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NERVOUS AND VASCULAR INFLUENCE ON LONGITUDINAL GROWTH OF BONE

AN EXPERIMENTAL STUDY ON RABBITS

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INTRODUCTION

The shape of a bone is predetermined for the species and the bone in question, and independent of the other parts of the organism *(Fell; Fell & Robinson; Selby & Murray; Selye)*. The length (and the girth) of a bone, however, is modified by hormonal, nutritional, mechanical, nervous, and vascular factors.

Apparently, *Stephen Hales (1727)* was the first to note that longitudinal growth of long bones takes place only at the epiphyses, though he did not recognize the importance of his discovery, which he mentions only in passing. His observation was soon confirmed by *DuHamel, Haller* and *Hunter;* none of them, however, seems to have understood the rôle of the epiphysial cartilage plate, nor the process of enchondral ossification, first mentioned by *Miescher*, and described in detail by *H. Müller*. *DuHamel* believed that interstitial growth of bone also occurred; it is now established that longitudinal growth of long bones occurs almost exclusively at the epiphysial cartilage plate *(Banks & Compere; Bisgard & Bisgard; Boerema; Dubreuil; Gatewood & Mullen; Haas 1917a; Helferich 1877; Humphry 1861; Maass; Silfverskiöld; Vahlquist; Wegner)*, though there have been a few dissenters *(Hellstadius 1947; Hellstadius 1951; Kornew; Latarjet; Policard; Wolff)*.

Gigantism and dwarfism due to pituitary disturbances are a well-known feature in affections of the pituitary and hypothalamus in childhood *(Ford)*; dwarfing can be produced experimentally by early hypophysectomy, and gigantism by administration of pituitary growth hormone. Growth, however, is also governed by the thyroid, the gonads, and possibly by the thymus. It has been suggested that the pituitary growth hormone need be present in only very small amounts to ensure comparatively normal growth, provided there is an adequate supply of thyroid and sex hormones *(Matson)*. On the other hand, an excess of both thyroid and estrogen caused retardation of growth in the rat *(Suzuki)*.

Lack of certain nutritional factors, such as vitamin D, retards longitudinal growth *(Duthie; Sissons)*, as does a quantitatively deficient diet *(Pratt & McCance; Winters & al.)*; however, longitudinal growth is less

retarded than other processes of growth. A wasting disease may also cause under-development *(Elo)*.

Under normal circumstances, longitudinal growth is very little affected by mechanical factors, but severely increased pressure perpendicular to the growth cartilage does cause slowing of growth *(Strobino & al.)*; lengthening has been produced with a traction apparatus applied to the epiphysis and the metaphysis *(Ring 1958a; Smith & Cunningham)*, though lengthening was attributed more to distraction of the epiphysial line than to facilitation of growth *(Ring 1958a)*. *Gelbke*, however, thinks traction deleterious to growth. According to a much favoured theory, reduced pressure on the epiphysial cartilage plate furthers growth *(Arkin & Katz; Ghillini; Hueter; W. Müller; Volkmann)*. According to another view, pressure *(Thoma; Wolff)*, and muscular action *(Bunak & Klebanova; Geiser)* is a stimulus for epiphysial growth, and inactivity slows down growth *(Barr; Elo; Evans; Helferich 1887; v. Langenbeck; Ollier; Phemister)*. According to a third view *(Eggers & al.; Jansen)* a certain degree of pressure stimulates bone growth, though excessive pressure is deleterious. The pressure needed to arrest growth at the epiphysial cartilage plate is high *(Gelbke; Strobino & al.)*, but there seems to be no definite threshold, since even slight pressure may induce changes in the growth cartilage if consistently applied *(Arkin & Katz; Maass)*.

Nervous and vascular factors influencing longitudinal growth of bone will be discussed in the following chapter.

HISTORICAL REVIEW

Nervous influence on longitudinal growth of bone

There is some anatomical basis for the assumption that the nervous system has a direct influence on bone growth: it is well known that bone marrow contains many nerve fibres, which apparently enter it with blood vessels, and nerve endings have been observed in close contact with osteoblasts in young animals *(DeCastro)*, and in compact bone in adults *(Hurrell; Ignatov)*. It has even been thought that trophic centres regulating bone growth exist in the spinal cord *(Curcio)*, though the experimental method supporting this view is open to criticism.

Much experimental work has been done on the effect of denervation on bone growth; most investigators have used peripheral nerve section, and a few have performed nerve root section. A glance at a sketch of the spinal cord and the nerve roots of the lower limb in relation to the sympathetic nervous system (Fig. 1) shows that there is a considerable difference between denervation achieved by peripheral nerve section and denervation achieved by nerve root section. Section of peripheral nerves produces complete denervation if extensive enough, whereas motor or

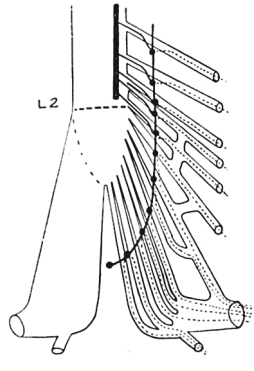

Fig. 1. Diagram of sympathetic innervation of lower limb in man; redrawn after *White & Smithwick*.
Preganglionic fibres ———— Postganglionic fibres --------

sensory nerve root section abolishes only part of the innervation of a limb; even if section of both motor and sensory nerve roots is performed, the sympathetic nerve supply remains (Fig. 2).

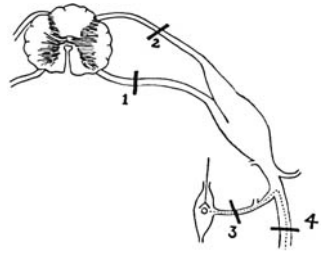

Fig. 2. Diagram showing relation of sympathetic chain to spinal cord below the level of L2 in man.
Preganglionic fibres ———— Postganglionic fibres --------
1 — section of motor nerve root; sympathetic fibres not affected.
2 — section of sensory nerve root; sympathetic fibres not affected.
1 + 2 — section of motor and sensory nerve roots; sympathetic fibres not affected.
3 — section of sympathetic postganglionic fibres.
4 — section of peripheral nerve; sympathetic fibres also severed.

After peripheral nerve section, the most general finding has been atrophy of bones with slight retardation of longitudinal growth *(Allison & Brooks; Armstrong; Bergmann; Ghillini; Howell; Pottorf)*. It is, however, stated by some *(Allison & Brooks; Grey & Carr; Joseph)* that the atrophy caused by simple immobilization of a limb is as severe as that caused by nerve section. Others have found hypertrophy and acceleration of growth after nerve section *(Ghillini; Kassowitz; Milne Edwards; Nasse; Ring 1961; Schiff; Vulpian)*; *Vulpian* stated that hypertrophy is due to an increased susceptibility to trauma in a limb lacking normal protective sensation, and *Ghillini* suggested that the different results were due to differences in the amount of running about allowed to the experimental animals. The denervation experiment which produced hypertrophy of the lower jaw *(Milne Edwards; Schiff)*, i.e. section of the mandibular nerve, was repeated by *Ollier* and *Sulger* with negative results; they attributed the hypertrophy previously reported to infection.

A few claim that denervation has a definite retarding effect on longitudinal growth *(Fischer; v. Langenbeck; Ollier; Samuel)*, but *Fischer* merely asserts this, *Ollier* gives no actual measurements, *Samuel's* results apply to regeneration of feathers in doves, and *v. Langenbeck* stresses the individual variations in the response to peripheral nerve section.

Peripheral nerve injuries in children are reported to cause retardation of growth *(Combes & al.; Ford; Remak)*, and in a case of syringomyelia in a child, slight shortening of the affected arm was reported *(Bermann)*.

Thus, at least in experimental work, peripheral nerve section seems to have very little effect on longitudinal growth of bone, and some *(Borel; Friedl & Schinz; Heřt; Kapsammer; Selye & Bajusz)* even go so far as to deny that peripheral nerve section has any influence on longitudinal growth of bone.

There are a few papers dealing with the effect of experimental nerve root section on bone *(Corbin; Corbin & Hinsey; Eloesser; Gillespie; Grey & Carr; Kuré; Ring 1961; Tower)*, but none of these experiments have been performed on very young animals. *Corbin, Corbin & Hinsey,* and *Eloesser* were concerned with the joint changes found after section of sensory nerve roots: *Gillespie* and *Grey & Carr* found atrophy and slight retardation of growth after motor nerve root section, but not after sensory root section; *Ring's (1961)* results refer to the length of the diaphysis only; *Tower* found atrophy after posterior root section combined with section of the spinal cord above and below the segments subjected to rhizotomy, but longitudinal growth seemed unaffected.

Kuré alone claimed that section of nerve roots had a direct influence on bone, but he gave no measurements, and his experiments were mainly concerned with the regeneration of the 5th toe in the Japanese giant salamander.

It is interesting that experimental nerve section, whether section of peripheral nerves or of nerve roots, should have so slight an effect on longitudinal growth of bones in view of the retardation of growth so often seen after paralysis of a lower limb by poliomyelitis. This retardation can be correlated with the degree of paralysis *(Gullickson & al. 1950; Humphry 1862; Lindholm; Ratliff; Ring 1957; Ring 1958a; Ring 1958b; Stinchfield & al.)* but all agree that individual cases show considerable deviation from the statistical mean.

Ring (1957) says that paralysis is not the direct cause of retardation of growth, and postulates a chronic vascular factor, perhaps brought about by muscle loss; *Barr* seems to favour a similar view; retardation of growth in a limb with congenital deficiency of vasomotor control has been described by *Krepler*. Blood flow in a limb is reported to be decreased by abolished muscle function *(Ollier; Sulger)*, immobilization *(Hultén)* and denervation *(Kemp & al.)*; changes in the pattern and decrease in the size of the blood vessels have been found after section of the sciatic nerve in rabbits *(Ferguson & Akahoshi)*. Several investiga-

tions, however, have given quite contrary results *(Hulth & Olerud; Imig & al.; Schröder & Seyfarth)*, and arteriovenous anastomoses have been demonstrated in legs paralyzed by poliomyelitis *(Braibanti; Piulachs & Vidal-Barraquer)*. Furthermore, there is no evidence that a cold and clammy limb is particularly likely to develop retardation of growth *(Ratliff)*, nor have any consistent changes in peripheral blood flow been found after poliomyelitis *(Abramson & al.; Dohn; McPherson & Kessel; Wiggins & al.)*.

At present, therefore, evidence on this subject is highly controversial, and it should be remembered that the possibility of direct damage to vessel walls by the poliomyelitis virus has been suggested *(Prick)*.

Since increased blood flow may lead to overgrowth of a limb (see page 13), sympathectomy might be expected to cause overgrowth. Increased blood flow has been demonstrated in muscles after sympathectomy on dogs *(Lowenstein & al.)*; increased growth of auricular hair after cervical sympathectomy on rabbits has been found *(Pye-Smith; Stirling)*, as well as enhanced regeneration after injury to a sympathectomized ear *(Liek)*. Stimulation of the sympathetic chain in dogs caused slight retardation of growth of bones *(Gullickson & al. 1951)*. Increased growth of bones, however, has generally not been found *(Bacq; Bisgard 1931; Bisgard 1933; Cannon & al.; McCullagh & al.; Ollier; Ring 1961; Simon)*, nor was increased growth seen after unilateral sympathectomy on a monkey with bilateral paralysis of the lower limbs after poliomyelitis *(Bisgard 1933)*. *Goetz & al., Gullickson & al. (1951)*, and *Kishikawa* are the only ones who have found increased growth of bones after sympathectomy on experimental animals (rabbits and dogs). *Goetz & al.* consider earlier failures to be due to the fact that only lumbar sympathectomy was performed, which does not cause sympathectic denervation above the knee; when they measured the bones of the foot, evidence of increased bone growth after lumbar sympathectomy was found. This tallies with reports on increased growth of short legs after sympathectomy in man *(Barr; Barr & al.; R. I. Harris; Harris & McDonald; Robertson)*, as well as with reports on increased growth after the use of sympathicolytic drugs *(Kottke & al.)*. There are, however, discordant views *(Bombelli; Fahey)* and some *(Ogilvie; Robertson)* do not think that sympathectomy is justified as a treatment for retardation of growth.

The difference between the effect of experimental nerve section and the effect of poliomyelitis may be related to the function of the autonomous nervous system; affection of the autonomous nervous system is a well-recognized feature in poliomyelitis *(Collins & al.; Fanconi; Hagel-*

stam; Kottke & al.; McPherson & Kessel; Lundbaek; Moldaver; Sabin; Sabin & Ward; Smith & al.; Spencer & al.; Steindler; Stenport; Zimányi & al.). *Fanconi* claimed that the skin and temperature changes in acute poliomyelitis are caused by lack of movement, destruction of lateral horn cells, and arterial hypoplasia *(Piulachs & Vidal-Barraquer; Telford & Stopford).* He also thought that retardation of growth is caused by a lesion of trophic nerves, apparently referring to the observation of *DeCastro* that nerve fibres can be found in close contact with osteoblasts in young animals. On the other hand, a sympathetic paralysis should cause vasodilatation, whereas the poliomyelitic limb is notoriously cold, blue and clammy, suggesting overfunction of the vasoconstrictive fibres; this vasoconstriction is reported to be absent in the acute stage *(Trott & al. 1956; Trott & al. 1958).*

Vascular influence on longitudinal growth of bone

Since an adequate supply of nutriment is essential for the growing cells of the growth cartilage, it is self-evident that circulation is an important factor in the longitudinal growth of bone. Many authors *(Brodin; Silfverskiöld; Trueta)* have stressed this point, but others *(H. A. Harris; Pease; Wilson & Thompson)* have thought functional factors equally important, and it has been maintained that stasis or moderate ischaemia have no effect on longitudinal growth *(Borel; Dickinson; Helferich 1887; Grey & Carr).*

Experimental research has been mainly directed towards finding measures for increasing bone growth, apparently because of the practical value that a reliable procedure for stimulating growth would have.

Longitudinal growth of bone has been stimulated experimentally by many methods: — trauma to the medullary cavity *(Ferguson; Greville & Janes; Kishikawa; Levander);* plugging of the medullary cavity *(Carpenter & Dalton; Trueta);* foreign material near epiphysial plates *(Chapchal & Zeldenrust; Ford & Canales; Pease; Wu & Miltner);* stripping of periosteum *(Brodin; Compere & Adams; Elo; Lacroix; Langenskiöld 1957; Ollier; Wu & Miltner);* arteriovenous fistula *(Doerr & Janes; Janes & Musgrove: Kelly & al.);* venous stasis *(Bergmann; Janes & Musgrove; Kishikawa; Pearse & Morton 1930; Servelle; Wu & Miltner);* electrolysis *(Wilson & Percy);* and heating *(Richards & Stofer).* Negative reports on the effect of venous stasis *(Borel; Dickinson; Grey & Carr; Helferich 1887;),* implantation of foreign material *(Bohlman; Haas 1958; Herndon & Spencer; Meisenbach; Montgomery & In-*

gram), and heating *(Ring & Lee)* have been published; the effect of ultrasound in moderate doses is reported to be nil *(Vaughen & Bender)*, and retardation of growth is found after higher dosage *(De Forest & al.)*.

It has been suggested that the common denominator for procedures resulting in stimulation of bone growth is an increase in the blood flow *(Janes & Musgrove)*, though blood flow is decreased immediately distal to an arteriovenous fistula, at least at first *(Pauporte & al.)*. *Arkin & Katz* thought that immobilization of the operated limb, with consequent decrease of pressure on growth cartilages, is an important factor in accelerating growth.

A few studies have been concerned with the effect of decreased vascular supply to bones: ligation of the femoral artery *(Friedl & Schinz; Latarjet; Milne Edwards; Ollier; Pearse & Morton 1931; Sousa Pereira)*, sometimes combined with peripheral nerve section *(Borel; Friedl & Schinz)* or division of the nutrient artery of the bone *(Latarjet)*. The results have generally been insignificant. Some shortening at the end of the growth period was seen after plugging of the nutrient canal in new-born rabbits *(Brookes)*, and extensive excision of the femoral and popliteal artery caused retarded healing of experimental fractures *(Pearse & Morton 1931)*. Retardation of growth in metacarpal and metatarsal bones after direct stripping of periosteum and perichondrium has been found *(Haas 1917b; Sousa Pereira)*, and it is well known that transplanted growth cartilages, being deprived of their blood supply, lose a considerable part of their growth potential *(Aron & Simon; Axhausen; Brücke; Enderlen; Haas 1916; Heikel 1959; Heikel 1960b, Helferich 1899)*; the contrary results of *Rehn & Wakabayashi* stand alone and are perhaps due to some error in the interpretation of the histological observations. The effect of interrupting either the epiphysial or the metaphysial blood supply to the epiphysial cartilage plate has been studied; in many cases epiphysiodesis was induced with closure of the epiphysial line *(Trueta & Amato)*.

These methods, which led to retardation of growth, all imply direct handling of the growth cartilage, and, however gentle these methods may have been, the possibility that the direct trauma had some effect cannot be excluded.

There seem to be no studies concerned with the effect of temporary ischaemia on growing limbs; it is stated that the time limit for operating in a bloodless field in the limbs is 2 hours *(Bunnell)*, and this time-limit can be extended *(Mason & Bell)*. In the dog, experimental studies have demonstrated neuromuscular functional impairment and delayed return of the normal temperature of the skin and muscles after

ischaemia of more than 4 hours' duration *(Paletta & al.; Walker & al.)*. Since growth processes are particularly active in the growth cartilage, which is thought to be very sensitive to disturbances in nutrition, the effect of temporary ischaemia on the growth cartilage seemed worth studying.

THE PURPOSE OF THE INVESTIGATION

The mechanism which causes retardation of longitudinal growth of bone in paralytic poliomyelitis is obscure. Experimental investigations have not been performed systematically, and the evidence gathered from clinical studies is conflicting. It is known that section of peripheral nerves causes only slight retardation of longitudinal growth, if any, and section of motor nerve roots, according to *Gillespie*, leads to slight retardation of longitudinal bone-growth in half-grown animals. *Ring (1961)* found an increase in diaphysial growth after section of motor nerve roots in puppies.

The purpose of my experiments was:

to investigate the effects of different kinds of partial denervation of the hind limb on longitudinal growth of bone in young animals;

to investigate the effect of severe devascularization on longitudinal growth of bone, avoiding direct trauma to the growth cartilages.

The latter part of the investigation is supplemented by studies on the effect of sympathectomy on severely devascularized limbs, and studies on the effect of temporary ischaemia on longitudinal growth of bone.

GENERAL PLANNING OF EXPERIMENTS

Rabbits 13—17 days old were used, generally weighing 140—270 grams; the comparatively large size of rabbits facilitates early intervention and good roentgenograms; their quick growth ensures that any changes produced by experimental procedures show soon.

To avoid as far as possible the effect of secondary contractures on longitudinal growth *(Bunnell; Kritter & Blount; v. Langenbeck; Schubert)*, rather short follow-up periods were used.

The bones selected for measuring were the femur and the tibia, which are the longest bones of the rabbit; thus, minor errors in measuring were not likely to influence the results unduly. Since most of the operations performed turned out to have caused some contracture, at least in the hip, symmetrical positioning of the hind limbs for roentgenographic measuring in the live animal was difficult. The use of roentgenographic measurements was therefore rejected, and the femur and the tibia were measured with calipers when the animals had been killed and the bones removed and cleaned; this method of measurement is exact to 0.5 mm *(Greville & Janes)*. The length of the femur was measured from the upper articular surface of the femoral head to the articular surface of the medial condyle; the length of the tibia was measured from the eminentia intercondylica to the incisure between the two articular surfaces of the lower end of the tibia. All measurements were corrected to the nearest 0.5 mm.

As the denervation procedures were found to be technically easier on the left side, the left hind limb was used as the experimental limb throughout the investigation, the right hind limb serving as a control. Autopsy was performed on all animals subjected to section of nerve roots, to check which roots had been severed.

The bones studied were preserved in formalin; they were usually decalcified in a 10 per cent solution of the disodium salt of diethylenetetraacetic acid, and stained with haematoxylin – van Gieson.

DENERVATION

Anatomy

The hind limb of the rabbit is innervated mainly by the nerve roots L6—L7—S1—S2 *(Goetz & al.; Krause)*, of which L7 and S1 are generally the thickest ones. In the rabbit the spinal cord extends well into the sacral canal; thus the nerve roots are short and easily identifiable.

Technique

Under local anaesthesia with lidocaine the lumbar spine was exposed through a dorsal midline incision. General anaesthesia with ether was then induced, and a suitable low lumbar hemilaminectomy was performed with some unroofing of the sacral canal. The nerve roots were exposed, identified, and cut. Penicillin was introduced into the wound, which was then closed in two layers with catgut.

In the course of preliminary experiments, not reported upon here, it was found both at operation and at autopsy that there were usually two nerve roots thicker than the others; these nerve roots were, on the whole, identifiable at autopsy as L7 and S1, though in some cases pre- or postfixation was found. In the animals with a four-root section these two thick roots were cut, as well as one root cranial and another caudal to them. In the animals with a three-root section the two thick nerve roots were cut, and one root caudal to them. In one group of two-root sections, the more caudal of the two thick nerve roots, and the one caudal to this, were cut; in another group of two-root sections, the more cranial of the two thick nerve roots, and the one immediately cranial to this, were cut.

Useful data were obtained from 45 animals.

Section of 4 motor nerve roots

After section of 4 motor nerve roots, complete or almost complete paralysis of the denervated limb was observed in all animals; no animal

LENGTH OF FEMUR AND TIBIA IN THE RABBIT
after section of
4 lumbosacral motor nerve roots

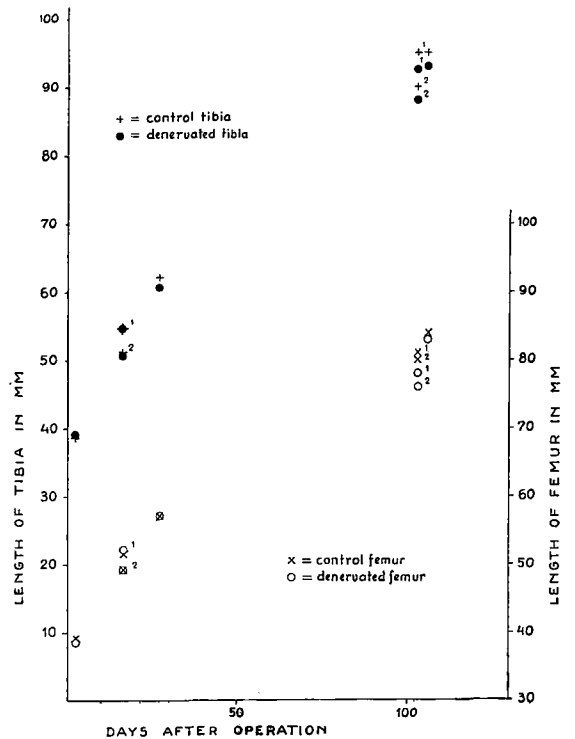

Fig. 3. Indices(1,2) denote different rabbits killed the same number of days after operation.

LENGTH OF FEMUR AND TIBIA IN THE RABBIT
after section of
3 lumbosacral motor nerve roots

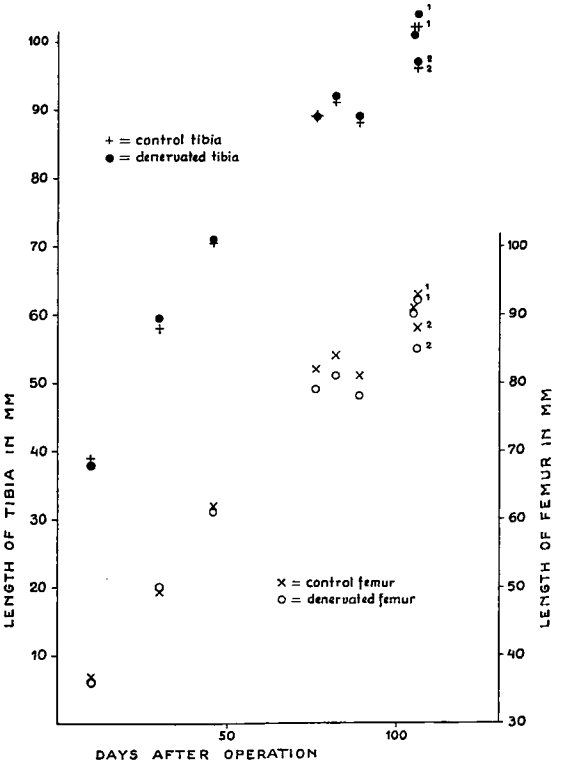

Fig. 4. Indices(1,2) denote different rabbits killed the same number of days after operation.

was able to use the denervated limb, although in some instances there was flexion and adduction in the hip. There was severe atrophy of the muscles of the denervated limb after 4 weeks.

The effect of section of 4 lumbosacral motor nerve roots on the length of the femur and the tibia is shown in Fig. 3.

Section of 3 motor nerve roots

After section of 3 motor nerve roots, paralysis, almost as severe as occurred after section of 4 motor nerve roots, was observed in the denervated limb; no animal was able to use the denervated limb. In the muscles, atrophy of the same degree as occurred in the group with section of 4 motor nerve roots was found.

The effect of section of 3 lumbosacral motor nerve roots on the length of the femur and the tibia is shown in Fig. 4.

Section of 2 motor nerve roots

Section of 2 motor nerve roots was performed in 8 animals. Two variants of this — see paragraph on technique, page 18 — were used, each on 4 animals. However, as no difference was found between these two groups either on postoperative examination, roentgenological examination, or by measurement, they are reported upon as a single group.

In all animals weakness of the denervated limb was found immediately after operation, but all animals were able to use the denervated limb well, possibly because of overlapping innervation.

The effect of section of 2 motor nerve roots on the length of the femur and the tibia is shown in Fig. 5.

Section of 4 motor and 4 sensory nerve roots

After section of 4 motor and 4 sensory lumbosacral nerve roots, complete or almost complete paralysis of the denervated hind limb was found, as in the group subjected to section of 4 motor nerve roots. No animal was able to use the denervated limb.

The effect of section of 4 motor and 4 sensory lumbosacral nerve roots on the length of the femur and the tibia is shown in Fig. 6.

Section of 4 sensory nerve roots

In the postoperative examinations of the animals subjected to sensory denervation, it was found that they retained a crude motility in the

LENGTH OF FEMUR AND TIBIA IN THE RABBIT
after section of
2 lumbosacral motor nerve roots

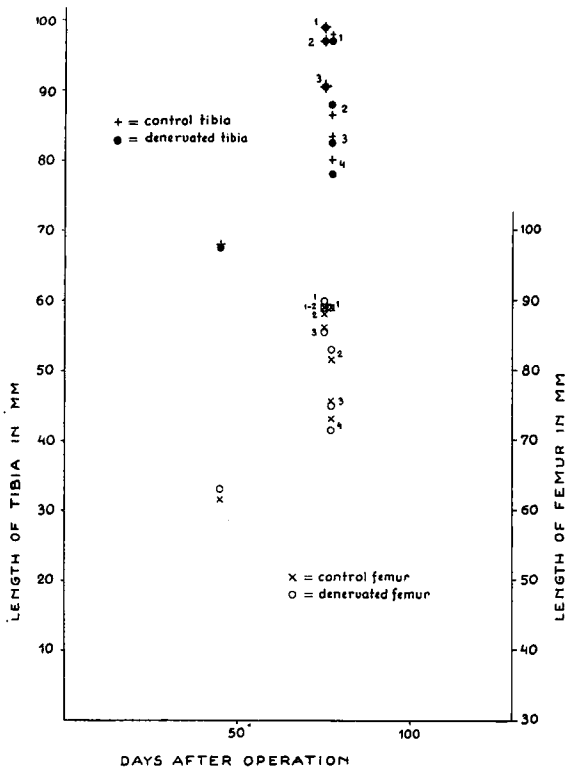

Fig. 5. Indices(1,2,3,4) denote different rabbits killed the same number of days after operation.

LENGTH OF FEMUR AND TIBIA IN THE RABBIT
after section of
4 motor and 4 sensory lumbosacral nerve roots

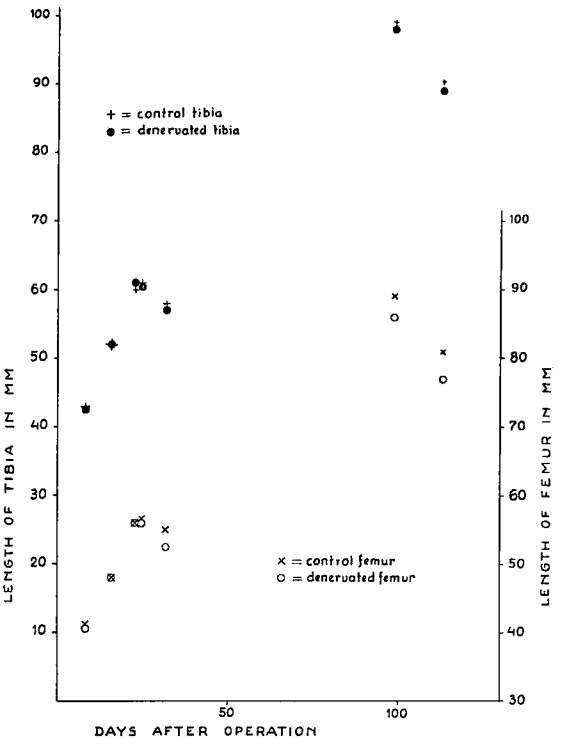

Fig. 6.

denervated limb *(Lassek & Moyer)*, though they could not move the limb in a purposeful manner. When lifted by the nape of the neck they would wriggle violently, kicking with both legs and stretching them forward, but the denervated limb would then sink down, apparently owing to lack of positional sense.

At autopsy, subluxation of the hip was found in some cases. Muscle atrophy was much less severe than in limbs subjected to motor denervation. Any grazing was generally found around the ankles, as a rule bilaterally.

The effect of section of 4 sensory nerve roots on the length of the femur and the tibia is shown in Fig. 7. There was considerable retardation of growth of the denervated femur, but not of the tibia. In all animals surviving operation for 25 days or more, considerable thickening of the lower epiphysis of the femur was observed, with distortion of the epiphysial line. The roentgenological and histological findings (see page 24 and 30) suggest that the retardation of growth was due to the rabbit's inadvertently injuring an anaesthetic limb. This assumption seems to tally with the experimental production of Charcot joints by sensory denervation *(Eloesser)* and with the bone changes associated with syringomyelia in children *(Ford)*. In no case was any sign of purulent arthritis found in the knee joint at autopsy.

To prove the assumption that retardation of growth of the femur, found after sensory denervation, was due to traumatization of an anaesthetic limb, experiments were performed with the denervated limb protected. Insertion of the denervated and skinned limb under the abdominal skin was considered a suitable method. Five animals were subjected to sensory denervation at 14 days of age; a fortnight later the denervated limb was skinned and inserted under the abdominal skin. At this stage, check roentgenograms showed no appreciable changes in the bones of the denervated limbs. The results of this two-stage procedure are shown in Fig. 8. Growth in the femur was much less retarded than in the series subjected to sensory denervation only, and in the tibia acceleration of growth was even seen.

Roentgenological observations

After section of 4 motor nerve roots, osteoporosis of the bones of the denervated limb (Fig. 9) was seen in all but two of the animals; of these two, one died 2 days after operation and one was killed 16 days after operation; another animal killed 16 days after operation already showed slight osteoporosis.

LENGTH OF FEMUR AND TIBIA IN THE RABBIT
after section of
4 lumbosacral sensory nerve roots

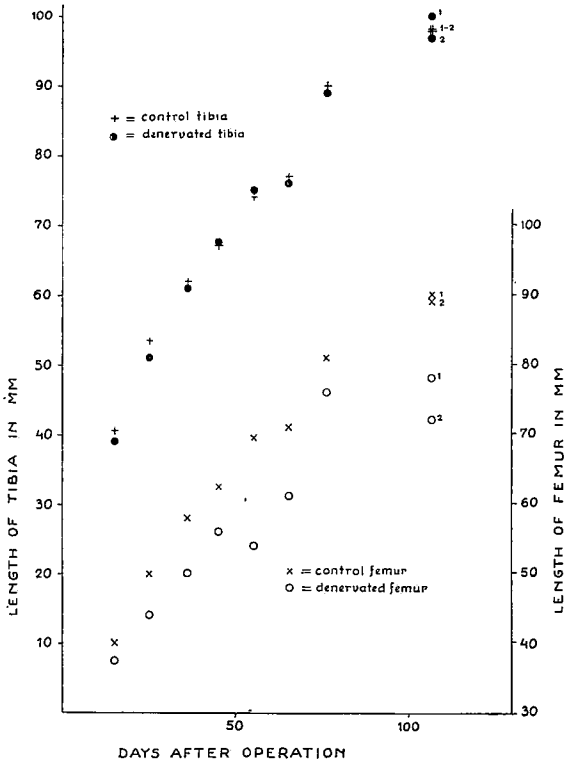

Fig. 7. Indices(1,2) denote different rabbits killed the same number of days after operation.

LENGTH OF FEMUR AND TIBIA IN THE RABBIT
after section of 4 lumbosacral sensory nerve roots
and insertion of the denervated limb under the abdominal skin

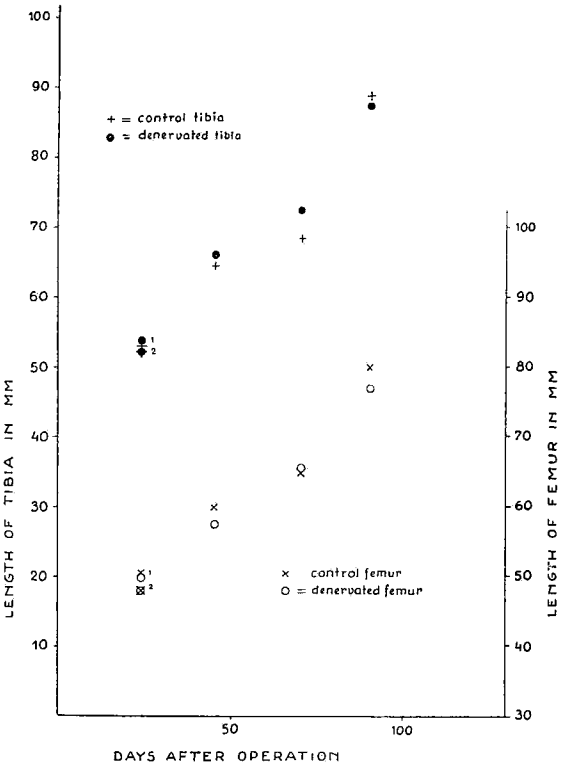

Fig. 8. Indices(1,2) denote different rabbits killed the same number of days after operation. Insertion of the denervated limb under the abdominal skin was performed 14 days after denervation (see page 00).

In the group subjected to section of 3 motor nerve roots, definite osteoporosis was seen from the 30th day after operation, and one animal had already shown osteoporosis in the denervated limb 9 days after operation (Fig. 10).

In the group subjected to section of 2 motor nerve roots, osteoporosis was observed in the denervated limb in all animals, despite the very slight and transient changes in the function of the limb (Fig. 11).

After section of 4 motor and 4 sensory nerve roots, osteoporosis was observed in the denervated limb in all but one of the animals; this animal died 8 days after operation.

As mentioned previously, retardation of growth and thickening was found in the denervated femur of the rabbits subjected to section of 4 sensory nerve roots. Fifteen days after operation, an area of decreased density was seen in the distal epiphysis of the denervated femur and the proximal epiphysis of the denervated tibia (Fig. 12); 25 days after operation, there was distortion and thickening of the distal end of the femur, and the epiphysial line appeared widened and blurred, with spurs of bony overgrowth at the margins (Fig. 13). Thirty-six days after operation the roentgenological findings were similar to those observed 25 days after operation, and, in addition, the proximal end of the denervated tibia was somewhat thicker than it was in the control limb, as was the cortex of the bone. In the animals killed 45, 55, 65, 76, and 106 days after operation, these changes had progressed, with considerable stunting of the longitudinal growth of the femur, and thickening of the upper end of the tibia. From the 45th day after operation, it was seen that the structure in the upper end of the femur had become progressively more irregular (Fig. 14). This may, possibly, be explained by the subluxation found in some cases.

These roentgenological observations suggest that deformity and retardation of longitudinal growth of the femur was due to inadvertent traumatization of an anaesthetic limb. Roentgenological examination of the animals in the control series, in which the denervated limb was skinned and tucked under the abdominal skin 14 days after denervation, showed much fewer changes. Roentgenograms of these animals immediately before the second operation showed no definite changes; roentgenograms of the 2 animals that died 24 days after denervation (10 days after the second procedure) showed some decrease in density in the lower femoral epiphysis; in the animal that died 45 days after denervation, there was thickening of the lower femoral epiphysis, the trabeculae of which seemed coarse (Fig. 15); the trabecular arrangement in the upper end of the femur

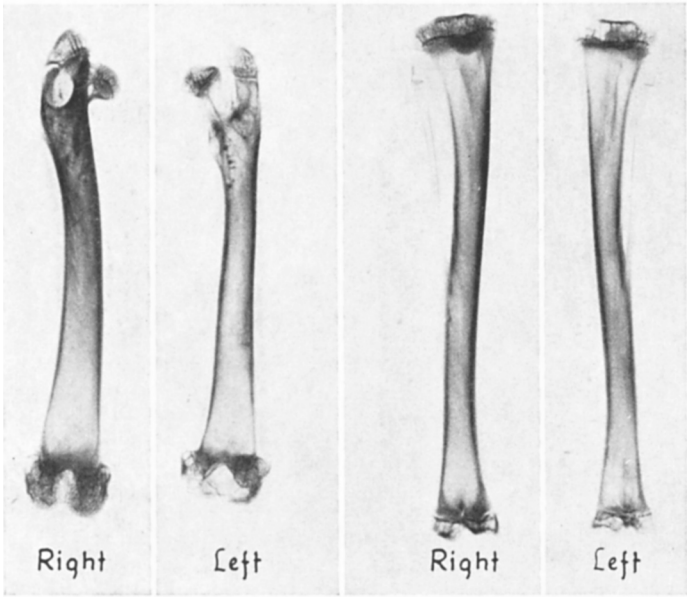

Fig. 9. Femora and tibiae of rabbit subjected to section of 4 lumbosacral motor nerve roots on the left at 17 days of age, and killed 103 days after operation. Left femur 4 mm shorter than right, left tibia 2 mm shorter than right. Considerable osteoporosis of left femur and tibia.

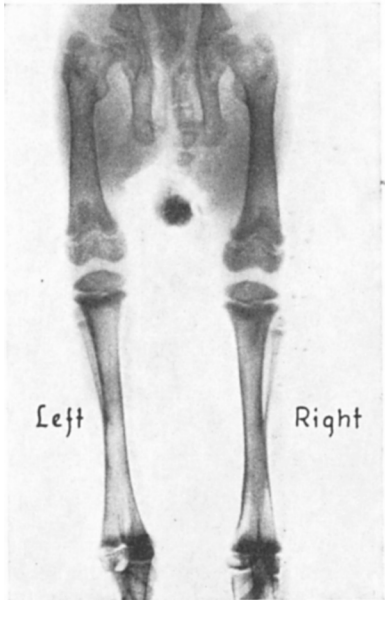

Fig. 10. Femora and tibiae of rabbit subjected to section of 3 lumbosacral motor nerve roots on the left at 15 days of age; roentgenogram of live animal 9 days after operation. Osteoporosis of left femur and tibia.

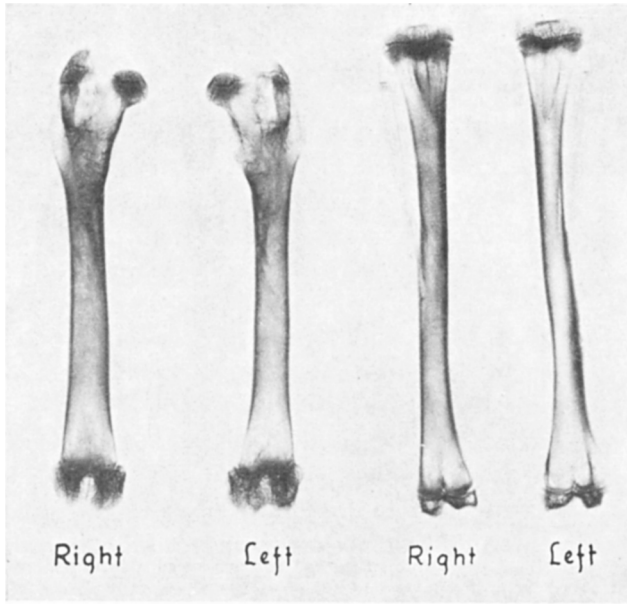

Fig. 11. Femora and tibiae of rabbit subjected to section of 2 sacral motor nerve roots on the left at 15 days of age, and killed 75 days after operation. Left femur 0.5 mm shorter than right, tibiae of equal length. Slight osteoporosis of left femur and tibia.

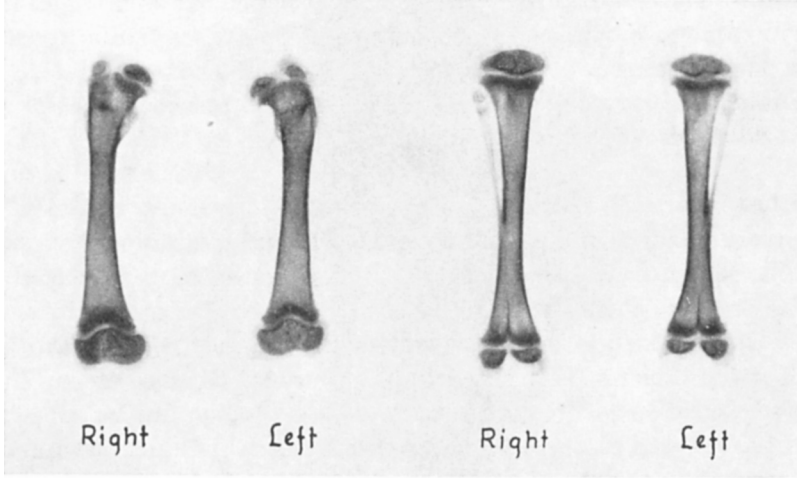

Fig. 12. Femora and tibiae of rabbit subjected to section of 4 lumbosacral sensory nerve roots on the left at 15 days of age, and killed 15 days after operation. Osteoporosis of lower femoral and upper tibial epiphysis.

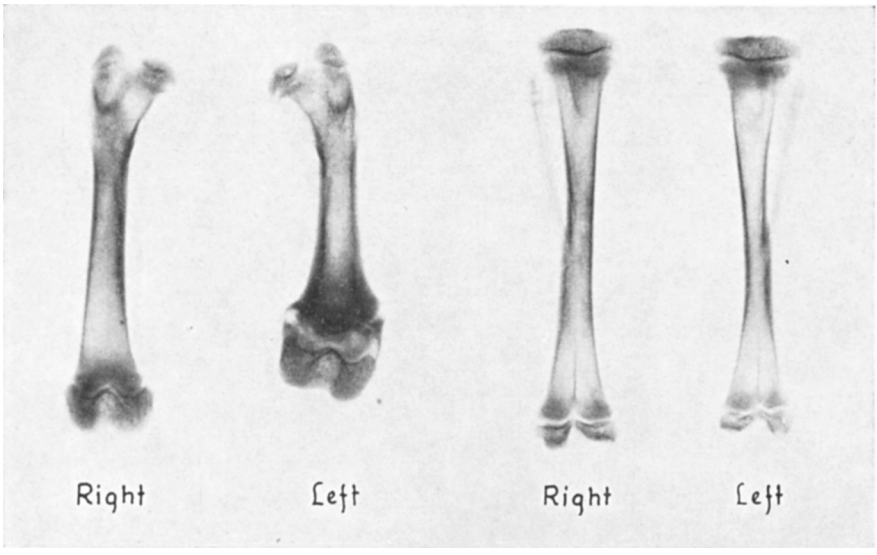

Fig. 13. Femora and tibiae of rabbit subjected to section of 4 lumbosacral sensory nerve roots on the left at 15 days of age, and killed 25 days after operation. Left femur 6 mm shorter than right; left tibia 2.5 mm shorter than right. Thickening of lower femoral epiphysis, epiphysial line blurred, bony overgrowth at lateral margins. Osteoporosis in lower femoral and upper tibial epiphysis. Compare Fig. 17, page 29.

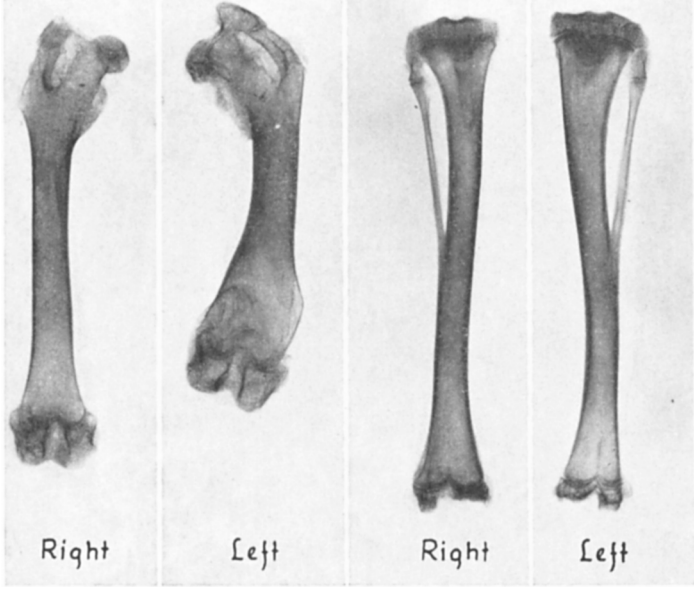

Fig. 14. Femora and tibiae of rabbit subjected to section of 4 lumbosacral sensory nerve roots on the left at 14 days of age, and killed 106 days after operation. Left femur 17 mm shorter than right; left tibia 2 mm longer than right. Thickening and distortion of left femur; slight thickening of upper end of tibia.

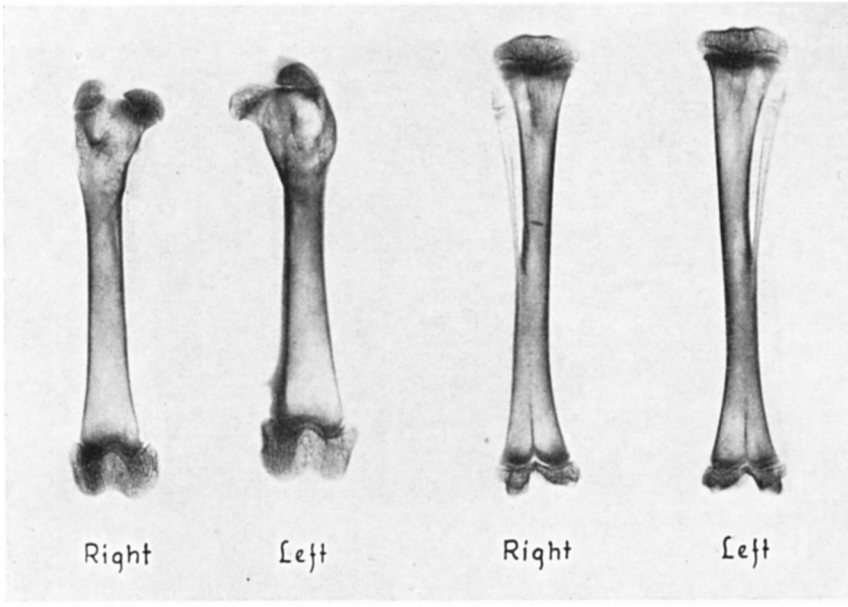

Fig. 15. Femora and tibiae of rabbit subjected to section of 4 lumbosacral sensory nerve roots on the left at 14 days of age; denervated limb inserted under the abdominal skin at 28 days of age; animal died 45 days after denervation. Left femur 2.5 mm shorter than right; left tibia 1.5 mm longer than right. Slight thickening of left femur; osteoporosis in femoral head and lower femoral epiphysis; very slight osteoporosis of left tibia.

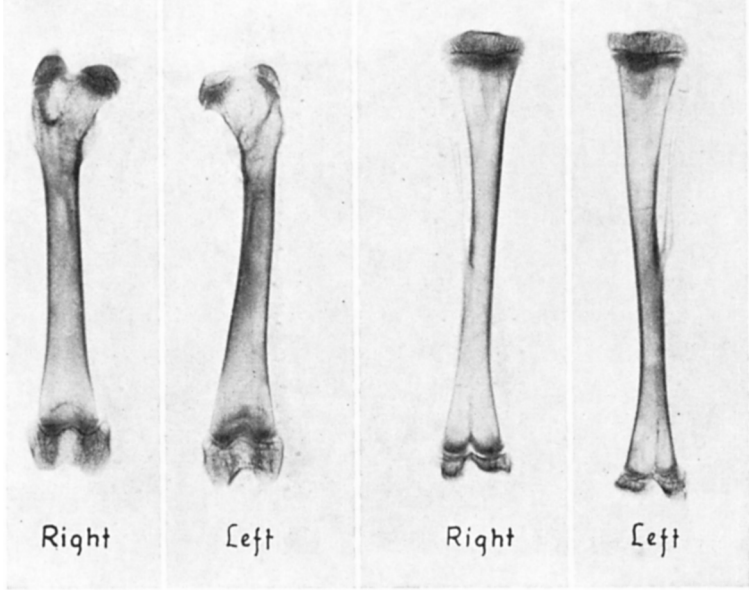

Fig. 16. Femora and tibiae of rabbit subjected to section of 4 lumbosacral sensory nerve roots on the left at 14 days of age; denervated limb inserted under abdominal skin at 28 days of age; animal killed 70 days after denervation. Left femur 0.5 mm longer than right; left tibia 4 mm longer than right. Osteoporosis of left femoral head and lower femoral epiphysis, and of tibia.

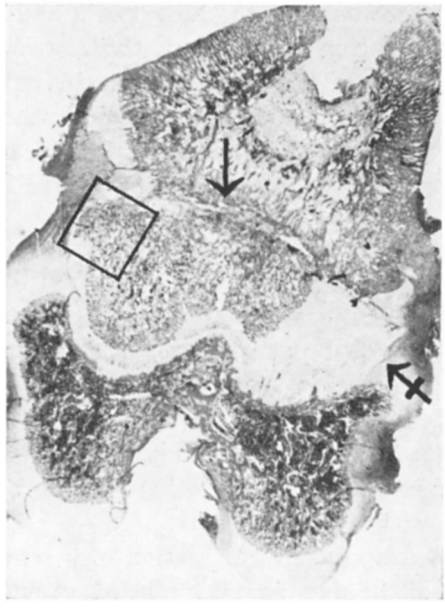

Fig. 17 a. Section of lower end of femur in rabbit subjected to severance of 4 sensory nerve roots on the left at 15 days of age, and killed 25 days after operation. Transverse fracture in metaphysis (↓); delayed ossification of growth cartilage (⇞). × 3.5.

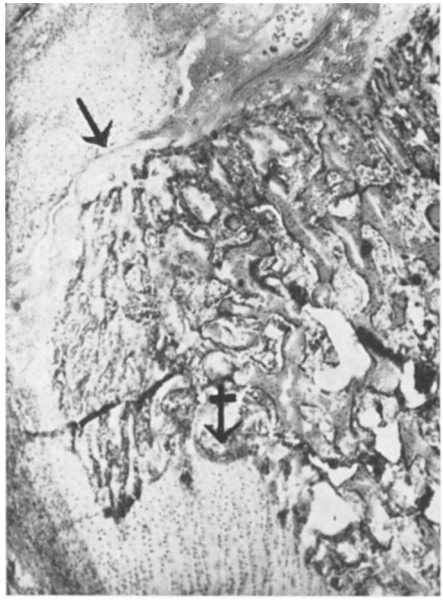

Fig. 17 b. Magnification of square marked on Fig. 17 a. Crack in metaphysis at top (↓); growth cartilage at bottom (⇟) × 25.

was also disturbed, as was that of the upper tibial epiphysis. In the animal killed 70 days after denervation, there was also thickening of the femur, in particular of the lower epiphysis, but the femora were equal in length, and the trabecular arrangement in the denervated femur (Fig. 16) was by no means as disturbed as in the case of the animal killed 76 days after denervation without insertion of the limb under the abdominal skin.

Histological observations

Only in the groups subjected to sensory denervation were changes found that could be called pathological; other groups are not worth reporting on.

In the rabbit killed 15 days after severance of 4 sensory nerve roots, slight disarrangement of the cellular columns in the lower femoral growth cartilage was found in some sections; otherwise the histological findings in this animal were within normal limits.

In the rabbit killed 25 days after section of 4 sensory nerve roots, a transverse fracture of the metaphysis of the lower end of the denervated femur was found; there was some disarrangement of the growth cartilage, and it seemed probable that the fracture had affected the growth cartilage (Fig. 17). Such a fracture provides a natural explanation for the retardation of growth observed after sensory denervation; it also explains the thickening.

A similar fracture was observed in the animals killed 36, 55 and 65 days after operation; in the other animals only very slight disarrangement of the cell columns in the growth cartilage was found, and it is doubtful whether such changes could be called pathological.

The animals that had had the denervated limb inserted under the abdominal skin showed no pathological changes in the growth cartilages.

DEVASCULARIZATION

Anatomy

The hind limb of the rabbit is mainly fed through the femoral artery *(Brookes & Harrison; Krause)*. Arteries branching off from this are the medial circumflex artery immediately below the inguinal groove, and the lateral circumflex artery a little further down, which in turn gives rise to the nutrient artery of the femur. The line of devascularization was generally distal to this latter artery.

Technique

Since several investigators had found that ligature of the femoral artery did not appreciably affect longitudinal growth *(Friedl & Schinz; Latarjet; Milne Edwards; Ollier; Pearse & Morton 1930; Sousa Pereira)*, presumably because of the quick development of collateral blood supply, a more drastic method of devascularization seemed necessary to retard growth. A further consideration was the avoidance of direct trauma to the growth cartilages.

The following procedure was evolved:

A transverse skin incision was made distal to the left groin under local anaesthesia with lidocaine. The lateral femoral muscles were cut in the mid-thigh, the sciatic nerve was identified, and the muscles posterior to it were severed. Next, the medial femoral muscles were cut, the femoral vessels were freed from the femoral nerve, tied, and severed. It was then ascertained that no remnants of muscle posterior to the femur remained undivided. In the first few animals of the series, the femoral nerve was sometimes accidentally severed. Penicillin was introduced into the wound and the skin was closed with catgut. Thus, the femur, the femoral and the sciatic nerves, and the skin were left intact, whereas all vessels and muscles in the thigh were cut. By means of this procedure the blood supply of the tibia was severely reduced, and, since the multiplying cells of the growth cartilage are apparently nourished from the epiphysial side *(Trueta & Amato; Trueta & Little; Trueta & Morgan)*, the lower femoral growth cartilage also suffered ischaemia.

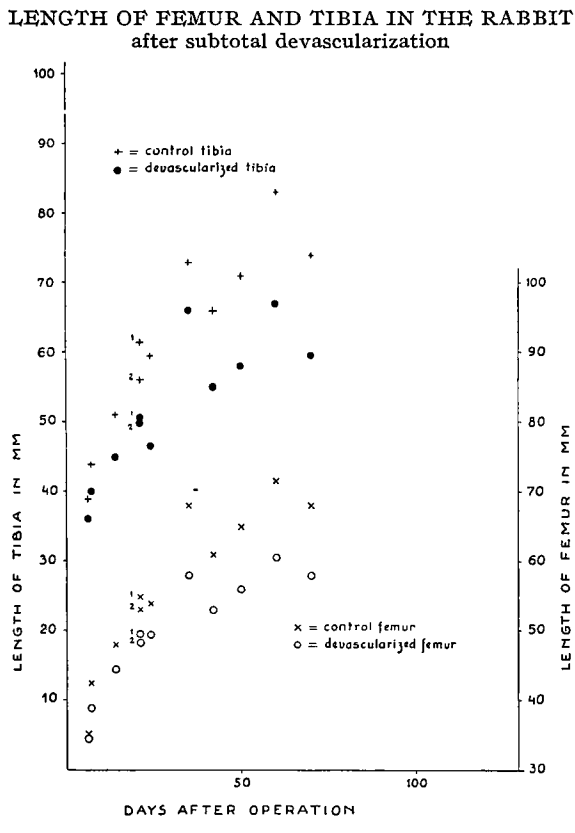

Fig. 18. Indices(1,2) denote different rabbits killed the same number of days after operation.

To produce temporary ischaemia, a piece of thin rubber tubing was tied tightly around the left thigh of the rabbit for 2—7 hours. Only the tibiae were studied; since the femur of the rabbit is well protected by muscles, the rubber tubing had to be applied just above the knee and it is possible that blood was still being supplied to the lower femoral epiphysis. These animals were killed 1, 4, 7, 14, and 40—42 days after the period of ischaemia.

All the animals were killed by decapitation; immediately afterwards a barium sulphate suspension was injected into the thoracic aorta. No attempt was made to measure the pressure of the injection, since perfectly adequate angiograms can be obtained without such a device *(Morgan)*. Roentgenograms were then taken of the lower part of the animal in the prone and supine positions, the hind limbs were skinned

LENGTH OF FEMUR AND TIBIA IN THE RABBIT
immediately after subtotal devascularization

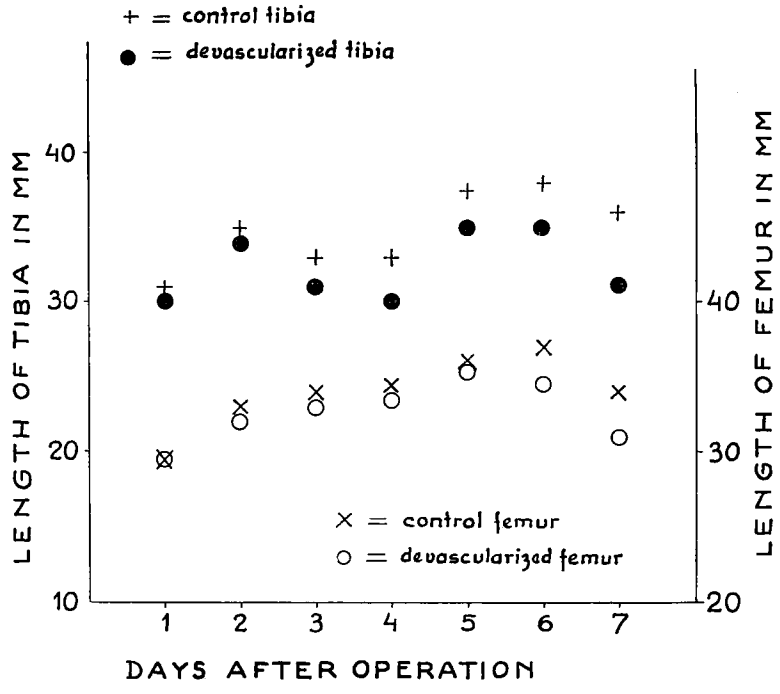

Fig. 19.

and exarticulated at the hip, and new roentgenograms taken of the skinned limbs.

The growth cartilages affected by operation were subjected to histological examination; however, the distal tibial growth cartilage was examined in a few instances only.

Useful data were obtained from 87 animals.

Subtotal devascularization

Little active motility remained in the devascularized limbs, which were mostly held in a position of flexion, adduction, and internal rotation at the hip, flexion at the knee, and extension at the ankle. In animals surviving operation for more than 21 days, the upper articular surface of the tibia was distorted and cup-shaped.

The effect of subtotal devascularization on longitudinal growth of the

femur and the tibia is shown in Fig. 18; there is considerable retardation of growth in both femur and tibia, more so in the tibia.

The immediate effect of subtotal devascularization on longitudinal growth was studied in a litter of 7 rabbits, killed 1—7 days after operation (Fig. 19). It was found that the epiphyses affected by devascularization were rather loose in comparison with those in the control limb.

Control procedures

Subtotal devascularization causes considerable injury to a growing limb, and it might be argued that the injury itself contributes to the stunting. Consequently, in one control group, the muscles of the thigh were severed as outlined in *"Technique"* (page 31), but the femoral vessels were preserved intact. The results of this control procedure are shown in Fig. 20. There seemed to be a slight retardation of femoral growth immediately after operation, but longitudinal growth of the tibia seemed unaffected by this procedure.

In a second control group, the femoral vessels only were divided. The results of this operation are shown in Fig. 21. It seemed to have had no effect on the longitudinal growth of the femur; growth of the tibia may have been slightly retarded.

Subtotal devascularization and sympathectomy

Since there is considerable disagreement concerning the effect of sympathectomy on the growth of bone (see page 12), it was thought that sympathectomy on limbs stunted by means of subtotal devascularization might shed further light on this problem.

Six litters of rabbits were subjected to subtotal devascularization; a fortnight later, half of these animals were subjected to left lumbar sympathectomy by the transperitoneal approach. Naturally, sympathectomy might have been more effective in the early revascularization period (see *"Roentgenological observations"*, page 43), but these were major operations for such young animals, and indeed, mortality was high.

The results of subtotal devascularization and lumbar sympathectomy are given in Table 1. Since lumbar sympathectomy does not affect the thigh in rabbits *(Goetz & al.)* only the lengths of the tibiae are given. The figures suggest that lumbar sympathectomy does not increase the longitudinal growth of limbs subjected to subtotal devascularization; this is supported by the statistical analysis kindly carried out by Mr. H. Lindroos.

LENGTH OF FEMUR AND TIBIA IN THE RABBIT
after severing of muscles in the mid-thigh

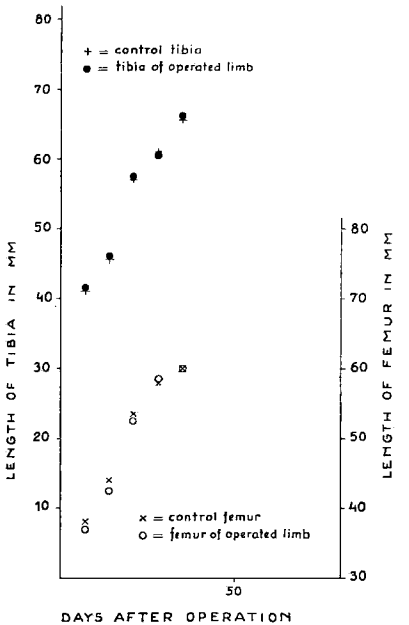

Fig. 20.

LENGTH OF FEMUR AND TIBIA IN THE RABBIT
after severing of femoral vessels

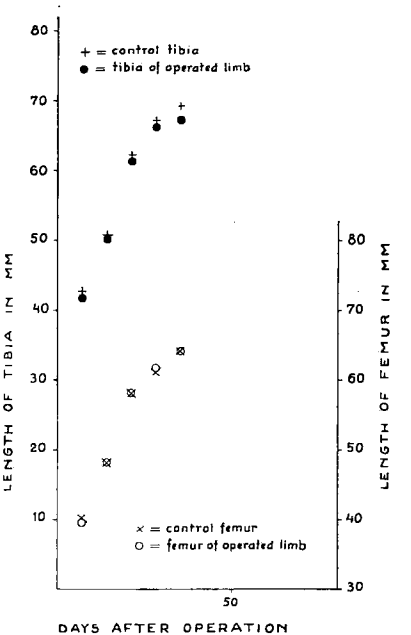

Fig. 21.

The sample mean of the difference in length between the control and experimental tibiae is 25.4 per cent in the group (x) subjected to subtotal devascularization and lumbar sympathectomy, and 21.8 per cent in the group (y) subjected to subtotal devascularization only. The variance is 49.4 and 75.9, respectively. If the sample means are assumed to be normally distributed, and if it is assumed that sympathectomy would increase longitudinal growth by approximately 2 per cent during the time these animals lived after sympathectomy *(Goetz & al.)*, the null hypothesis is $m_y - m_x = 2$. Then the variable $z = \bar{y} - \bar{x} - 2$ is normally distributed with the mean value = 0, and the variance $\sigma_z^2 = \frac{1}{8}(49.4 + 75.9)$, i.e. the standard deviation is 4.0.

The probability of a deviation of $z \leqq -5.6$ is $P(z \leqq -5.6) = P(\frac{z}{4.0} \leqq -1.4) = 1 - \Phi(1.4)$, or $P(z \leqq -5.6) = 0.08$. The null hypothesis can thus be rejected with a probability of 92 per cent.

TABLE 1. Difference in length of tibiae of rabbit after subtotal devascularization of left hind limb and left lumbar sympathectomy.

Animal	Length of right tibia	Length of left tibia	Difference as percentage of control limb
111	71 mm	53 mm	25
121	68 ,,	51.5 ,,	24
122	74 ,,	55 ,,	26
141	66 ,,	48 ,,	27
142	63 ,,	47 ,,	25
151	58 ,,	46 ,,	21
153	57 ,,	34 ,,	40
161	62 ,,	53 ,,	15

Difference in length of tibiae of rabbit after subtotal devascularization of left hind limb

Animal	Length of right tibia	Length of left tibia	Difference as percentage of control limb
112	73 mm	58 mm	21
113	73 ,,	59 ,,	19
123	70 ,,	40.5 ,,	42
124	73.5 ,,	59 ,,	20
131	78 ,,	62 ,,	21
132	75 ,,	59 ,,	21
152	67 ,,	56 ,,	18
162	69 ,,	61 ,,	12

All animals were killed 41 or 42 days after devascularization (27 or 28 days after sympathectomy). In the numbering, the first two digits denote the litter, the last digit the individual.

Temporary ischaemia

The effect of increasingly long periods of ischaemia on longitudinal growth of the tibia of the rabbit is shown in Fig. 22. It will be seen that ischaemia lasting up to 5 hours caused no significant retardation of growth, though changes in the other tissues of the limb were apparent. These changes tally well with the observations of *Paletta & al.*, since, even 6 weeks after the period of ischaemia, the animals were not able to use the injured limb normally.

The postmortal angiograms (see *"Roentgenological observations"*, page 43) make it clear that even short-term ischaemia produces changes in the peripheral vessels of the limb; since it was impossible to differentiate between the effect of temporary ischaemia and the effect of the vascular injury, this line of investigation was abandoned.

RETARDATION OF GROWTH OF TIBIA IN THE RABBIT
seen 40—42 days after 2—7 hours of ischaemia
(given as percentage of length of control limb)

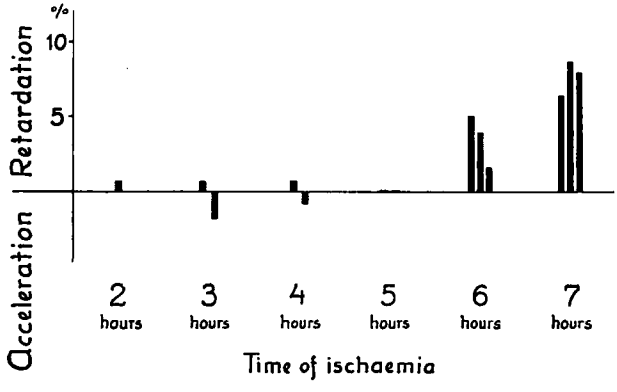

Fig. 22. Each column represents one rabbit.

Roentgenological observations

Widening of the lower femoral and both tibial epiphysial lines was seen 24 hours after subtotal devascularization (Fig. 23). This widening persisted throughout the first postoperative week; it gradually diminished, and narrowing of the lower femoral epiphysial line was already found 9 days after operation. Fourteen days after devascularization, narrowing of all epiphysial lines was seen (Fig. 24); narrowing was also seen 21, 35, 42, 50, 60, and 70 days after operation (Fig. 25). In many cases a streak of

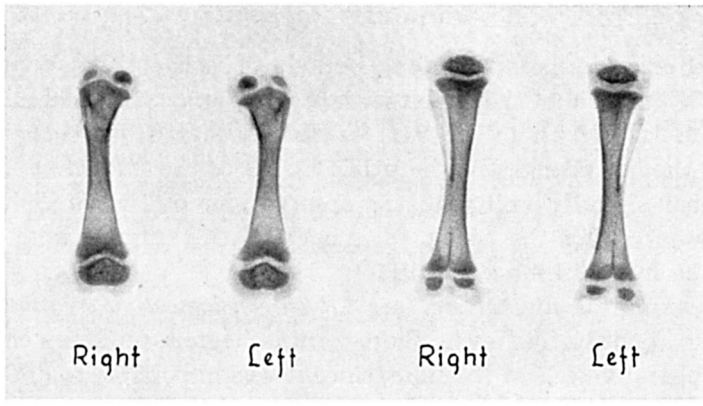

Fig. 23. Femora and tibiae of rabbit subjected to subtotal devascularization of left hind limb at 15 days of age, and killed 24 hours after operation. Femora of equal length; left tibia 1 mm shorter than right. Widening of lower epiphysial line of left femur and of both epiphysial lines of left tibia.

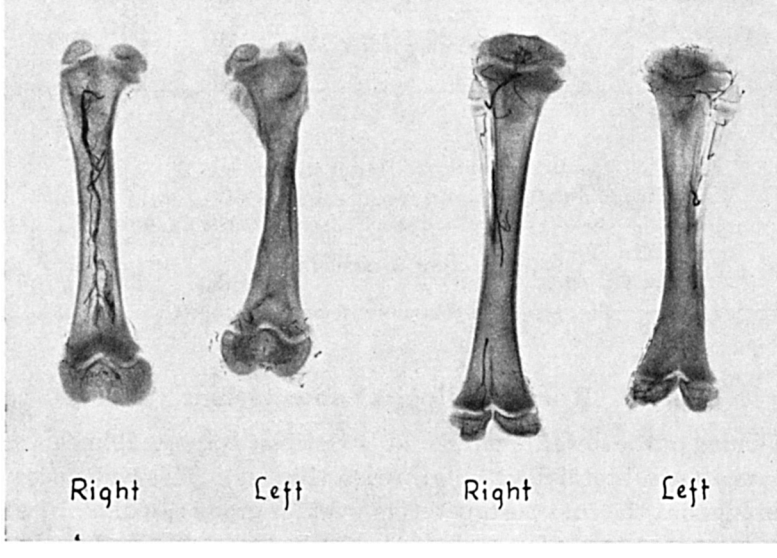

Fig. 24. Femora and tibiae of rabbit subjected to subtotal devascularization of left hind limb at 17 days of age, and killed 14 days after operation. Left femur 3.5 mm shorter than right; left tibia 6 mm shorter than right. Osteoporosis of left femur and tibia; narrowing of lower epiphysial line of left femur and of both epiphysial lines of left tibia.

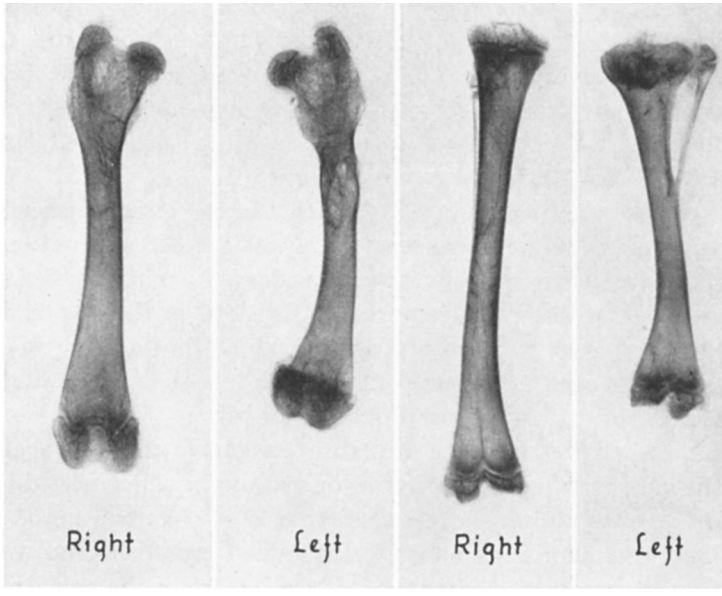

Fig. 25. Femora and tibiae of rabbit subjected to subtotal devascularization of left hind limb at 15 days of age, and killed 70 days after operation. Left femur 10 mm shorter than right; left tibia 14.5 mm shorter than right. Left femur slightly bent into varus; distortion of left upper tibial articular surface, streak of bony tissue crossing epiphysial line; narrowing of lower epiphysial line of left femur and of both epiphysial lines of left tibia.

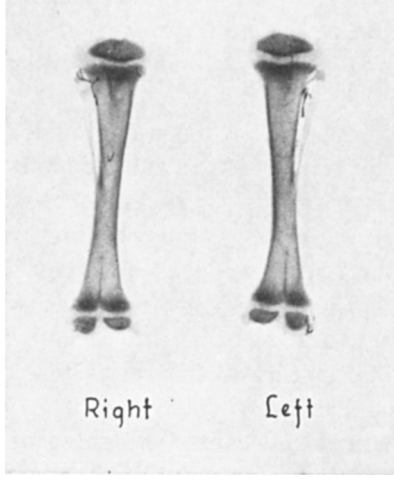

Fig. 26. Tibiae of rabbit subjected to temporary ischaemia of left leg for 4 hours at 14 days of age, and killed 24 hours after release of constricting band. Widening of both epiphysial lines of left tibia. Compare histological findings, Fig. 41 and Fig. 42.

bony tissue crossing the epiphysial line from the epiphysis to the metaphysis was seen (Fig. 25); histological examination corroborated this. In almost all cases, overgrowth of the fibula in relation to the tibia was observed; this was probably due to the bony central epiphysiodesis always found in the tibia after subtotal devascularization but not in the fibula (see *"Histological observations"*, page 54).

In the control group subjected to division of the femoral muscles with preservation of the femoral vessels, there was periosteal reaction in the femur at the site of operation, just as in the devascularized limbs (Fig. 24), and some osteoporosis of the femur and tibia, but in the epiphysial lines no changes were seen. The osteoporosis was attributable to inactivity, since the rabbits used the injured limb much less than the control limb.

In the control group subjected to section of the femoral vessels, no recognizable changes were seen in plain roentgenograms, not even in the case of the animal killed 35 days after operation, in which the tibia of the operated limb was 2 mm shorter than that of the control limb.

Temporary ischaemia of 2 hours' duration had caused no changes visible in plain roentgenograms 24 hours after the release of the rubber band; 4 days after the period of ischaemia, there was slight osteoporosis in the upper tibial epiphysis, possibly due to the inactivity of the limb.

Three hours of ischaemia had caused some widening of the upper tibial epiphysial line 24 hours after the release of the rubber band. Four days after the period of ischaemia this was no longer seen, though some swelling of cells in the growth cartilage was found at histological examination (see page 53). Forty and 41 days after operation, there was some osteoporosis in the upper tibial epiphysis, and periosteal reaction in the femur where the rubber band had been.

Four hours of ischaemia had caused widening of both tibial epiphysial lines 24 hours after the release of the rubber band (Fig. 26), and slight widening was seen 4 days after the period of ischaemia. Seven days after the procedure no widening of the epiphysial lines was seen, but osteoporosis of the upper tibial epiphysis was observed. Fourteen days after the period of ischaemia there was no difference between the experimental and the control limb; 40 and 41 days after ischaemia there was osteoporosis of the upper tibial epiphysis, and periosteal reaction in the femur where the rubber band had been.

Five hours of ischaemia had caused widening of the tibial epiphysial lines 24 hours after the release of the rubber band; widening was also seen 4 days after the period of ischaemia. There was no widening of the epiphysial lines 7 days after the procedure, but some osteoporosis of the

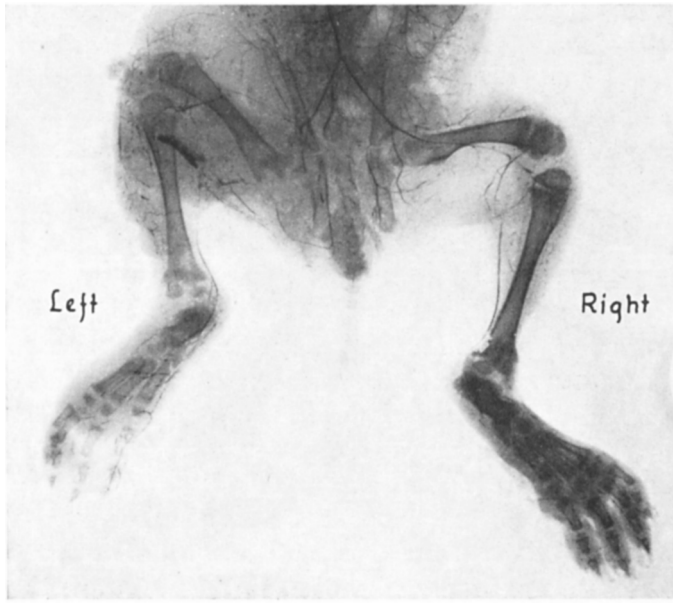

Fig. 27. Postmortal angiogram of lower part of rabbit subjected to subtotal devascularization of left hind limb at 15 days of age, and killed 72 hours after operation. Considerable revascularization in operated limb; compare roentgenogram of skinned limb (Fig. 28).

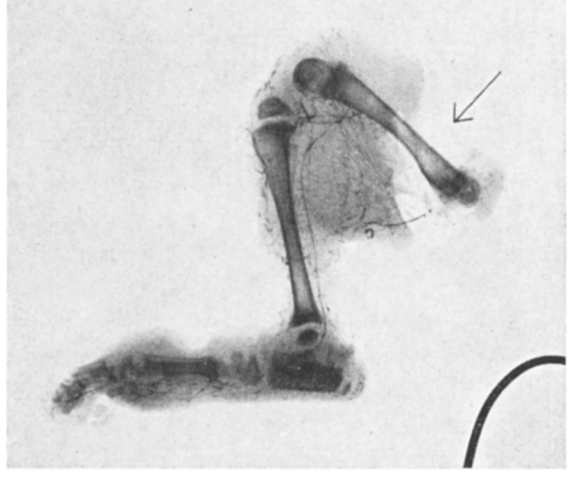

Fig. 28. Lateral roentgenogram of skinned left hind limb of rabbit subjected to subtotal devascularization at 15 days of age, and killed 72 hours after operation. Line of division of femoral muscles shows well (↓); considerable revascularization of muscles of leg. Compare Fig. 27.

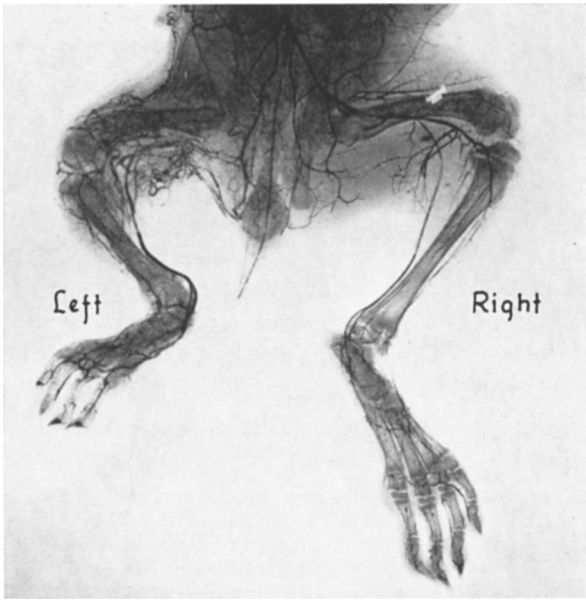

Fig. 29. Postmortal angiogram of lower part of rabbit subjected to subtotal devascularization of left hind limb at 17 days of age, and killed 14 days after operation. Increased vascularity in left thigh; distal vessels in operated limb thick and tortuous.

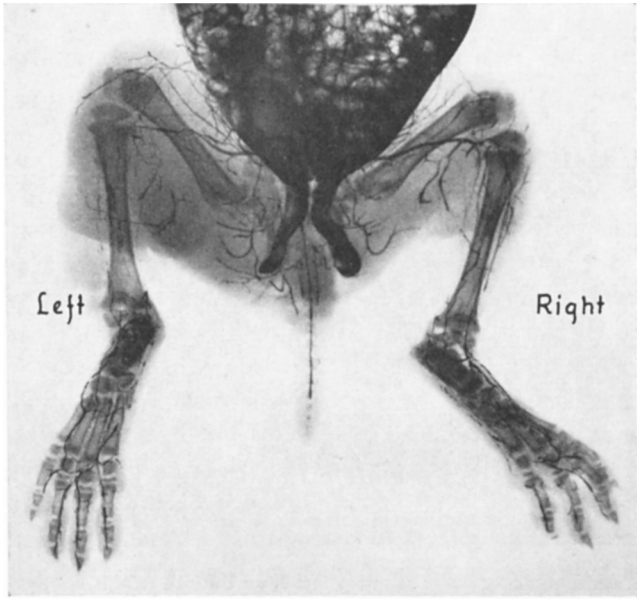

Fig. 30. Postmortal angiogram of lower part of rabbit subjected to temporary ischaemia of left leg for 2 hours at 14 days of age, and killed 24 hours after release of constricting band. Impaired filling of vessels in left leg.

upper tibial epiphysis was seen. Fourteen days after the period of ischaemia there was no widening, and 40 days after ischaemia osteoporosis of the upper tibial epiphysis was seen.

Six and 7 hours of ischaemia had caused widening of the epiphysial lines 24 hours after the procedure, which was still seen on the 7th, but not on the 14th day after the period of ischaemia; no widening of the epiphysial lines was seen 40—42 days after ischaemia.

Postmortal angiograms

After subtotal devascularization, no filling of vessels distal to the line of division of muscles and vessels was seen on the 1st or 2nd day after operation. On the 3rd postoperative day, there was already considerable revascularization with fine vessels in the muscles of the leg and in the foot (Fig. 27 and Fig. 28). In those few cases where only slight or no filling of distal vessels was seen in the operated limb after the 3rd postoperative day, it was assumed that changes in the composition of the barium sulphate suspension were responsible, since histological examination showed good regeneration.

Fourteen days after devascularization, there was intense hypervascularization around the line of incision in the thigh and also around the knee, apparently because of collateral vessels. The more peripheral vessels were somewhat tortuous and showed changes in calibre (Fig. 29) reminiscent of those seen after section of peripheral nerves *(Ferguson & Akahoshi; Hulth & Olerud)*. The over-all peripheral vascularization, however, seemed fairly good. Postmortal angiograms of animals killed 21, 35, 42, 50, 60, and 70 days after subtotal devascularization showed similar results.

In the control series subjected to division of femoral muscles, with preservation of the femoral vessels, the peripheral vascularization was intact; there was hypervascularization around the line of incision in the thigh.

In the control series subjected to section of the femoral vessels in the thigh, the peripheral vessels filled well through collaterals; the proximal part of the femoral artery did not fill, naturally enough.

Temporary ischaemia of 2 hours' duration had caused a slight decrease in vascularization in the leg, and narrowing of the femoral artery at the site of constriction in the thigh, 24 hours after the release of the rubber band (Fig. 30). Four days after the period of ischaemia, no changes were seen in the peripheral vessels, but there was narrowing of the femoral

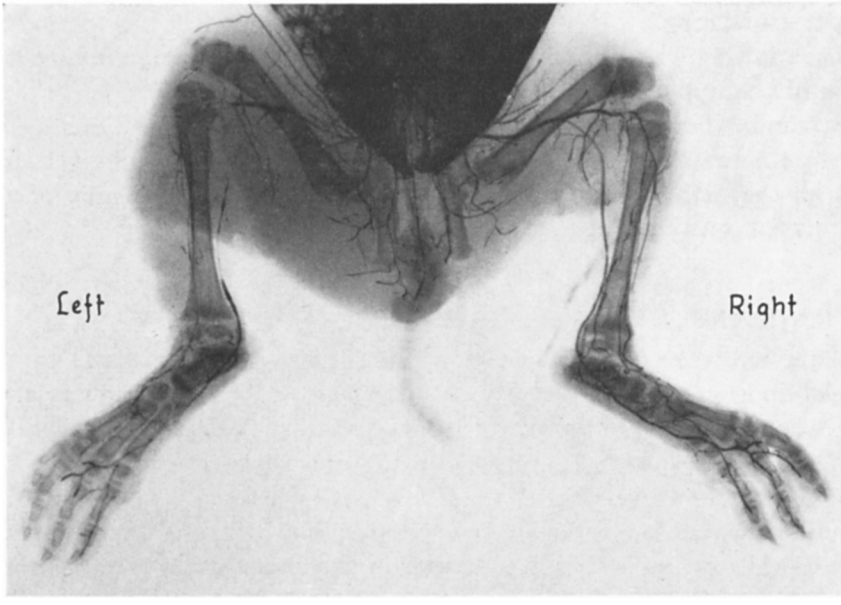

Fig. 31. Postmortal angiogram of lower part of rabbit subjected to temporary ischaemia of left leg for 4 hours at 14 days of age, and killed 24 hours after release of constricting band. Impaired filling of vessels in left leg.

artery at the site of constriction, a condition also seen 41 days after the period of ischaemia.

After 3 hours of ischaemia, the peripheral vessels in the injured limb were slightly narrower than in the control limb, and the femoral artery was narrow at the site of constriction, 24 hours after the release of the rubber band. Four days after the period of ischaemia, narrowing of peripheral arteries was still seen, but this was not seen in animals killed 7, 14, or 40 and 41 days after the procedure.

Four hours of ischaemia led to a decrease in peripheral vascularization and calibre variation in peripheral vessels, seen 24 hours after the release of the rubber band (Fig. 31). This was not seen in an animal killed 4 days after the procedure, but in animals killed 7 and 14 days after ischaemia, calibre variations were again observed. No changes, however, were seen 40 and 41 days after ischaemia, except narrowing of the femoral artery where the rubber band had been.

Five hours of ischaemia led to decreased peripheral vascularization and irregular filling of main arteries, seen 24 hours after the release of the rubber band; no pathological changes were seen in the animal killed 4

days after the period of ischaemia, but in animals killed 7 and 14 days after the procedure, there was increased vascularization around the knee and again irregular filling of peripheral arteries.

Six and 7 hours of ischaemia caused similar, but more severe, changes.

However, at this point of the investigation it was apparent that a short period of ischaemia, i.e. up to 5 hours, had caused no significant retardation of growth 6 weeks after the procedure (Fig. 22, page 37). The growth cartilages, though suffering damage (see page 53), apparently had an astonishing power of regeneration, more so than other tissues (skin, nerves, muscles) in the limb, which remained permanently damaged after a period of ischaemia lasting 4 hours or more. The angiograms showed that after 2 hours or more of ischaemia, definite changes in the vascularization of the injured limb had occurred, persisting up to 14 days after the injury. Consequently, no differentiation could be made between the effects of temporary ischaemia and the effects of more permanent vascular damage. It seemed that the difference in effect between long periods of ischaemia and subtotal devascularization was quantitative rather the qualitative; it was therefore thought unnecessary to pursue this line of investigation further.

Histological observations

Twenty-four hours after subtotal devascularization, the cells in the distal femoral growth cartilage showed signs of severe injury: swelling and poor staining of cells, and disarrangement of cell columns. In the upper tibial growth cartilage changes even more severe were seen (Fig. 32). In a few instances a transverse split in the growth cartilage was observed (Fig. 32; Fig. 33); no similar transverse splits were seen in the control limbs.

Forty-eight hours after devascularization there were already signs of regeneration, and 72 hours after devascularization regeneration was well under way (Fig. 34). This tallies well with the angiographic findings (page 43). Regeneration then proceeded at a steady rate, more and more new cells appearing towards the centre of the cartilage plate (Fig. 35).

A forked growth cartilage was seen in several animals (Fig. 36); it resembled the patterns observed by *Heikel (1960b)* in his work on epiphysial transplantation. The cells bordering this wedge-shaped piece of bone on the metaphysial side seemed to be necrotic, unossified cartilage

cells. Similar patterns were seen in the animals killed 9, 11, and 12 days after operation.

Nine days after devascularization, a streak of ossifying tissue was seen stretching across the growth cartilage from epiphysis to metaphysis. Twelve (Fig. 37) and 14 days (Fig. 38) after operation, this bony bridge was well developed. In animals with such an epiphysiodesis the upper articular surface of the tibia was bowl-shaped. In two instances the upper tibial epiphysis was not distorted (animals 161 and 162, page 36); histological examination showed narrow bony bridges (Fig. 39), the retardation of growth of the tibia being less severe than was usual after subtotal devascularization. In two instances (animals 123 and 153, page 36,) where growth was severely retarded, this bowl shape of the upper tibial epiphysis was not seen, and histological examination showed that the upper tibial growth cartilage had been almost completely destroyed.

In the lower growth cartilage of the femur, bony bridges between epiphysis and metaphysis were seen in many cases (Fig. 37), but these were on the whole rather narrow and there was no distortion of the distal articular surface. The bending of the femur into a varus position may have been caused by the different rates of growth of the condyles, though it is not clear why it was the medial condyle that always grew more slowly.

In animals killed more than 14 days after subtotal devascularization, the lower femoral growth cartilage had regenerated well, with small or no bony bridges between epiphysis and metaphysis; in the upper tibial growth cartilage (and sometimes in the lower) broad epiphysiodesis was seen, the growth cartilage plate being much thinner than in the control limb. In the animals killed 50, 60, and 70 days after operation, the upper tibial growth cartilage already seemed near the end of the period of growth (Fig. 40).

In the animals killed 7 and 14 days after severance of the femoral muscles (page 34), delayed ossification was observed in the centre of the lower femoral growth cartilage, but this was not seen later. No pathological changes were seen in the tibial growth cartilages. Apparently, therefore, severance of the femoral muscles had interfered with the blood supply of the lower femoral growth cartilage, but not with the blood supply of the tibia.

After section of femoral vessels no pathological changes were seen in the growth cartilages.

Temporary ischaemia of 2 hours' duration had led to no visible changes in the upper growth cartilage of the tibia 24 hours after the release of the rubber band, nor did any pathological changes develop later.

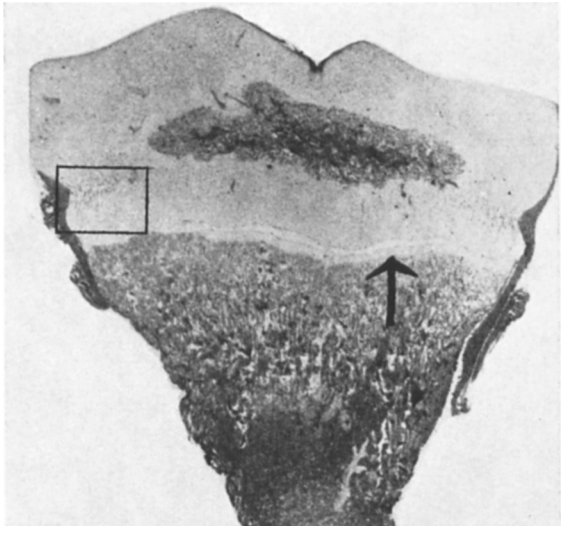

Fig. 32 a. Section of upper end of left tibia of rabbit subjected to subtotal devascularization of left hind limb at 15 days of age, and killed 24 hours after operation. Swelling of growth cartilage; transverse split in cartilage plate (↑). Compare Fig. 23. × 7.5.

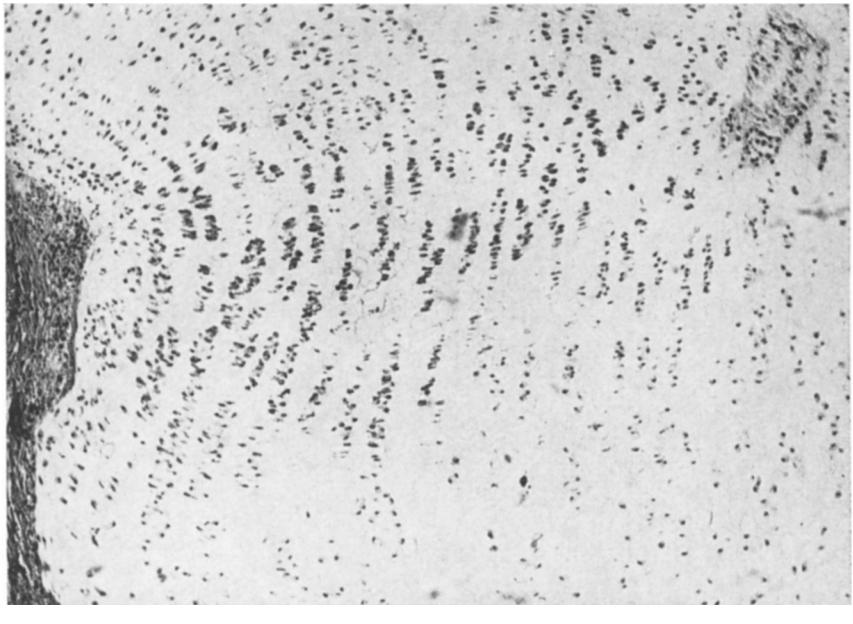

Fig. 32 b. Magnification of square marked on Fig. 32 a. Poor staining and swelling of cells; disarrangement of cell columns. × 70.

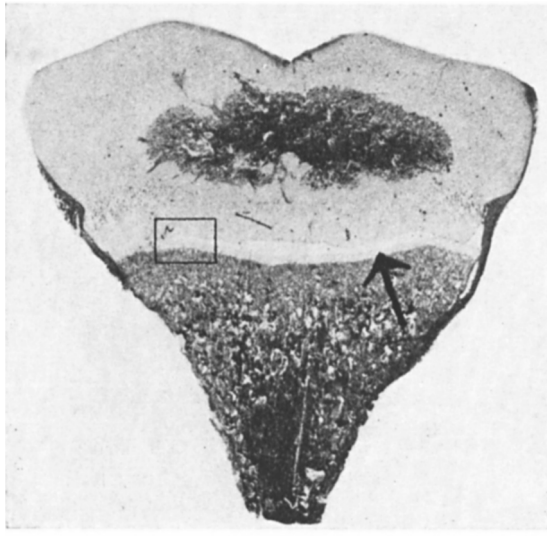

Fig. 33 a. Section of upper end of left tibia of rabbit subjected to subtotal devascularization of left hind limb at 15 days of age, and killed 24 hours after operation. Swelling of growth cartilage; transverse split in cartilage plate (↑). × 5.

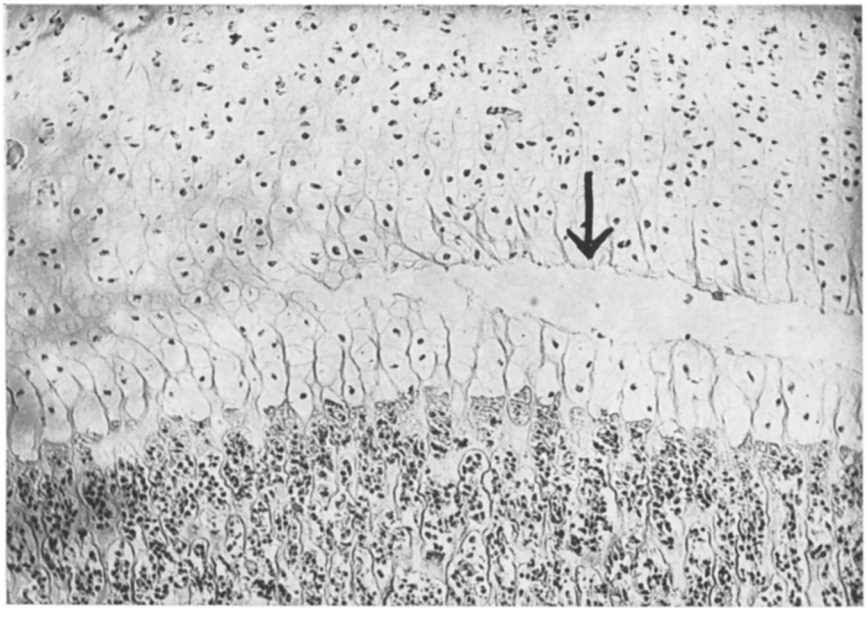

Fig. 33 b. Magnification of square marked on Fig. 33 a. Transverse split clearly shown (↓). × 70.

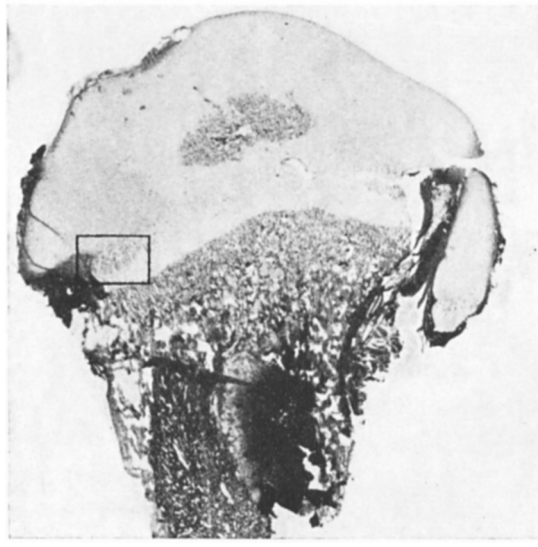

Fig. 34 a. Section of upper end of left tibia of rabbit subjected to subtotal devascularization of left hind limb at 15 days of age, and killed 72 hours after operation. Swelling of growth cartilage. × 6.

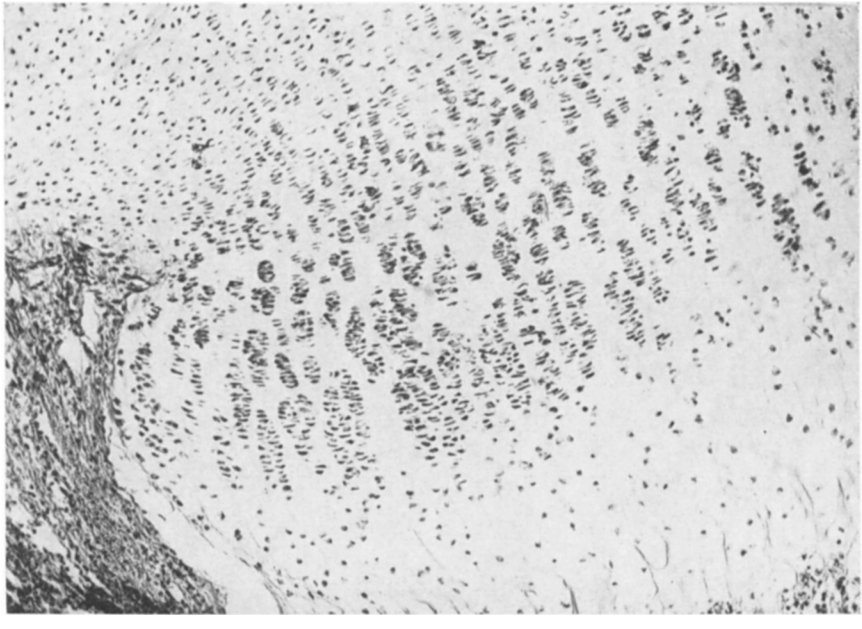

Fig. 34 b. Magnification of square marked on Fig. 34 a. Regenerating cells near the encoche; necrotic cells at bottom right. × 70.

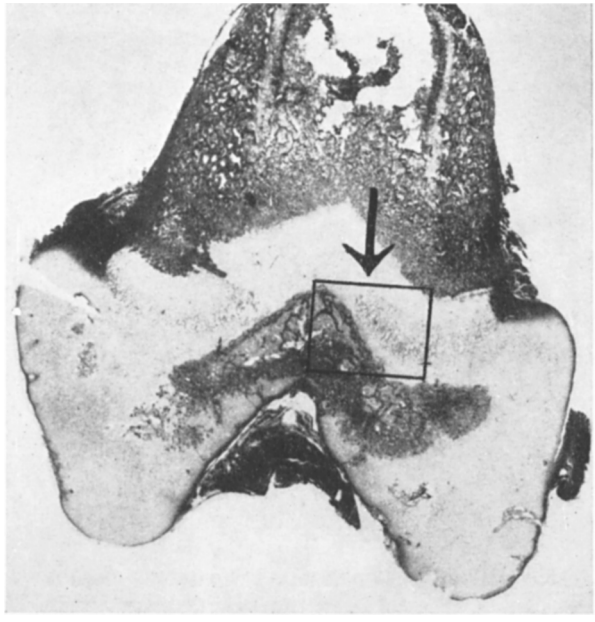

Fig. 35 a. Section of lower end of left femur of rabbit subjected to subtotal devascularization of left hind limb at 14 days of age, and killed 7 days after operation. Regenerating cells near centre of cartilage plate (↓). × 5.

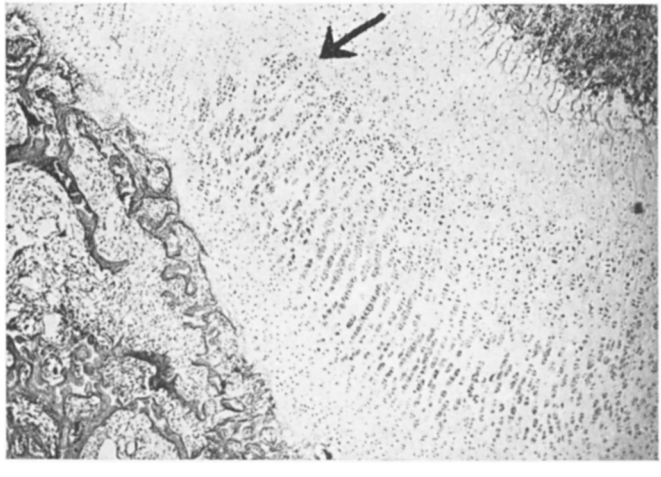

Fig. 35 b. Magnification of square marked on Fig. 35 a. Regenerating cartilage cells in centre of picture (←); necrotic cells at top right. × 26.

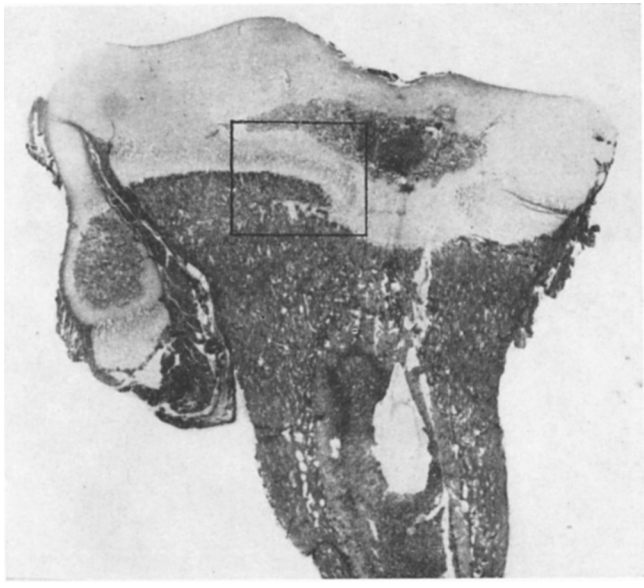

Fig. 36 a. Section of upper end of left tibia of rabbit subjected to subtotal devascularization of left hind limb at 14 days of age, and killed 7 days after operation. Forked growth cartilage formed by regenerating cells growing towards centre of cartilage plate. × 6.

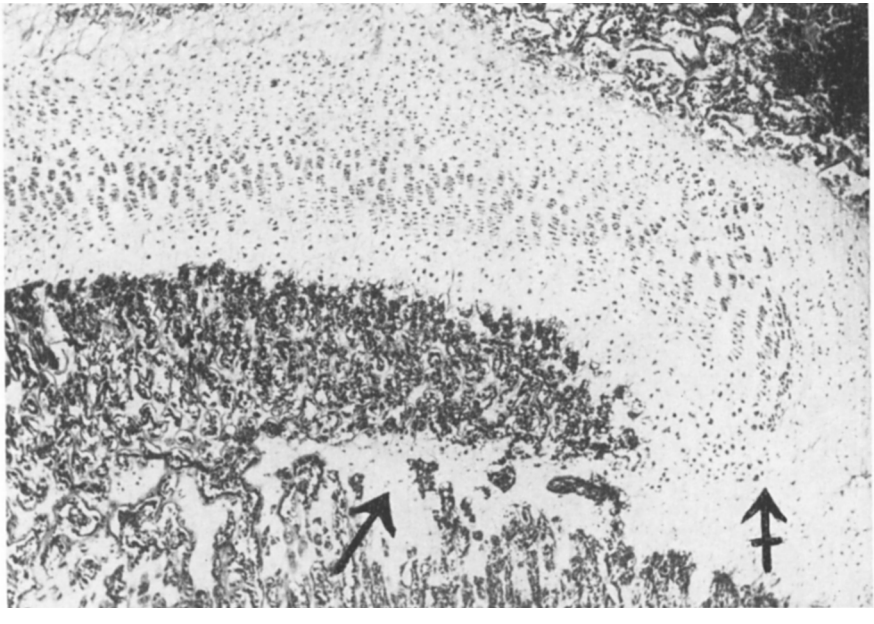

Fig. 36 b. Magnification of square marked on Fig. 36 a. Edge of cluster of regenerating cells (↟); necrotic and unossified cartilage cells (↑). × 35.

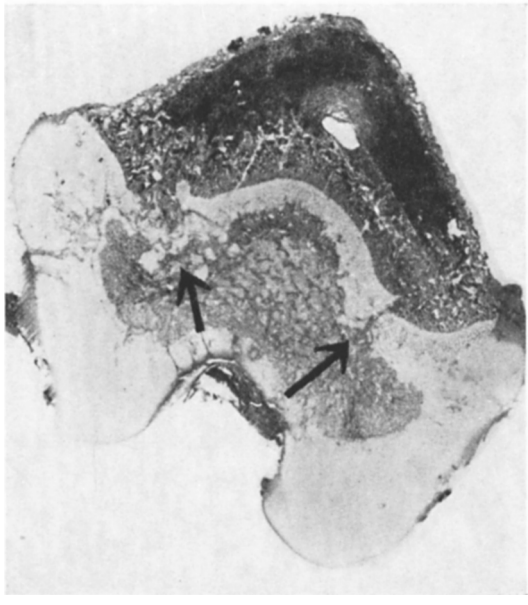

Fig. 37. Section of lower end of left femur of rabbit subjected to subtotal devascularization of left hind limb at 14 days of age, and killed 12 days after operation. Two bony bridges uniting epiphysis to metaphysis (↑ ↑). × 7.

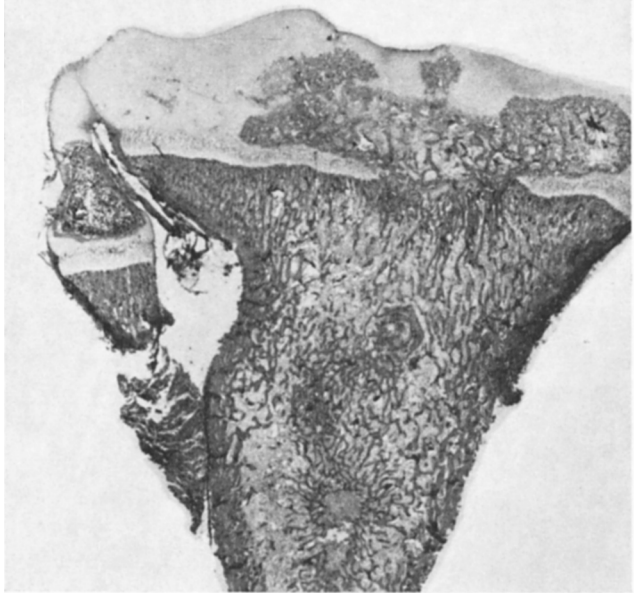

Fig. 38. Section of upper end of left tibia of rabbit subjected to subtotal devascularization of left hind limb at 17 days of age, and killed 14 days after operation. Bony bridge uniting epiphysis to metaphysis in centre of growth cartilage. × 6.5.

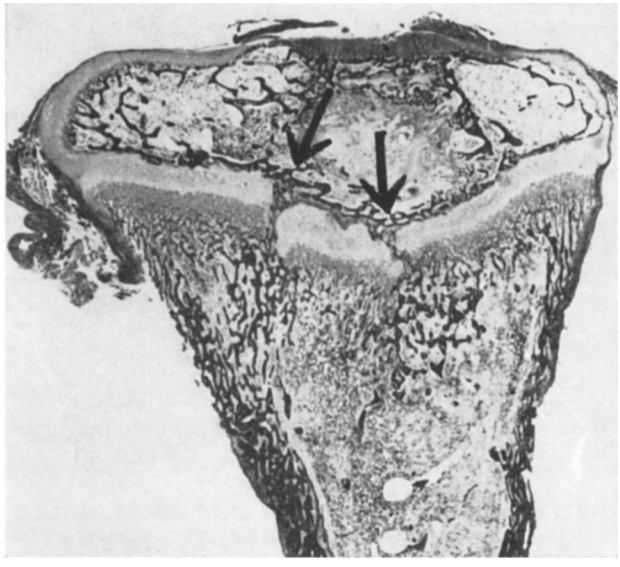

Fig. 39. Section of upper end of left tibia of rabbit subjected to subtotal devascularization of left hind limb at 15 days of age, and killed 41 days after operation. Growth cartilage well regenerated; narrow bony bridges (↓ ↓) uniting epiphysis to metaphysis; interference with growth unexpectedly mild (animal 162, page 36). × 7.

Three hours of ischaemia had led to some swelling of cartilage cells 24 hours after the release of the rubber band, which tallies well with the widening of the epiphysial line seen in roentgenograms; slight changes were still visible 4 days after the period of ischaemia, though the roentgenograms were normal by this time.

Four hours of ischaemia had caused swelling of cells and disarrangement of cell columns 24 hours after the release of the rubber band (Fig. 41); these changes were still visible 4 days after the period of ischaemia, but had disappeared by the 7th day.

Five, 6, and 7 hours of ischaemia caused even more severe changes in the growth cartilage, but these persisted for at most a week.

At this point these experiments were discontinued for the reasons given in the section on postmortal angiograms (page 45).

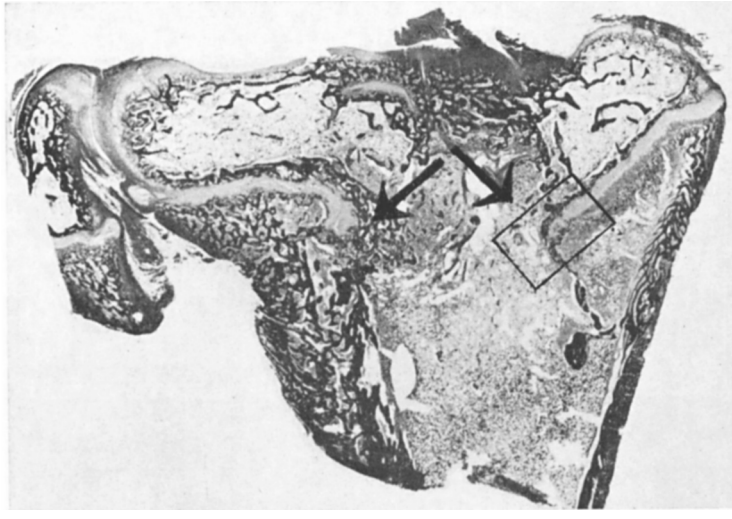

Fig. 40 a. Section of upper end of left tibia of rabbit subjected to subtotal devascularization of left hind limb at 14 days of age, and killed 60 days after operation. Broad bony bridge uniting epiphysis to metaphysis. Arrows (↓ ↓) show interrupted growth cartilage. × 4.

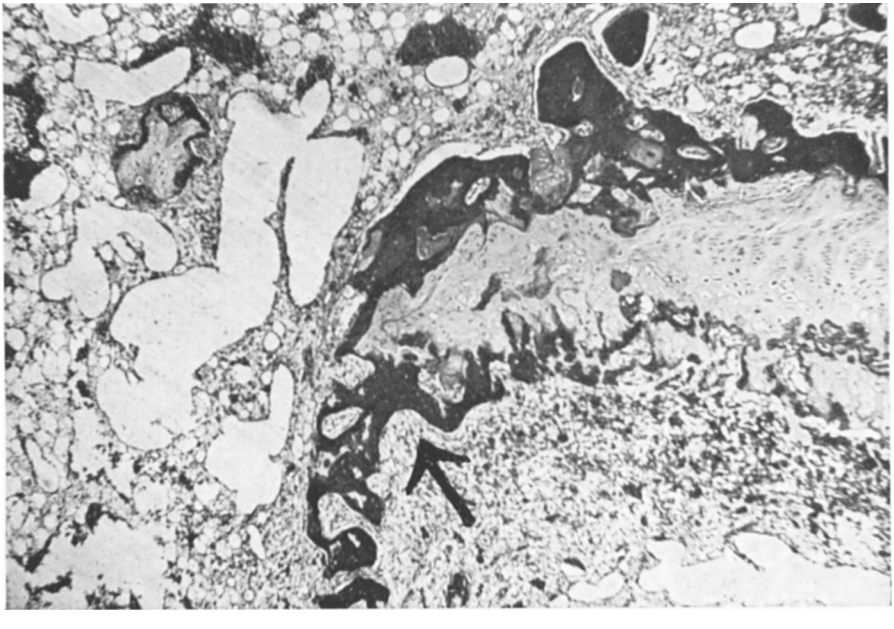

Fig. 40 b. Magnification of square marked on Fig. 40 a. Growth cartilage narrow and distorted (↑), apparently nearing end of period of growth. × 35.

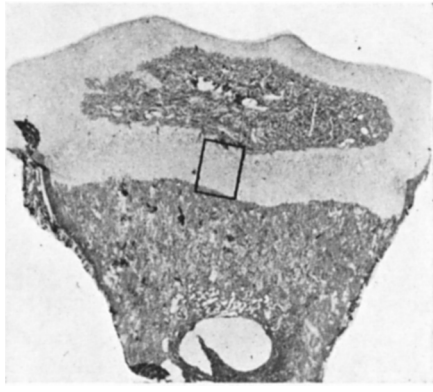

Fig. 41 a. Section of upper end of left tibia of rabbit subjected to 4 hours of ischaemia at 14 days of age, and killed 24 hours after release of constricting band. Swelling of growth cartilage; compare Fig. 42 a. × 5.

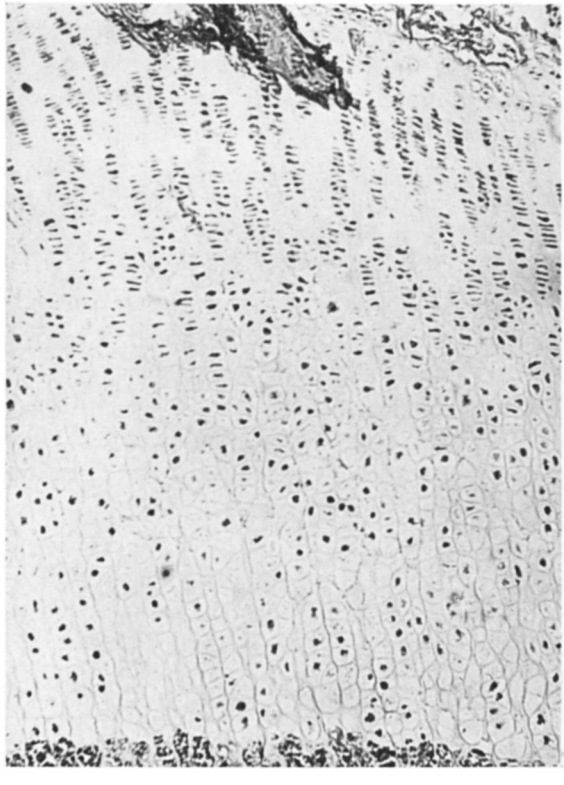

Fig. 41 b. Magnification of square marked on Fig. 42 a. Swelling of cartilage cells; compare Fig. 42 b. × 64.

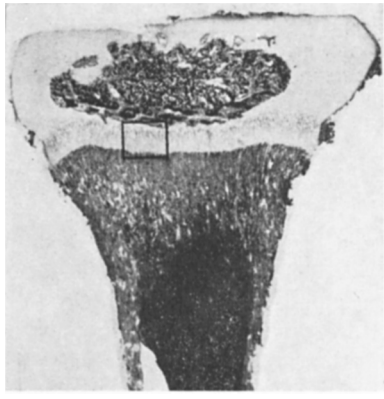

Fig. 42 a. Section of upper end of right tibia (control) of rabbit 15 days of age. Compare Fig. 41 a. × 4.5.

Fig. 42 b. Magnification of square marked on Fig. 42 a. Compare Fig. 41 b. × 63.

DISCUSSION

Even after extensive motor denervation of the hind limb of the rabbit, retardation of longitudinal growth of the femur and tibia was slight. Section of 4 motor nerve roots caused complete or almost complete paralysis of a hind limb, nevertheless the denervated femur was at most 5 per cent shorter than the control femur, and the denervated tibia at most 2.5 per cent shorter. Section of 3 motor nerve roots caused almost complete paralysis of the hind limb, but the denervated femur was at most 3.5 per cent shorter than the control femur. Section of 2 motor nerve roots (one of the main motor nerve roots was always spared) caused transient paresis, but all these animals later used the denervated limb well; atrophy of muscles and osteoporosis was present in all cases, yet no significant retardation of growth was found in either the femur or the tibia. Section of 4 sensory as well as 4 motor nerve roots produced results similar to those observed after section of 4 motor nerve roots.

After paresis caused by poliomyelitis in man, growth of a lower limb is often retarded by 5 per cent (i.e. about 4 cm in the average adult). Such a shortening is a handicap and an indication for treatment in order to correct the inequality in length; more severe shortening is not uncommon. The discrepancy between the degree of retardation of growth so often caused by poliomyelitis, and the degree of retardation seen after experimental motor nerve root section, suggests that paralysis per se leads to only slight retardation; indeed, lengthening of the diaphysis after motor nerve root section in puppies has been found *(Ring 1961)*.

Admittedly, the experimental animals did not live to maturity, but by the time a rabbit is 110—120 days old, the tibia has reached about 90 per cent of its final length *(Heikel 1960 a)*, and in these experiments the tibia had tripled its length from the time of rhizotomy. Another difference between retardation of growth caused by motor nerve root section, and retardation caused by poliomyelitis, is that poliomyelitis generally affects growth in the more distal parts of the limb, whereas in my experiments the femur seemed to be more affected than the tibia. However, any retarding effect of rhizotomy on the growth of the tibia may have been partially counteracted by the effect of abolition of weight-bearing *(Arkin & Katz)*.

The rôle of the vascular disturbances caused by poliomyelitis in producing retardation of growth has been emphasized by many authors (see page 13), and my experimental results support to some degree the theory that, when retardation of growth is seen in a paralyzed limb, it is caused by interference with its vascular supply. It is not known, however, whether poliomyelitis causes disturbances in the blood supply through direct injury to vessel walls, or through injury to the autonomous nervous system; conceivably, wasting of paralyzed muscles may contribute to vascular disturbances.

The arteriovenous anastomoses described in limbs paralyzed by poliomyelitis *(Braibanti; Piulachs & Vidal-Barraquer)* do not refute this theory of vascular disturbances if it is assumed that the growth cartilages suffer hypoxia, since the blood flow in the paralyzed limb is diverted through these arteriovenous shunts. An increase in the longitudinal growth of a paralyzed limb after the surgical creation of an arteriovenous fistula has been reported *(Cooley & al.; Hiertonn);* but there may be a difference in the effect of one large arteriovenous shunt and that of diffuse arteriovenous anastomoses. The original acceleration of growth in paralyzed limbs *(Lerique; Ollier; Ring & Ward; Seeligmüller)* may be due to the diversion of the blood flow from the paralyzed muscles to the bones before the arteriovenous anastomoses have opened up; it may also be due to abolition of weight-bearing.

Unexpectedly, retardation of growth and thickening of the femora subjected to sensory denervation were observed. The difference in length was 6—21 per cent, and some retardation of growth was already seen 15 days after denervation. The tibiae of these animals did not, however, suffer much, nor were they significantly shorter; indeed the denervated tibia was longer than the control tibia in some animals.

The short femora were grossly deformed and thickened in many instances, and the roentgenological appearance suggested the effect of trauma. This assumption was supported by the discovery of a transverse fracture in the metaphysis of some animals; it seemed probable that the fracture had involved the growth cartilage. Such a fracture could easily explain both the deformity and the retardation of growth, since an anaesthetic limb is subjected to inadvertent traumatization when the motor supply is preserved. Arthritic changes have been experimentally produced in adult animals through sensory denervation *(Corbin; Corbin & Hinsey; Eloesser)*, and changes similar to those reported here have been observed in patients with syringomyelia *(Ford);* indeed, it has been maintained that as long as there is a defect in sensory percep-

tion with the motor supply remaining intact, traumatization results *(Potts)*.

Insertion of the denervated and skinned limb under the abdominal skin was thought a suitable method for the protection of the denervated limb. Sensory denervation was carried out on a series of 5 animals, the denervated limb being inserted under the abdominal skin a fortnight after denervation. There was, at most, slight deformity of the lower femoral epiphysis, and no retardation of growth comparable in severity to that seen after sensory denervation without protective measures. In the protected limb of the animal killed 70 days after denervation, considerable lengthening of the tibia was found; such lengthening has been observed after the insertion of a sound but skinned forelimb of the rabbit under the skin of the trunk *(W. Müller)*. It can thus be considered likely that the changes in the lower end of the femur after sensory denervation were the result of inadvertent traumatization of an anaesthetic limb.

It could not be determined why only the lower end of the femur was affected, but, since the animals dragged the denervated limb along the floor of the cage, the lower end of the femur may have been the part of the limb mostly subjected to trauma. This assumption, however, was not supported by the fact that grazing, when present, was generally seen around the ankles.

Subtotal devascularization by severance of muscles and vessels in the thigh caused ischaemia of the limb distal to the line of incision, and considerable retardation of growth of the femur and the tibia. The devascularized femur was generally 10—15 per cent shorter and the devascularized tibia some 20 per cent shorter than the femur and tibia of the control limb; however, there was considerable variation. The apparent shortening of the femur may to some degree have been due to bending of the bone into varus, though not all short femora were bent.

It seems probable that longitudinal growth was almost completely arrested during the first week after subtotal devascularization. In the graph showing the retardation of growth 1—7 days after operation (Fig. 19, page 33), the control tibia in the animal killed 24 hours after operation is 31 mm long; in the animal killed 7 days after operation, the control tibia is 36 mm long. At 14 days of age the rabbit tibia grows on an average 3/4 of a mm in 24 hours *(Heikel 1960a)*; consequently, it may be assumed that the tibiae of these two animals were of about the same length at the time of operation, and that growth of the devascularized tibia was almost completely arrested during the first week. This tallies well with results seen after epiphysial transplantation *(Heikel 1960b)*.

In postmortal angiograms, quick recovery of the severely devascularized limbs was seen; the rapid growth of these animals was manifested also in repair after injury, and good filling of vessels peripheral to the line of incision in the thigh was already found 72 hours after operation. This compares well with studies on vascular regeneration *(Reichert)*.

Plain roentgenograms showed widening of the epiphysial line during the first week after operation; this widening was mostly attributed to swelling of necrotic cartilage cells, but has also been attributed to residual growth of injured cells *(Heikel 1960b)*. The epiphysial lines affected by devascularization were all narrower than those in the control limb on the 14th day after operation; this tallies well with the histological findings in the present series and with observations of transplanted growth cartilages *(Heikel 1960b)*.

Histological examination of the growth cartilages, 24 hours after operation, showed that the cartilage cells were severely injured, but good regeneration was seen 72 hours after operation. The quickest regeneration took place at the lateral borders of the growth cartilage; it seemed probable that resting cartilage cells survived there, and that, when revascularization had provided the blood supply, they started to multiply, pushing inwards to the centre of the cartilage plate. On the 7th postoperative day a forked growth cartilage was observed; from observations in this and other animals, it seemed probable that this double epiphysial line had been formed by regenerating cells pushing inwards to the centre of the growth cartilage, leaving the necrotic cells of the primary growth cartilage behind in the metaphysis, and that ossification of the necrotic cartilage cells had then been delayed long enough for this forked growth cartilage to become visible. The histological findings in my animals were similar to those of *Heikel (1960b)* in his experiments on epiphysial transplantation, and the appearance of the wedge-shaped fragment found in some disorders of the growth cartilage *(Langenskiöld 1952; Pylkkänen)* was well illustrated.

In some instances a transverse split in the zone of hypertrophied cells was seen during the first week after devascularization; no similar split was seen in sections from the control limb. Although trauma caused by the cleaning of the bones may have contributed, it was thought that these splits represented a pathological weakness in the devascularized growth cartilages, since no similar split was seen in the control limb. Moreover, macroscopic examination showed that the epiphyses in the devascularized limbs were rather loose during the first week after operation.

Widening, and later, slipping, of the upper tibial epiphysis in rabbits after ultrasonic injury has been described *(De Forest & al.)*. In experimental work on epiphysiolysis of the head of the femur in rabbits, it has been shown that after direct injury, slipping occurs most easily between the zone of hypertrophied cells and the zone of provisional calcification *(Harris & Hobson)*. The fact that a transverse split in the growth cartilage may occur after vascular injury, as seen in my experiments, may have some bearing upon the pathological processes in slipped upper femoral epiphysis, particularly as the vascular supply of this epiphysis is easily impaired.

From the 9th day after subtotal devascularization, a streak of ossifying tissue was seen in the tibia, bridging the epiphysis and the metaphysis across the centre of the growth cartilage — i.e., a central bony epiphysiodesis. Some kind of epiphysiodesis was found in all devascularized tibiae. In many cases two narrow bony bridges were found in the femur, the growth cartilage of which is partly divided in two; in some instances, however, the growth cartilage of the femur had regenerated so well that no epiphysiodesis was found. Similar bony bridges have been induced by direct experimental surgical trauma *(Campbell & al.; Ford & Key; Haas 1917a; Nové-Josserand)*; after experimental epiphysial fractures a central "dip" in the growth cartilage has been described *(Brashear)*, which may be construed as a minor epiphysiodesis not seriously impeding longitudinal growth.

Plain roentgenograms showed a blurred epiphysial line and distortion of the upper end of the tibia in the devascularized limb; this was seen 21 days after operation, and it was also seen in the animal surviving for 70 days. No comparable distortion was ever seen in the femur, the lower end of the tibia, or the upper end of the fibula. The bending of the femur seen in some animals may have been due to different rates of growth of the two condyles, though it is not clear why it was always the medial condyle that grew at a slower rate. Histological examination of the distorted upper tibial epiphyses always revealed a broad epiphysiodesis; a narrow epiphysiodesis, as in case 162 (page 36), apparently did not cause distortion. It was assumed that the cupped shape of the distorted upper tibial epiphyses was due to hindrance of growth in the centre of the growth cartilage, which retained some of its growth potential at the lateral borders. Distortion of the articular surface of the epiphysis was not induced in the experiments of *Campbell & al.*; the epiphysiodesis induced by their methods probably was so complete that growth was arrested. Some continuation of growth at the borders of the growth car-

tilage seems to be the essential factor that causes the upper articular surface of the tibia to assume a bowl shape. It must be taken into consideration, however, that the abnormal posture of the devascularized limb may also have played a part.

Similar epiphysiodeses have been found in epiphysial transplantation *(Heikel 1960b)* and in the course of investigations on the blood supply of the growth cartilage *(Trueta & Amato);* is seems, however, that in these cases there was complete inhibition of growth after the formation of a bony bridge between epiphysis and metaphysis. This may be compared to my results in cases 123 and 153 (page 36), in which histological examination revealed that the upper tibial growth cartilage was so severely damaged that only remnants were found; in these cases no bowl-shape was seen in the upper tibial epiphysis.

The regular occurrence of these bony bridges between epiphysis and metaphysis, at least in the tibia, lends some support to the view that regeneration starts from resting cells surviving at the borders of the growth cartilage; epiphysiodesis would then be caused by different rates of regeneration in different tissues. It also seems that the smaller the growth cartilage is in size, the better is it regenerated after vascular injury, such as devascularization or transplantation, and the smaller is the risk of a central epiphysiodesis. Consequently, if epiphysial transplantation is used in clinical orthopaedic surgery, it should be performed as early as possible.

The series of rabbits subjected to lumbar sympathectomy after devascularization is too small to permit any very reliable conclusions, but it seems that sympathectomy cannot compensate, even partially, for the retardation of growth caused by devascularization.

In the study on the effect of temporary ischaemia on longitudinal growth, it was found that 3 hours of ischaemia had already caused widening of the epiphysial lines 24 hours after the release of the constricting band; histologically, swelling of cells and disarrangement of the cell columns was seen. Four, 5, 6, and 7 hours of ischaemia produced even more pronounced changes, observed 24 hours after the procedure, both roentgenologically and histologically. However, 5 hours of ischaemia had caused no retardation of growth of the tibia 6 weeks after the procedure, and no pathological changes in the growth cartilages were observed at histological examination. Six hours of ischaemia led to some retardation of growth, and 7 hours of ischaemia had caused even more retardation 6 weeks after the period of ischaemia; however, no definite pathological changes were seen in the growth cartilages of these limbs,

the other tissues of which (skin, nerves, muscles) were seen to be severely damaged at macroscopic examination.

Postmortal angiograms showed impaired filling of peripheral vessels after 2 hours of ischaemia; the changes after this period of ischaemia were slight, but with increasing length of the period of ischaemia, it became more and more apparent that changes in the peripheral vessels persisted for up to 14 days. Consequently, the retardation of growth found after 6 and 7 hours of ischaemia cannot be attributed solely to the period of ischaemia, since impaired circulation for some time after this period probably played a part in producing the injury to the growth cartilages. It was not considered necessary to pursue these experiments further; it had been established that 7 hours of ischaemia were needed to cause definite retardation of growth, seen 6 weeks after the procedure; it had also been established that such a period of ischaemia caused impaired filling of peripheral vessels, which probably contributed to the retardation of growth.

It seems that, even if the growth cartilage cells are as sensitive to ischaemia as other tissues, they possess a remarkable power of regeneration, which makes it possible for them to stand vascular injuries with a surprisingly small loss of growth potential, whereas other tissues in the limbs are definitely and irreparably injured under similar circumstances.

CONCLUSIONS

Conclusions based on the results of my experiments on rabbits are as follows:

motor, and combined motor and sensory, denervation by spinal nerve root section leads at most to slight retardation of growth in the hind limb;

sensory denervation by means of section of posterior nerve roots leads to retardation of growth of the femur through traumatization of an anaesthetic limb where the motor supply is intact;

subtotal devascularization by severance of the large vessels and the muscles in the thigh causes severe interference with longitudinal growth through necrosis of the growth cartilages, and, particularly in the upper end of the tibia, it causes the establishment of bony epiphysiodesis; this retardation of growth does not seem to be affected by lumbar sympathectomy;

temporary ischaemia must be maintained for 6—7 hours to cause significant retardation of growth; though the cells of the growth cartilages are injured, they apparently possess a high power of regeneration.

The mechanism by which retardation of growth occurs in limbs paralyzed by poliomyelitis still remains obscure; this study suggests that motor paralysis (with the sympathetic nerve supply intact) plays a very minor part in the retardation of growth. From clinical evidence it seems probable that chronic vascular insufficiency with consequent hypoxia of growth cartilage cells is an important factor in poliomyelitic retardation of growth; the possibility of a direct affection of the blood vessels should be kept in mind.

SUMMARY

Paralytic poliomyelitis has been a common cause of inequality of length in the lower limbs. There is a correlation between the degree of paresis and the retardation of growth, though considerable deviations are observed, and the rôle of the vascular disturbances in causing retardation of growth in poliomyelitic limbs has been emphasized. However, the pathogenesis of the disturbances in growth occurring after poliomyelitis is still obscure. In many other diseases the influence of nervous and vascular factors on bone growth can be demonstrated. *The purpose of this investigation was to shed further light on the relation between the longitudinal growth and the nervous and vascular supply of bone.*

The effects of the following procedures on longitudinal growth of the femur and tibia in the rabbit were investigated:

1) motor denervation;
2) motor and sensory denervation;
3) sensory denervation;
4) sensory denervation and protection of the denervated limb by insertion under the abdominal skin;
5) subtotal devascularization by severance of the muscles and vessels in the thigh;
6) severance of all muscles in the thigh;
7) severance of the femoral vessels;
8) subtotal devascularization and lumbar sympathectomy;
9) temporary ischaemia.

The experiments were carried out on rabbits 13—17 days old. Denervation was performed by severing nerve roots of the left hind limb, devascularization by severing all muscles, and all vessels except those of the skin, in the left thigh. Sympathectomy was performed by the transabdominal route. Temporary ischaemia was maintained by tying a piece of rubber tubing round the left thigh. Useful data were obtained from 132 animals.

Motor denervation by means of section of 3 or 4 lumbosacral motor

nerve roots caused almost complete paralysis of one hind limb, and severe atrophy of muscles, but only slight retardation of growth of the femur (5 per cent or less), and almost none of the tibia (Fig. 3, page 19; Fig. 4, page 19). Section of 2 lumbosacral motor nerve roots caused definite atrophy of muscles and osteoporosis in the affected limb, but no significant retardation of growth was found (Fig. 5, page 21). The results of section of 4 motor and 4 sensory nerve roots were similar to the results of section of 4 motor nerve roots.

Section of 4 sensory nerve roots had caused deformation and retardation of growth of the femur 25 days after operation (Fig. 7, page 23); the roentgenological examination of the bones suggested the effect of trauma (Fig. 13, page 27), and histological examination revealed a metaphysial fracture (Fig. 17, page 29) in some instances; the fracture probably involved the growth cartilage. As it was assumed that the fracture was the result of inadvertent traumatization of an anaesthetic limb, the denervated limb was protected in one series of animals by being inserted under the abdominal skin. No fractures, and at most slight retardation of growth (Fig. 8, page 23), were seen in these animals.

Subtotal devascularization by severance of muscles and vessels in the thigh caused retardation of growth: 10—15 per cent in the femur, and more in the tibia (Fig. 18, page 32). Histological examination revealed necrosis of the cells of the growth cartilages 24 hours after operation (Fig. 32, page 47); 48 hours later considerable regeneration was found at the periphery of the growth cartilage (Fig. 34, page 49); regeneration then proceeded towards the centre of the cartilage plate (Fig. 35, page 50). Rapid revascularization was seen in postmortal angiograms (Fig. 27, page 41; Fig. 28, page 41). On the 7th day after devascularization a forked growth cartilage was seen (Fig. 36, page 51); this was due to necrotic cartilage cells being left behind in the metaphysis.

In some devascularized bones, histological examination revealed a split in the growth cartilage in the zone of hypertrophied cells (Fig. 33, page 48). It was thought that the split represented a pathological weakness in a growth cartilage deprived of its blood supply; consequently, this finding might have some bearing upon the pathological processes in slipped upper femoral epiphysis.

In animals surviving subtotal devascularization for more than 21 days, the upper articular surface of the tibia was usually distorted and cup-shaped; in these distorted bones a broad central bony bridge joined the epiphysis and the metaphysis (Fig. 40, page 54). In some instances the upper end of the tibia was not distorted; histological examination revealed

narrow bony bridges and these did not retard longitudinal growth so severely (Fig. 39, page 53).

Lumbar sympathectomy did not compensate for the retardation of growth in devascularized limbs.

Temporary ischaemia did not significantly affect longitudinal growth if maintained for up to 5 hours; 6 hours of ischaemia produced some retardation of growth, and 7 hours even more (Fig. 22, page 37). Postmortal angiograms revealed vascular changes after the period of ischaemia (Fig. 31, page 44). It was realized that the retardation of longitudinal growth might be due not only to the period of ischaemia, but also to a more lasting impairment of blood supply caused by vascular injury.

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