not find this effect after sectioning of three posterior nerve roots. The measurements of length after this intervention rather indicate a somewhat accelerated growth during the sixth post-operative day. No significant difference compared with sham-operated animals, however, is found. An explanation of the suggested effect could perhaps be sought in the operational procedure. The opening of the dura and the division of a number of posterior roots probably cause also some traumatization of corresponding anterior roots without this being seen at the post-operative appraisal of the motility. This non-visible motor injury can be the cause of the slight growth stimulation. Possibly an increased traumatization through the sensory loss can also be a contributing factor.

An injury to sensory roots could be thought to influence the skeletal growth by way of changed activity of the vascular smooth muscles through the interruption that occurs to dorsal-root vasodilator fibres and to their transmission of so-called antidromic vasodilator impulses. However, the results of an extensive research in this field, summarized by UVNÄS (1961), point to these fibres probably being pain fibres, completely lacking functional relations with vasodilator centres in the central nervous system and localized, as are other pain fibres in the extremities, mainly in the skin.

At fluorescence microscopy, the picture of the autonomic innervation apparatus of the intra-osseous arteries on the operated side completely agreed, as expected, with the findings on the control side.

The next step in the separation of the possibly active components at the growth acceleration after peripheral nerve sectioning was a selective *sympathectomy*. Considerable disagreement prevails whether the sympathetic nervous system exerts any influence on normal skeletal growth, and most experimental as well as clinical investigations fail to verify that a sympathectomy stimulates growth. One reason for the diverging results at

earlier investigations has probably been the limited possibilities for post-operative control of the extent of the sympathectomy. Even an extensive resection of paravertebral ganglions can result in an incomplete sympathectomy because of the great variation both in number and in localization of intermediate sympathetic ganglions (WRETE 1941, MONRO 1959).

A method that in each individual instance permits a control of the extent of such an intervention, as well as of the effect from other measures (peripheral nerve sectioning, anterior root sectioning) implying an interference with sympathetic structures, must therefore be considered of fundamental importance. The author has carried out this control by studying the noradrenalin content in the autonomic ground plexus of the intramedullary blood vessels. This was done with the well-documented fluorescence method according to FALCK & HILLARP, and based upon the disappearance of the nordadrenalin after postganglionic denervation (EULER & PURKHOLD 1951). As mentioned above, no preganglionic injury is reflected in the fluorescence.

A mapping of *the postganglionic outflow to the rabbit tibia* indicates that the lowest outflow occurs from the S_2-ganglion in the paravertebral trunk and that the ganglions between the L_5 and S_2 levels must be resected in order to obtain a complete interruption of the postganglionic connexions to the intramedullary arteries of the tibia. These results do not agree with earlier ones obtained by GOETZ *et al.* (1955). According to these authors, a removal of the ganglions L_4—L_6 is sufficient to perform sympathectomy on the hind limb of rabbit. They recorded the effect of the intervention by noting differences in the rate of warming of the hind limbs after precooling. An existing difference in this rate, however, need not mean a complete sympathectomy; moreover, it is not completely made clear to what extent thermal changes can directly affect the vessel musculature (HELLON 1963). Furthermore, the mentioned authors provide no information about where on the extremity these measurements were carried out. Seen against the background of the present author's finding, it is hardly probable that the recordings were made from the more peripheral parts of the limb, where at the same time an accelerated skeletal growth could be observed.

Lumbosacral ganglionectomy followed by control that the tibia on the same side has been completely sympathectomized has no significant effect on the growth in length of this bone according to present investigations. However, it must be noted that the observations, for reasons given above, did not begin until the third post-operative day and thus do not reveal any possible effect during an earlier post-operative period.

Because there are investigations including circulatory changes after sympathectomy indicating that an effect of the intervention remains for a longer time (TROTMAN & KELLY 1963) sometimes for several weeks (LOWENSTEIN *et al.* 1958), the observation period was extended to include

eighteen days. However, no effect whatsoever on the growth in length is noted during this period. The author's finding is thus comparable with that of most other investigators.

When the results of thet experiments referred to are compiled, it seems to be the motor component that is the underlying cause of the observed growth stimulation in tibia. A destroyed motor function means a muscular function reduced in varying degrees, an immobilization, and possibly also a disturbance of a trophic influence on the skeletal tissues by way of somatic nerves.

The question of a possible trophic influence on the skeleton attracted interest primarily in the beginning of this century (see CASSIRER 1912) but has also later been the object of discussion (HURREL 1938, MILLER & KASAHARA 1963, HULTH & OLERUD 1965).

In order to be able to illustrate the problem involved, the present author partially immobilized a group of experimental animals with intact innervation and studied the longitudinal bone growth after this measure, which means *division and partial resection of the Achilles tendon.* The endochondral growth in the proximal tibial metaphysis occurs at a higher rate (highly significant) on operated side than on control side post-operatively. The accelerated growth is most pronounced during the sixth post-operative day; concerning time, thus agreeing with the growth stimulation after denervation. After this time point, the acceleration reduces considerably, and the difference between the two sides is no longer significant during the eighteenth post-operative day. Contrary to the growth conditions after peripheral nerve sectioning, no growth retardation is ever developed here. This was interpreted as a result of the reducing immobilization that could be observed towards the end of the observation period, when the function in the calf musculature began to return.

In the literature, there is at present little evidence for the existence of trophic influence on bone growth from the nervous system. The present results show that such an influence at least plays no dominant role for the growth acceleration that follows on peripheral nerve sectioning or division of anterior nerve roots. The immobilization appears to have the incomparably greatest importance here. However, it seems as though only quantitative importance could be ascribed to the immobilization method. The present results agree with the growth acceleration at immobilization earlier observed by, *inter al.*, ARKIN & KATZ (1956), and RING (1961).

There is much argument that at immobilization of varying extent of an extremity, the static and dynamic conditions that normally prevail between the bones and the musculature is disturbed. Ever since HUETER-VOLKMANN (1862), many investigators have studied the effects on the growth in

skeletal length that a changed mechanical influence can exert by means of changed pressure and tension conditions at an inactivation of the musculature. However, no unanimity has been reached about this.

A haemodynamic balance exists also between skeleton and musculature, which can be affected by immobilization. Numerous morphological investigations have thus established the rich vascularization of the bone tissue. The arterial supply to musculature and skeleton occurs from the same arterial main trunks, and the functional relation between the two arterial areas is very well developed, especially in the muscle insertions at the end of a long bone. That, too, concerns the venous outflow, which through an even larger abundance of anastomizing channels is connected with surrounding intramuscular veins and with the deep vein trunks of the extremity. Under these circumstances, bone and cartilage, with surrounding musculature, are regarded as one functional unit (GEISER 1957, BROOKES 1958, TRUETA 1961, 1964, RING 1961) where the muscular activity is of decisive importance for the blood flow in the bone (BLAIR 1938, HEALD 1951, MORGAN 1959).

It must be noted that earlier investigators (MÜLLER 1924, 1928, STINCHFIELD *et al.* 1949, GELBKE 1951, ARKIN & KATZ 1956) have not always taken into consideration that these two factors—changed pressure and tension conditions, and disturbed musculo-skeletal circulation—at the same time occur at an immobilization and can be of importance for a changed skeletal growth.

They have both been interpreted by the present author as probable causes of the growth acceleration that has been observed.

It is in the nature of things that no parameter of the mechanical element can be found. The author has, instead, tried to observe the circulatory events in the bone under varying experimental conditions.

For the estimation of *the skeletal blood flow*, it was desirable to find a method that allows repeated registrations from one and the same animal. This has earlier only been done by using intra-osseous pressure measurements (CUTHBERTSON *et al.* 1964 b, KECK & KELLY 1965) and by using radio-isotope depot clearance technique (LOWENSTEIN *et al.* 1958). Both methods mean a traumatization of the bone marrow tissue. The measurements, which thus hardly reflect physiological conditions, can for this reason only be made a few times, and usually not in the same tissue section, a matter that makes it impossible to standardize the measuring conditions. The results of intra-osseous pressure measuring is further obtained from an artificial blood pool; this creates great difficulties in interpretation. Moreover, the fact that the measurements must always be made on anaesthetized experimental animals further limits the number of registrations. Thermo-

electric method could be applied in a similar manner, but with the same reservations regarding its usefulness. However, this method has been used in other connexions for observations covering a long period by permanently implanting the measuring unit in tissues (BETZ & HENSEL 1962, BETZ & BENZING 1963, BETZ *et al.* 1966). Here, repeated measurements can be made on fully conscious animals, and the measurement values are always obtained from the identical tissue area where, after a sufficient healing period, conditions prevail that to some extent reflect physiological ones. The author chose this method for registering skeletal blood flow. The physical properties of the bone tissue, however, offer considerable difficulties concerning construction and qualities in the measuring probes, and none of the types earlier used at permanent application could be employed here. The probes must be characterized by indifference to tissue, great strength, high sensitivity, and small dimension. These qualities are found in the type of measuring probes constructed for the purpose of and used in the present investigation.

The measurement principle is based on changes in the heat clearance of a tissue (apparent thermal conductivity), conditioned by changes in the blood flow of the tissue. Justified criticism (*inter al.* BILL 1962) has been directed to reports (*inter al.* GRAYSON 1952) that sought to assert that a linear relation prevails between heat clearance and blood flow. That this is not the case has been clearly shown by several investigations (BILL 1962, GOLENHOFEN *et al.* 1963, BETZ *et al.* 1966). The method is, in itself, purely qualitative, but can by calibration with the probe *in situ* be made semiquantitative (GOLENHOFEN *et al.* 1963) or even quantitative (BETZ *et al.* 1966). In the present investigation, it was used exclusively qualitatively.

Shortcomings in the measurement method lie in the fact that the measuring probe is only affected by the heat transport in a small surrounding tissue area with a few mm radius (GRAYSON 1952, GOLENHOFEN *et al.* 1963). The registrations therefore reflect highly local blood flow changes, which are probably not representative of the total blood flow through the tissue involved. It is reasonable, however, to presume that qualitative changes here follow one another.

An inhomogeneity in the local heat clearance can simulate a blood flow change (LINZELL 1953). This source of error has greatest importance at long-term measurements. If it exists at the registration of rapid courses, it manifests itself as a displacement of the base line, which is usually easy to discover. The fault, moreover, shows itself as a temperature gradient in the unheated probe, and can be altogether eliminated at individual registra-

tions of heat clearance values by checking the temperature-balance both before and after such registrations.
GRAYSON (1952) showed that a linear relation exists between the water content in a medium and its thermal conductivity. A change in the fluid content of a tissue can therefore considerably affect the results at measurements of heat clearance over long periods. The importance of this fact for the present long-term measurements is further discussed below.

The present investigation proved that the heat clearance value, in terms of heat conductivity, practically never agrees in the two tibial epiphyses in one and the same animal (pre-operative values). The explanation lies largely in the fact that the registrations cannot be made from identical points. The proximity of the probes to blood vessels can vary as can also the calibre of these vessels, which must necessarily influence the thermal conductivity. After application of the measuring probes, scar tissue is formed around them. A difference in the size and structure of this on the two sides must also essentially contribute to the difference in conductivity. The same phenomenon was observed at measurements in other tissues (GRAF & ROSELL 1958, BETZ 1965). This fact results in percentage calculations of the clearance-changes on the two sides not being comparable.

The mean value of heat clearance in the proximal tibial epiphysis in rabbit was calculated from the control sides in twenty animals. The measuring probes were at these registrations applied in the bone for about 4 weeks. The mean value was found to be $12.45 \pm 0.37 \times 10^{-4}$ cal/cm · sec · °C. The obtained mean value is higher than that noted by GRAF & STEIN (1957) in the sternum of patients (11.4 ± 2.95). It is true that, owing to the earlier-mentioned error caused by convectional heat transport via the conductors of the measuring probe, the present mean value is somewhat too high. However, GRAF & STEIN appear to have disregarded this error. Therefore, a difference in the fat content of the bone marrow in growing rabbit and in *homo* (the authors do not state the age of these) is, besides a possible species heterogeneity, a probable cause of the difference in the two mean values. The marked difference in the spreading of the two mean values is most likely due to the entirely different measurement conditions. GRAF & STEIN have recorded from pools of blood with greater or lesser element of coagulation; the present author obtained the measurement values from intra-osseous tissue with stationary vascular conditions.

The mean value of the intra-osseous temperature, calculated at the same time and from the same material as the above heat clearance value, measured on the control sides, amounts to $+39.61 \pm 0.23°$ C. The relatively large spreading is caused by the registrations probably not originating from

identical points. The physiological temperature field of an extremity (BRÜCK & HENSEL 1953), characterized by both an axial and a radial temperature fall, is decisive here.

An occlusion of the femoral artery results, as expected, in a lowering of heat clearance indicating a reduced intra-osseous blood flow. An unmistakable lowering is also obtained by partial blockade of the venous backflow in the extremity by the *occlusion of the femoral vein.* The latter observation agrees fully with the findings of most other investigators (*inter al.* PETRAKIS 1954, TRUETA 1964, VALDERRAMA & TRUETA 1965 (intra-osseous pressure measurements), WHITE & STEIN 1965 (chrome-labelled erythrocytes)). However, according to MCPHERSON *et al.* (1961) and SHAW (1963, 1964) that procedure results in an increased skeletal blood flow. These investigators have used the same method as the present author, but with the measuring probes only temporarily applied in the bones, which can have had decisive importance for the observed changes. The increase in intra-osseous pressure through the venous occlusion can, under these experimental conditions, cause displacements of the probes, simulating an increased heat clearance, a source of error that some authors (MCPHERSON *et al.*) themselves have pointed out.

Stimulation of the peripheral cut ends of *the femoral and the sciatic nerves* produces changes in heat clearance indicating qualitatively similar changes of the intra-osseous blood flow. Repeated *isotonic* muscle *contractions* (1/sec) cause a scarcely detectable lowering of heat clearance, whereas a clearly marked lowering is noticeable at similar *isometric contractions.* It is reasonable to presume that at both types of contractions, an increased blood flow of comparable size through the musculature occurs; thus, a different diversion of blood from bone, based on vascular resistance differences in the musculature in the two cases, can hardly be the cause of the observed change in heat clearance. At isometric contraction, the mechanical influence on both periosteal vessels and on musculo-osseous vascular connexions is probably greater; therefore an interference most likely occurs here, especially with the venous outflow from the bone. This interpretation agrees with what others have found at intra-osseous pressure measurement (TRUETA 1964, VALDERRAMA & TRUETA 1965).

A *tetanus* of the musculature lasting for a few seconds is, after an initial brief increase, followed by a marked lowering of intra-osseous heat clearance. This rapidly becomes normal after the stimulus has ceased. The suggested initial flow increase probably represents a venous backflow, because the compressing strength of surrounding musculature primarily acts on the

venous extra-osseous vascular bed (cf. VALDERRAMA & TRUETA 1965). The absence of valves in many outflow channels from bone is the morphological condition that renders possible such a backflow. CUTHBERTSON *et al.* (1965) have also demonstrated by means of röntgenographic and tracer technique in dog that the femoral medullary cavity can function as a collateral venous drainage channel.

The reduction of the bone blood flow that then follows is presumably an expression of the increasing mechanical interference to which the extra-osseous vessels are subjected. The rapid return to the original level by the blood flow indicates that the large muscular flow that characterizes a post-exercise-hyperaemia does not seem to disturb the inflow to the bone.

After 8—10 repetitive tetanic stimulations with about 5-second intervals, the flow pattern in the bone was slightly modified. After the stimulation had ceased, the bone blood flow in this case did not immediately return to the control level, but showed a further reduction for a few seconds. It seems probable that skeletal muscle blood flow after such a period of heavy work is relatively more increased than in previous experiments. In situations of this nature, a marked peak flow occurs for a few seconds immediately after cessation of exercise; the flow then falls rapidly to a fairly steady level. This temporary over-shoot of flow in skeletal muscle might very well lead to a transient redistribution of flow from bone to skeletal muscle and hence explain the above phenomenon.

After a rest period of 15—20 minutes, the effect of a renewed tetanic stimulation is again identical with that first mentioned.

Results that are completely identical with those described are obtained at stimulation experiments after previous blockade of the adrenergic fibres with bretylium, which indicates that the observed flow changes do not depend on the sympathetic system.

Against the background of the results referred to, the skeletal blood flow appears in more ways than one to be sensitive to influences from activity in surrounding musculature. Purely mechanical interference with the extra-osseous vascular bed seems to be important. The possibility of changed distribution conditions of the blood concerning vascular beds in skeleton and in musculature must also be taken into account.

The author has found an obvious increase of heat clearance on operated sides after *peripheral nerve sectioning.* The statistical analysis shows that the change is mostly pronounced during the fifth and to a somewhat higher degree during the sixth post-operative day. Thereafter, heat clearance tends to fall and from the eighth to the thirteenth post-operative day, the clear-

ance values show an increasing tendency to become negative in relation to the control sides.

The question of the extent that heat clearance in these experiments reflects circulatory courses in the bone is of greatest importance. Earlier investigations indicate that bone tissue at immobilization shows signs of hypervascularization in the form of dilated vessels (LANDOFF 1942, GEISER 1957, GEISER & TRUETA 1958, VALDERRAMA & LITTLE 1965, SEMB 1966 b) more in number than is normal (GEISER & TRUETA 1958). This must mean, *inter alia*, that the blood quantity at a given time is increased. The fluid content in a tissue is important for its thermal conductivity, and it might be possible that the observed changes in heat clearance are due not so much to changes in the blood flow as to changes in the amount of blood. Against this hypothesis is set the following: the morphological changes mentioned above are first noted after one week; thereafter, they develop gradually up to and including the fourth week of immobilization, thus following a course that in regard to time is completely unrelated to the development of heat clearance observed by the author.

Because of the ability of the sinusoidal system to change volume (BRÅNEMARK 1959) the intra-osseous blood volume can perhaps increase also when the venous outflow from the extremity is suddenly held back, as happens at occlusion of the femoral vein. However, the author observed an unmistakable lowering of heat clearance as a result of this measure.

A series of earlier investigations with the use of other methods has mentioned increased blood flow at immobilization in both the entire extremity (KEMP *et al.* 1947, IMIG *et al.* 1953, HULTH & OLERUD 1960, SCHRÖDER & SEYFARTH 1960) and in individual skeletal parts (SEMB 1966 a, b, c, SHIM *et al.* 1966). Against the background of these findings and the above discussion, it must be considered most probable that observed changes in heat clearance represent qualitatively similar changes in the bone blood flow.

The investigation of medullary blood (rabbit) disclosed a significant increase of pH and oxygen saturation after 10 days of plaster immobilization (SEMB 1966 a, b) which was interpreted as signs of increased blood flow in bone. Plasma clearance of Sr^{85} by bone in dog suggested also increased intraosseous flow, but not until after two weeks of immobilization (SEMB 1966 c) carried out in the same manner. All observed changes thereafter remained for more than two weeks. With reference to flow increase, a probable explanation of the time differences that are found between these results and those of the author can lie in the altogether different immobilization methods. Peripheral nerve sectioning of the kind that the author uses should result in a considerably more extensive inactivation of the extremity and,

moreover, a disappearance of tonus in the affected musculature, whose counterpart is not found at plaster immobilization. The supposition is confirmed by the observations of other investigators. Measurements of bone blood flow, based on the initial short term bone clearance of Sr^{85} (dog, rabbit), reveal that plaster immobilization, after some initial reduction, results in a relative increase of the blood flow in tibia and calcaneus after two weeks of immobilization (SHIM 1966), whereas a significant flow increase can be observed as early as one week after division of the sciatic nerve (SHIM *et al.* 1966).

At a comparison of the development of heat clearance with the course of the growth curve after peripheral nerve division (Fig. 15, p. 97 and Fig. 4, p. 48) a parallelity appears clearly with some time-lag during the latter part of the observation period. The biggest increases in heat clearance occur mainly at the same time as growth maximum, whereas the clearance values from operated sides fall below those of the control sides 2—4 days before the time when the accelerated growth in length changes to a retardation. It is conceivable that the immobilization causes metabolic processes in the bone tissue, which induce an increased flow. However, if it is presumed that changes in heat clearance qualitatively reflect changes in the intraosseous blood flow, the obtained results thus point more to the fact that the increased blood flow is the primary and that this results in an increased longitudinal bone growth. The observed delay in this can be thought to be due to the stimulatory effect on the proliferating parts and on the hypertrophic-degenerative parts of the cartilaginous cell columns manifesting itself after periods of varying length. The deviation in the growth curve at day 9 has possibly the same background (Fig. 4, p. 48).

Present investigations indicate, moreover, that changes in the haemodynamic balance between skeleton and musculature can be underlying causes of the observed flow increase at immobilization by peripheral nerve sectioning. Muscular activity seems normally to lead to a decrease of intraosseous flow by mechanical interference with mainly the venous outflow from the bone. This indicates that the bone in an immobilized extremity would get more blood than a corresponding freely mobile extremity expressed in an increased flow rate.

Studies of the blood flow in skeletal musculature in dog HUDLICKÁ *et al.* 1959, BASS & HUDLICKÁ 1960, 1964) indicate that the flow after peripheral nerve division is increased as early as after 3—4 days and thereafter increases further in order, 35 days after the denervation, to be about 50 % higher than the flow in normally innervated musculature. This condition

can perhaps be thought to cause a re-distribution of blood in the extremity to the disadvantage of the bone tissue, whereby the above-mentioned flow increase is counteracted and replaced by a subnormal intra-osseous flow. The size of the flow increase in the musculature, however, makes this hypothesis scarcely credible: the author could see no influence on the bone blood flow by a moderate postexercise-hyperaemia, where the muscle flow considerably exceeds the percentage figure mentioned above.

A more probable explanation of the successively decreasing flow noted during the latter part of the observation period is that the rapid onset of the muscle atrophy mechanically interferes primarily with the outflow from the bone, thereby conditioning an intra-osseous stasis. The dilated vascular structures seen in bone tissue at immobilization (cf. GEISER 1957, GEISER & TRUETA 1958, SEMB 1966 b, *inter al.*) can be an expression for such a stasis. The markedly increased intramedullary pressure that SHAW (1964) observed in tibia in children with poliomyelitic extremity shortening can also confirm this hypothesis.

The intra-osseous temperature seems to provide no information about the skeletal flow conditions.

Sympathetic lumbosacral ganglionectomy, according to the present investigation, is followed by an increase of bone blood flow, indicated by a significant rise in heat clearance. The observed flow increase, however, is of very short-lived nature and a return to normal begins as early as after one day. LOWENSTEIN *et al.* (1958) and TROTMAN & KELLY (1963) using the radio-isotope method have also observed increased intra-osseous flow after sympathectomy. The question of the duration of the flow increase after sympathectomy (also concerning soft parts) has been the object of very lively discussion without unanimity being reached. The author's result agrees with those of several other investigators (BARCROFT & SWAN 1953, LAMBERT 1957, 1960, SCHÖNBACH 1964). These have also noted an increase in the flow (the entire extremity) but with return to the original level after a few hours or days.

The flow increase can be explained by the sudden disappearance of tonus of the intra-osseous vessels at the ganglionectomy, and a normalization of the flow probably indicates a return of vascular tonus presumably through a sensitizing of the vascular musculature for circulating noradrenalin (MACMILLAN *et al.* 1962). The basal vascular tonus, established by local mechanisms, which characterizes most vascular circuits, is perhaps of importance here too (FOLKOW 1964).

The results of the heat clearance measurements agree well also with the observed effect on the longitudinal skeletal growth. The effect of the brief

initial flow increase during the first day can be expected to be insufficient to be reflected in the first observations of the growth in length, which do not occur until the third post-operative day. After this, there are no signs either of increased blood flow or of increased growth.

The author could not find a satisfactory explanation for the noted higher intra-osseous temperature on sympathectomized sides.

General summary

Earlier investigations on accelerated skeletal growth are surveyed.

The longitudinal skeletal growth in rabbit tibia was studied with the aid of intravital labelling with oxytetracycline under varying experimental conditions, including interference with different nervous functions and partial immobilization by intact innervation.

The distribution of postganglionic sympathetic fibres to tibia in rabbit was mapped.

Earlier methods of measuring skeletal blood flow are discussed, and a method for long-term measurements of intra-osseous blood flow, based on the heated thermo-couple principle of GIBBS, was developed.

Qualitative blood flow measurements in the proximal tibial epiphysis were made. Here, was studied the relation between muscular activity and blood flow in the bone, as well as the haemodynamic changes in skeletal tissue at simultaneous stimulation of bone growth and after sympathectomy.

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Some aspects of longitudinal bone growth

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