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From the Department of Histology and the Department of Orthopaedics,
University of Lund, Sweden

DAILY GROWTH IN LENGTH OF DIAPHYSIS MEASURED BY OXYTETRACYCLINE IN RABBIT NORMALLY AND AFTER MEDULLARY PLUGGING

BY

LARS INGVAR HANSSON

MUNKSGAARD
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Translator: L. J. Brown

Statistical adviser: K. Svensson

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CARL BLOMS BOKTRYCKERI A.-B.

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INTRODUCTION

Numerous clinical and experimental investigations have been published on the effect of so-called growth stimulating operations on the growth in length of shaft bones (for surveys, see Goff 1960, Taillard and Morscher 1965), but only few have included simultaneous examination of the morphology of the growth zones. This was mainly because no methods were available for measuring the growth in length in various growth zones during a single day and at the same time allowing examination of the morphology of the growth zones.

The aim of the first part of the present investigation was to ascertain, whether it is possible to measure the daily growth in length from various growth zones with simultaneous morphological examination of the zones. The purpose of this was to find out whether intravital marking of the endochondral growth process of the diaphysis can be used to determine the growth in length from the growth plate of the diaphysis, which is mainly responsible for the growth in length of the shaft bones.

In the second part a so-called growth stimulating operation was studied for its effect on the growth in length of the diaphysis and the morphology of the relevant growth zones.

CHAPTER I

THE LONG BONE AND ITS GROWTH IN LENGTH

A. Introduction

Stephen Hales (1731, 1748) was the first to show that the long bones grow in length only at their extremities. With an awl Hales bored two holes in the shaft of a bone of one of the limbs presumably a tarso-metatarsal bone (Lacroix 1951) or tibia (Payton 1932, Bisgard and Bisgard 1935) in a chicken. Two months later the chicken was killed and the distance between the two markings had not increased in the meantime. On the other hand, other parts of the bone had increased considerably in length, especially the proximal part. Hales therefore concluded that growth in length occurs in the cartilaginous areas at the extremities of bones and especially in the area with a cartilage plate between the head and the shaft of the bone. Some years after this discovery by Hales, John Belchier (1736 a and b) discovered that dentine and bony tissue in pigs and bony tissue in chickens fed madder root stained red. Duhamel (1739, 1742, 1743 a, b and c) studied bone growth with the aid of madder and found that only the bony tissue formed when the animal was fed madder turned red, while that formed before and afterwards was of normal colour. Using different methods Duhamel confirmed Hales' finding that growth in length occurs at the extremities of the long bones but claimed that interstitial growth also occurs to a varying extent in diaphyses. He was also able to show that transverse growth of the shaft occurs by appositional bone formation from the periosteum and not by interstitial growth in the bone tissue.

John Hunter (1800, 1835) repeated Hales' and Duhamel's experiments and found that long bones grow only at their extremities. He also found that appositional bone formation is accompanied by resorption of previously formed bony tissue. According to Hunter, this co-occurrence of bone formation and resorption holds for longitudinal as well as for transverse growth of long bones.

Flourens (1841, 1842, 1845, 1847, 1861) confirmed Hunter's conclusion by studying the longitudinal and transverse growth by means of subperiosteally implanted pins, rings, balls, plates of metal and partly by experiments with madder root. He also found that resorption of the bony tissue is not confined to the endosteal aspect of the diaphysis but occurs also in most parts of bony tissue, which also applies to appositional bone formation.

Ollier (1867) believed that the long bones grow only at their extremities but pointed out that in young animals slight interstitial growth can occur in the metaphysis.

The assumption that long bones grow in length only at their extremities has since often been confirmed by different methods, which have also shown that the growth occurs mainly within the growth plate of the diaphysis and that the rest is due to growth of the epiphysis (Humphry 1858, 1861, 1862, Wegner 1874, Dubreuil 1913 a, b and c, Digby 1916, Keith 1919, 1920, Müller 1924, Haas 1926, Harris 1926, 1931, 1933, Gatewood and Mullen 1927, Payton 1932, 1933, 1934, Bisgard 1933, Brash 1934, Silfverskiöld 1934, Bisgard and Bisgard 1935, Banks and Compere 1941, Boerema 1942). In contrast to Policard (1941), Lacroix (1951) found perichondral ossification to play no essential part in the growth in length of long bones.

According to Enlow (1963), the previously mentioned cartilage plate had been demonstrated several hundred years before its function had been realised. The first detailed description of its morphology was given by Müller (1858). The endochondral growth process has since been described more or less exhaustively by several workers in this field (Müller 1924, Weidenreich 1930, Harris 1933, Bloom and Bloom 1940, McLean and Bloom 1940, Lacroix 1951, 1961, Weinmann and Sicher 1955, Trueta and Morgan 1960, Sissons 1961, Gardner 1961, Leblond and Greulich 1961, McLean and Urist 1961, Bloom and Fawcett 1962, Hall 1965, Ham and Leeson 1965, Siffert 1966).

B. Long Bones and Growth in Length

1. VARIOUS REGIONS

Long bones, because of their mode of growth, belong to the group of bones known as cartilage bones. They increase in size by chondral growth including perichondral and endochondral growth (Keith 1948,

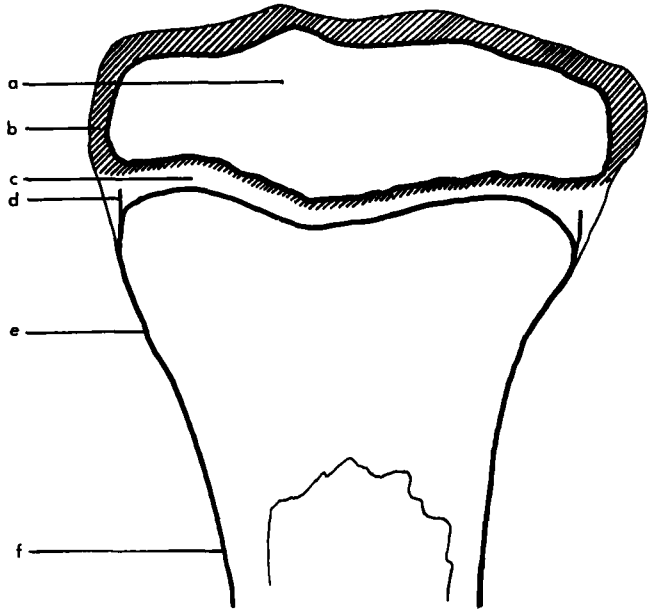

Fig. 1. Proximal tibia of 30 day old rabbit.

a. bony epiphysis
b. growth cartilage of epiphysis
c. growth plate of diaphysis
d. perichondral lamella
e. metaphysis
f. shaft proper

Gardner 1961, 1963, Bloom and Fawcett 1962, Bargmann 1964, Bucher 1965, Ham and Leeson 1965).

Long bones are divided into different parts according to their development and growth. But there appears to be no generally accepted nomenclature for this division, which has caused considerable confusion. It might therefore be convenient to define the names used for different parts of long bones discussed in the present investigation.

The long bone is divided into a middle portion which is called the diaphysis or the shaft (Harris 1933, Leblond et al. 1950, Lacroix 1951, Weinmann and Sicher 1955, Kopsch 1955, Bloom and Fawcett 1962, Copenhaver 1964, Ham and Leeson 1965, Hall 1965) and two end regions.

The shaft proper (Fig. 1) is the middle part of the diaphysis and is of practically uniform diameter (Lacroix 1951). The spongy part of the diaphysis is called the metaphysis (Fig. 1), which, as indicated by

the name, is situated close to the cartilaginous growth zone (Lacroix 1951, Ham and Leeson 1965, Hall 1965). This division of the diaphysis or the shaft appears more suitable than, for example, the cylinder and the funnel (Leblond et al. 1950, Leblond and Greulich 1961, Enlow 1963, Hall 1965), which refer only to the cortical bony tissue. In some investigations the shaft has been divided into diaphyseal and metaphyseal regions, the former part then including only the middle portion of shaft (Bhaskar et al. 1950, Brodin 1955, Leblond and Greulich 1961, Enlow 1963, Salter and Harris 1963). This division appears less suitable because the terms diaphysis and metaphysis describe areas relative to the growth zone.

The extremity of a long bone (Fig. 1) is divided into an epiphyseal area and a cartilaginous plate, which is situated between the above-mentioned diaphyseal and epiphyseal areas and is called the growth plate of the diaphysis, since it is responsible for the growth in length of the diaphysis.

There are different types of epiphyses, namely: pressure, traction (Salter and Harris 1963) and atavistic epiphyses (Hall 1965). Pressure epiphyses are often also called articular epiphyses (Salter and Harris 1963). Unlike pressure epiphyses (Salter and Harris 1963, Hall 1965) traction and atavistic epiphyses are nonarticular and usually do not take part in the growth in length of long bones and are therefore irrelevant.

During the first part of the growth period the entire epiphyseal area consists of a cartilaginous epiphysis. During the latter part of the period one or more epiphyseal or secondary ossification centres appear in the cartilaginous epiphysis (Bloom and Fawcett 1962, Copenhaver 1964), which occurs at different times in different epiphyseal areas (Weinmann and Sicher 1955, Gardner 1961, 1963). These epiphyses therefore consist of one or more central bony epiphyses and of surrounding remnants of the cartilaginous epiphysis (Fig. 1). These remnants form the growth cartilage of the epiphysis, and of the bony epiphysis. The epiphyseal part of the cartilage plate is part of the above-mentioned growth cartilage of the epiphysis. The superficial rests of the cartilaginous epiphysis bordering the joint cavity are usually called the joint or articular cartilage, while those parts covered by perichondrium are called the cartilage of the sides of the epiphysis (Lacroix 1951).

In the major long bones, to which belong the humerus, radius, ulna,

femur, tibia and fibula (Salter and Harris 1963) epiphyseal ossification centres appear, and thus cartilage plates, in both the proximal and distal cartilaginous end regions. In the other long bones of the limbs epiphyseal ossification centres and cartilage plates appear in only one of the two end regions. In the phalangeal bones, the first metacarpal and metatarsal bone, the cartilage plate is situated in the proximal end of the bone; in the other metacarpal and metatarsal bones, in the distal end (Lacroix 1951, Kopsch 1955). In the opposite end region of the above-mentioned bones no epiphyseal ossification centre or cartilage plate develops, and the diaphyseal endochondral ossification process continues in the cartilaginous epiphysis (Lacroix 1951).

The metaphyseal part of the cartilage plate (Fig. 1) does not belong to the epiphyseal region but forms a region between the epiphysis and diaphysis. It is from this region that the diaphysis grows in length and is therefore called the growth plate of the diaphysis (Levene 1964, Johnson 1964). This growth plate develops much earlier during the growth period than other parts of the cartilage plate (Gardner 1961, 1963) and can also occur in growth regions where no cartilage plate or bony epiphysis occurs (Siffert 1966).

The extremities of long bones have also been divided by other systems which for various reasons appear less suitable than those given above. According to some authors (Harris 1933, Weinmann and Sicher 1955, Bloom and Fawcett 1962, Hall 1965), the epiphysis does not include any part of the cartilage plate, while others (Leblond et al. 1950, Trueta and Morgan 1960, Salter and Harris 1963) claim that the epiphysis also comprises the entire cartilage plate. The epiphysis has sometimes (Lacroix 1951, Ring 1955, Goff 1960, Bevelander 1961) been regarded as identical with the bony epiphysis. In addition, it may be added that the epiphyseal cartilage is a term used to designate both the cartilaginous epiphysis (Gardner 1961, Bevelander 1961) and the cartilage plate (Dodds 1932, Dodds and Cameron 1934, Lacroix 1951, Weinmann and Sicher 1955, Ring 1955, Brodin 1955, McLean and Urist 1961). The term epiphyseal cartilage is therefore unsuitable (Bergenfeldt 1933, Salter and Harris 1963, Hall 1965).

As mentioned, the growth plate or disc has previously been used as a term for that part of the cartilage plate that is responsible for the growth of the diaphysis (Levene 1964, Johnson 1964). According to Siffert (1966), also the primary spongy bone of the metaphysis belongs to the growth plate of the diaphysis, but this classification appears less

suitable since the increase in length of the long bone is due exclusively to cartilaginous growth (Lütken 1947, Bhaskar et al. 1950, Weinmann and Sicher 1955).

The above-mentioned cartilage plate between the bony epiphysis and the diaphysis and here called the cartilage plate has been known under a wide variety of names such as the epiphyseal cartilage (Harris 1933, Lacroix 1951, Weinmann and Sicher 1955, Brodin 1955, Ring 1955, Elo 1960, Trueta and Morgan 1960, McLean and Urist 1961), the epiphyseal cartilage plate (Bhaskar et al. 1950, Kember 1960, Sissons 1961, Bloom and Fawcett 1962), the epiphyseal plate or disc (Leblond et al. 1950, Trueta and Morgan 1960, Messier and Leblond 1960, Leblond and Greulich 1961, Gardner 1961, 1963, Salter and Harris 1963, Ham and Leeson 1965, Harris et al. 1965, Hall 1965), the epiphyseal line (Harris 1933) or similar names (Trueta and Amato 1960, Hall 1965). These names are less suitable because they suggest that the cartilage plate belongs to the epiphyseal area, but say nothing about its importance for the diaphysis. In addition, the term the epiphyseal cartilage can, as mentioned, be confused with the cartilaginous epiphysis (Bergenfeldt 1933, Salter and Harris 1963, Hall 1965). The epiphyseal line corresponds, as a rule, to the synostosis forming between the epiphysis and the diaphysis at the end of the growth period (Copenhaver 1964). The cartilage plate has also been called the diaphyseal line (Keith 1919, 1920, 1948) and the diaphyseal cartilage (Macewen 1912) on the ground that it is involved with the growth in length of the diaphysis and does not take part to any appreciable extent in the growth of the epiphysis. Several other neutral names have also been used such as the growth cartilage (Trueta and Morgan 1960, Trueta and Little 1960, Lacroix 1961, Troupp 1961), the growth plate (Trueta and Trias 1961) or the growth disc (Keith 1919, 1920, 1948).

As mentioned previously, the above names refer to the cartilage plate, *i.e.* the cartilage plate between the bony epiphysis and diaphysis. It should, however, be mentioned that most of the above-mentioned investigations were carried out during the latter part of the growth period, by which time the growth of the epiphysis from the cartilage plate is concluded and the epiphyseal part of the cartilage plate has almost disappeared. Under these circumstances the cartilage plate, like the above-mentioned synonyms, will consist almost exclusively of the growth plate of the diaphysis [1] and be responsible only for the growth of the diaphysis.

[1] This plate is hereinafter referred to as the growth plate.

2. TOTAL GROWTH IN LENGTH

As mentioned previously, the growth in length of long bones is due to growth of the cartilaginous tissue at their extremities. In both extremities of major long bones, and in one extremity of minor long bones the above-mentioned growth in length occurs in the growth plate and in the growth cartilage of the epiphysis. In the opposite ends of minor long bones growth in length occurs in the cartilaginous tissue covering the end of the bone. Conversion of these cartilaginous areas to bony tissue does not increase the total length of the bone but results in a growth in length of the diaphysis and the bony epiphysis (Lütken 1947, Bhaskar et al. 1950, Weinmann and Sicher 1955). During the first part of the growth period the growth in length of the cartilaginous area is almost identical with that of the diaphysis and bony epiphysis, which means that the cartilaginous areas retain their thickness. During the latter part of the growth period the thickness of the cartilage gradually decreases because the rate in growth in the cartilaginous area during this period is somewhat lower than that of the diaphysis and bony epiphysis (Weinmann and Sicher 1955, Morscher et al. 1965).

a. *The Growth Plate*

The metaphyseal part of the cartilage plate (Fig. 2) is responsible for the major part of the growth in length of long bones. This growth is interstitial and occurs by division and hypertrophy of the cartilage cells and production of intercellular substance. The degenerative process, like the intercellular calcification, chondrolysis and the endochondral ossification in the metaphysis, does not increase the length of the bone but contributes to growth in length of the diaphysis. The intercellular calcification in the cartilage matrix results in an increase in the strength of the transition between cartilaginous tissue and metaphyseal bony tissue (McLean and Urist 1961, Bloom and Fawcett 1962).

This part of the cartilage plate also increases transversely by interstitial growth (Langenskiöld 1947, Weinmann and Sicher 1955) and by appositional growth from the perichondrium (Weinmann and Sicher 1955, Solomon 1966) and possibly also from the ossification groove (Lacroix 1951, 1961) which is a very cellular area in the adjacent part of the periosteum.

b. *The Growth Cartilage of the Epiphysis*

The epiphyseal part of the cartilage plate (Fig. 2) is responsible for a minor part of the growth in length of the bone in the same way

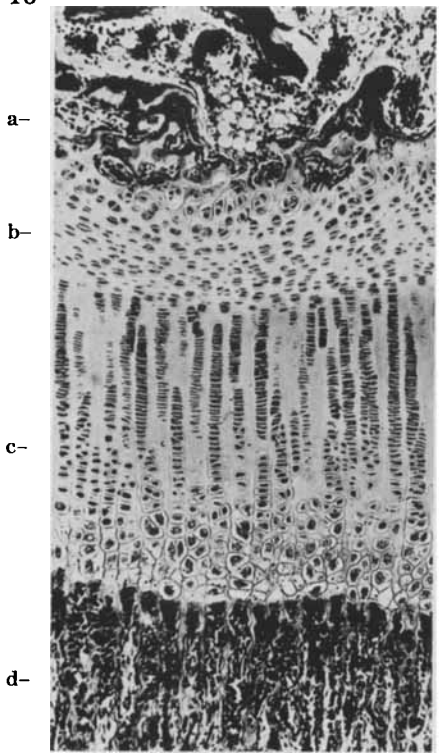

Fig. 2 A. Distal radius of 20 day old rabbit (75×)

a. bony epiphysis
b. growth cartilage of epiphysis
c. growth plate of diaphysis
d. metaphysis

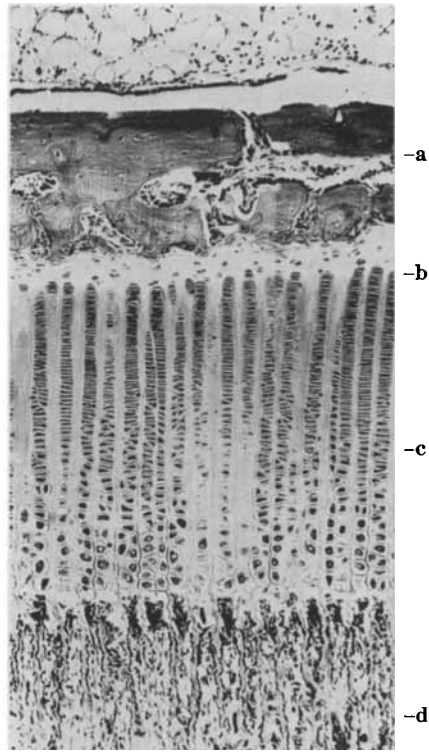

Fig. 2 B. Distal radius of 70 day old rabbit (75×)

a. bony epiphysis
b. remnants of growth cartilage of epiphysis
c. growth plate of diaphysis
d. metaphysis

as the metaphyseal part. At the same time the bony epiphysis grows in diaphyseal direction from this cartilaginous area in the same way as the diaphysis grows from the metaphyseal part. Growth of the long bone and the bony epiphysis from this area occurs only during the earliest part of the growth period (Lacroix 1951, Ring 1955, Ham and Leeson 1965). Payton (1933) and Guldhammer (1963) believe to have shown the occurrence of resorption of the bony epiphysis in this area during the latter part of the growth period which, however, could not be verified by even thorough investigations (Lacroix 1951, Trueta and Morgan 1960, Ham and Leeson 1965).

Those parts of the cartilaginous epiphysis which cover the peripheral part of the bony epiphysis (Fig. 1) are responsible for the rest of the growth in length of long bones. If the cartilaginous area borders the joint cavity and is called joint cartilage, growth occurs interstitially, and if the hyaline cartilage is covered by perichondrium, also appositionally (Weinmann and Sicher 1955). Growth of the bony epiphysis in peripheral-longitudinal direction occurs in the same way as that previously described for the diaphysis (Müller 1924, Lacroix 1951, Weinmann and Sicher 1955, Ham and Leeson 1965, Hall 1965). The cartilaginous area alone is responsible for the growth in length at the extremities of the minor long bones, which have no cartilage plate (Roche 1965).

C. Growth Apparatus of Diaphysis

This section is concerned with the growth plate and the adjacent zone of endochondral ossification in the metaphysis (Fig. 3) which together form the growth apparatus of the diaphysis (McLean and Urist 1961, Siffert 1966).

1. MORPHOLOGY

a. *The Growth Plate*

The above-mentioned cartilaginous plate is conventionally divided into different zones according to the appearance and function of the cartilage cells. It has also been divided according to the intercellular calcification of the cartilage matrix which, however, is not limited to the growth plate but also occurs in the zone of endochondral ossification in the metaphysis (Gardner 1961, Bevelander 1961). Some authors have used a combination of various classification grounds. In the present investigation the growth plate is divided according to the function of the cartilage cells into the following zones: the zone of germinative cells, the zone of proliferative cells, the zone of hypertrophic cells, and the zone of degenerative cells (Fig. 3), which is situated adjacent to the metaphysis. The above-mentioned names indicate the function of the cartilage cells and appear to be preferable to a descriptive classification in this investigation.

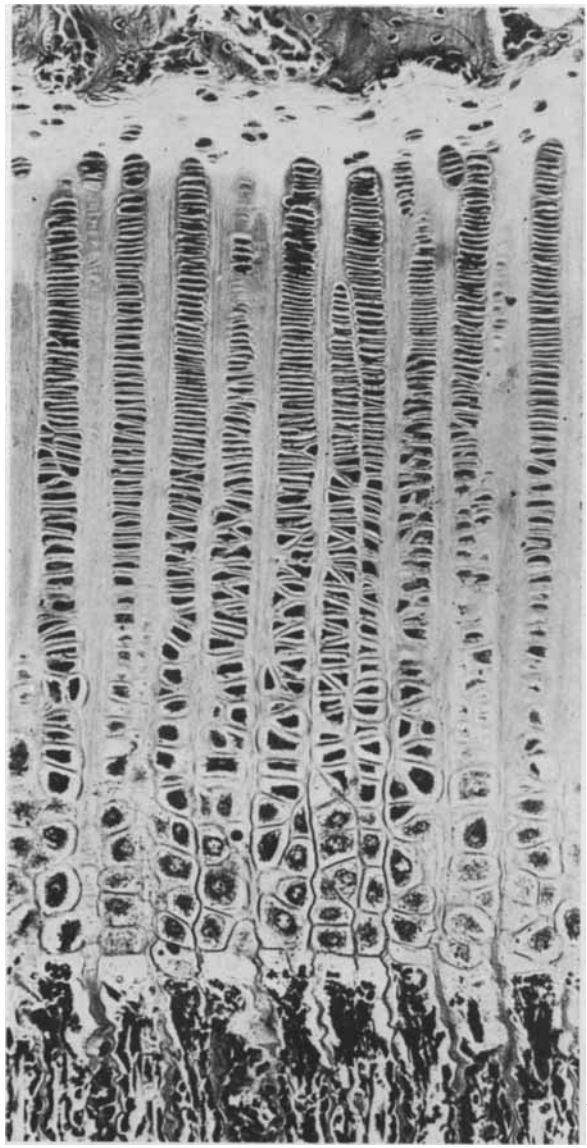

Fig. 3. Distal radius of 70 day old rabbit. Higher magnification of growth apparatus of diaphysis shown in Fig. 2 B (190×).

THE ZONE OF GERMINATIVE CELLS

The zone of germinative cells (Fig. 3) comprises the most epiphyseal part of the growth plate.

The cartilage cells are sometimes single and sometimes grouped (Trueta and Morgan 1960) and usually irregularly distributed (Hall 1965) in the zone. The cell is, as a rule, elongated and ellipsoid with a small, round, dense and usually eccentric nucleus (Berthold 1954).

The zone is widest during the former part of the growth period. With increasing age the zone decreases in height and is finally seen as a narrow zone (Dodds 1930, Dodds and Cameron 1934, Copenhaver 1964, Hall 1965).

In this part both matrix (Duthie and Barker 1955) and cartilage cells (Dodds 1930, Copenhaver 1964) are produced. There are fairly numerous mitoses of which only a few can be seen in longitudinal sections of the bone, while many can be observed in transverse sections (Lacroix 1951, Rigal 1962). According to Rigal (1962) the mitotic activity varies from species to species. In rats mitoses are few, while in rabbits they are numerous.

There appears to be various synonyms for the zone of germinative cells *e.g.* the zone of reserve cells (Dodds and Cameron 1934, Lacroix 1951, Berthold 1954), the reserve zone (Ring 1955), the zone of reserve cartilage (Copenhaver 1964), the zone of resting cartilage cells (Trueta and Morgan 1960), the zone of resting cartilage (Ham and Leeson 1965), the zone of resting cells (Trueta and Little 1960), the zone of germinal cells (Trueta and Morgan 1960), the zone of undifferentiated cartilage cells (Trueta and Morgan 1960) and the basal zone (Lacroix 1951, 1961).

THE ZONE OF PROLIFERATIVE CELLS

The zone of proliferative cells (Fig. 3) is situated metaphyseally to the zone of germinative cells (Trueta and Morgan 1960). The former zone is, as a rule, wider than the latter (Hall 1965) and these two zones together form about half of the height of the growth plate (Trueta and Morgan 1960).

The cartilage cells in the zone of proliferative cells are, as a rule, arranged in columns of different length (Lacroix 1951, 1961, Weinmann and Sicher 1955, Trueta and Morgan 1960, McLean and Urist 1961, Ham and Leeson 1965) or in groups of varying size (Dodds 1930, Dodds and Cameron 1934, Berthold 1954, Weinmann and Sicher 1955, McLean and Urist 1961, Salter and Harris 1963). The columns of cells extend in longitudinal direction of the bone (Weinmann and Sicher 1955, McLean and Urist 1961, Bevelander 1961, Ham and Leeson 1965). These columns usually start at the border of the zone of germinative

cells and extend through the growth plate to the metaphysis. Shorter columns are also seen starting at different levels in the zone and extending a varying distance towards the metaphysis (Dodds 1930, Dodds and Cameron 1934, Berthold 1954, Weinmann and Sicher 1955).

The cartilage cells, which lie in small clusters, are round and, as a rule, have a larger and looser nucleus than the cells in the previous zone. These clusters occur in the epiphyseal part of the zone where they form a transition area between the zone of germinative cells and the area with cell columns (Berthold 1954). Sometimes the entire zone is made up largely of this type of cartilage (Salter and Harris 1963), which is, however, usually replaced by cartilage built up of cellular columns (Kember 1960, Trueta and Morgan 1960).

The cartilage cells in the columns are flattened to a varying extent. The amount of cytoplasm appears to be smaller than earlier. The cell nucleus is situated close to the cell membrane. The cartilage cell therefore appears smaller than in the zone of germinative cells (Berthold 1954, Hall 1965). In the columns these cells are situated on top of one another so that the thickest part of the cell lies against the flattened part of the next cell (Dodds 1930, Lacroix 1951, 1961). The shape and size of the cells vary only little in the different parts of the columns in the zone of proliferative cells (Trueta and Morgan 1960). The number of proliferative cells in a column varies widely for different reasons (Harris 1933, Trueta and Morgan 1960, Hall 1965).

Between the columns is a cartilage matrix in the form of longitudinal septa of varying size (Weinmann and Sicher 1955, McLean and Urist 1961, Bloom and Fawcett 1962). The same type of septa can be seen between the different groups of cartilage cells. Between the cells in a column are transverse septa (Lacroix 1951, Weinmann and Sicher 1955, Bloom and Fawcett 1962). These transverse septa, however, are not always perpendicular to the longitudinal axis of the cellular column (Lacroix 1951). The thickness of the septa in the cellular column varies (Dodds 1930, Dodds and Cameron 1934, Weinmann and Sicher 1955).

Cartilage cells form by mitosis also in this zone. The activity is much higher than in the zone of germinative cells. As a rule, mitosis occurs in a plane perpendicular to the longitudinal direction of the column of cells (Dodds 1930, Lacroix 1951, 1961, Kember 1960, Hall 1965), and these mitosis are difficult to observe in longitudinal sections through the columns (Lacroix 1951). During mitosis each cellular column functions as a unit, which means that cells in a given column divide almost simultaneously but at different times in different columns

(Dodds 1930, Trueta and Little 1960, Rigal 1962, Hall 1965). Rigal (1962) observed a relationship between mitosis in the column and in that cell in the zone of germinative cells, which is situated closest to the epiphyseal part of the cellular column. When this cell is undergoing division the cells in the column are in the resting phase. Kember (1960), who used tritiated thymidine autoradiography in the rat, found that the generation time of the most active cells situated in the middle part of the zone is between 1 and 3 days. The generation time of the other proliferative cells increases in epiphyseal respectively metaphyseal direction, which means that the activity is lowest near the borders of the zone. The time for those that are nearest the epiphysis was up to 4 times as long as that for the most active cells. This part of the column therefore represents a zone of reserve cells (Kember 1960). In addition, as in many other tissues, the frequency of mitosis show diurnal variation (Simmons 1962, Simmons and McLean 1962).

The appearance and development of the cellular columns have been the subject of various investigations. The cells in a column are believed to derive from a single cell situated in the zone of germinative cells (Dodds 1930, Dodds and Cameron 1934, Weinmann and Sicher 1955, Lacroix 1961, McLean and Urist 1961). Later autoradiographic investigations suggest that a single mother cell gives rise to a large number of cells which continuously increase in number by repeated division in the zone of proliferative cells (Kember 1960, Messier and Leblond 1960, Rigal 1962). This means that only few mother cells are necessary.

In this zone the cartilage cells produce large amounts of cartilaginous matrix, which has been shown by autoradiography (Dziewiatkowski 1951, 1952, Duthie and Barker 1955, Leblond and Greulich 1961).

The zone of proliferative cells has also been called: the zone of palisade (Trueta and Morgan 1960), the zone of young proliferating cartilage (Ham and Leeson 1965), the zone of flattened cells and cell multiplication (Dodds and Cameron 1934), "die Schicht der Knorpelsäulen" (Taillard and Morscher 1965), the zone of cartilage cell columns (Morscher et al. 1965), the zone of proliferation (Lacroix 1951, Trueta and Morgan 1960), the proliferating zone (Lacroix 1961, Hall 1965) and the proliferative zone (Trueta and Little 1960, Rigal 1962).

THE ZONE OF HYPERTROPHIC CELLS

This zone is on the metaphyseal side of the zone of proliferative cells (Fig. 3). There is no distinct border between the proliferative and

the hypertrophic cells in a column because the maturation process is slow (Trueta and Little 1960). In addition, proliferative cells in a column are sometimes intermingled with hypertrophic cells in adjacent columns, presumably because the proliferative cells in one column divide at a different time than those in an adjacent column (Trueta and Little 1960, Rigal 1962).

In this zone, in contrast to the previously mentioned zone, the cartilage cells differ from one another in size. The smallest cells are situated in the epiphyseal part of the zone, the largest in the metaphyseal part (Dodds and Cameron 1934).

In the zone of hypertrophic cells the cartilage cells gradually mature during their passage through the zone towards the metaphysis (Kember 1960, Messier and Leblond 1960). At the same time the cartilage cells begin to increase in volume and loose their cuneiform appearance and become almost spherical or cubical. This expansion of the cells occurs mainly in longitudinal direction of the column. The transverse septa, which formerly ran obliquely to the longitudinal axis of the column, become more and more perpendicular. In some cases the septum turns in the opposite direction with the result that it finally runs parallel to the column and the hypertrophic cells then lie adjacent one another. Occasionally, though rarely, the cartilage cells retain their cuneiform appearance during maturation (Lacroix 1951, 1961).

As a result of hypertrophy the cartilage cells expand in the longitudinal direction of the column to about 4 times their original length. The cartilage cells also expand simultaneously transversely but not so markedly. Since the longitudinal septa between the cellular columns also become narrower simultaneously, the growth plate does not increase transversely. The amount of intercellular substance is, however, not reduced, but instead the longitudinal septa probably increase in length (Lacroix 1951, 1961). Trueta and Morgan (1960), on the other hand, believe that the intercellular substance is markedly reduced in association with hypertrophy of the cells and that the matrix becomes more compact than before and that the border between the intercellular substance and the cartilage cells becomes more distinct.

During maturation of the cartilage cells larger and larger vacuoles appear in the cytoplasm because of the deposition of glycogen (Lacroix 1951, 1961, Dixon and Perkins 1961, Ham and Leeson 1965) and fatty substances (Lacroix 1951, Bargmann 1964). During the hypertrophic stage the deposition is largest, after which it abruptly decreases with the result that the most mature cartilage cells in the zone of hyper-

trophic cells have no glycogen deposits (Dixon and Perkins 1961, Lacroix 1961). During the latter part of the maturation process fluid accumulates inside the cells with the result that the cytoplasm and the nucleus have an oedematous appearance (Weinmann and Sicher 1955, Trueta and Morgan 1960).

The nucleus, which in the zone of proliferative cells is situated peripherally in the cell, migrates towards the centre during the hypertrophic process (Trueta and Morgan 1960, Taillard and Morscher 1965). At the same time it increases in volume and its structure becomes looser than before (McLean and Urist 1961).

In this zone the production of cartilage matrix is more intense than in previously mentioned parts of the growth zone. This production is confined to that part of the zone of hypertrophic cells which is least mature and situated nearest the zone of proliferative cells. In the metaphyseal part of the zone there is probably no production of cartilage matrix (Duthie and Barker 1955, Leblond and Greulich 1961, Johnson 1964).

The zone of hypertrophic cells (Trueta and Morgan 1960, Trueta and Little 1960) has been known under various names such as the zone of cell growth (Dodds and Cameron 1934), the zone of maturing cartilage (Ham and Leeson 1965), the zone of maturation (Scott and Pease 1956), the hypertrophic zone (Lacroix 1951), the zone of progressive hypertrophy (Lacroix 1961), the hypertrophying zone (Hall 1965), the giant-cell zone (Trueta and Little 1960) and the zone of giant cells (Trueta and Morgan 1960).

THE ZONE OF DEGENERATIVE CELLS

Metaphyseally to the zone of hypertrophic cells is the zone of degenerative cells (Trueta and Morgan 1960, Trueta and Little 1960, Park 1964), which reaches the metaphysis.

This zone (Fig. 3) includes the cartilage cells nearest the metaphysis in each column. The border between the hypertrophic and the degenerative cells in a column is, as a rule, diffuse. The cartilage cells reach maximum size in the zone of hypertrophic cells. When these hypertrophic cells have reached the zone of degenerative cells, vacuolisation of the cytoplasm continues simultaneously as the nuclei begin to degenerate, and to swell and loose large amounts of chromatin (McLean and Urist 1961). This degeneration process finally results in the death and desintegration of the cells (Trueta and Morgan 1960, Sissons 1961).

The lacuna, which is situated close to the metaphysis, is often called the clear cell, because in many cases the lacuna appears to be empty (Irving 1964).

The number of cells in the zone of degenerative cells is small. According to Trueta and Morgan (1960), a column contains only one or occasionally two degenerative cartilage cells. However, Dodds and Cameron (1934), like Lacroix (1951, 1961), Ham and Leeson (1965) and McLean and Urist (1961), reported that as many as four degenerative cartilage cells may be seen in the column.

Also this zone has been known under different names such as the zone of fully grown cells (Dodds and Cameron 1934) and the zone of cell death (Messier and Leblond 1960). Synonyms referring to the intercellular calcification are also often used such as the zone of calcification (Dodds and Cameron 1934, Lacroix 1951) and the zone of calcifying or calcified cartilage (Lacroix 1961, Ham and Leeson 1965), but these names are not synonymous with the zone of degenerative cells for they refer to the intercellular calcification process.

The hypertrophic and the degenerative cartilage cells are often referred to as belonging to a single zone known under different names, such as the zone of edematous cells (Bhaskar et al. 1950), the zone of hypertrophic cells (Leblond et al. 1950) and the layer of hypertrophying or hypertrophied cells (Salter and Harris 1963).

b. *The Line of Erosion and the Metaphysis*

THE LINE OF EROSION

The border between the zone of degenerative cells and the metaphysis (Fig. 3) is well defined (Lacroix 1951). Chondrolysis of the degenerative zone occurs along this border. This process involves particularly the transverse septa, and then the cellular lacunae are opened but smaller parts of the longitudinal septa are also destroyed. Chondrolysis occurs by cellular activity. These chondrolytic cells are believed to be either uninuclear mesenchymal cells, such as capillary endothelial cells and connective tissue cells (Dodds 1932, Weinmann and Sicher 1955, Sissons 1961, Irving 1964) or multinuclear cells, such as osteoclasts (Dodds 1932). According to Trueta and Morgan (1960) chondrolysis occurs in association with a sort of microhaemorrhage from blood capillaries along the line of erosion.

This line or narrow zone has also been known under such names as the erosion line (Cameron 1961), the zone of cartilage removal (Dodds

and Cameron 1934, Gardner 1961, Copenhaver 1964), the base of cartilage plate (Kember 1960), the erosion zone (Morscher et al. 1965) and "die Eröffnungszone" (Bargmann 1964).

THE METAPHYSIS

As mentioned, the metaphysis (Fig. 3) is the spongy part of the diaphysis bordering the growth plate. Its upper border, which corresponds to the erosion line, is thus distinct but its lower one bordering the shaft proper is diffuse (Lacroix 1951).

The metaphysis is built up of spongy tissue, which is composed of two components, namely cartilaginous matrix and bony tissue. In longitudinal sections of the bone the metaphysis appears to consist of trabeculae which, as a rule, run in the direction of the longitudinal axis of the bone. The central part of the metaphyseal trabeculae consists of cartilaginous matrix which is covered on both sides by bony tissue with the exception of a narrow zone close to the growth plate (Robinson and Cameron 1956). The cartilaginous central part is directly connected with the longitudinal septa in the growth plate (Ham and Leeson 1965). Transverse sections through the metaphysis show no trabeculae but, instead, cartilaginous parts forming walls in longitudinal tunnels whose inner wall is lined by bony tissue. The type of the newformed endochondral bony tissue varies during the growth period but is always sooner or later replaced by lamellar bone (Lacroix 1951, Weinmann and Sicher 1955, Pritchard 1961, Enlow 1963, Frost 1963 b).

The spongy tissue of the metaphysis is made up of primary and secondary spongiosa. Primary spongiosa is situated nearest to the growth plate and comprises that part where osteogenesis is very active, and resorption of the longitudinal trabeculae is insignificant. The trabeculae are therefore delicate, dense and run uninterrupted through this part. In the secondary spongiosa osteogenesis is not so active, and many trabeculae are completely or partly resorbed. The trabeculae in the secondary spongiosa are therefore sparser, shorter and thicker than in the primary spongiosa (Sissons 1953, 1961).

The peripheral parts of the metaphysis have also undergone thorough reconstruction and its transverse diameter has been reduced and is the same as the other parts of the diaphysis, *i.e.* the shaft proper (Harris 1933, Lacroix 1951, Weinmann and Sicher 1955, Leblond and Greulich 1961, Enlow 1963). This results in elongation of the shaft proper at the same rate as the increase in length of the diaphysis by growth from the growth plate (Leblond and Greulich 1961).

c. *The Endochondral Calcification*

As previously mentioned, the intercellular substance is produced in the zone of germinative cells, the zone of proliferative cells and the adjacent part of the zone of hypertrophic cells, and is most active in the last mentioned zones. These areas show no signs of calcification in the intercellular substance. Robinson and Cameron (1956) therefore call this part the zone of uncalcified cartilage matrix. In the metaphyseal part of the zone of hypertrophic cells as well as in the zone of degenerative cells calcium salts are seen in the intercellular substance. Similar deposits are also seen in the cartilaginous parts of the trabeculae of the metaphysis.

The border between intercellular substance with and without calcium salts is said to be at the level of hypertrophic cartilage cells nearest the metaphysis (Durning 1958, Trueta and Morgan 1960, Trueta and Little 1960, Ham and Leeson 1965) or at the border between the hypertrophic and the degenerative cells (Dodds and Cameron 1934, Robinson and Cameron 1956, Takuma 1960).

As a rule, the calcified part of the growth plate includes 1—4 cells in each column of cartilage cells (Dodds and Cameron 1934, Bloom and Bloom 1940, McLean and Bloom 1940, Lacroix 1951, 1961, Robinson and Cameron 1956, Durning 1958, Ponlot 1959, Trueta and Morgan 1960, Trueta and Little 1960, McLean and Urist 1961, Ham and Leeson 1965). There is said to be a definite ratio between the above-mentioned number of cells and growth activity (Harris 1933, McLean and Bloom 1940, Bloom and Bloom 1940).

Robinson and Cameron (1956) divide the area with calcium salts in the cartilage matrix in two parts, namely the zone of cartilage undergoing calcification and the zone of completely calcified cartilage matrix. The border between these two zones can coencide with the erosion line (Leblond et al. 1950, Robinson and Cameron 1956), pass somewhat epiphyseal to the line in the growth plate (Bloom and Bloom 1940, McLean and Bloom 1940, Trueta and Morgan 1960, Trueta and Little 1960) or metaphyseally to the erosion line in the metaphysis (Gardner 1961, Bevelander 1961). This variation can be explained by the varying growth activity in different growth plates (McLean and Bloom 1940, Bloom and Bloom 1940, Gardner 1961).

The first sign of calcification in the intercellular substance along the border between the zone of uncalcified cartilage matrix and the zone of cartilage undergoing calcification occurs in the form of granules

situated singly or in small groups. These granules are situated pericellularly in the longitudinal septa, but are not contiguous with the cells (Robinson and Cameron 1956, Scott and Pease 1956, Takuma 1960, Lacroix 1961) and are numerous at the level where the longitudinal septa are crossed by the transverse septa (Trueta and Little 1960). Further metaphyseally the corresponding area in the longitudinal septa undergoes calcification, but also the central part of the septa which, however, is, as a rule, incompletely calcified (Robinson and Cameron 1956). Trueta and Little (1960) like Durning (1958) believe that it is the central parts of the septa that undergo complete calcification while the peripheral parts are not calcified.

Calcium salts are deposited also in the transverse septa but not to the same extent as in the longitudinal septa (Bloom and Bloom 1940, McLean and Bloom 1940, Lacroix 1951, Weinmann and Sicher 1955, Scott and Pease 1956, Durning 1958, Ponlot 1959, Trueta and Little 1960, Ham and Leeson 1965). This opinion is not shared by Dodds (1930, 1932), Dodds and Cameron (1934) and Robinson and Cameron (1956), who found no signs of calcification in these septa.

The metaphyseal calcified part of the growth plate is known by different names, very often as the zone of calcification (Dodds and Cameron 1934, Bloom and Bloom 1940, McLean and Bloom 1940, Lacroix 1951, Weinmann and Sicher 1955, Trueta and Morgan 1960, Trueta and Little 1960, Copenhaver 1964, Morscher et al. 1965) or the zone of provinsial or preparatory calcification (McLean and Bloom 1940, Cameron 1961, McLean and Urist 1961, Bloom and Fawcett 1962, Salter and Harris 1963). Other names have also been used such as the zone of calcifying cartilage (Ham and Leeson 1965, Hall 1965) and the zone of calcified cartilage (Leblond et al. 1950, Gardner 1961, Leblond and Greulich 1961, Lacroix 1961).

In the present investigation the following terms are used: the zone of uncalcified cartilage, the zone of cartilage undergoing calcification and the zone of completely calcified cartilage.

d. *The Epiphyseal Part of the Cartilage Plate*

Though the epiphyseal part of the cartilage plate (Fig. 2) does not belong to the growth apparatus of the diaphysis, it might not be out of place to dwell on this area because it is of significance in the nutrition of the growth plate.

The epiphyseal part of the cartilage plate develops, as mentioned,

in association with the appearance of bone nuclei in the cartilaginous epiphysis. In the beginning this part is well developed (Fig. 2 A), but decreases with time in thickness and finally consists of only a narrow zone (Fig. 2 B) situated just under the terminal plate of bone which develops in the bony epiphysis. This development occurs parallel with the growth activity, which decreases rapidly with age (Siegling 1941, Boerema 1942, Lacroix 1951, Ring 1955).

During the first part of the growth period the zones differ distinctly in respect of development of the cells. The zone of germinative cells borders the corresponding zone in the growth plate after which follow the other zones towards the bony epiphysis. Cartilage cells in these zones are usually situated in clusters of a varying number of cells (Dodds 1930, Bargmann 1964) but in some cases they form short, irregular columns (Ring 1955) which reach the erosion line at the border of the bony epiphysis.

Also this cartilaginous area can be divided into different zones according to the endochondral calcification process in the cartilaginous matrix. The area containing calcium salts in the epiphyseal part comprises only a few cells situated nearest the bony epiphysis in the group or column of cells (Leblond et al. 1950, Ring 1955, Copenhaver 1964).

During the latter part of the growth period the epiphyseal part of the cartilage plate is very narrow. It consists partly of an uncalcified part near the growth plate and of a calcified part near the bony epiphysis. The uncalcified part comprises largely only the remaining part of the zone of germinative cells. The calcified part, which is also irregular and narrow, consists only of a few cartilage cells, which appear to be hypertrophic and degenerative (Copenhaver 1964), but sometimes it has no cells at all (Trueta and Little 1960). During this period there is a terminal plate of bone in the bony epiphysis, which with time becomes thicker because of deposition of bone lamellae (Lacroix 1951, 1961, Weinmann and Sicher 1955, Ring 1955, Trueta and Morgan 1960).

e. *The Perichondrium and the Periosteum*

The bony tissue of the diaphysis, like large parts of the cartilaginous tissue in the end region, is separated from surrounding structures by a fibro-elastic membrane. In the former case the membrane is called periosteum and in the latter perichondrium. During the growth period the perichondrium develops into periosteum. As for the periosteum, it

grows in length at the same rate as the diaphysis, but this growth is largely interstitial (Lacroix 1951).

The metaphyseal part of the growth plate and the endochondral bone tissue of the metaphysis are lined by a lamella consisting of the perichondral bony tissue (Fig. 1), which corresponds to the end part of the primary bone collar and is the result of the perichondral ossification (Policard 1941, Lacroix 1951, 1961, Sissons 1961, Gardner 1961, Solomon 1966). This bone lamella forms a perichondral ring which may be more or less complete and vary in sharpness of outline in different growth regions (Lacroix 1951, Gardner 1961). The outside of the perichondral ring is lined by periosteum, which in certain areas is rich in cells, particularly epiphyseally. This cellular area is known as the ossification groove and is believed to have various functions (Weidenreich 1930, Kollath 1932, Langenskiöld 1947, Langenskiöld and Edgren 1949, Lacroix 1951, Rigal 1962, Solomon 1966).

2. NUTRITION

Blood vessels reach the growth plate in three areas (Fig. 4) and are therefore divided into epiphyseal, metaphyseal and perichondral blood vessels (Brodin 1955, Morgan 1959, Trueta and Morgan 1960, McLean and Urist 1961, Irving 1964, Hall 1965).

a. *The Epiphyseal Vessels*

The arteries (Fig. 4) usually enter those parts of the epiphysis that are lined with periosteum or perichondrium (Morgan 1959, Salter and Harris 1963, Ham and Leeson 1965, Hall 1965). In the bony epiphysis the vessels branch and form a network with many anastomoses. Some of these vessels reach and enter the epiphyseal part of the cartilage plate. In young individuals they run deep into the cartilage plate and appear to reach the area of transition between its epiphyseal and metaphyseal part. During the latter part of the period of growth the vessels do not penetrate so deep but then the epiphyseal part is considerably narrower so that the vessels still reach the growth plate (Harris et al. 1965).

The pattern of the epiphyseal vessels is thus similar during the former and the latter part of the growth period, but it has been studied more extensively during the latter period. The courses of the vessels during this period will therefore be discussed in what follows.

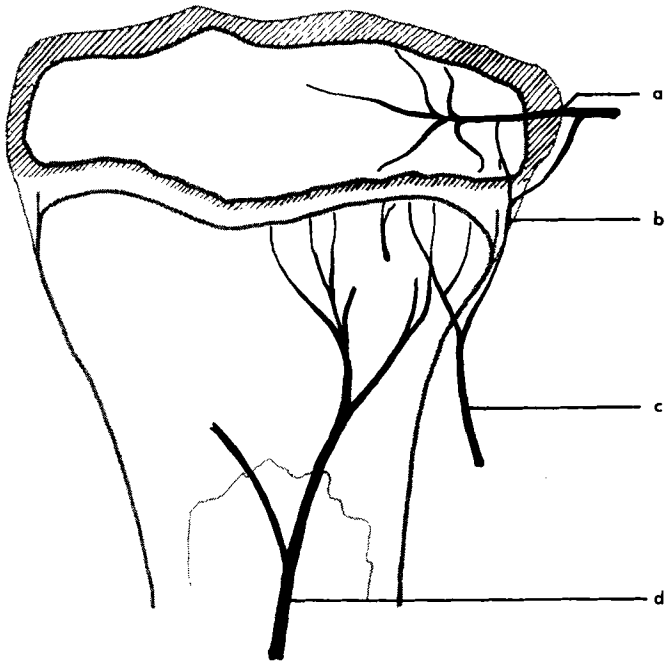

Fig. 4. Arteries in proximal tibia of 30 day old rabbit.

a. epiphyseal arteries
b. perichondral arteries
c. metaphyseal perforating arteries
d. nutrient artery

During this period, by when the growth of the bony epiphysis from the epiphyseal part of the cartilage plate is complete, the epiphyseal vessels run first through the terminal plate of bone and then divide into short vessels which in turn divide into smaller ones that branch off perpendicularly and run parallel to the epiphyseal aspect of the cartilage plate. After a short distance each vessel divides into capillaries. This means that each artery that passes through the terminal plate of bone gives rise to about 24 terminal capillaries. No anastomoses are seen between these vessels when they have passed the terminal plate of bone, in contrast to the numerous anastomoses between vessels within the other parts of the bony epiphysis. The terminal capillaries extend through the calcified zone, where they form a flattened end region before they pass into the venous part which often but not always turns back through the same canal in the terminal plate of bone. The flattened part of the capillary covers an area comprising

four to twelve columns of cells whose epiphyseal ends usually converge towards the capillaries. The capillary endothelium often lies close to the tops of the columns of cells or adjacent to the germinative cartilage cells. The venous capillaries empty into venous vessels, which after having passed the terminal plate of bone, empty into the venous sinusoids in the bony epiphysis (Morgan 1959, Trueta and Morgan 1960, Irving 1964, Hall 1965). The sinusoids in the bony epiphysis empty via veins, which leave the epiphysis in the same areas as those entered by the arteries (Nelson et al. 1960).

b. *The Metaphyseal Vessels*

The vessels that reach the metaphyseal border of the growth plate come from two different vascular systems (Fig. 4).

The vessels that reach the growth plate through the central part of the metaphysis are branches of the nutrient artery. The nutrient artery passes into the bone marrow cavity through a canal which often runs obliquely to the longitudinal axis of the bone. The vessel divides in the marrow cavity into an ascending and a descending branch (Morgan 1959, Brånemark 1959, Göthman 1961). As a rule, there is only one nutrient artery, but in the femur there are often two (Trueta 1963). As to the tibia, after a short distance the ascending branch runs towards the proximal metaphyseal area of the diaphysis and divides, usually into two to four branches. This arborisation occurs a varying distance from the origin of the vessel (Göthman 1961). The branches run a straight course towards the metaphysis at the same time as they diverge. In the beginning they are situated in the posterior part of the bone marrow cavity, but closer to the metaphysis one branch bends forwards, while the others continue in the same direction. The main branch and the smaller branches give off small vessels to the cortex. The larger branches arborise richly, in the area of transition between the shaft proper and metaphysis. The central branches of the vessels run along the longitudinal axis of the bone towards the growth plate, while the branches nearer the cortex usually follow this wall in the metaphysis and give off longitudinal branches to the growth plate. In the secondary spongiosa of the metaphysis the vessels branch richly. In this area fine arterioles develop, which run in the primary spongiosa towards the growth plate. These vessels are straight and parallel and do not branch further but run as capillaries to the most metaphyseal degenerative cell in each column (Fig. 3), after which they turn and

run back to the bone marrow, where they empty in the sinusoids. As a rule, one capillary reaches only a single cell column. The capillary nearest the degenerative cell appears to be sinusoidally swollen and to consist only of a membrane and endothelial cells (Cameron 1961, Irving 1964). According to Trueta and Little (1960) and Trueta and Morgan (1960) the vessels are open.

The peripheral vessels of the metaphysis are formed by the periosteal vessels which in this area are called the metaphyseal perforating arteries. When these vessels have passed the periosteum of the metaphysis they break up into increasingly smaller vessels, which form an anastomosing network in the peripheral metaphysis before they finally form arterioles close to the growth plate which unite with capillaries running to the metaphyseal aspect of the growth plate.

There is no difference in the vessels reaching the peripheral part of the metaphysis and those in the central parts. Trueta and Morgan (1960) calculated that four-fifths of the above-mentioned capillaries that reach the growth plate in the proximal tibia in the rabbit derive from the nutrient artery and the remainder from the metaphyseal perforating arteries.

The venous vessels, which follow after the sinusoids, form a central vein which in the middle of the diaphysis unites with the central veins from other areas and leaves the bone marrow cavity through the emissary foramen (Morgan 1959). In addition, there are a smaller number of veins that leave the medullary cavity via the nutrient foramen. The veins of the metaphysis empty also through anastomoses into periosteal venous plexa.

c. *The Perichondral Vessels*

Besides the metaphyseal and the epiphyseal vessels there is a vascular system in the perichondrium round the growth plate (Fig. 4). This system consists of a circumferential artery which *via* anastomoses intercommunicate with the epiphyseal and the metaphyseal vessels (Morgan 1959, Trueta 1963). The circumferential vessels presumably correspond to the vessels belonging to the perichondral ring of the ossification groove (Morgan 1959) and have therefore been called the perichondral vessels (Hall 1965).

d. *Occurrence of Vessels in the Growth Plate*

The growth plate is usually believed to be avascular (Harris 1933, Haraldsson 1959, Trueta and Morgan 1960, Bloom and Fawcett 1962, Irving 1964, Hall 1965, Harris et al. 1965). In various species, however, vessels have been observed in the growth plate prenatally and during the early postnatal growth period. As a rule, the vessels come from the epiphyseal vessels or from the perichondrium (Weinmann and Sicher 1955, Trueta 1957, Tilling 1958, Brookes 1958, Levene 1964).

Significance of Various Vascular Systems

The significance of the various vascular systems (Fig. 4) for the nutrition of the growth plate has been the subject of several investigations. The epiphyseal vessels are believed to be responsible for the nutrition of the chondrocytes in the zone of germinative cells and the zone of proliferative cells and probably also for other cartilage cells (Trueta and Morgan 1960, Trueta and Amato 1960, McLean and Urist 1961, Trueta 1963, Salter and Harris 1963, Ham and Leeson 1965, Hall 1965). The parts adjacent to the perichondrium probably receive their nutrition from the perichondral vessels (Brodin 1955, Morgan 1959, McLean and Urist 1961, Irving 1964). On the other hand, the metaphyseal vessels are believed not to be of any importance for the nutrition of the cartilage cells, but to be important for the endochondral calcification and chondrolysis along the erosion line. In addition, these vessels are responsible for the nutrition of the cells taking part in the endochondral osteogenesis (Trueta and Morgan 1960, Trueta and Little 1960, Trueta and Amato 1960, McLean and Urist 1961, Salter and Harris 1963, Trueta 1963). Brodin (1955) and Irving (1964), on the other hand, claim that the cartilage cells in the growth plate may also receive their nutrition from the metaphyseal vessels.

CHAPTER II

METHOD FOR DETERMINING GROWTH IN LENGTH OF THE DIAPHYSIS

A. Survey of Literature

Various methods have been used by different investigators for studying the growth in length of shaft bones. As a rule, macroscopic methods have been used, but microscopical methods have also been applied (see Table 1). Most of the methods, however, appear unsatisfactory for one or more of the following reasons.

1. The method does not allow determination of growth in length of the shaft bone from different growth regions.
2. The method allows measurement of growth in length only during periods of several days.
3. The method has traumatic effect on growth.
4. The method has a toxic effect on the growth process in association with intravital marking.
5. The method is not accurate enough for calculation of the growth rate.
6. The method does not allow simultaneous determination of the rate of growth and the morphology of the region of growth.
7. The method is expensive and laborious and its usefulness thereby limited.

Most of the available methods have one or more of the above disadvantages which render them less suitable for the purpose of the present investigation.

It was therefore decided to find out whether it might be possible to measure the growth in length of the diaphysis from the growth plate by intravital staining of the endochondral calcification process with some member of the tetracycline group.

The various tetracyclines[1] have been the subject of thorough investigations regarding their chemical and physical as well as pharmacological properties (Regna et al. 1951, Stephens et al. 1952, English et al. 1953, Boothe et al. 1953, Cunningham et al. 1953, Conover et al. 1953, Dowling 1955, Regna 1955, Lepper 1956, Musselman 1956, Kunin et al. 1959, Kunin and Finland 1961, Spitzy 1962).

The tetracycline molecule consists of a naphthacene ring, which has radicals that vary in a definite way from one member to the other of the tetracycline group. On comparison with TC it will be found that CTC, like DCTC, contains a chlorine atom, while OTC contains a hydroxyl group. Moreover, DCTC lacks a methyl group present in the other tetracyclines.

The tetracyclines are amphoteric compounds in neutral environments and usually not readily soluble. Hydrochloride compound is partly soluble in water but then forms an acid solution which limits its use (Regna et al. 1951, Stephens et al. 1952, Conover et al. 1953, Regna 1955, Spitzy 1962). Therefore various compounds with suitable properties have been manufactured (Spitzy 1962).

Tetracyclines can build compounds with a large number of other substances (Frost et al. 1961a, Spitzy 1962). They can form complex compounds by chelation with a large number of polyvalent metal ions (Regna et al. 1951, Boothe et al. 1953, Albert 1953, Albert and Rees 1956, Weinberg 1957, Kohn 1961, Ibsen and Urist 1962, Urist et al. 1962a and b, Ibsen et al. 1963) such as Ca^{+2}, Mg^{+2}, Cu^{+2}, Sr^{+2}, Fe^{+2} and Zn^{+2}. The affinity of the various metal ions to the tetracycline group varies. Ca^{+2} is said to have a lower affinity than other metal ions occurring in the blood (Urist et al. 1962a). On comparison between the different tetracyclines it has been found that the affinity to the same metal ion varies considerably and it is usually stated that OTC has the lowest affinity (Owen 1963). The stability of the complex compounds varies considerably, while the solubility in water is, as a rule, poor in neutral or basic environments (Weinberg 1957, Ibsen and Urist 1962, Ibsen et al. 1963). The tetracyclines bind the metal ions in certain proportions (Albert 1953, Albert and Rees 1956, Weinberg 1957, Urist et al. 1962a

[1] The following abbreviations will be used according to Johnson (1964):

CTC — chlortetracycline
OTC — oxytetracycline
TC — tetracycline
DCTC — demethylchlortetracycline

and b, Ibsen and Urist 1962, Urist and Ibsen 1963, Ibsen et al. 1963). Investigations of the binding of Ca^{+2} and Mg^{+2} to tetracyclines suggest that the binding occurs stepwise to certain definite parts of the tetracycline molecule depending on the ratio between the concentration of the metal ions and that of the tetracycline molecules (Weinberg 1957, Ibsen and Urist 1962, Myers 1965). In addition, the tetracyclines form complex compounds with various types of proteins (Loo et al. 1957, Titus et al. 1958, Frost et al. 1961 a, Kohn 1961, Spitzy 1962, Urist et al. 1962a, Urist and Ibsen 1963).

In investigations of the distribution of the tetracyclines in the blood it has been found that between 20 and 75 % is bound to proteins, while the rest is free and can form chelates with metal ions occurring in the blood. The affinity between the proteins and the various tetracyclines varies in such a way that CTC is bound in the largest quantity and then in descending order DCTC, TC and OTC (Kunin et al. 1959, Kunin and Finland 1961, Marget and Kienitz 1964). The binding to proteins is reversible (Kelly and Buyske 1960, Spitzy 1962, Urist et al. 1962a, Urist and Ibsen 1963, Marget and Kienitz 1964).

The ability of the tetracyclines to fluoresce when exposed to ultraviolet light was observed early and has been the subject of thorough investigation (Salzman 1950, Udenfriend et al. 1957, Udenfriend 1962). The tetracyclines show characteristic absorption and fluorescence curves. The position of these curves in the spectrum are influenced by different factors such as the solvent, pH and the chemical structure (De Lerma 1958, Frost et al. 1961a, Udenfriend 1962). The magnitude of the absorption or excitation and the intensity of the fluorescence are influenced by the concentration of the tetracyclines, the solvent, pH, temperature, intensity of the exciting light, duration of illumination and chemical structure (De Lerma 1958, Udenfriend 1962). The band of excitation of CTC, OTC, TC and DCTC in neutral and basic water solutions lies between 3500 and 4400 Å (Frost et al. 1961a, Udenfriend 1962, Ibsen et al. 1963). The various tetracyclines differ only slightly in excitation and fluorescence bands. OTC and TC show practically identical values while excitation and fluorescence are shifted somewhat towards longer waves of the spectrum for CTC, probably because of the aromatic chlorine atom (Regna et al. 1951, Stephens et al. 1952, Conover et al. 1953). CTC is labile in basic and neutral environments and then iso-CTC is formed, which is the reason why the excitation and fluorescence maxima are often given as 3550 respectively 4450 Å (Udenfriend 1962).

The above-mentioned factors affect the excitation and fluorescence of the tetracyclines to a varying degree. The occurrence of metal ions results in a bathochromic and bathofloric effect (Salzman 1950, Udenfriend et al. 1957, Ibsen et al. 1963). In the presence of Ca^{+2} ions this occurs in a characteristic way in that the excitation band becomes narrower and is situated between 4200 and 4600 Å, while the fluorescence band extends between 4800 and 6500 Å. The same change is said to occur when the tetracyclines are dissolved in nonpolar solvents such as carbon tetrachloride and xylol-containing substances as mounting agents for histological sections (Frost et al. 1961a).

Helander and Böttiger (Helander and Böttiger 1953, Böttiger 1955a and b) were the first to examine the rate of uptake, distribution, accumulation, retention and excretion in experimental animals with fluorescence microscopy and reported that high concentrations transiently occurred in the reticulo-endothelial system, liver and kidneys. After 3 days only small amounts were found in the bone marrow.

In 1956 the first investigation was published where it was demonstrated that the tetracyclines are bound to bone tissue and the teeth. This examination was carried out by André, who injected tritiated tetracycline into mice and determined the deposition with the autoradiographic method. André showed that in pregnant mice tetracycline passes through the placenta to the bone tissue in the foetus.

Later investigations with fluorescence microscopy have shown that tetracyclines are fixed to tissues undergoing mineralisation (Frost et al. 1961a, Urist and Ibsen 1963, Bevelander 1964, Johnson 1964). Deposition of tetracyclines in association with the ossification process has been the subject of several investigations (Milch et al. 1957, 1958, Rall et al. 1957, Titus et al. 1958, Bevelander et al. 1959a and b, 1960b, Frost 1960a, Frost et al. 1960a and b, 1961a and b, Harris 1960a and b, Muzii 1961, Harris et al. 1962, Hulth and Olerud 1962, Vanderhoeft et al. 1962a and b, Cohlan et al. 1962, 1963, Bevelander 1964, Tapp 1966, Puranen 1966). Deposition in association with endochondral growth has, however, not been studied extensively and the investigations have given varying results (Frost et al. 1960a and b, 1961a and b, Bevelander et al. 1960b, Hulth and Olerud 1962, Coutelier et al. 1963, Urist and Ibsen 1963, Steendijk 1964a and b, Hansson 1964, 1966, Tapp 1966). The ability of callus to bind tetracyclines in association with calcification and ossification of the tissue has received attention (Malek and Kolc 1960, Hulth and Olerud 1964).

The deposition of tetracyclines in dental tissue has been extensively

studied and mostly in dentine (Milch et al. 1957, 1958, Rall et al. 1957, Zipkin and Larson 1960. Boyne and Miller 1961, Bevelander et al. 1961, Owen 1961, Grøn and Johannessen 1961, Zegarelli et al. 1961).

Degenerating and necrotising tissues, undergoing calcification bind large amounts of tetracyclines (Häkkinen 1959, Vassar et al. 1960, Mustakallio 1962). The tetracyclines are also bound to a varying extent to certain types of tumours, presumably to the proteins of the tumour cells (Rall et al. 1957, Loo et al. 1957, McLeay 1958, Milch et al. 1961, Yabe et al. 1964) or to calcium salts in and around the tumour (Vassar et al. 1960, Malek and Kolc 1960, Owen 1961, 1965) or in association with resorption of necrotic areas (Vassar et al. 1960, Mustakallio 1962).

The fluorescence of mineralised tissues containing tetracycline is usually yellow, but may vary between orange and green-yellow. There appears to be only a slight difference between the different types of tetracyclines in that the colour of the fluorescence of DCTC is said to be orange, that of CTC to be golden-yellow, that of TC yellow, and that of OTC green-yellow (Harris 1960a and b, Harris et al. 1962). These differences are demonstrable only when the tetracyclines are present in high concentration so that the colour cannot always be regarded as specific of the various tetracyclines (Frost et al. 1961a).

In a large number of investigations the various tetracyclines have been used for intravital marking of the position of calcification process at different times. This method has been used for determining lamellar bone formation (Harris 1960a and b, Frost et al. 1961b, Harris et al. 1962, Hulth and Olerud 1962, Vanderhoeft et al. 1962a, Lee 1963, 1964, 1965, Landeros and Frost 1964, Lee et al. 1965, Tapp 1966) and dentine and enamel formation (Bevelander et al. 1961, Bevelander 1964, Bevelander and Nakahara 1966). The use of the tetracyclines for calculating the rate of endochondral calcification has been proposed by Hulth and Olerud (1962) and has since been used by Hansson (1964, 1966) and Tapp (1966).

In therapeutic and larger doses the tetracyclines can have a toxic effect on the calcification process in cartilage and bony tissue as well as on enamel and dentine, with the result that tetracycline-containing hypomineralised areas may develop (Bevelander et al. 1959a and b, 1960b, 1961, Harris 1960a, Cohlan et al. 1962, 1963, Rolle et al. 1962, Harris et al. 1962, Urist and Ibsen 1963, Owen 1963, Kienitz 1964, Nylén et al. 1964, Löfgren et al. 1965, Omnell et al. 1966). It is claimed

that the toxicity of the various tetracyclines varies and OTC is generally regarded as being least toxic (Harris 1960a, Harris et al. 1962, Owen 1963, 1965, Chu et al. 1964, Kienitz 1964, 1965) probably because of its affinity for mineralised tissues is low compared with that of other tetracyclines (Owen 1963, 1965).

In addition, it might be mentioned that, when used in small doses, the tetracyclines, like other substances belonging to the antibiotic group, have a stimulating effect on growth (Johnson 1964, Owen 1965). This effect has been demonstrated in experimental animals (Owen 1965) and in children (Jollife et al. 1956, Johnson 1964) and is believed to be due to the tetracyclines improving the nutrition in different ways (Owen 1965).

In previous investigations of the deposition of the tetracyclines in mineralised tissues complicated and laborious methods have generally been used for preparing sections for fluorescence microscopy. Different types of fixation have been tried such as chemical fixation in formalin, methyl and ethyl alcohol (Harris 1960a and b, Plaza-Roca 1960, Muzii 1961, Harris et al. 1962, Hulth and Olerud 1962), physical fixation by freezing (Milch et al. 1957, 1958, 1961, Hulth and Olerud 1962) and in some cases no fixation (Frost 1960a and b, Frost et al. 1960a and b, 1961a and b, Vanderhoeft et al. 1962a and b). In certain cases the preparation was embedded in methyl metacrylate before sectioning (Harris 1960a and b, Muzii 1961, Harris et al. 1962, Hulth and Olerud 1962). Various methods have been applied for sectioning such as freeze-sectioning (Milch et al. 1957, 1958, 1961) and cutting in a microtome (Bevelander et al. 1960b, Rolle et al. 1962) or in a milling machine (Harris 1960a and b, Harris et al. 1962). In several cases the preparation has instead been sawn in slices, which were afterwards ground to suitable thickness (Frost 1958, 1960a, Frost et al. 1960a, 1961a and b, Hulth and Olerud 1962).

B. Author's Investigations

1. DEPOSITION OF TETRACYCLINES IN ASSOCIATION WITH ENDOCHONDRAL CALCIFICATION

It is clear from the survey of the literature that the deposition of the tetracyclines in association with the chondral growth process has been studied in only a few, usually brief investigations. This applies to the

endochondral calcification as well as the endochondral ossification, but the only observations reported on the deposition of tetracyclines in association with perichondral ossification are those published by Hansson (1964).

In the present investigation the deposition of the tetracyclines in association with the endochondral calcification was studied as well as the way in which this deposition is affected by various factors.

Of the various tetracyclines, OTC (Terramycin®) was chosen because this compound is regarded less toxic than the other tetracyclines and secondly because it is available in a form suitable for parenteral use.

The following factors were studied for their effect on the deposition of OTC in endochondral calcification:

1. Time, *i.e.,* interval between administration of the drug and sacrifice of the experimental animal.
2. Growth region. The deposition was studied in the growth regions of the tibia, fibula and radius as well as in the distal growth region of the ulna. The investigation thus included both slow and rapidly growing regions.
3. The dose of OTC per kg bodyweight. In the previous investigations the parenteral doses used usually ranged between 10—50 mg per kg bodyweight. In the present investigation large doses of 10 and 25 mg per kg bodyweight were used and relatively small doses of 0.5 and 1.0 mg per kg bodyweight. This wide range was used to find out the suitable dose for further work.
4. The age of experimental animals.

In this investigation only the intravenous route was used, because administration by this route produces a rapid, transient blood concentration of OTC and thereby deposition, which is desired in intravital marking for assessment of growth.

A search was also made for pathological changes which might occur in association with the deposition of OTC. In this investigation it was thought important to use a dose without any definite toxic effect on growth in length.

The methods used previously for preparing sections for examination for deposition of tetracyclines in mineralised tissues were often complicated. This might very well to a great extent explain the discrepancy between the results obtained in the previous investigations of the deposition in association with endochondral calcification. Attempts were therefore made to find out whether it was possible to reduce the number

of sources of error attending preparation of sections for fluorescence microscopy. In the present investigation the preparation of the sections was simplified as far as possible including the fixation and the actual sectioning.

a. *Material*

Previous investigations (Lacroix 1951, Sissons 1953, Brodin 1955, Langenskiöld 1957, Trueta and Morgan 1960, Elo 1960, Heikel 1960, Troupp 1961, Ryöppy 1965) have shown that rabbits are suitable for the investigation of growth of long bones and these animals were therefore used in the present study. In order to obtain an animal series as uniform as possible, only white rabbits from a single breeder were used. After arrival at the department the animals were observed for a few days. The experimental animals were kept together with their mothers both before and during the experimental period. Notes were made of the date of birth, sex, and weight of the animals before and during the experimental period. Only young rabbits, which weighed more than 250 grams at 20 days and 400 grams at 30 days and appeared normal and healthy were used. All animals that fell ill before or during the experimental period were excluded from the investigation. The animals were fed on pellets (Fors, Ewos) *ad libitum* and roots, usually carrots in amounts sufficient for the animal to have a sufficient amount of water. The experimental animals were kept in well ventilated room with daylight-illumination in fairly large netcages with a floor of network to prevent contact with faeces and urine. Average temperature was 16—20°C and relative humidity 40—50 %.

The solution of Terramycin®[1] was diluted before used with isotonic saline to suitable concentration. In the investigation 4 dilutions were used, namely: 25, 10, 1.0 and 0.5 mg OTC per ml solution. These dilutions were used for injection for at most 2 weeks, during which they were kept, like the original solutions, in the dark at a temperature of +4°C.

The diluted OTC-containing solution was given intravenously. The rate of injection was 2—4 ml solution per minute. The injections were always given at mid-day so that any effect of diurnal variation would be the same for all of the animals.

Experimental animals were killed at the end of the experimental

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[1] The author is much indebted to Pfizer for kindly supplying the Terramycin® used in this study.

period by a rapid intravenous injection of 6 % mebumal sodium solution (ACO) in a dose of 1.0—1.5 ml per kg bodyweight.

In the investigation of the deposition of OTC in cartilaginous and bony tissue after intravenous injection all together 93 rabbits were used, which were 5—6 weeks old at the beginning of the experimental period.

1. Fifteen animals were given 25 mg OTC per kg bodyweight intravenously. The animals were killed after following intervals: 1, 2, 5, 10, 15, 30 minutes, 1, 3, 6, 12 hours, and 1, 2, 4, 6 and 8 days.

2. Eight animals were given 10 mg OTC per kg bodyweight intravenously. Of these, 2 were killed 1 respectively 10 minutes after the injection. The remaining 6 animals were killed after an interval of 6 and 12 hours, and 1, 2, 4 and 8 days.

3. Of 4 animals, two were given 0.5 and two 1.0 mg OTC per kg bodyweight intravenously. The animals were killed 10 minutes after the injection.

4. Four rabbits of a single litter were given different doses of OTC intravenously and killed 1 day later. The doses were 25, 10, 1.0 and 0.5 mg per kg bodyweight.

5. In order to study the frequency of pathological changes in association with the deposition of OTC, 4 groups of 15 animals each were given 25, 10, 1.0 and 0.5 mg OTC per kg bodyweight intravenously and killed 1 day later.

6. The normal autofluorescence of the growth regions of the long bones were studied in two rabbits, who were given no OTC. The animals were killed at 5 weeks of age.

In the investigation of the endochondral growth per day from different growth plates at different ages (see p. 90) it was also studied whether the deposition of OTC was affected by age. The total number of animals was 35, each of which was given 2 intravenous injections of 1.0 mg of OTC per kg bodyweight. The 2 injections were given at an interval of 24 hours and the animals were killed 10 minutes after the last injection in the usual way. The animals were killed at 20, 30, 40, 50, 60 and 70 days of age.

b. *Method*

PREPARATION

Immediately after killing the animal, the ends of the long bones were dissected free. The various bones were sawn through the diaphysis at

a level of about 10 mm from the growth plate, which could be readily identified. When being sawn the bone was held firmly in the region of the diaphysis to protect the ends of the bones from being traumatised. The proximal tibia like the distal tibio-fibula was divided also into a tibial and a fibular half.

FIXATION

Immediately after the preparation the ends of the bones to be examined by fluorescence microscopy were fixed in an abundant amount of absolute ethyl alcohol and thereby also dehydrated. As a rule, the fixation period was 12—48 hours, preliminary studies having shown this period to be most suitable.

SECTIONING

After the specimens had been fixed the end regions were cut with a razorblade. The various parts of the end region could be readily identified with the naked eye or under a loupe. Both longitudinal and transverse sections were cut.

Longitudinal sections: In the proximal tibia the sagittal cut surface was used for orienting the frontal section along the longitudinal, metaphyseal trabeculae in the central part of the metaphysis. These sections were thus frontal sections in the longitudinal direction of the shaft bone. In the proximal and distal fibula, as in the distal tibia, corresponding frontal sections were cut in the same way through the central part of the metaphysis. In the proximal and distal radius as well as in the distal ulna the superficial parts were first cut off in a plane parallel to the ulnar surface of the radius and the radial surface of the ulna, respectively. It was then easy to cut sections through the central part of the metaphysis and along the trabeculae of the metaphysis, the sections being placed perpendicular to the above-mentioned cut surface.

Transverse sections: These sections were taken at various levels of the growth plate and the metaphysis in the proximal tibia. In this case the sections were cut in a plane perpendicular to the central trabeculae of the metaphysis. These transverse sections were used only in the examination of the deposition of OTC in the various parts of the growth region.

The thickness of the sections was determined with the aid of a micrometer of Zeiss Standard-microscope by focusing on the upper and

the lower surface of the section. The thickness of the longitudinal sections suitable for fluorescence microscopy varied between 10 and 80 μ, but most of the sections were about 40 μ.

MOUNTING

The sections were then transferred directly to xylol where they were allowed to remain until they were translucent which, as a rule, took 5—10 minutes. The sections were mounted in DePeX (Gurr, London), recommended by Mayersbach (1958). The mounted sections were then left for 12—24 hours in faint light after which they were examined under fluorescence microscope.

FLUORESCENCE MICROSCOPY

In the fluorescence microscopic examination a conventional binocular microscope was used with Zeiss large fluorescence fitting (Zeiss, Oberkochen). The source of radiation consisted of a mercury high pressure lamp of type Osram HBO 200 W. As a primary filter a combination of a BG 12/4 and a BG 38/2.5 was used. The filter allowed the transmission only of light of wavelength between 3300—5000 Å. As a secondary filter a barrier filter 47 was used, which allowed the passage only of light of a wavelength longer than 4700 Å. In those cases where it was desirable to filter off autofluorescence, barrier filter 53 was used. In most cases the examination was made with the aid of a dark field condensor (NA 0.65/0.85) together with planachromatic objective with 6.3× (NA 0.16) and 10× (NA 0.22) magnification. To obtain higher magnification use was made of a planachromatic objective 16× (NA 0.32) and 25× (NA 0.45) magnification with a dark field condensor with NA 0.85/0.95.

On some occasions an ordinary light field condensor with a wide open diaphragm was used together with planachromatic objectives 2.5×, 6.3× and 10×. In this combination the secondary filter was, as a rule, a barrier filter 53 to filter off direct light from the lamp with a wavelength of less than 5300 Å. C8× or C12× was used as ocular.

In the length measurement in the microscope use was made of an ocular Kpl 12.5× equipped with a micrometer 10 mm long and graduated into 100 parts. This micrometer was used together with a planachromatic objective 10× (NA 0.22) and was calibrated with the aid of an objective micrometer (Reichert) in such a way that every part on the scale corresponded to 10 μ.

FLUORESCENCE MICROPHOTOGRAPHY

The above-mentioned microscope was fitted with a camera (24× 36 mm). For microphotography the same objectives were used and the same condensors as those described previously and as ocular Kpl 8×. For colour photos Ektachrome EHB 135/20 (Kodak) was used and for photography in black-white Scientia 50 B 65 (Gevaert).

c. *Results*

GENERAL

In the fluorescence microscopic examination of the growth area in normal non-treated animals, autofluorescence of varying intensity and colour was noted. In areas without signs of calcification this autofluorescence was different shades of blue.

In areas with cartilage matrix containing calcium salts, as in the growth plate and the metaphyseal trabeculae the autofluorescence was much more intense and blue-white or pale blue. The autofluorescence in the bony tissue in the metaphysis was less intense and clear blue with blue-white streaks. Many cells in the cartilaginous growth zone were seen because of the light blue autofluorescence of the nuclei which were surrounded by dark blue fluorescent cytoplasm.

In animals that had received OTC the normal autofluorescence was seen only in certain areas. The other parts showed secondary fluorescence[1] with varying intensity because of its content of OTC.

In some of the areas the fluorescence was intense and golden-yellow, while in others it was faint and green-yellow.

The fluorescing OTC thus occurred in varying concentration in different parts of the growth regions of the shaft bone.

1. A very large amount was deposited in the cartilage matrix in association with the endochondral calcification process in the end regions of the shaft bone.
2. In the diaphysis and bony epiphysis OTC appeared in varying concentration in association with the endochondral ossification.
3. The perichondral ossification process bound large amounts of OTC.
4. Distinct fluorescence was observed on the surfaces undergoing lacunar resorption.

[1] In what follows fluorescence is to be understood as secondary fluorescence.

5. In addition, diffuse and weak fluorescence was often seen in older bony tissue and in superficial parts of the periosteum, perichondrium and joint cartilage.

In what follows only the deposition of OTC in association with the endochondral calcification process will be discussed. The rest of the deposition is not discussed because it is not necessary for determination of the growth in length of the diaphysis.

DEPOSITION IN ASSOCIATION WITH THE ENDOCHONDRAL CALCIFICATION

The following section deals with the deposition of OTC in different growth regions and at different intervals after the injection. The animals that were used for this investigation were 5—6 weeks old at the beginning of the experimental period.

Deposition in Different Growth Regions and at Different Intervals after Intravenous Injection of 25 mg OTC per kg Bodyweight

Proximal Tibia

1 minute

The injected fluorophore was observed already 1 minute after the beginning of the intravenous injection in a band situated largely within the growth plate and partly in the adjacent part of metaphysis (Figs. 5, 9). The fluorescent band was 120—160 μ wide. The metaphyseal front was well defined and either coincided with the line of erosion or ran parallel to that line in the metaphysis at a distance of at most 40 μ from the growth plate. The front of the fluorescing band closest the perichondral region was situated further in the metaphysis, but as a rule at most 80 μ.

The intensity of the fluorescent band was highest in a roughly 50 μ wide area in that part situated closest to metaphyseal front, and the fluorescence was intense golden-yellow. In those parts closer to the epiphysis the colour of the band gradually became paler yellow and in the most epiphyseal parts it was pale green-yellow. The metaphyseal border of the fluorescent band was thus sharply defined while its epiphyseal border was diffuse and difficult to identify. Epiphyseally to the band in the growth plate and metaphyseally to the band in the cartilaginous part of the metaphyseal trabeculae there was normal autofluorescence.

The yellow fluorescence in the fluorescing band was due to the OTC-content of cartilage matrix in both longitudinal and transverse septa (Fig. 7). No deposition was found with certainty in the cartilage cells, which usually showed pale blue autofluorescence of the nucleus and dark blue autofluorescence of the cytoplasm. The largest amounts of OTC were found in the longitudinal septa and especially in those parts connected with the transverse septa. The most intense fluorescence was seen in the periphery of the septa adjacent to the cartilage cells. The fluorescence in the central parts of the septa was, as a rule, faint but showed wide variations.

In the narrowest longitudinal septa the central, less strongly fluorescent part was often so narrow that it appeared as if the intensely fluorescing more peripheral parts joined one another.

In the broader longitudinal septa no signs of deposition of OTC were found in the central parts of the septa and particularly when this central part was widened which was the case in those parts adjacent to the transverse septa. This central area was, as a rule, narrowest in the most metaphyseal part of the band and continued into the autofluorescing cartilaginous part of the trabeculae of the metaphysis. In the more epiphyseal parts the central area became broader at the same time as the more peripherally situated parts of the fluorescing septa became narrower and less fluorescent.

In the transverse septa there was also yellow fluorescence, but of much lower intensity than that in the longitudinal septa. Moreover, these septa were thinner than the longitudinal septa so that the intensity appeared to be the same in the entire septum. In the area where the septa were in connection with the longitudinal septa, the fluorescent line divided y-shaped and the two limbs continued directly in the intensively fluorescing parts in the longitudinal septa. These fluorescing transverse septa occurred only in the most metaphyseal part of the growth plate.

The fluorescent band comprised 4—8 cartilage cells situated closest to the metaphysis in each column of cells. Of these cells, 2—5 situated close to the metaphysis in each column were surrounded entirely by fluorescent cartilage matrix in both the longitudinal and the transverse septa. The other more epiphyseally located cells were outlined only by fluorescing cartilage matrix in the longitudinal septa.

In those parts of the growth plate which were adjacent to the perichondral ring, yellow fluorescence was seen in the cartilage matrix further in the growth plate than in other areas in both longitudinal

and transverse septa and the fluorescence often appeared to be most intense near this bony lamella. The result was that many cells in the columns adjacent to the perichondral region were partly or entirely surrounded by fluorescent cartilage matrix.

2—15 minutes

During the next few minutes the fluorescence appeared to be the same as after one minute. During this period the fluorescence increased in intensity and appeared to reach a maximum at the end of this period. Then the metaphyseal front of the band was more marked than before.

30 minutes—6 hours

The fluorescent band became increasingly wider during the following 6 hours. Its metaphyseal border which just after the injection was at the border between the growth plate and the metaphysis, was situated further down in the metaphysis. This was possible to observe because of, among other things, the metaphyseal vessels, which extend to the line of erosion, were brown and readily identified, especially in the area adjacent to the growth plate.

It was therefore possible to ascertain that one hour after injection the metaphyseal front of the fluorescent band was some tens of microns further from the growth plate compared with the position just after the injection. Three hours after the injection the distance was 60—80 μ and after 6 hours 120—150 μ.

As before the fluorescence was most intense in the most metaphyseal part of the fluorescent band while it decreased gradually in the epiphyseal part. The strongest fluorescent part during this period became some tens of microns wider.

The maximal width was noted about 3 hours after the injection. Also the epiphyseal less fluorescent part of the band became broader

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Fig. 5. Localisation of OTC in association with endochondral calcification 1 minute after intravenous injection of 25 mg per kg bodyweight. Section from proximal tibia (60×).

Fig. 6. Localisation of OTC in association with endochondral calcification 12 hours after intravenous injection of 25 mg per kg bodyweight. Section from proximal tibia (60×).

Fig. 7. Localisation of OTC in association with endochondral calcification 10 minutes after intravenous injection of 10 mg per kg bodyweight. Section from proximal tibia (150×).

Fig. 8. Localisation of OTC in association with endochondral calcification 24 hours after intravenous injection of 25 mg per kg bodyweight. Pathological changes in fluorescent band. Section from distal ulna (60×).

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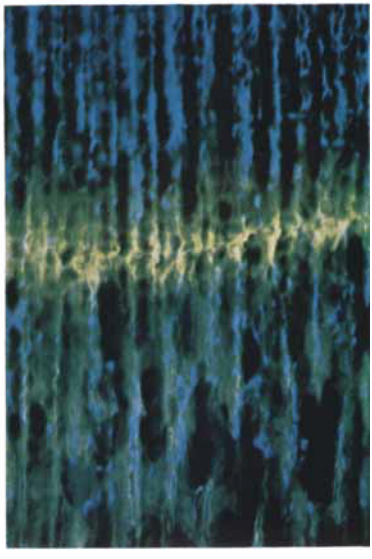


8

and 6 hours after the injection it still extended far into the growth plate, as a rule about 100 μ.

The fluorescent OTC was, as before, localised to the longitudinal and to the transverse septa in the way described previously.

12 hours—8 days

The front of the fluorescent band was localised further into the metaphysis with increasing interval after the injection. Between 6 and 12 hours the less fluorescent epiphyseal part of the band lost contact with the growth plate and then the fluorescent band was situated entirely in the metaphysis (Figs. 6, 10). The distance between the front and the line of erosion 24 hours after the injection was about 500 μ, while it was about 1980 μ in the animal that was killed 4 days after the injection. In these experimental animals the distance tended to increase by about 500 μ per day on the average. But the breadth of the fluorescent band did not increase. It appeared instead to decrease between 6 and 12 hours after the injection and then to persist between 120—150 μ. That part of the fluorescent band, which appeared to decrease in width was the epiphyseal weakly fluorescent part, while the stronger fluorescent part remained unchanged.

It was noted that the fluorescent band became less and less marked the greater the distance from the growth plate. This appeared to be because large parts of the band had gradually been resorbed and caused increasingly large spaces in the trabecular system. This resorption of the band was only slight during the first day and then increased as the band became localised further from the growth plate. Especially in two regions this resorption was marked, namely under the periosteum and near the bone marrow cavity.

During the first few days the peripheral parts of the band adjacent to the perichondral ring remained unchanged but then these parts became weaker and finally disappeared in association with the narrowing of the metaphysis. The situation was the same in the middle parts of the fluorescing band. During the first days after the injection, when the band was close to the growth plate, this weakening was only slight. With increasing interval the band became weaker in the middle part and 4—6 days after the injection it disappeared and was replaced by the expanding bone marrow cavity. Eight days after the injection there was only a short distance of the fluorescent band on either side of the bone marrow cavity. The intensity in these parts was lower than immediately after the injection because many of the OTC-containing cartilaginous trabeculae had been resorbed.

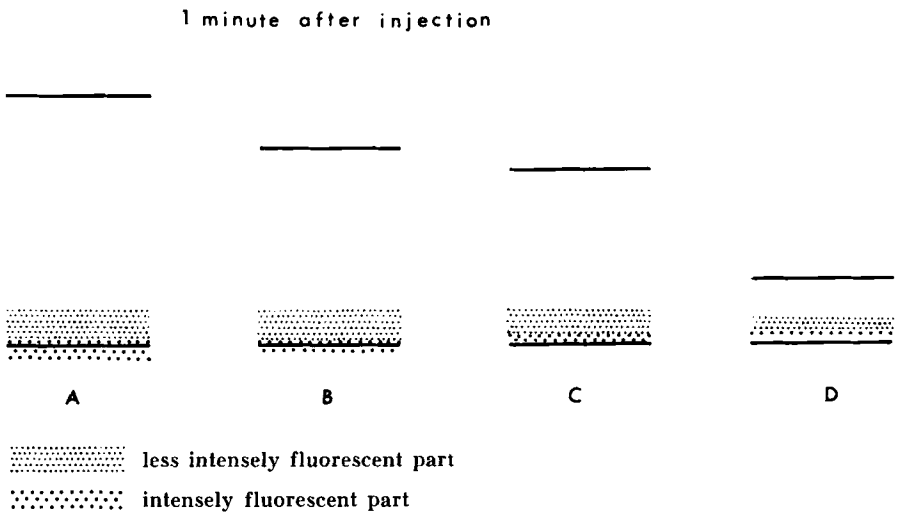


Fig. 9. Localisation of OTC in association with endochondral calcification in different growth regions 1 minute after intravenous injection of 25 mg per kg bodyweight. A: distal ulna, B: proximal tibia, C: distal radius and D: proximal radius. Distance between bold lines indicates growth plate and below lower bold line, metaphysis.

As previously mentioned, the fluorescent OTC was deposited in association with the injection in the longitudinal and the transverse septa. Even when the fluorescent band was situated in the metaphysis the localisation of the fluorescence was the same in the longitudinal septa and adjacent rests of the transverse septa. In the fluorescent cartilage matrix of the trabeculae were distinct indentations after resorbed cartilage cells.

In the parts of the metaphysis which were situated diaphyseally to the band, only blue-white and pale blue autofluorescence was seen in the parts of the trabeculae that consisted of cartilage matrix. In that part of the metaphysis which was gradually situated epiphyseally to the fluorescent band, a transitional area of fluorescent green was seen adjacent to the band indicating only slight deposition of OTC, while the rest showed ordinary autofluorescence.

In the metaphyseal part of the growth plate most cases showed small amounts of OTC in spite of the fact that the fluorescent band was far in the metaphysis with an autofluorescent area between itself and the growth plate. The intensity of the fluorescence was low and the colour was often pale green-yellow. This weak fluorescent area extended, as

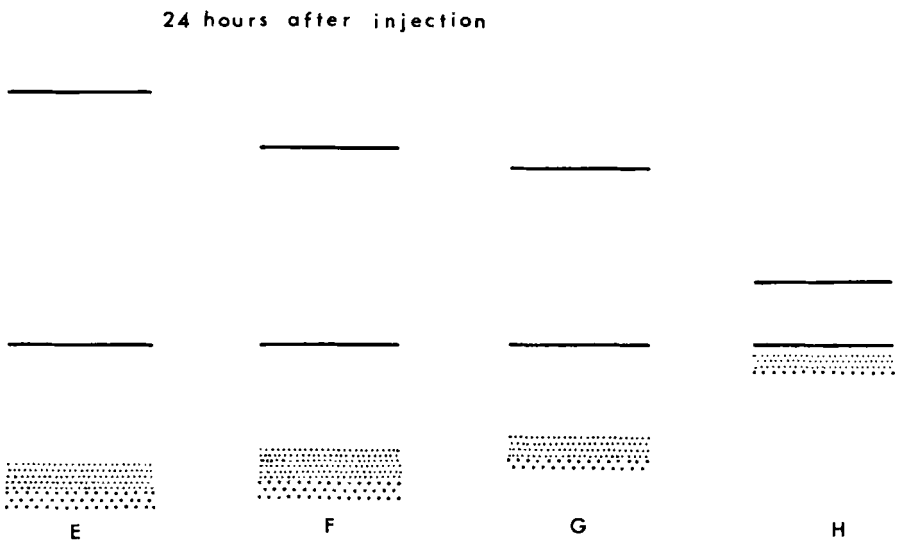


Fig. 10. Localisation of OTC in association with endochondral calcification in different growth regions 24 hours after intravenous injection of 25 mg per kg bodyweight. E: distal ulna, F: proximal tibia, G: distal radius and H: proximal radius. Otherwise same scheme as in Fig. 9.

a rule, at most 100 μ into the growth plate (Fig. 6). The exact localisation was mainly in the most metaphyseal parts of the longitudinal septa and to a smaller extent in the transverse septa nearest the metaphysis. The localisation was thus about the same as immediately after an injection of OTC.

Distal Ulna

The deposition of OTC in the growth plate and the metaphysis of the distal ulna resembled that in the proximal tibia more than that in other regions studied. The similarity was not complete and therefore the deposition in the distal ulna will be discussed separately.

The fluorescent band that appeared very soon after the injection was 120—160 μ broad (Fig. 9). Its metaphyseal border was situated 30—60 μ in the metaphysis in the central part of the growth region and a few tens of microns further in the metaphysis in the areas adjacent to the perichondral ring. The stronger fluorescent metaphyseal part of the band was, as a rule, 50—70 μ broad and extended about 20 μ into the growth plate in the central part of the growth zone. Epiphyseally thereto the fluorescence gradually decreased and ceased diffusely after about 60 μ.

Six hours after the injection the metaphyseal front of the band was situated about 160 μ from the line of erosion. Some time between 6 and 12 hours after the injection the band was situated entirely in the metaphysis. The fluorescent band was then most often only 120—150 μ broad with a roughly 60 μ broad, intensely fluorescent area. The distance between the front of the band and the growth plate increased with time. One day after the injection it was about 580 μ (Fig. 10) and 4 days after the injection about 2100 μ.

Three to four days after the injection large parts of the fluorescent band were resorbed in association with expansion of the bone marrow cavity. Since also the peripheral parts adjacent the periosteum were gradually resorbed there remained only two short parts of the fluorescent band on each side of the bone marrow cavity.

Also in the distal ulna there was a weak green-yellow fluorescence at most 100 μ broad in the metaphyseal part of the growth plate and in the cartilaginous matrix of trabeculae in the adjacent part of the metaphysis although the fluorescent band was situated further in the metaphysis. That part situated in the metaphysis was obscured from view partly by the metaphyseal vessels and therefore appeared weaker than the part in the growth plate.

Proximal Fibula, Distal Tibio-Fibula and Distal Radius

The deposition of OTC in these regions showed several features in common and will therefore be delt with together.

The fluorescent band, which developed as in the previously mentioned regions immediately after the injection, appeared to be confined to the metaphyseal part of the growth plate. The metaphyseal front seemed to coincide with the erosion line. In the area adjacent to the perichondral ring the front was often situated some tens of microns inside the metaphysis. The fluorescent band in the various growth zones the first few minutes after the injection were between 100—120 μ broad and their fluorescence was most intense in an area 40—50 μ broad nearest the metaphysis and then became fainter towards the epiphysis where it ceased with a diffuse outline (Fig. 9).

The deposition of OTC in the cartilage matrix was the same as in the proximal tibia, thus largely in the longitudinal septa and only to a small extent in the most metaphyseal transverse septa in each cell column.

During the first hours after the injection the fluorescent band became broader and its front became situated further into the metaphysis. Six hours after the injection this front was 100—110 μ in the meta-

physis. In the band the most metaphyseally situated part showed the strongest fluorescence and was about 60 μ broad. The epiphyseal outline was diffuse and difficult to determine but appeared to extend 50—100 μ into the growth plate. The fluorescent band in these growth zones lost contact with the growth plate between 6 and 12 hours after the injection as in the proximal tibia.

The breadth of the various fluorescent bands then remained constant between 100—120 μ and were much narrower than 6 hours after the injection. As before, the weakly fluorescent part of the band, which tapered, while the intensely fluorescent band, as before, was 50—60 μ broad.

The distance between the front of the band and the line of erosion increased by 420—460 μ per day (Fig. 10) varying somewhat from one experimental animal to another. This distance was, as a rule, largest in the distal radius, somewhat less in the proximal fibula and smallest in the distal tibio-fibula on comparison between the various growth zones in one of the animals.

Large parts of the fluorescent band were resorbed entirely or partly according to the same pattern as that described previously. Resorption of the middle part of the fluorescent band usually occurred 3—5 days after the injection.

During the period when the fluorescent band was situated entirely in the metaphysis, there was usually a weak green-yellow fluorescence in the metaphyseal part of the growth plate as a rule 60—80 μ broad. This weak fluorescence was of the same type in all of the above-mentioned growth zones.

Proximal Radius

1—15 minutes

Within one minute after the injection large amounts of OTC were deposited in the growth plate of the proximal radius in the metaphyseal part. Here, too, an intensely fluorescent band developed with golden-yellow colour whose intensity increased during the following minutes. The band was situated inside the growth plate, separated entirely from the metaphysis in contrast to what was seen in the other regions studied. The fluorescing band was 30—60 μ broad and was separated from the metaphysis by a 10—30 μ broad area with autofluorescence (Fig. 9).

The fluorescing band was most intense in the most metaphyseal part in a 20 μ broad band while the yellow fluorescence in the rest of the fluorescing band decreased relatively rapidly in epiphyseal direction.

The metaphyseal front of the band was sharply outlined and was more sharply defined than in the proximal tibia.

The largest amount of OTC-containing cartilage matrix was found in the longitudinal septa and especially in the peripheral parts of the septa. In the metaphyseal part of the fluorescent band there was also deposition in a few transverse septa. 1—2 of the cells in each cell column and always the most metaphyseally situated ones were outlined by golden fluorescent transverse septa. In the areas adjacent to the perichondral ring the fluorescent band approached the metaphysis and furthest out to the perichondral ring the metaphyseal vessels extend a short distance into the band, but as a rule at most 20 μ.

30 minutes—12 hours

During the first few hours the fluorescent band became broader and the autofluorescent area situated between the band and the metaphysis became narrower and finally disappeared. Three hours after the injection this area had disappeared and had been replaced by intense golden-yellow fluorescence at the same time as the intensely fluorescent part had become about 20 μ broader than just after the injection. Six hours after the injection the front of the fluorescing band was situated in the metaphysis and about 20 μ from the growth plate. The stronger fluorescent part extended about 10 μ into the growth plate. The total breadth of the fluorescent band appeared to be about 60 μ and its epiphyseal border was more diffuse and more difficult to delineate than before. At 12 hours after the injection the front of the band was situated about 40 μ in the metaphysis. The largest part of the band was then inside the metaphysis, while a minor part some tens of microns in breadth was situated within the growth plate adjacent to the metaphysis. That part of the fluorescent band that was situated in the metaphysis fluoresced much weaker than before because of the brown coloured metaphyseal vessels which obscured the OTC-containing cartilaginous trabeculae.

1—8 days

Between 12 and 24 hours after the injection the fluorescent band lost contact with the growth plate and was then situated entirely in the metaphysis. The fluorescent band on that occasion was between 40—60 μ broad and did not vary appreciably in breadth during the following period. One day after the injection the distance between front of the fluorescent band and the line of erosion was about 100 μ (Fig. 10).

This distance appeared to increase by about 100 μ per day in the experimental animals and was, for example, 4 days after the injection 420 μ.

As previously mentioned in the description of the other growth zones, large parts of the fluorescent band were resorbed entirely or partly when it was situated within the metaphysis. The expansion of the bone marrow cavity in epiphyseal direction resulted in the middle part of the fluorescent band disappearing entirely within 6 days after the injection. In addition the peripheral parts were resorbed in association with the narrowing of the metaphysis.

In those cases where the fluorescent band was situated within the metaphysis small amounts of OTC were sometimes also seen in the growth plate. Also in these cases autofluorescence occurred in the growth plate adjacent to the metaphysis. Autofluorescence also occurred in the cartilage matrix in the trabeculae situated both diaphyseally and epiphyseally to the strongly fluorescent band in the metaphysis in the same way as in the proximal tibia.

Deposition after Intravenous Injection of Various Amounts of OTC

In the preceding sections the deposition of OTC at a varying interval after intravenous injection of 25 mg OTC per kg bodyweight was discussed. Below an account is given of the deposition of OTC 10 minutes and 24 hours after intravenous injection of a varying amount of OTC. The appearance of the fluorescent band in the proximal tibia was studied after injection of the following doses: 25, 10, 1.0 and 0.5 mg OTC per kg bodyweight.

10 minutes

Administration of the dose was always followed by the appearance of a band situated largely within the metaphyseal part of the growth plate and to a certain extent in the adjacent part of the metaphysis. The fluorescent band, which appeared after 25 mg per kg was 120—160 μ broad while the breadth after injection of 10 mg per kg appeared to be some tens of microns less. The corresponding breadth of the band after 1.0 and 0.5 mg per kg bodyweight was 80—100 μ. The metaphyseal front of the fluorescent band appeared to be identical after all doses but not the epiphyseal border. The metaphyseal border of the fluorescent band was more distinct when the dose was large than when it was small.

The intensity of the fluorescence was highest after injection of 25 mg per kg and weakest after 0.5 mg per kg bodyweight and irrespective of

the size of the dose it was always most intense in the metaphyseal part and decreased in epiphyseal direction.

The colour in the most metaphyseal part of the band after injection of 25 mg per kg was intense golden-yellow and after 10 mg per kg somewhat paler yellow. After smaller doses 1.0 and 0.5 mg per kg the colour was pale yellow respectively green-yellow and the fluorescence after the latter dose decreased and almost disappeared in a few minutes. The corresponding difference in intensity and colour occurred also in the epiphyseal part of the fluorescent band.

24 hours

The fluorescent band was now situated far in the metaphysis after all doses. The distance between the front of the band and the line of erosion was always between 500—510 μ, but the breadth of the band varied widely. After 25 mg per kg the breadth was 120—150 μ and after 10 mg per kg it was 100—120 μ. The fluorescent band appearing after the small doses was much narrower and was between 30—60 μ and appeared to be somewhat broader at 1.0 than at 0.5 mg per kg.

The difference in intensity and colour with the various doses appeared to be about the same as just after the injection. The more strongly fluorescing part of the band appeared to have increased by some tens of microns in breadth after 25 mg per kg, while its breadth after injection of the smaller dose 0.5 mg per kg was the same as immediately after the injection. The epiphyseal border of the fluorescent band after all doses and especially after small doses was more difficult to recognise than immediately after the injection.

The part of the metaphysis which was situated in the fluorescent band and the line of erosion was much narrower after 25 mg per kg than after 0.5 mg per kg. In this area blue or blue-white autofluorescence was seen in the cartilaginous parts of the trabeculae. This autofluorescence occurred after all doses in that part nearest the line of erosion and extended furthest into the metaphysis after a small dose because the fluorescent band was then much narrower than after injection of a large dose.

In the metaphyseal part of the growth plate small amounts of OTC were seen after injection of all doses.

Variation of Deposits of OTC with Animal's Age

In the previous section an account was given of the deposits of OTC at an age of 5—6 weeks as well as the effect of various factors on the

deposition. In what follows the effect of age on the deposition after an intravenous injection of 1.0 mg OTC per kg bodyweight is discussed in association with the endochondral calcification process in the proximal tibia.

10 minutes

The fluorescent band was much broader in animals 20—30 days old than in older animals. In 20—30 day old animals the breadth was about 120 μ compared with 60 μ in 70 day old animals. This also applies to the more brightly and less brightly fluorescent part of the band. The more brightly fluorescent part in the younger animals was about 70 μ against only 30 μ in 70 day old animals. The weak fluorescent part decreased also with age but only by some tens of microns during the entire period.

As previously mentioned, the fluorescent band extended up to 40 μ into the metaphysis at 5—6 weeks of age. In young animals this was more pronounced and at 20 days of age the fluorescent band usually extended 30—50 μ metaphyseally on the erosion line. On the other hand, the fluorescent band as a whole was situated in the metaphyseal part of the growth plate at 60—70 days of age and then its front coincided with the erosion line. In addition the metaphyseal front appeared to be better outlined with age, which to a certain extent holds also for the epiphyseal outline of the band.

Also the intensity of the fluorescent band changed with age. The intensity at 20 days of age was relatively low, and in some animals it was difficult to record photographically. The intensity increased with age and particularly between 20 and 40 days. It was highest at 70 days when the colour of the fluorescent band was clear yellow compared with weak green-yellow at 20 days in the strongest fluorescent part.

Changes of the same type were seen in other growth zones studied with the result that the fluorescent band became narrower with increasing age and more strongly fluorescent, at the same time as its metaphyseal border shifted in epiphyseal direction.

24 hours

The observations described above were also made 24 hours after the injection. Thus the breadth of the fluorescent band decreased with age. It was about 80 μ at 20 days and 50 μ at 70 days in the proximal tibia. This decrease was caused mainly by the fact that the more strongly fluorescent part which occupied the major portion of the band diminished with increasing age, while it was not possible to observe any

corresponding decrease of the weakly fluorescing part since it was only 10—20 μ broad during the period of life covered by the investigation.

The position of the fluorescent band changed with age and with time was situated closer to the growth plate. The distance between the front of the fluorescent band and the erosion line at 30 days of age was about 590 μ against about 400 μ at 70 days. On the other hand, this distance appeared to be somewhat shorter at 20 days than at 30 days.

The intensity of the fluorescent band which at 24 hours appeared to be slightly stronger than at 10 minutes after the injection increased with age in the way described previously.

The above-mentioned changes in the breadth, position and intensity of the fluorescent band were observed also in the other growth zones examined. It should be mentioned that the fluorescent band in the proximal radius in some animals aged 60—70 days was 24 hours after the injection still situated entirely or partly within the growth plate which was not the case in other growth zones studied.

Pathological Changes

In the growth zones in the animals that had received OTC intravenously in a dose of 25 mg per kg bodyweight deviations from the normal appearance of the metaphysis and the growth plate were often seen a short time after the injection.

The appearances of the changes seen in the proximal tibia are described below. It should, however, be mentioned that corresponding changes occurred also in other regions studied.

As a rule, the changes made their appearance at the earliest during the first hour after the injection in the strongest fluorescent part of the band at the border between the metaphysis and the growth plate. The appearance of the changes varied considerably. The slightest changes occurred in the form of slight bending of the OTC-containing cartilaginous trabeculae in the most fluorescent part of the band. When the changes were severe, the cartilaginous trabeculae were interrupted in the fluorescent zone (Fig. 8). In addition, especially when the changes were severe, the epiphyseal and diaphyseal parts of the cartilaginous trabeculae were displaced to a varying extent in relation to one another both transversally and longitudinally. The result was that the fluorescent band in the area with these changes was often narrower than in adjacent normal areas and the breadth of the fluorescent band was sometimes only half of normal. In areas with these changes this

was generally most pronounced in the central part, peripherally the changes decreased diffusely and disappeared. In some cases changes were seen in all parts of the fluorescent band.

The severest changes in a growth zone were often closest to the perichondral ring. In this region the epiphyseal parts of the cartilaginous trabeculae were usually displaced, in direction towards the perichondral lamella, in relation to the diaphyseal parts of the trabeculae. This shift of the cartilaginous trabeculae was more pronounced in those parts where the trabeculae converged in diaphyseal direction than in those parts where the trabeculae ran more or less parallel to the central trabeculae. In some cases the part of the perichondral ring which was situated epiphyseally to the fluorescent band was displaced peripherally in relation to the diaphyseal part.

In the growth zone of the proximal tibia crude changes were also seen in the central part. In this part the change often consisted of a change in the longitudinal direction of the trabeculae and sometimes, though rarely, in transverse direction.

In other parts of the fluorescent band between the area adjacent to the perichondral ring and the central area, there were rarely any changes. In those cases where changes were seen they consisted of curving and compression of the cartilaginous trabeculae.

The above-mentioned changes developed, as mentioned above, already during the first few hours after the injection. Both the normal and the changed cartilaginous trabeculae in the fluorescent band were covered by bony tissue on the first day. On the following days parts of the band were resorbed within both the normal and the changed part. Within the remaining parts of the band changed cartilaginous parts were often seen in the metaphyseal trabeculae at the end of the experimental period.

In the area with severe changes, changes were seen on the first day after the injection, which were probably secondary to changes within the band (Fig. 11). In the area with curved and broken cartilaginous trabeculae an autofluorescent tongue of cartilage cells from the growth plate was seen. In adjacent areas, where the fluorescent band appeared to be normal, no corresponding cartilage cells were seen. The tongue of cartilage cells during the first few hours after the injection was often rectangular, but later, after it had increased further in length, it became more and more triangular with the apex pointing in diaphyseal direction. This was because the tongue of cartilage cells was resorbed in the peripheral parts, which was marked in the area closest to the

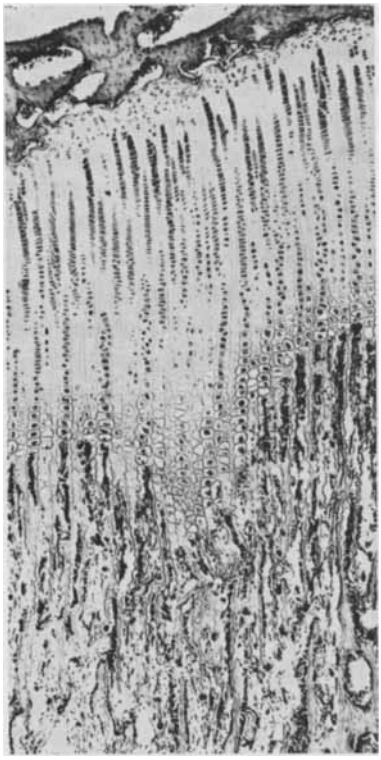


Fig. 11 A. Pathological changes in form of curved and broken metaphyseal trabeculae and a tongue of cartilage cells extending into metaphysis 24 hours after intravenous injection of 10 mg OTC per kg bodyweight. Paraffin section (about 10 μ) from proximal tibia from decalcified material fixed in Bouin's fluid. Haematoxylin-eosin according to Ehrlich (50×).

fluorescent band. The remaining triangle was then broken down in epiphyseal direction and replaced by metaphyseal trabeculae with intermediate vessels. The tongue of cartilage cells therefore often disappeared 1—2 days after the injection. The metaphyseal trabeculae, which replaced the central parts of the cartilage tongue, often ran in a largely straight line, while the more peripheral trabeculae closest to the fluorescent band converged towards the centre and further epiphyseally again became straight.

Occasionally it was observed a few days after the injection single or small clusters of cartilage cells epiphyseally to and adjacent to the fluorescing band in the area with severe changes. In addition, the metaphyseal vessels in this area ran more irregularly than in cases with slight changes. In the area with slight changes the metaphyseal vessels were traced through the fluorescent band right to the most metaphyseal cartilage cells in the growth plate. In the area with pronounced changes the metaphyseal vessels ran to the fluorescent

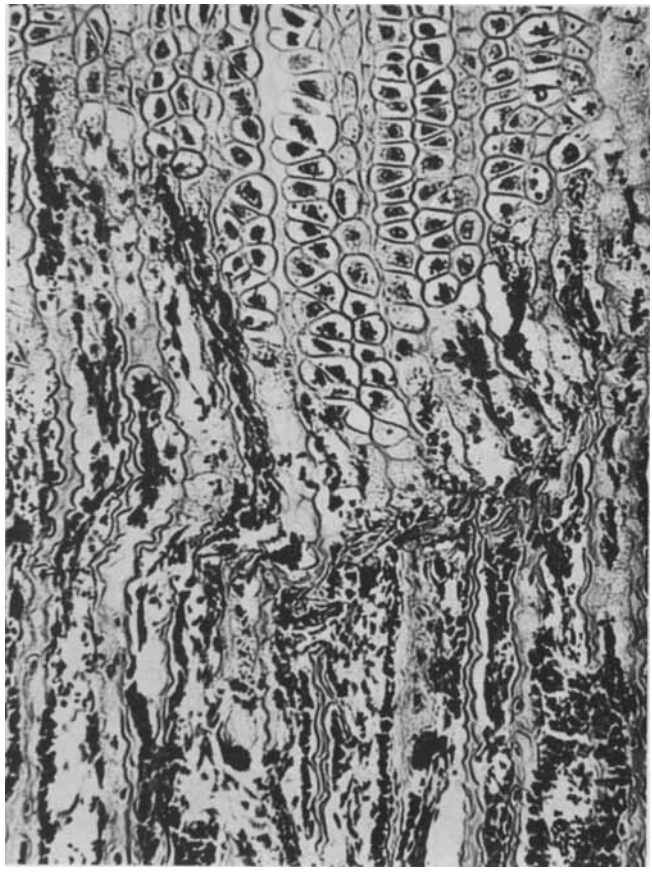


Fig. 11 B. Higher magnification of area with pathological changes in Fig. 11 A. (155×).

band. Further epiphyseally, between the cartilage cells adjacent to the fluorescent band and the remaining part of the tongue of cartilage cells, were brown metaphyseal vessels which appeared to be in communication with the metaphyseal vessels in the adjacent areas.

In the investigation of the frequency of the pathological changes it was found that the growth zone of the proximal tibia showed signs of pathological changes of varying severity in all 15 animals killed 24 hours after the injection of 25 mg OTC per kg bodyweight. In some animals the changes dominated and in others they were seen in only a small part of the fluorescent band. In the present material were 4 cases with only slight curving of the cartilaginous trabeculae, 6 with severe bending and 5 with pronounced changes with bent and broken trabeculae.

In 15 experimental animals which had been given 10 mg OTC intravenously and were killed 24 hours after the injection the frequency and the severity of the changes were lower. In 11 of these animals there were no signs with certainty, while a slight effect was noted in 4 animals.

In 15 experimental animals which had been given 1.0 mg OTC changes in the form of deformed trabeculae occurred in 1 case. These changes were confined to a small area adjacent to the perichondral lamella.

The occurrence of changes one day after an intravenous dose of 0.5 mg OTC was studied in the same way in a similar series of 15 experimental animals. In these animals the proximal tibia showed no changes with certainty.

Changes occurred also in the other growth regions studied after intravenous injection of 10 and 25 mg OTC per kg bodyweight. As in the proximal tibia, the changes appeared to be located primarily in the most fluorescent part of the band.

The changes were, as a rule, somewhat more common in the distal ulna than in the proximal tibia. Similar changes also occurred in the distal tibio-fibula and proximal radius but were not so common or so severe as in the former regions. Such changes were seldom seen in the proximal fibula and distal radius.

It was also observed that in those cases where changes occurred in a growth zone similar changes occurred in most cases also in the growth zone on the contralateral side.

d. *Discussion*

Previous methods used for the production of sections for fluorescence microscopic examination of the deposition of the tetracyclines in the growth regions of the shaft bone have usually been more complicated and laborious than the one used here. In the present investigation it proved possible to simplify the fixation, dehydration and sectioning and to exclude embedment of the preparation, apparently without introducing new sources of error.

It has previously been shown that the results of the examination depend to no small extent on the method of fixation used. Hulth and Olerud (1962) observed on comparison of different fixation methods that freeze-fixation combined with freeze-drying, like fixation in absolute methyl or ethyl alcohol for 1—2 days does not cause any demon-

strable dissolution of bound tetracyclines which, however, occurs on fixation in aqueous solutions. It has also been shown that long fixation periods in aqueous fixatives as well as in absolute methyl or ethyl alcohol results in increased dissolution of tetracyclines bound to mineralised tissues (Frost 1960 a, Frost et al. 1960 a and b, Muzii 1961) and, secondly, that the risk of dissolution is greatest during the first few days after the injection when tetracyclines are believed to be less firmly bound than later (Frost et al. 1960 a, 1961 a, Hulth and Olerud 1962).

In the present investigation, however, it was observed that also fixation in absolute ethyl alcohol can give rise to dissolution of bound tetracyclines, since green-yellow fluorescence is seen in the zone of cartilage undergoing calcification in spite of the fact that several hours and days have elapsed between the injection and the sacrifice of the animal. Similar green-yellow fluorescence has previously been observed by Harris et al. (1962) and Lee (1963, 1965) in the zone of calcification in lamellar bone formation, but they considered this fluorescence not to come from deposited tetracyclines but instead to be identical with the autofluorescence of osteoid tissue. The reason why this deposition is believed to be largely postvital is that the cartilaginous tissues which are situated between the zone of cartilage undergoing calcification and the intensely fluorescent band in the metaphysis usually contain no demonstrable amounts of tetracyclines, which they should if the above-mentioned deposition had occurred intravitally. The postvital deposition is probably also the reason why the fluorescent band is somewhat wider some hours after the injection than later, when the band is situated in the metaphysis. It is also probable that the diffuse occurrence of tetracyclines, like their deposition in the form of surface stain in bone tissue (Ghosez 1959, Ponlot 1959, Frost et al. 1960 b, 1961 a, Harris 1960 a, Harris et al. 1962, Hulth and Olerud 1962, Steendijk 1964 a and b, Tapp et al. 1965), occurs largely postvitally.

As the degree of mineralisation in the cartilaginous and osseous trabeculae is very high (Pritchard 1961), it is not probable that the fixation in absolute ethyl alcohol causes shrinkage of the mineralised tissue of the growth zone. On the other hand, the fixation causes appreciable shrinkage of the non-mineralised tissues (Romeis 1948, Baker 1955, Burck 1966).

At the same time as the preparation is fixed in absolute ethyl alcohol it undergoes dehydration so that it need only be placed in a single

fluid before mounting, which according to Hulth and Olerud (1962) markedly reduces the dissolution of the bound tetracyclines.

The preparation of sections from the fixed and un-embedded material is not complicated and requires no expensive equipment, which it often does when the previous used sectioning methods are employed (Milch et al. 1957, 1958, 1961, Plaza-Roca 1960, Harris 1960 a and b, Hulth and Olerud 1962, Harris et al. 1962, Urist et al. 1962, Lee 1963, 1964, 1965, Steendijk 1964 a and b, Lee et al. 1965). In addition, the preparations in the previous investigations were often embedded in methacrylate (Muzii 1961, Hulth and Olerud 1962, Smeenk et al. 1963, Lee 1963, 1964, 1965, van der Sluys Veer et al. 1964, Lee et al. 1965) which is usually a laborious process. The method used enables fluorescence microscopic examination of sections already about 15 minutes after the preparation has been removed from the fixing fluid and thus almost within 12 hours of the death of the experimental animal so that the method appears to be less laborious and more rapid than many of those used previously. This method also gives good complete sections which facilitates the fluorescence microscopic examination.

In the present investigation it was observed that the resolving power is highest in the thin sections of about 10 μ and lowest when the sections are more than 80 μ thick. The intensity of the fluorescence is much lower in the thin sections, especially when the dose given is as low as 0.5 mg OTC per kg bodyweight. It would appear that the best thickness for the sections is between 30—60 μ when the resolving power is excellent at the same time as the intensity of the fluorescence is sufficient to determine the deposition of OTC after both large and small doses. Frost et al. (1961 a) have previously tried to find out the most suitable thickness of the sections and give it as about 50 μ. They observed, as in this investigation, that neither thinner sections nor thicker ones have any advantage with certainty. A thickness of about 50 μ has previously been used by Harcourt et al. (1962), Landeros and Frost (1964) like by Amprino and Marotti (1964), but also thinner (Milch et al. 1957, 1958, 1961, Bevelander et al. 1960 b, Smeenk et al. 1963, Lee 1963, 1964, 1965, van der Sluys Veer et al. 1964, Gregg and Avery 1964, Steendijk 1964 a and b, Lee et al. 1965, Tapp et al. 1965) as well as thicker sections (Harris 1960 a and b, Harris et al. 1962, Hulth and Olerud 1962, Urist et al. 1962 b, Vanderhoeft 1964). These differences in the thickness of the sections used can probably help to explain the discrepancy between the reports on the deposition of tetracyclines in mineralised tissues.

In the present investigation the fluorescence does not appear to change in colour or intensity after mounting of the sections. This does not corroborate the finding of Boiko (1959) and Frost et al. (1960 a and b). In their investigations the changes were probably due to the fact that the sections were mounted without previous dehydration. It has previously been shown that the fluorescence of tetracyclines is quenched and becomes more shortwaved in polar and aqueous solvents but, on the other hand, not in nonpolar and nonaqueous solvents such as xylol and various xylol-containing mounting compounds (Frost et al. 1961 a, Hattner and Frost 1962). On the other hand, it was observed that the resolving power during fluorescence microscopy increases the first few hours after the sections have been mounted, which is probably due to the penetration of the mounting compound into the section.

For various reasons it was apparent in the present investigation that OTC is deposited in the endochondral calcification process in a well defined band. The deposition agrees well with the area of deposition of the radioactive isotopes Ca^{45} (Ponlot 1959, Lacroix 1960 a and b, 1962) and P^{32} (Leblond et al. 1950, Leblond and Greulich 1961), which are deposited in the zone of cartilage undergoing calcification. In the broad longitudinal septa the deposition of OTC agrees with the results obtained in electron microscopic examinations of the endochondral calcification process by Robinson and Cameron (1956), Scott and Pease (1956) as well as by Takuma (1960), while the deposition in the narrow longitudinal septa agrees with the assumption of Durning (1958) and Trueta and Little (1960). The somewhat varying deposition in the longitudinal septa is therefore an expression of the normal variation in a growth region. The differences in opinions may be due to the use of material from different growth zones, species and animals of different ages in the previous investigations referred to above.

The deposition of OTC in the transverse septa is, as is apparent from the results, much less than that in the longitudinal septa. This agrees well with previous investigations (Bloom and Bloom 1940, McLean and Bloom 1940, Lacroix 1951, Scott and Pease 1956, Durning 1958, Trueta and Little 1960) which were performed with different methods. The reason why Dodds (1932) and Dodds and Cameron (1934) were unable to find signs of deposition of calcium salts may be due to the sensitivity of the method used having been too low. It might also be an expression of a normal variation, because Robinson and Cameron (1956) were

unable to show deposition of calcium salts in the transverse septa with the electron microscopic method.

It is clear from the above that deposition of OTC in the endochondral calcification process agrees with the results of the autoradiographic and electron microscopic examinations. This argues strongly for the tetracyclines being deposited in association with the development of the apatite crystals in the zone of cartilage undergoing calcification. This also gains support from the fact that already within a few years after isolation of the tetracyclines it was observed that polyvalent metal ions such as Ca^{+2} can form complex compounds or chelates with the tetracyclines (Regna et al. 1951, Boothe et al. 1953, Albert 1953, Albert and Rees 1956, Weinberg 1957). It has since been proved that these chelates either can be bound to the surface of the apatite crystals or develop on the surface of the apatite crystals (Urist et al. 1962 b, Ibsen and Urist 1962, Ibsen et al. 1963, Urist and Ibsen 1963, Steendijk 1964 a and b, Myers 1965). It is also known that the decalcification process releases tetracycline molecules from tetracycline-containing areas (Titus et al. 1958, Boiko 1959, Ibsen et al. 1963, Urist and Ibsen 1963), supporting the existence of such a bond.

It has previously been shown that the tetracyclines can also be bound directly to the organic components in different types of tissue (Loo et al. 1957, Titus et al. 1958, Frost et al. 1961 a, Du Buy and Showacre 1961, Spitzy 1962, Urist et al. 1962 b, Urist and Ibsen 1963, Yabe et al. 1964), which in many cases is believed to occur via metal ions (Titus et al. 1958, Kohn 1961). The binding of tetracycline molecules directly to the organic components in the cartilage matrix in the endochondral calcification process is probably of no importance, which agrees with the results of previous investigations (Frost et al. 1960 a and b, 1961 a, Hulth and Olerud 1962, Urist and Ibsen 1963, Steendijk 1964 a and b).

It was found that the fluorescent band, which immediately after the injection marks the zone of cartilage undergoing calcification, varies considerably in position in relation to the line of erosion and also in width and intensity. It was shown that the rate of the endochondral growth process influences the fluorescent band in different ways. Thus, the metaphyseal border of the calcification zone is situated far into the metaphysis in the growth zones which have a high growth rate, as in the distal ulna and the proximal tibia, while this border runs epiphyseally to the line of erosion and thus within the growth plate in the proximal radius where the endochondral growth process is relatively

slow. In addition the position of the fluorescent band varies in the same growth region so that the metaphyseal front extends further in metaphyseal direction in those parts of the growth region situated closest to the perichondral lamella. Moreover, the zone of cartilage undergoing calcification is wider in the growth zones with a high rate of growth than in zones with a low rate of growth.

It was found that the deposition of tetracyclines is influenced not only by the rate of the growth process but also by the age of the experimental animals. Concerning the position and width of the fluorescent band no difference was found with certainty with age but the intensity of the fluorescence appeared to increase with age, in contrast with the findings in enamel and dentine (Bevelander and Nakahara 1966). It was found that the intensity in the different parts of the fluorescent band gradually increases with age which appears to be uniform in all the growth zones.

The amount of OTC injected was not found to influence the position of the metaphyseal front with certainty, probably because the cartilaginous matrix metaphyseally to this borderline is completely mineralised (Pritchard 1961). On the other hand, it was observed that the intensity in the fluorescent band decreases with decreasing dose of OTC, because the blood concentration is lower with consequent smaller deposition of OTC in the areas undergoing mineralisation (Milch et al. 1957, 1958, Buyske et al. 1960, Kelly and Buyske 1960, Frost et al. 1961 a, Bevelander and Nakahara 1966). Also the width of the fluorescent band was influenced by the amount of OTC and this was observed by the fact that the epiphyseal border of the band when large doses are given extends further in epiphyseal direction than when low doses are given. This may be ascribed to the assumption that the affinity in the band is greatest metaphyseally and decreases slowly in epiphyseal direction. The affinity most epiphyseally is therefore very weak with the result that only minute amounts of OTC are deposited in this part when a small dose is given, so that the intensity of the fluorescence will be very low and difficult to recognise.

The deposition of the tetracyclines during the endochondral calcification process has previously been the subject of various investigations which have given discrepant and apparently contradictory results. The previous investigations have, as a rule, been focused on the question whether the deposition of the tetracyclines occurs in the calcium-containing part of the growth plate of whether the deposition occurs only in the metaphysis. Thus Frost et al. (1960 a and b, 1961 a and b),

Frost (1961), Urist and Ibsen (1963) like Steendijk (1964 a and b), claimed that the tetracyclines are deposited in the growth plate, while Milch et al. (1957, 1958, 1961), Bevelander et al. (1960 b), like Holmes (1963), stated that normally no deposition occurs in the growth plate but that deposition of large amounts of tetracyclines occurs in the adjacent part of the metaphysis. According to Hulth and Olerud (1962) the largest amounts of tetracyclines are deposited in the metaphysis nearest the growth plate, while small amounts are deposited in the cartilaginous matrix in the growth plate. They stated, however, that the former deposition is not related to the endochondral calcification but to the activity of the osteoblasts in this region. Similar deposition partly in the growth plate and partly in the metaphysis has also been observed by Coutelier et al. (1963), but they claimed that the deposition in the two areas is related to the endochondral calcification process.

On closer analysis of these investigations it was realised that the experimental animals in some of the studies (Holmes 1963, Coutelier et al. 1963) have not been sacrificed until several hours after the injection of the tetracyclines, so that the localisation of the tetracyclines in these cases does not correspond to the zone of cartilage undergoing calcification. The reason why Milch et al. (1957, 1958, 1961) found no deposition in the growth plate under normal conditions but fluorescent tetracyclines in the cartilaginous matrix in an osteochondroma is difficult to understand. The light bluish-gray fluorescence they observed in the metaphyseal part of the growth plate was interpreted as autofluorescence. This finding might depend on too long an interval between the injection and sacrifice of the experimental animals, with the result that these observations do not apply to the zone of cartilage undergoing calcification. It may also be due to the sections not being adequately dehydrated. The reason may also be that the above-mentioned investigations were carried out in the growth zone of the distal femur which belongs to one of the most rapidly growing regions and if so, the area with bluish-gray autofluorescence would correspond to the epiphyseal and faintly fluorescent part of the fluorescent band. It is also concievable that the method for preparing the sections influenced the deposition in such a way that the bound tetracycline was dissolved since the tetracycline is initially only very loosely bound (Frost et al. 1960 a, 1961 a, Hulth and Olerud 1962). In addition, Milch et al. (1957, 1958) stated that only occasional sections were satisfactory for histological examination owing to fragmentation of the specimen which may be due to the freeze-fixation and freeze-sectioning method used, which

surely caused difficulties and influenced the localisation of the deposited tetracycline. The varying deposition of the tetracyclines in the endochondral calcification process in the other investigations (Frost et al. 1960 a and b, 1961 a and b, Bevelander et al. 1960 b, Frost 1961, Hulth and Olerud 1962, Urist and Ibsen 1963, Steendijk 1964 a and b) can probably be explained in part by the fact that these investigations were carried out in different growth regions, in animals of different ages and in material from different species. The method for preparing sections may also have influenced the possibilities of localising the fluorophore.

During the first few minutes after the intravenous injection of OTC it was observed that the intensity in the most intense part of the fluorescent band increased and after at most half an hour it reached its maximum value. At the same time the colour changed and became more yellow. This was presumably because the OTC-concentration in the cartilaginous matrix increased and reached its maximum during the latter part of the above-mentioned period.

During the following hours after the injection the fluorescent band became wider because the endochondral calcification process continuously moved in epiphyseal direction. The tetracyclines continued to be deposited in the zone of cartilage undergoing calcification as long as there were circulating tetracyclines in the blood. It was found that the deposition after intravenous injection of 25 mg OTC per kg bodyweight almost stopped after 3—6 hours, while the deposition after smaller doses ceased earlier. This was because the smaller doses produced a lower blood concentration and were eliminated earlier from the blood than the larger doses (Buyske et al. 1960, Kelly and Buyske 1960).

After the above-mentioned period only slight vital deposition of tetracyclines occurred in the zone of cartilage undergoing calcification. The fluorescent band has then reached its final width. The fluorescent band becomes localised more and more metaphyseally during the following periods. This is because the zone of cartilage undergoing calcification like the growth plate shifts in epiphyseal direction, while the fluorescent band is situated at the same site the whole time because the tetracyclines are bound to the mineralised cartilaginous matrix. It was found that the fluorescent band looses contact with the growth plate at different times after injection in different growth regions. In the growth regions with high rate of growth as in the distal ulna and the proximal tibia it occurs much earlier than in the growth zones with slow rate of growth such as proximal radius,

because of the difference in position of the calcification zone in relation to the line of erosion.

The fluorescent band becomes fainter to a certain extent with time, which is due to the resorption processes in the metaphysis. It was observed that the fluorescent band comes into contact with the bone marrow cavity after 3—6 days in the different growth zones, which suggests that the distance between the growth plate and the bone marrow cavity is related to the rate of the endochondral growth process (Lacroix 1951, Sissons 1953).

After deposition of the tetracyclines in the zone of cartilage undergoing calcification has ceased, the width of the fluorescent band varies not only with the rate of growth process but also with the dose of tetracyclines and the method by which it is given. Intraperitoneal injection gives the widest band, since elimination of the tetracyclines from the blood occurs much slower. In addition the width of the fluorescent band is affected also by the duration of the administration and probably also by the type of tetracycline used, since different tetracyclines are eliminated at a different rate from the blood (Kunin et al. 1959, Kunin and Finland 1961, Owen 1965).

The literature contains no information on the causes of the varying width of the fluorescent band in the endochondral calcification process, but it has been shown that the fluorescent band occurring in association with lamellar bone formation is influenced by the rate of growth as well as the duration of the administration of the fluorescent substance (Frost et al. 1960 a and b, 1961 a, Lee 1963, 1964, Landeros and Frost 1964), while the dose and mode of administration are not described as influencing the width of the fluorescent band (Frost et al. 1960 a and b, 1961 a), which may be explained by the fact that these variations are small and therefore difficult to record.

The intensity of the fluorescence in the brightest part of the fluorescent band depends on the dose of OTC, because a large dose results in a higher concentration of the OTC in the blood than the small dose (Kunin et al. 1959, Buyske et al. 1960, Kunin and Finland 1961, Naumann 1962, Owen 1965) and thereby larger deposition of fluorophore (Buyske et al. 1960, Frost et al. 1960 a, Harris 1960 a, Harris et al. 1962). The metaphyseal front of the fluorescent band is better outlined after intravenous injection than after intraperitoneal injection of the same dose, because the blood concentration reaches its maximum value quicker after intravenous injection and because this value is higher than after intraperitoneal injection (Boothe et al. 1953, Dowling

1955, Buyske et al. 1960, Knothe and Mahler 1960). Compared with these two methods of injection an oral dose results in a much lower blood concentration (Buyske et al. 1960, Kelly and Buyske 1960, Knothe and Mahler 1960).

The intensity also increases with age, which is difficult to explain with certainty. This may argue for the assumption that the affinity in the zone of cartilage undergoing calcification increases or that the same dose produces a higher concentration in the blood in older experimental animals than in younger ones.

The intensity is said to vary with the type of tetracycline. The most effective fluorophore is said to be DCTC followed by CTC, TC and OTC in the order given (Harris 1960 a, Frost et al. 1961 a, Hattner and Frost 1962, Harris et al. 1962, Owen 1963, Johnson 1964). On injection of corresponding doses the varying affinity of the Ca-ions to the different tetracyclines must also be considered (Owen 1961, 1963, 1965, Kienitz 1965), as well as the varying concentration of tetracyclines in the blood after the same dose (Kunin et al. 1959, Kunin and Finland 1961, Kienitz 1965).

In the present investigation it was observed that the colour of the fluorescent band varies with the concentration of OTC from golden-yellow when the concentration is high, to green-yellow when it is low. The colour of fluorescence thus varies considerably with the concentration of fluorophore with the result that the colour is not specific of the tetracyclines (Frost et al. 1961 a), which was formerly believed to be the case (Harris 1960 a, Harris et al. 1962, Owen 1963, 1965). This above-mentioned specificity holds only for the same concentration of fluorophore in the fluorescent band.

As previously mentioned, the intensity of the fluorescence decreases with decreasing dose and at the same time the colour becomes more green-yellow, which is probably due to the disturbing blue or blue-white autofluorescence in the area of deposition. With further decrease of the dose it becomes more difficult to demonstrate the deposition. The intensity of the fluorescence also depends on the exposure time because of photodecomposition of the fluorophore (Udenfriend 1962). In previous investigations (Milch et al. 1957, 1958, Boiko 1959) it has been reported that the deposition after intraperitoneal doses of down to 0.25 mg per kg bodyweight can be demonstrated by fluorescence microscopy. It is very probable that the deposition after smaller doses given intravenously can be demonstrated. Moreover, in the present investigation a deposition was observed after an intravenous dose of 0.5 mg OTC

per kg bodyweight without difficulty. But these small doses are of no practical value for developing the method for determination of growth since the intensity of the fluorescence decreases so rapidly during the fluorescent microscopic examination that photographic recordings are difficult to obtain.

The resorption process of tetracycline-containing cartilage and bone tissue surely releases considerable amounts of tetracyclines, but this occurs during a long period and therefore results in a very low blood concentration. This circulating tetracycline is bound like the tetracycline injected only to a small extent (Frost et al. 1961 a), less than 10 % (Buyske et al. 1960, Kelly and Buyske 1960), while the rest is eliminated in the urine and faeces (Urist and Ibsen 1963). Corresponding observations have previously been made in autoradiographic experiments with Ca^{45} (Ponlot 1959, Lacroix 1960 a, 1962). Such a deposition probably explains the extremely weak fluorescence sometimes seen in the cartilage matrix situated epiphyseally to the fluorescent band.

In this investigation it was observed that pathological changes develop within the fluorescent band already during the first hours after the intravenous injection of 25 mg OTC per kg bodyweight. The changes appear primarily to develop in the most intensely fluorescent part of the band in the form of bent or fragmented cartilaginous trabeculae. This deformation surely occurs intravitally, which has been shown by the fact that the deformed cartilaginous parts are enclosed in bone tissue during the following period and that secondary changes occur in the neighbouring part of the growth plate and the metaphysis.

The above-mentioned deformation occurs, as mentioned, already during the first few hours after the injection and probably during the period when the most intensely fluorescent part of the band is situated at the level of or immediately metaphyseally to the line of erosion when the OTC-containing cartilaginous matrix is not covered by mineralised endochondral bone tissue. It is observed earliest in the growth regions, where the rate of the endochondral growth process is high and latest in the growth regions with low rate of growth.

The secondary changes occur during the following days in the area situated epiphyseally to those parts of the fluorescent band which are deformed most. Here the resorption of the degenerative cartilage cells is clearly disturbed with the result that the growth plate becomes thicker in this area. A corresponding tongue of cartilage has previously been reported in rachitis (Dodds and Cameron 1934, Trueta and Buhr 1963), after compression of the growth area (Trueta and Trias 1961)

and after destruction of the metaphyseal vessels (Trueta 1958, Trueta and Amato 1960, Trueta 1963, Hansson and Wiberg 1963, Wiberg 1964). Bevelander et al. (1959 a and b, 1960 b) have previously observed a similar cartilaginous tongue after treatment with tetracyclines and reported that it is caused by the fact that the tetracyclines disturbed the endochondral calcification process.

The primary cause of the development of the cartilaginous tongue is surely the disturbance of the endochondral calcification process by the tetracyclines, which in turn results in deformation of the cartilaginous trabeculae in the fluorescent band. The deformation prevents the metaphyseal vessels from proceeding further in epiphyseal direction. It is also conceivable that the resorption process is disturbed by the fact that the mineralisation of the cartilaginous matrix is incomplete, but this appeared less likely because the cartilaginous tongue develops only epiphyseally to the most deformed parts of the fluorescent band.

Much suggests that the changes in the most intensely fluorescent part of the band are primarily caused by the tetracyclines disturbing the endochondral calcification process and the cartilage matrix in this area being hypomineralised. This assumption is supported by the previous observation that the tetracycline-containing area, which develops in association with lamellar bone formation (Harris 1960 a, Harris et al. 1962) as well as with the formation of enamel and dentine (Bevelander et al. 1961, Grøn and Johannessen 1961, Bevelander 1964, Nylén et al. 1964, Löfgren et al. 1965, Omnell et al. 1966) can, under certain circumstances, be hypomineralised. Moreover, Bevelander et al. (1960 a) observed corresponding hypomineralised, tetracycline-containing areas in the calcite-shell of larval sand dollars, which lived in water containing tetracyclines (Bevelander 1964). Similar hypomineralisation has also been recorded in other testaceans after treatment with tetracyclines, and it has been shown that calcite-crystals are smaller in tetracycline-containing areas (Bevelander and Goss 1962, Bevelander 1963). In addition Saxén (1965, 1966) found hypomineralisation and growth retardation in tissue cultures of shaft bones in the presence of tetracyclines in concentrations of 1—10 mcg per ml.

Grøn and Johannessen (1961) found that an intraperitoneal injection of 100 mg of OTC per kg bodyweight to young rats resulted in tetracycline-containing hypomineralised bands in the dentine, while a dose of 10 mg produced no microradiographically demonstrable hypomineralisation. Bevelander et al. (1961) observed that an intramuscular injection of 150 mg TC per kg bodyweight (Cohlan et al. 1963) to

weanling rats resulted in hypomineralised bands in both the dentine and the enamel (Bevelander 1964). On examination of deposition of different types of tetracyclines in lamellar bone formation in dogs it has been observed (Harris 1960 a, Harris et al. 1962) that microradiographically demonstrable hypomineralised bands occur after intravenous injection of various tetracyclines in doses of more than about 60 mg per kg bodyweight, while only CTC results in hypomineralised bands when given in doses of 33 mg per kg bodyweight.

Cohlan et al. (1962, 1963) claim that the growth in length of the fibula is reduced by, on the average, 40 % in prematures after a daily oral dose of 4×25 mg TC per bodyweight for 9—12 days and that the retardation is less after 4×7 mg TC. They also state that the growth during the following period is somewhat higher than normal (Bevelander 1964). They used, however, a roentgenological method for determining the rate of growth which means that they registered the growth in length of the diaphysis since at this age there are no bony epiphyses in the fibula. This suggests that during treatment with tetracyclines they measured retardation of the endochondral and perichondral calcification and during the period after treatment recorded the temporarily increased rate of the calcification process. In a similar investigation Chu et al. (1964), on the other hand, could not show any definite disturbance of the growth process, which according to Chu et al. could be explained by the fact that OTC is a weaker chelating agent than the other tetracyclines. The prematures were in this case given 2×7.5 mg OTC daily per kg bodyweight intramuscularly for 10 days.

Owen (1963) claims to have demonstrated that therapeutic oral doses of different tetracyclines for a long period do not disturb the growth in length of the shaft bones, while large doses of CTC retard growth but that growth becomes normal on withdrawal of treatment.

Moreover, Bevelander et al. (1959 a and b, 1960 b) like Smith and Chapman (1963) showed that the injection of 0.1—0.5 mg TC into the yolk sac of an egg does not affect the growth of chick embryos in contrast to doses of 2.5—5.0 mg TC, which retard growth, inhibit mineralisation and result in partial deformation of the skeleton (Bevelander 1964, Johnson 1964). This type of injection results in a much higher blood concentration for a long time since the tetracycline is not lost which enables deposition of larger amounts of the injected tetracyclines than in mammals which bind at most 10 % of the dose injected (Buyske et al. 1960, Kelly and Buyske 1960, Urist and Ibsen 1963).

Summarising, the toxic effect of tetracyclines on the calcification process has in the previous investigations only been demonstrated after parenteral doses of more than about 30 mg per kg bodyweight except when the tetracyclines were given in such a way that the blood concentration was high for a long time.

In the present investigation no deformations were observed after intravenous injection of 0.5 mg OTC per kg bodyweight. Only in one case a slight deformation was observed after administration of 1.0 mg OTC, while deformities were common after doses of 10 and 25 mg OTC. It is therefore probable that the critical level for development of deformities caused by the toxic effect of OTC on the endochondral calcification process is about 1.0 mg OTC per kg bodyweight under the above-mentioned conditions.

This does not exclude the possibility that OTC can have a slight toxic effect on the endochondral calcification process when given in doses below 1.0 mg OTC because hypomineralisation is a primary effect, while deformation of the tetracycline-containing cartilaginous trabeculae is a secondary effect. However, it appears hardly likely that doses of 1.0 mg OTC and less have any retarding effect on the rate of the growth process with certainty because these intravenous doses result in low and transient blood concentration (Dowling 1955, Musselman 1956, Naumann 1962).

In previous investigations (Bevelander et al. 1959 a, 1960 b, Cohlan et al. 1962, 1963, Owen 1963, 1965), Smith and Chapman 1963, Bevelander 1964) it has been shown that large doses of tetracyclines retard the proliferation and hypertrophy of the cartilage cells. This results in reduction of the rate of growth of the shaft bone. On the other hand, no such retardation could be demonstrated after small doses given for a corresponding period (Bevelander et al. 1959 a, 1960 b, Cohlan et al. 1962, 1963, Rolle et al. 1962, Owen 1963, 1965, Chu et al. 1964). In addition Taguchi (1963) has shown that a concentration of tetracycline less than 10 mcg per ml does not affect the growth of cartilage and bone cells in tissue culture while a concentration of 50 mcg per ml does.

It therefore appears that intravenous doses of 0.5 and 1.0 mg OTC per kg bodyweight do not with certainty affect the proliferation and hypertrophy of cartilage cells but such an effect cannot be excluded after intravenous doses of 10 and 25 mg OTC per kg bodyweight since the blood concentration after these doses achieves a high maximum and the tetracyclines persist in the blood stream for several hours (Hunt

et al. 1952, Waddington et al. 1954, Dowling 1955, Musselman 1956, Buyske et al. 1960, Knothe and Mahler 1960, Naumann 1962).

Neither is it probable that the growth process is stimulated by intravenous doses of 0.5—25 mg OTC per kg bodyweight. In the previous investigations (Joliffe et al. 1956, Litchfield et al. 1958, Loughlin et al. 1958, Bunn 1958, Da Veiga 1960, Vanderhoeft 1964, Johnson 1964, Owen 1965) this has usually been demonstrated in children and experimental animals, who were, for some reason, undernourished and received small oral doses for long periods. This effect, which is also produced by other types of antibiotics, is presumably due to the action on the intestinal flora (Bunn 1958, Owen 1965) and is thus only of secondary importance in the investigation of the growth process.

e. *Summary*

This part of the investigation was concerned with the deposition of OTC in association with the endochondral calcification process in the growth regions of the diaphysis.

The experimental material consisted of white rabbits, aged 20—70 days, mostly about 40 days. Each of the animals received an intravenous injection of 0.5—25 mg OTC per kg bodyweight and were killed a varying period after the injection. The growth regions of the shaft bones were fixed, dehydrated in absolute ethyl alcohol, after which the unbedded preparations were sectioned by hand and mounted. The deposition of OTC was studied with the aid of fluorescence microscopy.

It was found that OTC immediately after the injection is deposited in association with endochondral calcification in the zone of cartilage undergoing calcification as a fluorescent band. The distinctly outlined metaphyseal front corresponds to the border between the zone of cartilage undergoing calcification and the zone of completely calcified cartilage at the time of the injection. This border is situated metaphyseally to the line of erosion in the growth zone with high rate of growth and epiphyseally to the line of erosion in the growth zone with low rate of growth.

During the first few hours after the injection the fluorescent band becomes somewhat broader and more intense. The fluorescent band then gradually loses contact with the zone of cartilage undergoing calcification and becomes situated further in the metaphysis. This shift is not true but only apparent because the fluorescent band is stationary, while the zone of cartilage undergoing calcification and the growth

plate are continually migrating in epiphyseal direction at rates which vary with the region of growth and with the age of the experimental animals. The width of the fluorescent band after intravenous injection was found to vary with the amount of OTC and the rate of growth in the region. The intensity varied with the amount of OTC and with the age of the animals.

Amounts of bound OTC are released the first period after the time of injection because OTC-containing cartilage and bone tissue is entirely or partly resorbed.

Intravenous injections of doses of more than 1.0 mg OTC per kg bodyweight often produced more or less deformed cartilaginous trabeculae in and epiphyseally to the fluorescent band. These pathological changes increased in frequency, extension and severity with increasing dose of OTC and with the rate of growth and were probably affected also by the degree of loading of the growth zone. The primary cause was probably that the OTC had a toxic effect on the endochondral calcification process which gives rise to a hypomineralised fluorescent band, which is readily deformed. It cannot be excluded that a certain hypomineralisation occurs after parenteral doses of 0.5 and 1.0 mg OTC per kg bodyweight but such small doses are presumably not enough to reduce the rate of the growth process.

2. DETERMINATION OF RATE OF GROWTH FROM THE GROWTH PLATE

The fluorescent OTC is bound, as previously mentioned, to the areas in the growth region of the diaphysis, which are undergoing endochondral calcification at the time of the injection. The site of the calcification process can therefore be marked at different times by repeated injections, each producing a fluorescent band which can be used for determining the rate of endochondral growth, since the line of erosion and the endochondral calcification process accompany one another.

It appears best to use the metaphyseal front of the fluorescent band for this determination because this front marks the border between the zone of completely calcified cartilage and the zone of cartilage undergoing calcification at the time of the injection. The growth in length from the growth plate during the interval between 2 injections thus corresponds to the distance between the metaphyseal fronts of the fluorescent bands.

It appears advisable to give intravenous injections of 1.0 mg OTC

per kg bodyweight since the marking then occurs rapidly and is distinct at the same time as OTC in this dose has no definite retarding effect on the rate of the growth process.

It is possible to determine the growth in length for such short intervals as a few hours as well as for periods of several days. In the following investigation of the growth in length it was decided to determine the growth per day, since any diurnal variation of the rate of growth in this way will not affect the result. The interval between the last injection of OTC and the sacrifice of the animal was 10 minutes since this short period is sufficient to give exact marking.

In the following part of the investigation the mean error of the determination of growth in length per day from different growth plates was determined because it is necessary to assess the reliability of the method.

a. *Material*

In the investigation of the error of the method 3 rabbits were used, which were 35 days old at the end of the experimental period. These animals were given 1.0 mg OTC per kg bodyweight intravenously on 2 occasions at an interval of 24 hours. The animals were killed 10 minutes after the last injection in the usual way.

The following growth regions were used for determination of the error of the method: proximal tibia (fibular part), proximal and distal fibula from both halves of the body.

b. *Method*

Longitudinal sections of the shaft bone were prepared and the fluorescent microscopic examinations were carried out in the way previously described (see page 42).

The growth from the growth plates was determined by measuring the distance along the metaphyseal trabeculae in the direction of the shaft bone between the metaphyseal fronts of the two fluorescent bands (Fig. 12). The measurement was done only in the region of the metaphysis where the metaphyseal trabeculae meet the growth plate almost at right angles. This measuring area was therefore well defined in all of the regions studied. The measurements were made on sections where the metaphyseal trabeculae could be traced for hundreds of microns in metaphyseal direction from the line of erosion. The distance between the metaphyseal fronts of the fluorescent bands was deter-

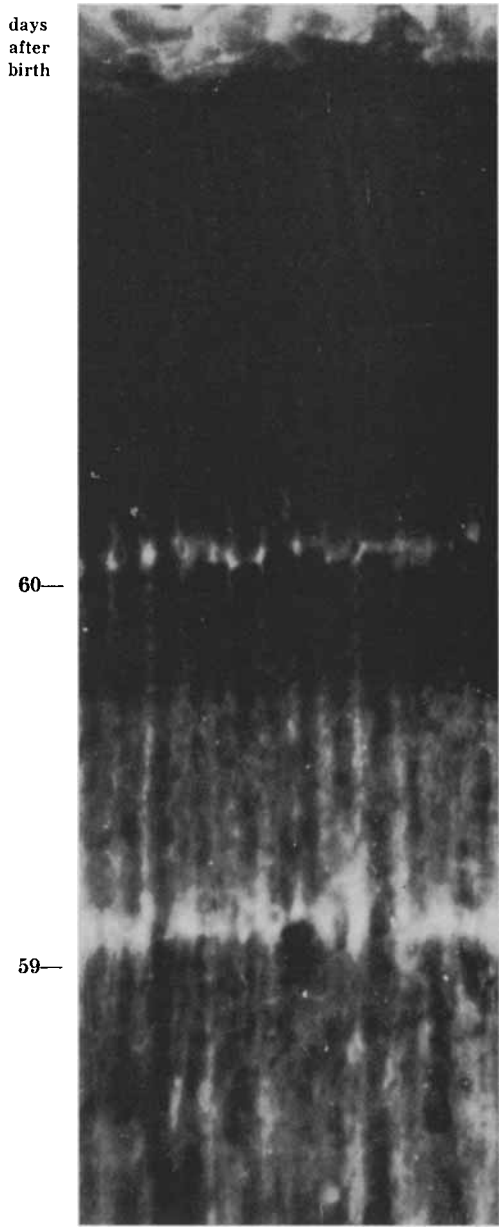


Fig. 12. Localisation of OTC in association with endochondral calcification after intravenous injections of 1.0 mg per kg bodyweight at 59 days (lower fluorescent band) and at 60 days of age (upper fluorescent band). Animal was killed 10 minutes after last injection. Distance between metaphyseal fronts of the two fluorescent bands indicates longitudinal growth during 60th day. Section from proximal fibula (120×).

mined in 2 parts of the above-mentioned area in each section and in 5 sections which thus gave a total of 10 measurements for each growth region.

The statistical calculations were performed according to conventional methods (Dixon and Massey 1957, Snedecor 1962).

Abbreviations:

M = arithmetic mean	MS = mean square
s = standard deviation	t = student's t-distribution
df = degrees of freedom	F = Snedecor's F-distribution
SS = sum of squares	p = probability

The following formulae were used in the statistical analysis:

$$s = \sqrt{\frac{\Sigma(x_i - M)^2}{n-1}}; \qquad t_{(n-1)} = \frac{M-\mu}{s/\sqrt{n}};$$

$$t_{(n-1)} = \frac{(M_1 - M_2) - (\mu_1 - \mu_2)}{s_o \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

$$\text{where } s_o = \sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1+n_2-2}}$$

The following levels were used in tests of significance:

(−) = insignificant	$p > 5\,\%$
* = almost significant	$5\,\% > p > 1\,\%$
** = significant	$1\,\% > p > 0.1\,\%$
*** = highly significant	$0.1\,\% > p$

c. *Results*

The growth from the different growth plates was determined in the way described. Determinations were made of the growth on the sections on two occasions with one month's interval. The data obtained were treated statistically and the results obtained are given in tables 2, 3 and 4. The differences which are accounted for in the tables correspond to the error of the method as the assumption is made that under normal conditions there is probably no difference in the rate of growth between the right and left side.

The total mean error of a given measurement of the rate of growth from the proximal growth plate of the tibia (Table 2) was found to be 13.4 μ or 2.5 % of the average rate of growth per day of the experimental animals. The corresponding errors of the method for the proxi-

mal (Table 3) and distal fibula (Table 4) were 17.4 μ and 15.2 μ, respectively, corresponding to 3.8 % and 3.4 % of the average rate of growth per day from the growth plates in these growth regions.

The measuring error, *i.e.* the difference observed in the determination of the growth at different times constituted a considerable part of the total mean error and varied between 6.8 μ and 8.0 μ for the different growth regions. The other part of the total mean error was due to the use of sections from different growth regions and was reflected partly as a difference between sections from the same growth region and partly as a difference between the right and left sides. On the other hand, no difference was found with certainty between the different parts of the area measured in the section.

The values given above apply to the error of the method for a single determination. In the following examinations the mean value of 10 different determinations in a growth region was used with the result that the mean error, like its various components, was reduced.

Under these conditions the mean error of the determination of the rate of growth from the proximal growth plate of the tibia was 4.2 μ (0.8 %), while the corresponding values for the proximal and distal growth plates of the fibula were 5.5 μ (1.2 %) and 4.8 μ (1.1 %), respectively.

d. *Discussion*

It is thus possible to determine the rate of growth per day from different growth plates by repeated marking of the endochondral calcification process with the aid of the tetracyclines. This possibility has previously been suggested by Hulth and Olerud (1962), while Frost et al. 1960 a and b) claimed that the tetracylines are deposited only transiently during the endochondral calcification process and therefore cannot be used for determining the growth from the growth plate.

In previous investigations of lamellar bone the rate of growth has been determined from a single fluorescent band or with the aid of multiple fluorescent bands.

In the former case the width of the fluorescent band was determined, after which the rate of growth was calculated from the period of administration (Frost 1960 b, Landeros and Frost 1964) but the width of the calcification zone was not taken into consideration and since the deposition of the tetracyclines continues as long as the tetracyclines are

in the circulation, determinations by this method do not give correct values.

In the last-mentioned case, where the determination was made from multiple fluorescent bands, the growth was determined in different ways. Use was often made of the distance between the fluorescent bands or, in other words, the width of the intermediate autofluorescent area (Harris 1960 a, Landeros and Frost 1964, Sissons and Lee 1964, Lee 1965). This method has inherent sources of error because the two measuring points adjacent to the fluorescent bands do not correspond to one another. The measuring point at the band that appears first corresponds to the border between the calcification zone and the osteoid containing no calcium at the time when the demonstrable deposition after the first dose of tetracycline ceases, while the measuring point adjacent to the other fluorescent band corresponds to the border between the calcification zone and the completely calcified area when the second tetracycline dose is deposited. Lee (1963, 1964), like Tapp (1966), measured instead the distance between the midpoints or the most intense parts of the fluorescent bands but even then various factors can affect the results since such a calculation assums that the fluorescent bands correspond exactly to one another.

It is more correct to measure the distance between those parts of the fluorescent bands which mark the border between the entirely calcified area and the zone of cartilage undergoing calcification at the moment the deposition of tetracycline begins. In the endochondral calcification process this border corresponds to the metaphyseal fronts of the fluorescent bands. The distance between identical points is measured regardless of the dose of tetracycline, the type of tetracycline and the interval between the last administration and the removal of the preparation. It is essential, however, that the doses of tetracycline should be given in the same way.

The total error of the method in the determination of the growth from multiple fluorescent bands comprises several components which are related to the sections and to the reading in the fluorescence microscope.

The difference observed in the determination of the growth at different times consists entirely of a measuring error with certainty and is not a sign that the sections have changed with time.

No difference was found between the measurements in the different

parts of the area measured, which means that it is immatrial which part is measured in the study of growth.

On comparison between sections from the same growth region a definite difference was observed in the proximal tibia and distal fibula but no definite difference was found between sections from the right and left sides. In the proximal fibula, on the other hand, there was a difference between the two sides. The cause of this difference between sections from the same growth region and between sections from the right and left growth regions is surely due to the fact that the direction of the sections can vary within certain limits without this variation being observed with certainty in the microscope or that sections are taken from varying parts in the central area of the growth region.

On the other hand, it is not likely that the different steps in the preparation of sections are involved in these sources of error because the rate of growth is determined in mineralised parts of the growth region.

The two components of the total error of the method appear to be of largely equal magnitude and to vary but slightly from one growth region to another.

The reason why the mean error for the proximal fibula is somewhat larger than that for the proximal tibia, is that the former growth region is relatively small and that its growth plate runs a somewhat varying course. This implies that sectioning is more difficult and the measuring area smaller. This is also reflected in the comparison between the distal fibula and the proximal tibia.

The total error of the method in the determination of growth was calculated as about 15 μ against 5 μ for 10 determinations with a slight variation of the different growth regions. The reason why the percentage was relatively higher for the proximal fibula than for the proximal tibia was that the rate of growth is higher in the proximal tibia than in the proximal fibula. This also applies to comparisons between the proximal tibia and the distal fibula.

It was thus found that the error of the method is influenced only slightly by the choice of region of growth studied. This means that the error of the method is about the same in different growth regions, where the growth plate is practically at right angle to the longitudinal axis of the long bone and the central trabeculae. These conditions are also present in the distal ulna, proximal and distal radius as well as the distal tibia.

e. *Summary*

The sources of error occurring in the determination of the rate of growth from the growth plate with the aid of multiple marking of the endochondral calcification process with OTC were analysed.

The experimental animals were given 1.0 mg OTC per kg bodyweight intravenously on 2 occasions at an interval of 24 hours and the animals were killed 10 minutes after the last injection. The growth regions of long bones were treated and examined in the way described previously.

The growth in length from the growth plate during the interval between the injections, *i.e.* the distance between the metaphyseal fronts of the fluorescent bands in the longitudinal direction of the long bone was determined in well defined parts of the metaphyseal area.

The error of a single determination of growth from the growth plate was calculated as about 15 μ with slight variation from one growth region to another. This error of the method corresponded to 2.5 % of the growth per day in the proximal tibia, 3.8 % of the growth per day in the proximal fibula. This variation was due largely to the rate of growth being higher from the proximal growth plate of the tibia than from the proximal growth plate of the fibula.

The error of the method was due to an equal extent to the preparation of the sections and to the reading of the results in the fluorescence microscope.

Multiple marking of the endochondral calcification process with OTC thus allows a rapid, simple and at the same time exact determination of the growth from the growth plate for short periods without any toxic effect on the growth rate with certainty.

CHAPTER III

RATE OF GROWTH FROM THE GROWTH PLATE AND ITS RELATION TO THE MORPHOLOGY OF THE GROWTH PLATE IN NORMAL RABBITS

A. Survey of Literature

The first publication dealing with the rate of growth of a long bone from its end regions appears to be that published by Stephen Hales (1731, 1748). He studied the rate of growth of a long bone, probably a tarso-metatarsal bone (Lacroix 1951) or tibia (Payton 1932, Bisgard and Bisgard 1935), in chicks and found that the growth occurred mainly from its proximal end region. Similar experiments on the tibia of different animals were carried out some years later by Duhamel (1743b), who observed that the growth in this long bone occurred mainly from its proximal end region. These publications were later followed by a large number of investigations. Some of which were carried out only on the diaphysis, and some also included other parts of the end regions of the long bone. This section will be concerned with the growth in length of the diaphysis. It should, however, be pointed out that the difference between the total growth in length of the long bone and the rate of growth in length of the diaphysis is, as a rule, small.

From investigations on the rate of growth of different long bones it is known that the length of the long bones increases along an S-shaped curve during the prenatal and postnatal period of growth and this also holds for the growth in length of the different diaphyses. This means that the rate of growth during foetal life is first very slow and later increases to reach a maximum presumably during the middle and latter part of the foetal period after which it rapidly decreases before birth (Schulz 1960, Israelsohn 1960, Sissons 1961).

According to Israelsohn (1960), birth has only a slight influence on the growth curve. The rate of growth after birth shows the same evenly decreasing tendency as before and appears to be the same for both sexes. This postnatal period in man is said to comprise the first 3—5 years of life (Maresh and Deming 1939, Green and Anderson 1947,

Goff 1960, Anderson et al. 1963, Taillard and Morscher 1965). Bergmann (1931), on the other hand, claimed that there is a period of increased rate of growth starting at the age of 1 year and lasting for some years. Later the rate of growth again decreases.

The period of decreasing rate of growth is followed by a period of constant rate of growth, which in man comprises the ages of 6—9 years. After the rate of growth has become fairly steady it again begins to increase slowly and reaches its maximum between the ages of 10—12 years in females and between 12—14 years in males (Green and Anderson 1947, Anderson et al. 1963). Tupman (1962), who studied the rate of growth of the femur, tibia and vertebrae in man at a skeletal age of 7—17 years could not demonstrate any period with increased rate of growth and found that the rate of growth is constant until the skeletal age of about 12 years in girls and about 14 years in boys. On the other hand, he did observe a growth spurt for the vertebrae immediately before the skeletal age of 12 and 14 years in girls and boys, respectively, which results in a corresponding growth spurt for the total length of the body during this period.

During the following age period until the end of the period of growth the rate of growth decreases rapidly. In man this period consists of about 4 years for both sexes, which means that the growth ceases first in girls (Green and Anderson 1947, Tupman 1962, Anderson et al. 1963).

The rate of diaphyseal growth is not the same for all bones, but differs from one bone to another (Gill and Abbott 1942, Schmid and Künle 1958, Heikel 1960, Ryöppy 1965, Taillard and Morscher 1965). According to Gill and Abbott (1942) the rate of growth of the tibia is faster than that of the femur during the first part of the postnatal period, while the rate of growth during the latter part is higher for the femur than for the tibia, which ceases to grow somewhat earlier than the femur. It is also claimed that the rate of growth of the tibia is more even, than for the femur (Gill and Abbott 1942).

The variation of the rate of growth with age has not been studied so extensively in various experimental animals as in man and, as a rule, for only short periods of postnatal growth period and usually during the latter part. The results of these investigations (Ray et al. 1950, Bhaskar et al. 1950, Heikel 1960, Wray and Goodman 1961) agree well with the results of the above-mentioned investigations in man.

The growth from the different growth plates like that from the end regions also follows an S-shaped curve. The rate of growth from the different growth plates has not been given for the prenatal period,

even though this period has been investigated by Bisgard and Bisgard (1935) and Gill and Abbott (1942). Several investigations of the rate of growth after birth have shown that it decreases with age (Payton 1932, Bisgard and Bisgard 1935, Aries 1941, Gill and Abbott 1942, Sissons 1953, Heikel 1960, Tapp 1966). Bisgard and Bisgard (1935) claimed that this decrease does not begin until the latter part of the postnatal growth period and that the rate of growth during the first part of the postnatal period is constant. Payton (1932), on the other hand, believed that the rate of growth decreases with age during the major part of the postnatal growth period and that this decrease becomes more and more marked towards the end of the growth period. This decrease of the rate of growth is different for different growth plates. It is greatest for the most slowly growing growth plates, while the opposite holds for the growth plates with the highest rate of growth (Payton 1932, Bisgard and Bisgard 1935, Gill and Abbott 1942).

This means that the ratio between the growth from the proximal and distal growth plates of a long bone varies with age (Payton 1932, Brash 1934, Gill and Abbott 1942), as it does for the end regions of the long bone (Ollier 1867, Brodin 1955, Elo 1960). In a large number of investigations it has, however, been assumed that this ratio is constant throughout the growth period (Digby 1916, Bergmann 1929, 1931, Phemister 1933, 1935, Wilson and Thompson 1939, Blomqvist and Rudström 1943, Green and Anderson 1947, Heikel 1960, Salter and Harris 1963, Anderson et al. 1963, Guldhammer 1963, Nordentoft 1964, Ryöppy 1965). In several of these investigations large variations have been observed, which have, however, been explained by the error of the method and individual variations of the experimental material, while the age factor has, for various reasons, not been taken into consideration.

The distribution of the growth during the prenatal period appears to be more even than later, but to vary between different long bones. This is supported by the observations by Bisgard and Bisgard (1935) and by Gill and Abbott (1942). The last two authors observed in a new-born baby that the growth of the diaphysis of the tibia and of the femur was 50 and 46 %, respectively, from the proximal growth plate. Since they determined the rate of growth roentgenologically with the aid of transverse lines, which appeared in the child because the mother had during the last months of pregnancy been treated with bismuth, their findings hold for the last 5 months of intrauterine life. Bisgard and Bisgard (1935) obtained similar results, namely 49 and 38 % for

the proximal end of the tibia and of the femur in new-born goats, whose mother had received phosphorous during pregnancy. They also determined the proportions of growth according to Digby's method, but their results were less compatible which was due to the last method having serious sources of error shown by Ollier (1861, 1867), Payton (1934) and Hendryson (1945).

During the following postnatal growth period the proportions during the first part change slowly, and become more pronounced during the following period (Ollier 1867, Payton 1932, Bisgard and Bisgard 1935, Gill and Abbott 1942, Brodin 1955, Elo 1960). A large number of investigations have also been carried out on the postnatal period (Harris 1926, 1931, 1933, Bergmann 1929, 1931, Phemister 1933, Silfverskiöld 1934, Blomqvist and Rudström 1943, Vahlquist 1943, Green and Anderson 1947, Ring 1955, Heikel 1960, Anderson et al. 1963, Ryöppy 1965) with varying results because different age periods and different types of animals were studied. Moreover, different methods of determination were used.

The rate of growth is probably the same from the right and from the corresponding left growth plate in one and the same individual. In some cases a slight difference has been recorded (Ollier 1867, Bergmann 1931, Goff 1960), which, however, can be explained by the sources of the error of the method (Nordentoft 1964).

It has long been known that the growth of the major long bones of the lower extremities occurs mainly from the growth plates situated nearest the knee joint, while the corresponding growth plates in the upper limbs are situated furthest from the elbow joint (Humphry 1858, 1861, 1862, Flourens 1861, Ollier 1861, 1867, Digby 1916, Harris 1926, 1931, 1933, Bergmann 1929, 1931, Payton 1932, 1933, 1934, Phemister 1933, Brash 1934, Silfverskiöld 1934, Bisgard and Bisgard 1935, Aries 1941, Gill and Abbott 1942, Vahlquist 1943, Green and Anderson 1947, Weimann and Sicher 1955, Ring 1955, Heikel 1960, Salter and Harris 1963, Anderson et al. 1963, Ryöppy 1965, Taillard and Morscher 1965). It should however, be mentioned that Broca (see Ollier 1867) reported that the diaphysis of the fibula grows mainly distally. However, he used *foramen nutritium* to determine the proportion of growth from the various growth plates of the long bone, which according to later investigations gives unreliable results. Similar growth has also been reported for the tibia in homo (Bergmann 1929, Caffey 1937, Vahlquist 1943), but these investigations comprised only the earliest part of the growth period. Moreover, the growth of the diaphysis occurs

mainly from a growth plate situated near the earliest developed bone epiphysis and in the end region where fusion between the bone epiphysis and the diaphysis occurs latest (Humphry 1858, 1861, Flourens 1861, Ollier 1861, Payton 1932, 1933, Bisgard and Bisgard 1935, Phemister 1935, Weinmann and Sicher 1955). The reason why the proportion of growth from this growth plate is largest is believed to be due mainly to the higher rate of growth and only to a less extent to the growth in this region continuing during a longer period compared with the other growth plate of the long bone (Humphry 1861, Payton 1932, Bisgard and Bisgard 1935) though the opposite opinion has also been presented (Humphry 1858).

On comparison of the rate of growth in different long bones a definite pattern appeared. The distribution percentage between the proximal and the distal growth plates of the tibia and fibula is much more even than in the femur, in which growth appears to be more evenly distributed than in the radius, humerus and ulna (Bisgard and Bisgard 1935, Gill and Abbott 1942, Blount 1955, Weinmann and Sicher 1955). Under comparable conditions it has been shown that this holds for different species (Bisgard and Bisgard 1935, Gill and Abbott 1942, Blount 1955), but it should be mentioned that Ollier (1867) early observed that there exists a variation between different species. This finding has been confirmed by later investigations by Bisgard and Bisgard (1935) and Gill and Abbott (1942).

Concerning the distribution of growth during the entire prenatal and postnatal growth period, no reliable information is available even though experiments have been performed to assess this distribution (Digby 1916, Bisgard and Bisgard 1935, Phemister 1935). Gill and Abbott (1942), Lacroix (1951) like Taillard and Morscher (1965) have tried to calculate the growth from the different growth plates in per cent of the total growth in length of the diaphysis and arrived at the following percentages for the proximal growth plate: humerus 80, radius 25, ulna 20, femur 30, tibia 55 and fibula 55 %, but, as previously mentioned, these values are not quite reliable.

The thickness of the growth plate varies considerably with age. This was observed already during the 18th century by Duhamel (1743b). Its thickness is greatest in young individuals and during the latter part of the growth period it becomes thinner and finally disappears in association with fusion between the bone epiphysis and the diaphysis. In certain kinds of animals no fusion occurs and a cartilage plate persists (Dodds and Cameron 1934, Asling and Evans 1961).

The growth plate is equally developed in both sexes during the earliest part of the growth period. It is above all the thickness of the zone of germinative cells that is striking, but also the other zones are thicker than in older individuals (Harris et al. 1965, Morscher et al. 1965). During the earliest part of sexual maturation a sex difference begins to appear and then the growth plate becomes broader in males than in females (Morscher et al. 1965). Towards the end of the growth period the growth plate is broader in males than in females and disappears later in association with fusion between the bone epiphysis and diaphysis (Bargmann 1964, Bucher 1965).

The thickness and morphology of the growth plate varies considerably between different kinds of animals, which is due to some extent to differences in rate of growth (Harris 1933, Sissons 1953).

In one and the same individual the thickness and morphology of the growth plate varies with its site. According to several authors there is a proportional relationship between, on the one hand, rate of growth, and on the other, the thickness of the growth plate (Fahmy 1964, Taillard and Morscher 1965), the height of the different zones (Harris 1933, Elo 1960) as well as the number of cells in the cell column (Harris 1933) and in the different zones (Harris 1933, Trueta and Morgan 1960). Johnson (1964) found that the relationship between the morphology and rate of growth of the growth plate varies with age because the cartilage cells develop slower in older individuals than in younger ones. In addition, it has been observed that the distal growth plate of the fibula is thicker than its proximal plate, even though its rate of growth is slower (Ollier 1867).

B. Author's Investigations

It is clear from the survey of the literature that opinions differ concerning the normal growth process. This applies above all to the rate of growth and its relation to the morphology of the growth plate. The reason appears to be that, with but few exceptions, the methods used formerly for determining the rate of growth did not allow correlation between the rate of growth and morphology.

Therefore the rate of growth and its relation to the morphology of the growth plate were examined in the rabbit under normal conditions. The examination was concentrated on the age period between 20—70

days since the experimental investigations published were, as a rule, made during this period of life. The growth process was studied in regions which are readily accessible to operations and whose growth plates are oriented at right angles to the longitudinal axis of the bone. Factors, which might influence the normal rate of growth, such as sex, litters and nutrition were studied.

Since the investigation was concerned with the morphology of the growth plate it was decided to study the length of the cell columns. In the rabbit the growth plates contain numerous cell columns of which a large number extend from the border between the zone of germinative cells and the zone of proliferative cells through the following zones to the line of erosion (Fig. 2, 3). The length of these complete cell columns is thus an excellent measure of the active zones and of the capacity of the growth apparatus (Kember 1960, Trueta and Morgan 1960, Rigal 1962). It should be added that the zone of germinative cells which varies considerably in thickness in a growth plate, probably does not take any appreciable part in the activity of the growth apparatus compared with that of other zones (Kember 1960, Rigal 1962).

The length of the complete cell column is a better measure of the growth apparatus than the number of cells in the cell column and the number of cells in different zones of the cell column. One of the reasons is that the division of the cells in a cell column occurs almost simultaneously, but at various times in different cell columns. This means that the number of cells in the cell column varies considerably and that the borders between the stages of development in different columns vary, as pointed out by Trueta and Little (1960) and Rigal (1962). Another reason is that it appears to be difficult to determine with certainty the number of cells in the cell column because the cells in the cell column may be divided among several sections in the histological examination.

1. MATERIAL

The rate of growth and its relation to the growth plate was determined in all together 35 rabbits from 9 litters with 3—6 animals in each litter (see page 41). The growth was determined for the 20th, 30th, 40th, 50th, 60th and 70th day of life in 5, 10, 5, 5, 5 and 5 animals, respectively. The members of the litter were distributed over the experimental period in order to obtain as equal a distribution as

possible. The sex distribution of the animals was kept as even as possible to avoid any sex difference. Each of the experimental animals received two intravenous injections of 1.0 mg OTC per kg bodyweight at an interval of 24 hours and was killed 10 minutes after the last injection.

In association with the examination of the rate of growth and its relation to the morphology of the growth plate the error of the method for determining the length of the cell columns was determined. For this purpose use was made of 3 rabbits belonging to one and the same litter and the examination was carried out when the animals were 35 days old. Since the length of the cell columns in the latter part of the investigation were usually studied in the tibial part of the proximal growth plate of the tibia, the error of the method was studied for this area.

In addition it was studied whether there was any difference in rate of growth between litters and sexes and for this examination 6 litters of all together 36 animals (19 males and 17 females) were used. The distribution in the various litters was as follows: Litter 1. 2 males+3 females, litter 2. 4 males+2 females, litter 3. 2 males+5 females, litter 4. 3 males+2 females, litter 5. 5 males+2 females and litter 6. 3 males+3 females. All of the experimental animals were killed at 30 days of age after having received two intravenous injections of 1.0 mg OTC per kg bodyweight at an interval of 24 hours, the animals as usual were killed 10 minutes after the last injection.

2. METHOD

The following growth regions were used for fluorescent microscopic examination: proximal and distal growth regions of the left tibia, fibula and radius. In the proximal growth region of the tibia use was made of the fibular part. The preparation of the sections was done in the way given in Chapter II, page 42. The rate of growth from the different growth plates was studied in the way given in Chapter II, page 78.

The following growth regions were used for light microscopic examination of the morphology of the growth plate: proximal and distal growth regions of the right tibia and radius. Of the proximal tibia, the tibial part was used. The growth regions were fixed in the fresh stage in Bouin's solution for 24—48 hours according to size and age of the experimental animals. The bones were then decalcified in equal parts

of 7 % sodium formate and 40 % formic acid for 20—30 days. Paraffin sections about 10 μ thick were prepared with the help of a slide microtome. In the proximal growth region of the tibia frontal sections were placed in the middle of the growth plate and the plane of section was parallel to the longitudinal direction of the cell columns which was checked in the microscope during sectioning. This also holds for sectioning of the distal growth plate of the tibia. In the two end regions of the radius sections were cut from the central part of the growth plates parallel to the cell columns. The plane of the sections was placed at right angle to the ulnar surface of the radius.

Paraffin sections obtained from the above-mentioned regions were stretched on water (Clayden 1962) at a temperature of at most 35°C and then allowed to dry in the air at room temperature for 1 day. The sections were stained in haematoxylin-eosin according to Ehrlich and were mounted in DePeX (Gurr, London).

For the light microscopic examination use was made of the same type of microscope as that previously described with the same objective and ocular equipment.

For determining the length of the cell columns that area of the growth plate was chosen where the cell columns are practically perpendicular to the erosion line. The length of the cell columns was determined only in columns which were continuous from the border between the zone of germinative cells and the zone of proliferative cells right to the erosion line. In the above-mentioned area 4 cell columns were selected from each section which satisfied the conditions given and this determination was made in 5 sections from each growth plate so the length of 20 cell columns was determined in each growth plate.

3. RESULTS

a. *Rate of Growth from Different Growth Plates*

The rate of growth from the growth plates (Table 5) examined followed during the selected age period of 20—70 days definite curves which appeared to be characteristic of the different growth plates.

PROXIMAL TIBIA

The rate of growth per day from the growth plate of the proximal tibia (Fig. 13) showed a very regular curve. During the actual age

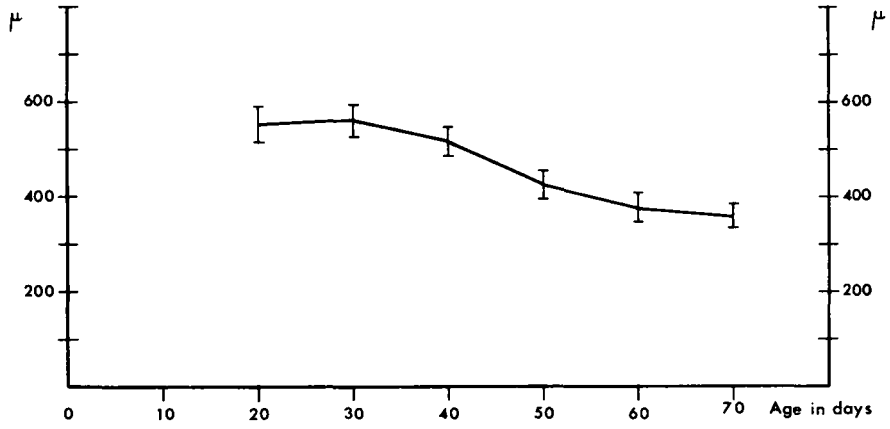


Fig. 13. Rate of growth per day from proximal growth plate of tibia (Table 5).

period the rate of growth was highest in the beginning of this period when it was 561 ± 30 μ per day at 30 days of age, which was slightly more than the growth rate at 20 days of age, when it was 554 ± 36 μ per day. During the following period the rate of growth gradually fell to 362 ± 26 μ per day at 70 days of age.

DISTAL TIBIA

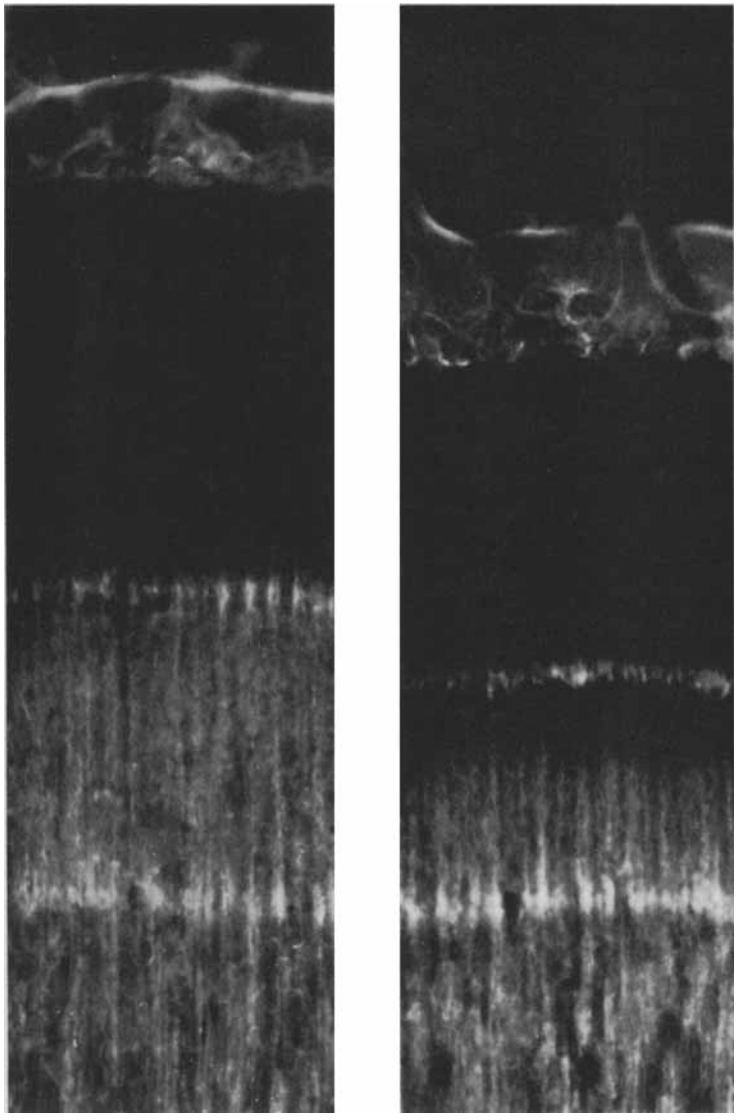
Also in this growth area the growth rate changed in a corresponding manner. Its highest value appeared to be reached at about 30 days of age with 489 ± 43 μ per day while the corresponding at 20 days of age was 480 ± 41 μ per day. The rate of growth then appeared to have reached its maximum and gradually decreased to 234 ± 27 μ per day at 70 days of age.

PROXIMAL FIBULA

The rate of growth from the growth plate of the proximal fibula was highest at 30 days of age when it was 479 ± 34 μ per day, while the corresponding value for 20 days of age was 448 ± 37 μ per day. After 30 days of age the rate of growth slowed down and was at 70 days of age 332 ± 22 μ per day.

DISTAL FIBULA

The rate of growth from the distal growth plate of the fibula was the same as that of the adjacent distal tibia throughout the investigation period.



A B

Fig. 14. Growth from distal growth plate of radius during 30th (A) and 70th (B) day after birth (80×).

PROXIMAL RADIUS

The rate of growth in the proximal radius was much lower than in other regions studied. It was highest at 20 days, when it was 170 ± 32 μ

per day. During the following age period the rate fell slowly, with the result that at 70 days it was only 30 ± 16 μ per day.

DISTAL RADIUS

The rate of growth from the distal growth plate was much higher than from the proximal growth plate. The highest rate was noted at 30 days (Fig. 14 A), when it was 486 ± 30 μ per day, against 444 ± 40 and 470 ± 39 μ per day at 20 and 40 days, respectively. During the following period the rate fell to 352 ± 19 μ per day at 70 days (Fig. 14 B).

COMPARISON BETWEEN DIFFERENT GROWTH PLATES AND DIAPHYSES

Comparison of the rate of growth from different growth plates showed that it was highest in the proximal tibia during the age period studied, and lowest in the proximal radius. The rate of growth in the other regions studied throughout the experimental period lay between the above-mentioned regions. At 20 days the rate of growth in the distal tibio-fibula was higher than in the proximal fibula and distal radius. At 30 days the above-mentioned regions were at about the same level. During the rest of the age period the rate of growth in the distal radius was higher than in the other regions but lower than that of the proximal tibia. The rate of growth from the growth plates of the proximal fibula and distal tibio-fibula was practically the same until about 40 days of age, after which the rate of growth for the proximal fibula began to be higher than that of the distal tibio-fibula and to approach the rate of growth for the distal radius and the proximal tibia.

The rate of growth of the various diaphyses showed the same tendency as that from the different growth plates. The rate of growth of all the diaphyses was highest in the beginning of the growth period and particularly at 30 days, after which it decreased, but at different rates for different diaphyses. During the entire age period the rate of growth of the diaphysis of the tibia was highest, while that of the fibula was somewhat lower and that of the radius lowest.

PERCENTAGE OF GROWTH IN LENGTH OF THE DIAPHYSIS FROM ITS PROXIMAL AND DISTAL GROWTH PLATES

The relative growth from the different growth plates varied with age in a regular manner (Table 5 and Fig. 15).

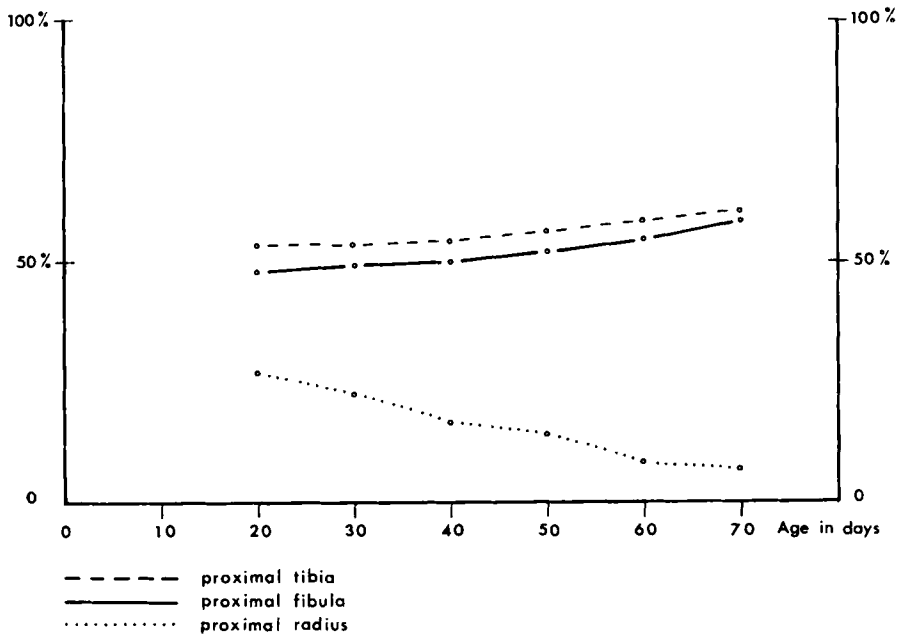


Fig. 15. Rate of growth per day from proximal growth plate of tibia, fibula and radius in per cent of rate of growth per day of diaphysis (Table 5).

Examination of the growth of the tibial diaphysis showed that in the beginning of the experimental period the growth from the proximal growth plate was 53.6 ± 0.7 %. This percentage increased so that at 50 days it was 56.1 ± 0.7 % and finally at 70 days 60.8 ± 1.9 %.

The same pattern was observed for the growth of the fibular diaphysis, though it was initially different. At 20 days the value for the proximal fibula was 48.0 ± 0.8 %, and increased gradually and at 30 days it was 49.5 ± 0.7 %, at 50 days 52.3 ± 0.7 % and finally at 70 days it was 58.7 ± 1.0 %. The rate of growth during the first part of the age period was thus higher distally than proximally, while the relationship changed between 30 and 40 days.

The growth of the radial diaphysis during the entire growth period occurred mainly from the distal growth plate, the proportion increasing with age. The value for the proximal radius at 20 days was 27.6 ± 3.9 %, but at 70 days it was 7.7 ± 3.5 %.

b. *Length of Cartilage Cell Columns in Different Growth Plates*

In all of the growth plates from animals, aged 20—70 days, there were numerous cell columns extending from the line of erosion to the border between the zone of germinative cells and the zone of proliferative cells. These columns thus provided a measure of the height of the zones of proliferative, hypertrophic and degenerative cells.

ANALYSIS OF THE ERROR OF THE METHOD

In the examination of the error of the method the growth plate of the proximal tibia was selected. The length of the cell columns in the preparations was examined twice at an interval of one month. The data were studied statistically and the results are given in Table 6.

The assumption was made that there is no difference between corresponding areas on the left and right sides. The differences between the length of the cell columns found on statistical analysis were therefore most probably due to the error of measurement.

The total error of the method was found to be 18.9 μ, which corresponded to 2.8 % of the average length of the cell column in the growth plate of the proximal tibia. The measuring error, which is identical with the residual value, proved to be the largest of the components of the error of the method. In the present investigation it was 17.8 μ or 2.6 % of the average length of the cell column.

The above-mentioned error of the method was calculated for a single determination and was much smaller when the mean of 20 different cell columns was used. Then the mean error was found to be 4.2 μ or 0.6 % of the average length of the cell column.

The statistical analysis of the length of the cell columns revealed an irregular variation in that the cell columns in some experimental animals were longer in the left growth plate than in the right growth plate, while the ratio was the reverse in other experimental animals. Otherwise no certain interaction was found between the different causes of the variation.

LENGTH OF CELL COLUMNS (TABLE 7)

Proximal Tibia

The curve for the length of the cell columns at different ages in the growth plate of the proximal tibia (Figs. 16, 17) resembled that for its

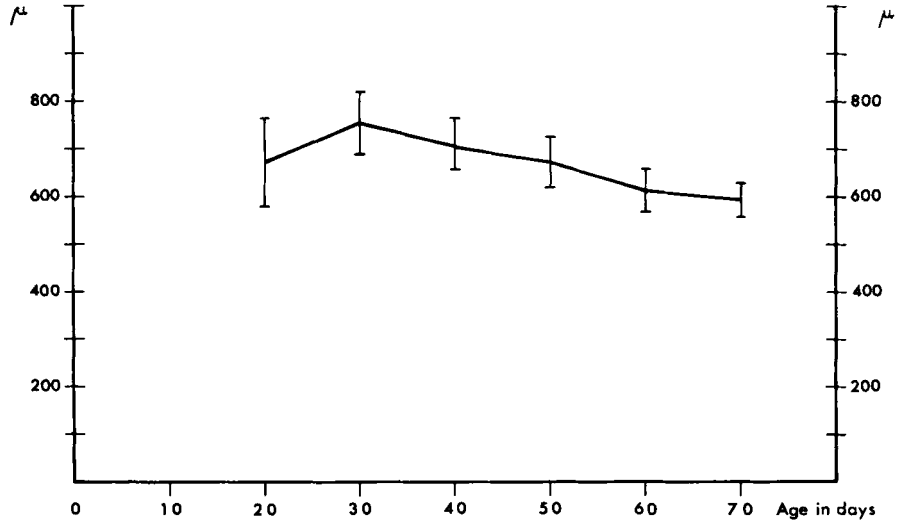


Fig. 16. Length of cartilage cell columns in proximal growth plate of tibia (Table 7).

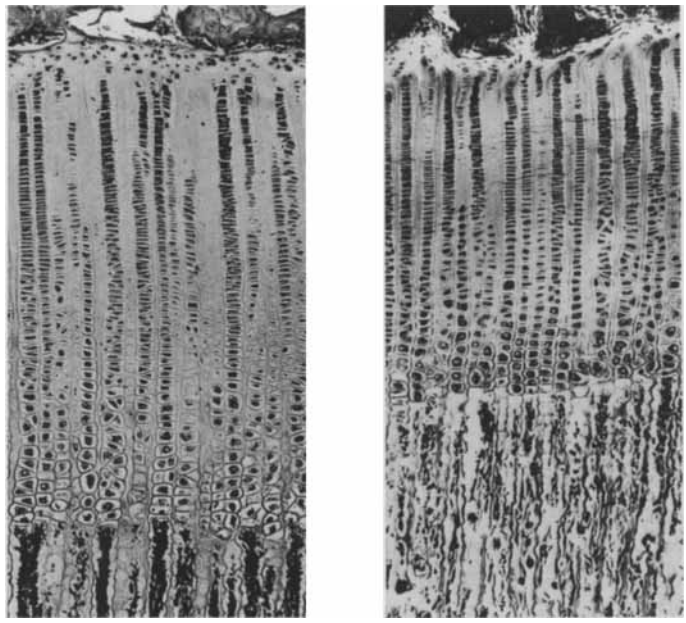
rate of growth. The cell columns were longest at 30 days, when they were 753 ± 66 μ, while they appeared to be shorter at 20 days. The length decreased relatively slowly with age and at 70 days it was 591 ± 35 μ.

Distal Tibia

This growth plate was examined only at 30, 50 and 70 days because it was much more difficult to obtain satisfactory sections from this region than from the other regions. In this growth plate most of the cell columns are more or less curved and are almost straight in only a small area which must be used for the examination. Here the same tendency was found as in the proximal growth plate. The length of the cell column decreased from 621 ± 18 μ at 30 days to 460 ± 10 μ at 70 days.

Proximal Radius

Compared with the other growth plates the cell columns in the growth plate of the proximal radius were short. At the beginning of the investigated age period they were at most 242 ± 16 μ at 20 days. They became shorter with age and at 70 days they were 171 ± 50 μ.



A B

Fig. 17. Proximal growth plate of tibia at 30 (A) and at 70 (B) days of age (75×).

Distal Radius

The cell columns in the growth plate of the distal radius (Fig. 2) were longest at 30 days when they were 660±39 μ. This was only slightly longer than during the next period. During the last part of the age period the length of the cell columns decreased markedly and they were then 542±45 μ at 70 days.

c. *Effect of Different Factors on Normal Rate of Growth*

This part of the investigation was concerned with the rate of growth from the proximal growth plate of tibia in both male and female animals from different litters. The statistical treatment of the data and the results obtained are given in Table 8.

No difference between sexes was found at the age of 30 days. On the other hand a highly significant difference was found between different litters. There was no interaction between the above-mentioned factors so that an irregular variation with sex can be excluded.

Statistical analysis of the relation between the weight of the experimental animals and the rate of growth distinctly showed that under normal conditions there is no correlation between these two factors since the correlation coefficient was ≈ 0.

4. DISCUSSION

The sources of error in the determinations of the rate of growth from the growth plate as determined with the aid of OTC were discussed in the previous chapter. On the other hand, it is necessary to discuss the sources of error that may occur in the determination of the length of the cell columns in the growth plate.

Free-preparation of the growth region probably implies no certain sources of error, since this is done as gently as possible concerning the growth plate and secondly as quickly as possible to avoid dehydration of the growth region (Tarkhan 1931, Sääf 1950).

The sources of error can be sought mainly in histological treatment of the preparations (Patten and Philpott 1921, Tarkhan 1931), sectioning and measuring of the length of the cell columns. In the present investigation, however, care was taken to treat all the material as uniformly as possible so that the sources of error were reduced and practically equal in the entire material (Sääf 1950).

The fresh preparations were fixed in Bouin's solution since this fluid provides a rapid and excellent fixation (Patten and Philpott 1921, Romeis 1948, Baker 1955, Clayden 1962, Burck 1966). In addition, this fixative results in only slight shrinkage, of at most a few per cent of the length (Patten and Philpott 1921, Romeis 1948, Baker 1955).

The following decalcification in formic acid and sodium formate probably does not affect the length of the cell columns since this decalcification fluid is weak and therefore has a mild effect (Kristensen 1948, Clayden 1962).

In previous investigations (Patten and Philpott 1921, Tarkhan 1931, Ries 1938, Romeis 1948, Baker 1955) it has been shown that the shrinkage usually occurs during dehydration and paraffin embedding of the preparation and is usually given as about 10—20 % of the original length after the embedding process. It is probable that the size of the shrinkage in the present investigation was less because it is known from experience that the shrinkage is less in post-

foetal material than in embryonic material. In previous investigations of the effect of shrinkage most investigators have used embryonic preparations. In addition dehydration and embedment were done by a more gentle method in the present investigation.

The remaining part of the histotechnical treatment probably resulted in no further shrinkage, as this was done carefully (Patten and Philpott 1921, Sääf 1950). It was observed in the preliminary investigations that the length of the cell columns was reduced considerably when the paraffin sections were exposed to temperatures above 40°—50°C and that the reduction was probably more pronounced when the sections were exposed to this temperature for several hours. Such findings have also been reported previously by Tarkhan (1931) and Sääf (1950).

The sources of error which can be expected in the histotechnical treatment probably cause a reduction of a few per cent of the true length of the cell column and these sources of error are largely eliminated in comparison between different determinations. Therefore, it did not appear necessary to correct the measured length of the columns in the different growth plates because of these sources of error.

It is apparent from the preceding sections that the histotechnical treatment probably did not cause the differences responsible for the error of the method because the histotechnical treatment probably affected all cell columns to the same extent.

Statistical analysis showed an irregular interaction in that the cell columns in sections from the right growth region were longer than in sections from the left growth region in some of the animals, while the relationship was the opposite in the other experimental animals. This difference is probably not a sign of a normal variation because there was no corresponding variation of rate of growth and because the difference could very well be explained by the sources of error in association with the sectioning. In preliminary investigations it was observed that the length of the cell columns in the growth plate of the proximal tibia varied with their position; the further dorsally the longer they were. Moreover, in frontal sections the central cell columns were shorter than the peripheral ones. This means a side difference in those cases where sections were not taken from identical parts of right and left growth regions or at the same angle. On the other hand, the statistical analysis showed no difference between sections from the same growth region with certainty, which can probably be explained by the fact that different sections were taken at practically the same angle and that the last section was taken at most 200 μ dorsally to the first section.

The error of the method is due largely to the determination of the length of the cell columns, since the length of the cell columns probably varies to a certain extent.

In this examination no certain difference was found between the determination of the length of the cell columns on different occasions, which suggests that the sections did not change with time and that the measurement of the length of the cell columns did not vary from one time of measurement to another.

The area used in the determination of the length of the cell columns in different growth plates studied probably varied only slightly because all were examined by the author. On the other hand, the source of error due to the sectioning surely varies to a certain extent from one growth region to another. In the proximal and distal growth plates of the radius this source of error was probably equally large as in the growth region of the proximal tibia, somewhat larger in the distal tibia since it is more difficult to obtain satisfactory sections from this growth region than from other growth regions examined.

This investigation was concerned with normal growth in the rabbit during the first part of the postnatal growth period, namely between 20 and 70 days. This age period corresponds in man roughly to the period between 2 and 8 years, Heikel (1960) having shown that 1 day in the rabbit corresponds to about 40 days in man.

During this age period there is probably no difference between experimental animals of different sexes partly because no certain difference was found with sex in the examination of 30 day old animals and, secondly, because a sex difference was not noted until after 9 years of age in homo (Maresh and Deming 1939, Green and Anderson 1947, Goff 1960, Anderson et al. 1963), which corresponds more than 80 days in the rabbit.

The investigation produced evidence for the assumption that the highest rate of growth during the age period studied occurs during the first part, but at different times in various growth regions. The rate of growth from the growth plate in the distal radius and proximal fibula is thus higher at 30 days than at 20 days, while the difference is only small in the proximal tibia and the distal tibio-fibula. The relation in the proximal radius is the opposite with a higher rate of growth at 20 days than at 30 days.

It appears probable that the above-mentioned differences in rate of growth between 20 and 30 days are due to the effect of local factors and,

secondly, to change in nutrition occurring during the age period between 20 and 30 days in the rabbit.

The occurrence of a similar variation of the rate of growth in man has been observed by Bergmann (1931) and in the rat by Tapp (1966), while a corresponding variation could not be shown in other investigations (Maresh and Deming 1939, Green and Anderson 1947, Goff 1960, Anderson et al. 1963, Taillard and Morscher 1965). This may be because the growth in previous investigations was recorded at long intervals with the result that transient changes in the rate of growth could not be demonstrated or because these investigations were carried out on the entire growth in length of the long bone and then the above-mentioned variation in rate of growth was partly or entirely eliminated. In addition, it is conceivable that there is a species variation which may have something to do with the varying nutrition during the age period studied.

During the following age period the rate of growth decreases at a different rate in different growth regions. The decrease is most pronounced between 40 and 50 days except in the proximal radius, where it is most pronounced between 30 and 40 days. After this age period the decrease becomes less with the result that the rate of growth during the age period between 60 and 70 days appears to be practically constant especially in the proximal fibula, proximal and distal radius, while it is somewhat less marked in the proximal tibia and distal tibiofibula.

The above-mentioned findings correspond well with the results from previous investigations in man (Maresh and Deming 1939, Gill and Abbott 1942, Green and Anderson 1947, Schmid and Künle 1958, Goff 1960, Tupman 1962, Anderson et al. 1963, Taillard and Morscher 1965) and in different experimental animals (Payton 1932, Bisgard and Bisgard 1935, Aries 1941, Sissons 1953, Heikel 1960), but, as a rule, the methods used did not allow recording of less pronounced variations in rate of growth.

In the present investigation it was observed that the rate of growth decreases at different rates in different growth regions, which has also been observed by previous investigators (Payton 1932, Bisgard and Bisgard 1935, Gill and Abbott 1942). In the last-mentioned investigations it was stated that the decrease occurred more rapidly in the growth regions that grew slowly but in the present investigation the decrease varied partly with growth region and partly with age.

It was shown that the percentage of the growth of the diaphysis

varied with age and from one growth region to another, which has also been shown by previous investigations (Ollier 1867, Payton 1932, Bisgard and Bisgard 1935, Gill and Abbott 1942, Brodin 1955, Elo 1960). In addition there is probably also a variation with species, which is apparent from comparisons of the results obtained in the above-mentioned investigations.

In the present investigation it was observed that the percentage of growth of the diaphysis from its two growth plates during the first part of the growth period studied is more evenly distributed than during the latter part. This has also been observed in previous investigations (Ollier 1867, Payton 1932, Gill and Abbott 1942, Brodin 1955, Elo 1960).

It has previously been assumed that the rate of growth in the major long bones of the lower limbs is highest from the growth plates, which are situated near the knee joint, while corresponding growth plates in the upper limbs are situated in the proximal humerus and in the distal radius and ulna. The present investigation showed that in rabbits this is the case first after about 35 days. Before this age growth of the diaphysis of the fibula occurs mainly from the distal growth plate. This has previously been reported by Broca (see Ollier 1867), who, however, used a less reliable method of determination. Similar growth has also been recorded for tibia during the prenatal and the earliest part of the postnatal growth period (Bergmann 1929, Bisgard and Bisgard 1935, Caffey 1937, Vahlquist 1943).

The rate of growth from different growth regions in the rabbit has been the subject of several investigations (Sissons 1953, Brodin 1955, Trueta and Amato 1960, Heikel 1960), which have given varying as well as somewhat low values compared with the results in the present investigation. This is certainly because different parts of the growth period and different parts of the growth region were examined in the previous investigations. In addition, it is conceivable that the experimental material varied from one investigation to another.

It is difficult to make comparisons between growth in different species because of differences in the duration of the growth period and skeletal development (Heikel 1960). The present investigation, however, showed that the rate of growth in the rabbit is generally higher than in other species previously examined, such as homo (Harris 1926, 1931, 1933, Gill and Abbott 1942, Green and Anderson 1947, Goff 1960, Anderson et al. 1963), dog (Ryöppy 1965), goat (Bisgard and Bisgard 1935), pig (Payton 1932, 1933, 1934) and rat (Dodds and Cameron

1934, Aries 1941, Leblond et al. 1950, Sissons 1953, Kember 1960, Tapp 1966). Rabbits therefore are suitable for investigation of the growth of the long bones.

As previously mentioned, in the rabbit there are numerous cell columns which extend through all the zones in the growth plate with the exception of the zone of germinative cells. This abundance of long cell columns is probably because the rabbit belongs to quickly growing species (Harris 1933, Sissons 1953). The length of the cell columns in the rabbit thus provided an excellent measure of the total thickness of the zones of proliferative, hypertrophic and degenerative cells during the growth period.

The present investigation showed that the length of the cell columns varies from one growth region to another and with the age of the experimental animal. In the proximal tibia and distal radius the columns at 30 days were longer than at 20 days, while cell columns in the growth plate of the proximal radius were of the same length on both occasions. During the following age period the length of the columns was reduced which occurred at a different rate in the different growth plates and during different parts of the age period studied.

In previous investigations it has been reported that there is a marked correlation between the rate of growth and morphology of the growth plate (Harris 1933, Trueta and Morgan 1960, Elo 1960, Fahmy 1964, Taillard and Morscher 1965). However, this correlation has not been analysed closely probably because the rate of growth in the above-mentioned investigations was determined with relatively crude methods.

In the present investigation the results suggested a marked, but at the same time somewhat varying correlation between the rate of growth and the morphology of the growth plate. This correlation varies also with species (Sissons 1953, Johnson 1964) and with age, as well as in different growth regions and in different parts of the growth plate. It was thus observed that the length of the cell columns varied with age and did not decrease to the same extent as the rate of growth. This suggests that the development of the cartilage cells in the growth plate in older animals is slower than in younger ones which has been shown previously by Johnson (1964).

It was found that the rate of growth, like the length of the cell columns, varied slightly from animal to animal of the same age. Thus in contrast to what Sissons (1953) believed to have shown in experiments on rabbits and rats there was probably no rapid variation.

The difference in the rate of growth between animals from different

litters suggests that the growth rate is influenced by hereditary factors. The growth rate is also affected by the state of nutrition, which probably explains why members of a large litter often grew somewhat slower than those in a small one. Poor nutrition, like disease, always reduces the rate of growth, usually with reduction in the thickness of the growth plate, as shown by Acheson (1960), Park (1964) and Pratt and McCance (1964).

Normally, there is probably no correlation between the weights of the experimental animals and the growth rate in a given litter because such members generally have the same possibilities of growth.

5. SUMMARY

In this part of the investigation determinations were made of the growth rate and its relation to the morphology of the growth plate in different growth regions between 20 and 70 days in the rabbit in order to obtain a control series for later comparison with growth under varying experimental conditions.

The animals were given 1.0 mg OTC per kg bodyweight intravenously on 2 occasions at an interval of 24 hours and were killed 10 minutes after the second injection. The rate of growth from the growth plates in the tibia, fibula and radius was measured in the way described previously, while the morphology of the growth plate was examined in paraffin sections of decalcified material fixed in Bouin's fluid.

It was found that the rate of growth from the growth plates described curves which appeared to be characteristic for different growth regions. The rate of growth was highest during the first part of the age period studied and the rate of decrease varied from growth plate to growth plate during different parts of the age period. The relative growth of the diaphysis from its different growth plates varied in a regular manner.

In the examination of the morphology of the growth plate determinations were made of the length of the cell columns extending from the border between the zone of germinative cells and the zone of proliferative cells to the line of erosion. A correlation was found between the rate of growth and the length of the cell columns. The correlation varied with age and from one growth region to another.

The rate of growth also varies to a certain extent between experimental animals from different litters, which is probably due to hereditary and nutritional factors. On the other hand, no variation with sex was found in 30 day old normal rabbits and no correlation between weight and rate of growth.

CHAPTER IV

EFFECT OF PLUGGING OF METAPHYSEAL MARROW CAVITY ON GROWTH IN LENGTH OF DIAPHYSIS

A. Survey of Literature

1. CLINICAL OBSERVATIONS OF STIMULATION

A large number of conditions of different aetiology resulting in anisomelia have been described in clinical investigations.

Several types of *congenital anomalies* causing increased growth are known (Taillard and Morscher 1965). Vascular anomalies such as arterio-venous fistula have been reported as the commonest cause (Janes and Musgrove 1949, Coursley et al. 1956, Ejrup and Hiertonn 1961, Goidanich and Campanacci 1962).

Already during the 19th century *infectious processes* in the neighbourhood of the growth regions of long bones have been observed stimulating growth (Humphry 1862, Ollier 1867), a finding which has later been confirmed (Bergmann 1929, 1931, Trueta 1953, 1958). According to Trueta, growth is stimulated particularly when the infectious process is situated in the shaft proper plugging the marrow cavity.

In several investigations stimulation of the growth of paretic limbs has been observed in poliomyelitis. This stimulation is believed to occur only during the first year after onset, after which growth of the paretic limb is retarded (Jansen 1957, Ring and Ward 1958, Ratliff 1959).

In this connection it should also be mentioned that inflammatory conditions of a joint usually act as growth stimulants (Brattström 1963, Taillard and Morscher 1965).

Stimulated growth has occasionally also been observed in patients with different types of *tumours* and especially tumours involving the metaphyseal region (Goff 1960, Taillard and Morscher 1965).

Trauma in the form of a diaphyseal fracture is probably the com-

monest cause of increased rate of growth of a long bone and has been the subject of extensive investigations (Ollier 1867, Truesdell 1921, Burdick and Siris 1923, Levander 1929, Bisgard 1936, Blomqvist and Rudström 1943, Hedberg 1945, Bertrand and Trillat 1948, Trueta 1953, 1958, Blount 1955, Greville and Ivens 1957, Emnéus 1957, Emnéus and Wiberg 1958, Chigot 1958, Barfod and Christensen 1959, Goff 1960, Guldhammer 1963, Emnéus and Hedström 1964). In several investigations (Burdick and Siris 1923, Trueta 1953, 1958, Goff 1960) it has been claimed that the stimulation of growth varies with the degree of dislocation, which, however, could not be verified by Emnéus (1957).

It is usually found that the stimulation of growth ceases 1—2 years after the occurrence of the fracture (Blount 1955, Emnéus 1957, Emnéus and Wiberg 1958, Goff 1960, Emnéus and Hedström 1964), but it should be mentioned that Blount (1955) and Goff (1960) observed that the growth ceases earlier on the fractured side than on the other side at the end of the growth period.

A fracture through the growth plate and through the epiphyseal region may cause marked reduction of growth from the traumatised area (Salter and Harris 1963), while growth from the opposite growth region is often stimulated (Macewen 1912, Blount 1955, 1958).

Also surgical intervention on various parts of a long bone may cause an increase in the rate of growth for some time after the operation (Humphry 1862, Ollier 1867, Compere and Adams 1937, Bertrand and Trillat 1948, Blount 1955, Breine and Johanson 1966).

In addition *mechanical factors* may stimulate growth. According to Heuter-Volkmann's theory, longitudinal compression of the growth zone retards growth while traction stimulates it. The retarding effect of compression has later been demonstrated both clinically and experimentally (Müller 1924, Gelbke 1951, Blount and Zeier 1952, Strobino et al. 1952, Siffert 1956, Arkin and Katz 1956, Amako and Honda 1957, Goff 1960, Trueta and Trias 1961). Longitudinal traction has been described as stimulating growth, but this appears uncertain since experimental investigations (Gelbke 1951, Smith and Cunningham 1957, Haas 1958, Ring 1958) have given contradictory results.

It is uncertain whether elimination of loading of the growth regions has a stimulating or retarding effect because investigations have given contradictory results (Humphry 1862, Ollier 1867, Macewen 1912, Müller 1924, Bergmann 1931, Compere and Adams 1937, Arkin and Katz 1956, Geiser 1957, Geiser and Trueta 1958, Ring 1961).

2. EXPERIMENTAL AND CLINICAL INVESTIGATIONS OF THE EFFECT OF SO-CALLED GROWTH STIMULATING OPERATIONS

The possibility of treating anisomelia by increasing the rate of growth of the shorter limb in relation to that of the longer contralateral limb has been the subject of a large number of clinical and experimental investigations. The methods used have generally been called growth stimulating, but, as mentioned, this is to be understood as the increased rate of growth compared with that on the contralateral side.

a. *Operations on Long Bone*

DIAPHYSEAL REGION

Operations on Periosteum

Experimental investigations: Ollier (1867) studied the growth stimulating effect of periosteal stripping in young rabbits and found that the tibia on the operated side was 2—5 mm longer than that on the other side 3 months after the operation.

The growth stimulating effect of periosteal stripping has since been demonstrated in several experimental investigations (Wu and Miltner 1937, Lacroix 1947, 1951, Bertrand and Trillat 1948, Brodin 1955, Langenskiöld 1957, Elo 1960, Solá et al. 1963). The strongest stimulation after periosteal stripping has usually been observed during the first weeks after the operation (Lacroix 1947, 1951, Brodin 1955, Langenskiöld 1957, Elo 1960). During the subsequent period the stimulation appears to be less marked (Lacroix 1947, 1951, Brodin 1955, Elo 1960). Brodin (1955) and later Elo (1960) showed that periosteal stripping of the proximal part of the tibial diaphysis retards the growth from the proximal growth region while it stimulates growth from the distal growth region.

Investigations by Wu and Miltner (1937) and Solá et al. (1963) suggest that repeated periosteal stripping probably does not increase the growth stimulating effect of the first operation.

Periosteal stripping with simultaneous implantation of foreign material (Langenskiöld 1957) or organic tissue (Elo 1960) between the periosteum and the cortex probably produces a slight increase in the growth rate in addition to that brought about by more periosteal stripping.

In those cases where the operation included not only periosteal

stripping but also traumatisation of the perichondrium no stimulation of growth occurred but instead a marked retardation (Ollier 1867, Elo 1960).

Clinical investigations: Periosteal stripping has often been used to stimulate growth since the operation is simple and risk-free. Such an operation usually produces an increase of about 1 cm during the first year after the operation (Bertrand and Trillat 1948, Vacirca and Canepa 1956, Taillard 1958, 1959, Contessa 1959, Taillard and Morscher 1965).

Operations on Cortical Bone Tissue

Only few investigations have been performed on the growth stimulating effect of operations on the cortical bone.

Experimental investigations: Bertrand and Trillat (1948) observed that trepanation of the cortical bone tissue in young guinea-pigs did not produce any stimulation over and above that of periosteal stripping.

Clinical investigations: There are probably no clinical investigations of the growth stimulating effect of operations on the corticalis.

On the other hand, a clinical investigation has been published by Frejka and Fait (1958), who performed stripping of the major part of the periosteum between the growth zones in the tibial diaphysis with exception of the region around the nutrient artery. This operation was combined with a number of longitudinal incisions in the corticalis in the middle part of the diaphysis. They observed that the postoperative rate of the growth was higher on the operated side compared with that of the contralateral tibia. The difference in length was 1—3 cm.

Operations on Marrow Cavity

Drilling of Marrow Cavity.

The literature contains several reports of the effect of drilling holes in the diaphysis. In some investigations large parts of the bone marrow were destroyed while the destruction was probably less in other investigations.

Experimental investigations: Kishikawa (1936) studied the stimulating effect of a number of drilled holes in the shaft proper and claimed to have demonstrated roentgenologically an increased rate of growth in the operated shaft bone the first few weeks after the operation. In later investigations of the effect of drilling holes (Compere and Adams 1937, Hutchinson and Burdeaux 1954, Hansson and Wiberg

1963, Wiberg 1964) and curettage (Wu and Miltner 1937) in the metaphyseal area or in the shaft proper have given different results and usually shown only an insignificant effect.

Clinical investigations: The operations referred to above have also been used in clinical investigations. Thus, Ferguson (1933) reported that drilling holes in combination with curettage of the corresponding part of the marrow cavity of the proximal tibia and fibula produced a demonstrable stimulation of growth.

Later investigations have shown that drilling holes in the diaphyseal region stimulates growth to the same extent as periosteal stripping (Contessa 1959, Taillard and Morscher 1965).

Implantation of Various Substances into the Marrow Cavity

A large number of investigations of the stimulating effect of implantation of different substances into the marrow cavity by different methods have been described and are surveyed below.

Experimental investigations: The first to use this growth stimulating method was probably von Langenbeck (1869) who implanted ivory pegs into metaphyseal regions of the tibia and femur in a dog. He found an increase in growth of about 0.5 cm of both the tibia and femur during the following months.

Later a large number of similar investigations were carried out with different substances (Meisenbach 1910, Bohlman 1929, Bergmann 1931, Kishikawa 1936, Wu and Miltner 1937, Chapchal and Zeldenrust 1948, Bertrand and Trillat 1948, Herndon and Spencer 1953, Wilson and Percy 1956, Haas 1958). The growth stimulating effect varied widely and sometimes even retardation was observed probably because the operation had traumatised the adjacent growth plate.

Trueta (1953, 1958) claimed the existence of a correlation between the growth stimulation after implantation of different substances in the marrow cavity and the grade of plugging of the marrow cavity as well as the grade of the periosteal trauma. He had previously found that growth stimulation after a diaphyseal fracture, like diaphyseal osteomyelitis, is usually greatest when the marrow cavity is plugged and that the growth stimulating effect disappears as the marrow cavity again begins to become normal. Trueta therefore studied the growth stimulating effect of surgical blocking of the marrow cavity and found that plugging of the marrow cavity with pieces of bone or inert metal with simultaneous traumatisation of the periosteum and destruction of a nutrient artery in the middle part of the diaphysis and

claimed that the operation usually resulted in definite stimulation of growth. Trueta (1958) found that denatured bone tissue gave better stimulating effect than vital bone tissue because the blocking of the marrow cavity is longer in the former case.

Similar operations have since been performed on rabbits by Langenskiöld (1957) and Elo (1960). The former blocked the marrow cavity of the tibia with the aid of a transplant consisting of the proximal fibula and at the same time performed periosteal stripping and implanted subperiosteally a piece of plastic film around the diaphysis. Elo, on the other hand, implanted a piece of skin into the marrow cavity of the proximal tibia of rabbit. After both types of growth stimulating operations the rate of growth of the tibia increased on the operated side the first few weeks after the operation.

Clinical investigations: Several of the foreign substances used in the above-mentioned investigations have also been tried clinically. The growth stimulating effect has varied widely (Bertrand and Trillat 1948, Pease 1952, Accinno and Parker 1954, Carpenter and Dalton 1956, Jansen 1957, Tupman 1960, Goff 1960, Nordentoft and Guldhammer 1964).

The growth stimulating effect of plugging of the marrow cavity has also been investigated clinically (Ståhl 1957, Trueta 1958, Contessa 1959, Taillard and Morscher 1965). The operation has usually comprised extensive periosteal stripping. In general, the growth stimulating effect was considerable during the first half year after the operation and then decreased relatively quickly (Taillard and Morscher 1965). In those where the operation also included lumbar sympathectomy the stimulation of growth lasted somewhat longer (Ståhl 1957).

Fracture and Osteotomy

The growth stimulating effect of the diaphyseal fracture has probably not been used for stimulating growth but in this respect has been substituted by diaphyseal osteotomy. On the other hand, the diaphyseal fracture has been used in experimental investigations. Therefore this type of trauma should be included in the survey of the literature of growth stimulating methods.

Experimental investigations: In several investigations it has been shown that the rate of growth of a shaft bone with a diaphyseal frac-

ture is much higher than that of the contralateral shaft bone (Levander 1929, Bisgard 1936, Bisgard and Martenson 1937, Compere and Adams 1937, Greville and Janes 1957, Wray and Goodman 1961). Bisgard (1936) like Compere and Adams (1937) claimed, in contrast with Levander (1929), that the stimulating effect occurs only in the fractured shaft bone while the other shaft bones of the same limb are not affected. Later Wray and Goodman (1961) showed that a diaphyseal fracture of the tibia of the rat results in a few days' retardation of growth of the femur of both the fractured and the control side on comparison with that in a comparable control series. During the following period, on the other hand, the rate of growth is higher than normal on both sides and especially on the fractured side. The final result is thus that the femur on the fractured side is longer than the corresponding shaft bone on the other side.

Clinical investigations: Goff (1960) reported that he had used splinter osteotomy as a growth stimulating operation, which resulted in increased growth in the operated shaft bone during the following 9—12 months. The difference achieved between the two sides was usually a few centimetres, Blount (1955) reported that multiple osteotomies has a stimulating effect on growth.

EPIPHYSEAL REGION

Experimental investigations: It has been shown that implantation of pieces of metal, for example, into the bone epiphysis has no growth stimulating effect but retards growth, particularly in those cases where the cartilage plate has been traumatised (Ford and Key 1956, Key and Ford 1958, Haas 1958, Ford and Canales 1960). Haas (1958) reported growth stimulation only in those cases in which he had implanted copper nails into the bony ephiphysis and an iron pin in the adjacent metaphysis.

Clinical investigations: Carpenter and Dalton (1956) implanted iron nails into the epiphyseal area in patients with poliomyelitis with anisomelia, but observed only an insignificant stimulating effect.

Since inflammation of a joint often has a stimulating effect on the growth of the adjacent regions, Bertrand and Trillat (1948) studied the effect of injection of blood into the joint cavity but found no demonstrable stimulating effect on growth.

b. *Operations on Vascular System*

ARTERIES

Implantation of Arteries

In recent years several experimental investigations have been carried out in which attempts have been made to increase the arterial flow to one of the growth regions of the shaft bone by implanting the femoral artery into the bone marrow cavity of the diaphysis or into the bony epiphysis of the shaft bone (Woodhouse 1963, Dickerson and Duthie 1963, Boyd and Ault 1965, Del Torto 1965, Dickerson 1966). In these investigations a significant increase in growth was obtained compared to that on the opposite side and to a group of control animals that had been subjected to an incomplete operation which did not include arterial implantation (Dickerson 1966).

Ligation of Nutrient Artery

Ollier (1867) like Wu and Miltner (1937) and Trueta (1953, 1958) found no definite stimulating effect of ligation of the nutrient artery. On the other hand, Brookes (1957) who occluded the nutrient foramen of the femur in newborn rabbits found this operation initially to retard growth on that side. This retardation was followed by stimulation and then later by retardation. The final result was a somewhat shorter femur on the operated side.

Later Troupp (1961) showed that a more proximal ligature such as ligation of the femoral artery usually caused retardation of the growth of the femur and tibia.

VEINS

Since the latter part of the 19th century several clinical experimental investigations have been carried out on the effect of venous stasis on the growth in length of the shaft bone. In these investigations venous stasis was produced either by ligation of the veins or by application of a tourniquet.

Experimental investigations: The stimulating effect on venous stasis appears uncertain judging from the results of experimental investigations. In various studies no stimulation has been demonstrated (Wu and Miltner 1937, Keck and Kelly 1965), while others have reported a slight stimulation (Pearse and Morton 1930, Bergmann 1931, Kishikawa 1936, Servelle 1948, Hutchinson and Burdeaux 1954, Colt and Iger 1963).

Clinical investigations: Only few clinical investigations (Helferich 1887, Schüller 1889) have been published on the stimulating effect of venous stasis. According to these investigations, venous stasis causes a slight increase in rate of growth.

ARTERIO-VENOUS FISTULAE

Since the 19th century it has been known that congenital and traumatic arterio-venous fistulae in a limb stimulate growth (Janes and Musgrove 1949, Coursley et al. 1956, Ejrup and Hiertonn 1961, Goidanich and Campanacci 1962).

Experimental investigations: The above-mentioned clinical observations were followed by experimental investigation of the stimulating effect of a surgically created fistula between the femoral artery and femoral vein on growth (Janes and Musgrove 1949, 1950, Kelly et al. 1959, Vanderhoeft et al. 1963 a and b, Keck and Kelly 1965). These investigations showed that shaft bones at the level of, and distally to, the fistula increased considerably in length and thickness compared with the control side.

Clinical investigations: A corresponding stimulation has also been observed in clinical investigations (Hiertonn 1957, 1961, Janes an Jennings 1961).

c. *Operations on Nervous System*

Surgical sympathectomy and pharmacological sympathetic block are regarded as growth stimulating methods but not operations on the motor and sensory nerves (Ring 1961, Troupp 1961) or on the peripheral nerves (Bergmann 1931, Troupp 1961) because such intervention causes irreparable damage.

Experimental investigations: The growth stimulating effect of lumbar and periarterial sympathectomy has been studied experimentally. In most of these investigations no appreciable growth stimulating effect has been found with certainty (Bacq 1930, Bergmann 1931, Bisgard 1931, 1933, Harris and McDonald 1936, Ring 1961, Troupp 1961), though a slight effect has been observed (Kishikawa 1936).

Clinical investigations: Lumbar sympathectomy has been used in the treatment of anisomelia after poliomyelitis. The growth stimulating effect of this operation, however, is said to be insignificant (Harris and McDonald 1936, Barr et al. 1950) or very uncertain (Nordentoft and Guldhammer 1964).

d. *Physical Methods*

ROENTGEN RADIATION

The effect of roentgen radiation on the chondral growth process has been widely studied experimentally. It has been shown that the effect varies with the dose, small doses to the growth regions often produce brief growth stimulation (Baunach 1935), while somewhat larger doses generally retard growth (Barr et al. 1943, Langenskiöld and Edgren 1949, Hulth and Westerborn 1960).

SHORT-WAVE DIATHERMY

Experimental investigations: Doyle and Smart (1963) reported that moderate doses of short-wave diathermy produce a significant stimulation of growth without injuring the cartilaginous growth zone. In other experimental investigations, however, no such stimulation has been found (Granberry and Janes 1963, Schneider 1963) but instead considerable retardation as well as pronounced injury to the cartilaginous growth zone (Wise et al. 1949).

Clinical investigations: Repeated treatment with short-wave diathermy has been tried clinically. The stimulation of growth was, however, only slight (Wilson and Thompson 1939, Bertrand and Trillat 1948).

OTHER PHYSICAL METHODS

In addition to the growth stimulating effect of internal heating (Ring and Lee 1958, Richards and Stofer 1959), ultrasonics (De Forest et al. 1953), galvanisation (Paschetta 1949) and direct heat (Taillard and Morscher 1965) have been tried, but only internal heating of the area of the diaphysis appears to have stimulating effect.

e. *Mechanical Methods*

As mentioned, according to Heuter-Volkmann's theory longitudinal traction of shaft bones stimulates growth. Haas (1958) and Ring (1958), studied this point experimentally but found only a slight increase in length. It was also found that the risk of complications by this type of method is considerable.

B. Author's Investigations

It is clear from the survey of the literature that several growth stimulating methods have been studied both experimentally and clinically. The differences between the results of these investigations are probably ascribable to differences not only between the stimulating methods used but also between the materials and the period of observation. In addition, various measuring methods with considerable sources of error have been employed (Table 1).

In the present investigation it was therefore decided to study how a growth stimulating method affects the normal growth process. Plugging of the marrow cavity of the proximal metaphysis of the tibia was used as growth stimulating operation, since this method has previously been used experimentally and clinically and is believed to have a considerable growth stimulating effect (Trueta 1953, 1958, Ståhl 1957, Taillard and Morscher 1965). The reason why the operation was performed on the proximal part of the tibial diaphysis was that it was possible to examine the effect of the operation on the proximal growth region with damaged vessels and the effect on the distal growth region with intact vessels.

1. MATERIAL AND METHOD

In this part of the investigation all together 173 rabbits were used, all of which at the beginning of the experimental period satisfied the conditions set forth on page 41. During the experimental period 51 animals were rejected because of complications after operation or because of disease. The remaining 122 animals were divided into 2 series, 92 being assigned to series A and 30 to series B.

During the experimental period all the animals received intravenous injections of 1.0 mg OTC per kg bodyweight at exactly 24 hours' interval between consecutive injections. Each animal in series A received all together 5 OTC-injections on 5 consecutive days during a period between 2 days before and 100 days after the operation. Each animal in series B received all together 6 OTC-injections on 6 consecutive days during a period between 29 and 34 days of age, *i.e.* in the operated animals between 1 day before and 4 days after the operation. In both series the animals that received OTC at the operation this was given immediately before the beginning of the operation. The animals were killed 10 minutes after the last OTC-injection with mebumal sodium.

Series A. All of the animals belonging to this series were operated

upon at 30 days of age with plugging of the marrow cavity with homologous cortical bone tissue. The operation was carried out on the left tibia in the middle part of the ventromedial surface of the proximal metaphysis at a distance of about 8—10 mm from the growth plate.

During the operation the animals were anaesthetised with ether on open mask after first having received an intraperitoneal injection of 0.5 ml 6 % mebumal sodium solution (ACO) per kg bodyweight. The operation area was first shaved and disinfected with Jodopax-solution (Ferrosan). Under aseptic precautions a 1.5 cm long, longitudinal incision was made through the skin, after which the periosteum was opened through a short, V-shaped incision so that the surface of the corticalis was seen. With the aid of a dental burr a hole with a diameter of about 5 mm was made through the corticalis. The bone marrow at the level of the hole was destroyed with the aid of the burr and of a metal nail with hooked tip and the marrow cavity was then filled with homologous bone tissue, which had been kept in absolute ethyl alcohol for about 1 week. The skin wound was sutured with Pehafil® sutures. The underlying periosteum was not sutured. The wound was left undressed.

Series B. The animals belonging to this series came from 5 litters, 5—7 members in each. These animals were distributed among 7 groups in order to eliminate differences due to variations between the litters. The various groups were treated at the age of 30 days as follows:

Group 1: This group was not operated upon and served as control group.

Group 2: In this group the metaphyseal marrow cavity in the proximal metaphysis of the left tibia was plugged in the way described above.

Group 3: In this group the animals were subjected to the same operation as described above with the exception of the plugging of the marrow cavity.

Group 4: In this group the operation consisted only of drilling of the cortical bone in the metaphysis of the proximal tibia, without destruction of the marrow cavity.

Group 5: In this group the operation consisted of a V-shaped incision through the periosteum in the previously described area.

Group 6: The operation consisted only of an incision through the skin without injury to the underlying periosteum.

Group 7: This group was not operated upon but only anaesthetised with mebumal sodium and ether.

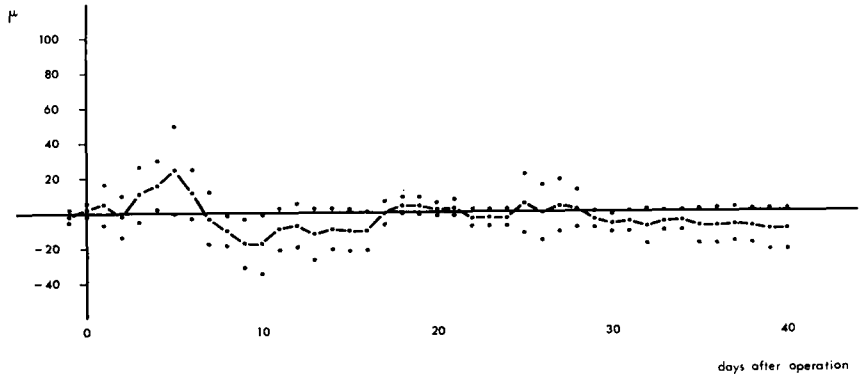


Fig. 18. Difference in growth per day between left and right proximal growth plate of tibia after medullary plugging (Table 9).

The growth per day in both series was determined in the way described on page 78 in sections from the following growth regions: left and right proximal tibia (fibular part), proximal and distal fibula and the left distal radius.

The length of the cell columns was determined in the way described on page 92 in sections of the growth plates in the following growth regions: left and right proximal (tibial part) and distal tibia and right distal radius.

The material was treated statistically according to the methods given on page 80.

2. RESULTS

a. *Difference in Rate of Growth and Morphology between Left and Right Sides after Unilateral Plugging of Metaphyseal Marrow Cavity*

RATE OF GROWTH

Proximal Tibia (Table 9 and Fig. 18)

On comparison between the left tibia (operated side) and the right tibia (control side) in experimental animals in which the metaphyseal marrow cavity had been plugged it was found that the rate of growth was influenced in a very regular way.

During the first 2 postoperative days no significant difference was demonstrable between the rate of growth on the two sides (Fig. 19). During the following period until the 6th day the growth per day was

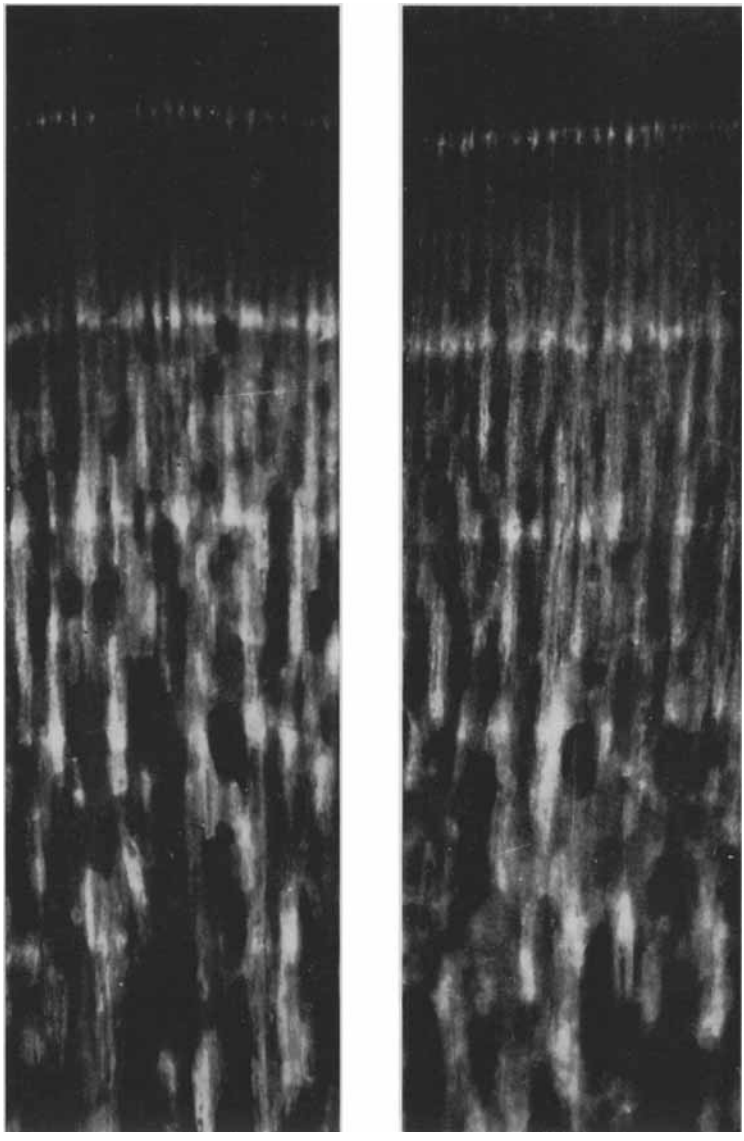


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Fig. 19. Growth from proximal growth plate of tibia on right and left side during 1st, 2nd, 3rd and 4th day after medullary plugging (50×).

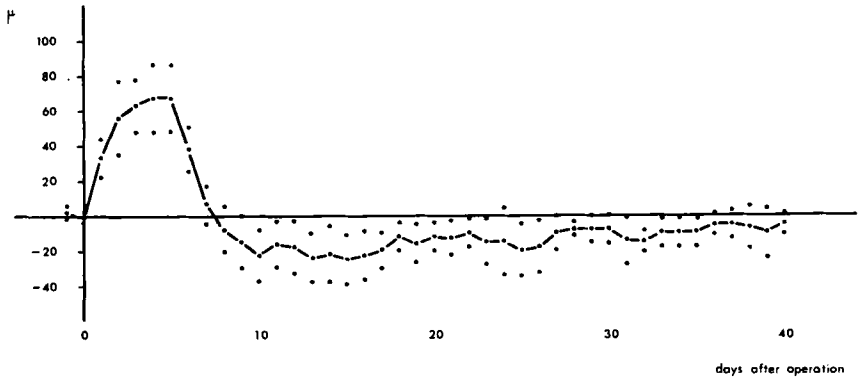

Fig. 20. Difference in growth per day between left and right proximal growth plate of fibula after medullary plugging (Table 10).

at most 25 ± 25 μ more on the operated side than on the control side. This corresponded to 4.9 % of the rate of growth on the control side. During the following 10 days, on the other hand, the rate of growth was some tens of microns slower on the operated side than on the control side. At 60 days and at 80 days after the operation the rate of growth on the operated side was probably lower than on the control side. This difference was about 10 % of the rate of growth on the control side.

On calculation of the total postoperative difference in growth between the operated and unoperated side it was found that after 6 days the total growth was between about 60—70 μ more on the operated side and this difference represented the maximum value. During the following period this difference diminished and the total growth on the control side gradually exceeded that on the operated side. Forty days after the operation the total difference was about 105 μ.

Proximal Fibula (Table 10 and Fig. 20)

Immediately after the operation a difference occurred in the rate of growth per day of the operated and unoperated side. The rate of growth on the operated side during the first 7 days was much higher than on the control side with a maximum difference of about 60—70 μ per day during the 3rd—6th day which corresponded to 14—16 % of the growth per day on the control side (Fig. 21).

During the 2nd and 3rd week the growth per day, on the other hand, was some tens of microns slower on the operated side than on

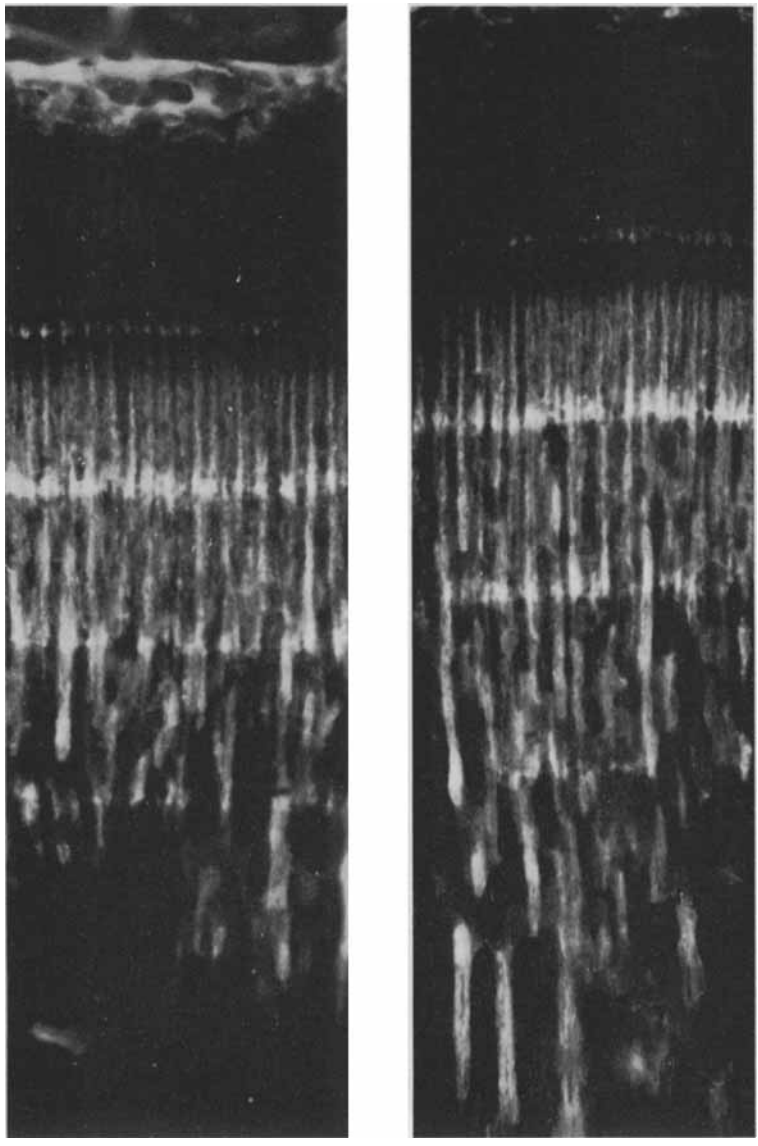

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Fig. 21. Growth from proximal growth plate of fibula on right and left side during 3rd, 4th, 5th and 6th day after medullary plugging (50×).

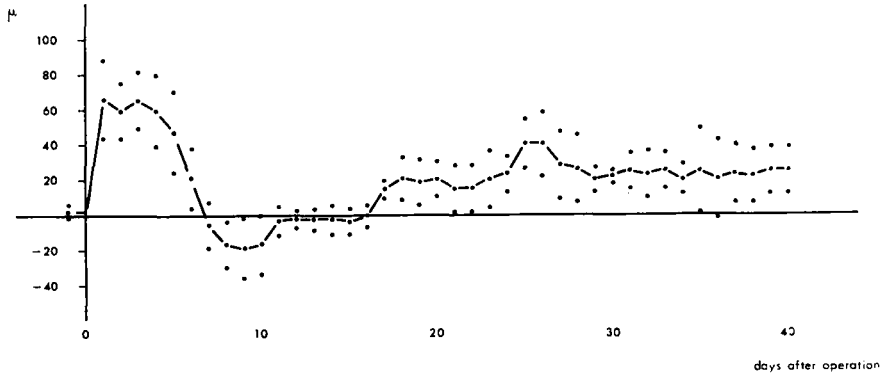

Fig. 22. Difference in growth per day between left and right distal growth plate of fibula after medullary plugging (Table 11).

the control side. During the rest of the observation period the ratio was similar, but the difference was, as a rule, not significant.

The higher rate of growth per day on the operated side resulted in a total postoperative difference of about 331 μ after 7 days. Then the difference diminished so that between 25 and 30 days after the operation the total growth was roughly the same on both sides. During the following period the total growth was larger on the control side than on the operated side and the difference was about 119 μ at 40 days after the operation.

Distal Fibula (Table 11 and Fig. 22)

The growth per day from the distal growth plate of the tibia was not recorded, but instead the growth per day from the distal growth plate of the fibula. It is probable that the rate of growth was the same from the distal growth plates of the tibia and of the fibula after the operation, as under normal conditions (see page 94). In addition, it has been shown previously that the distal tibia and fibula in the rabbit are connected by synostoses between the diaphyses and between the bone epiphyses of which the latter occurs later and at 30—45 days of age (Heikel 1960).

On comparison it was found that during the first postoperative days the rate of growth per day on the operated side was much higher than on the control side. The maximal difference in rate of growth was about 55—70 μ (13—15 %) and was noted during the first 4 days. This was followed by a short period with the reversed ratio. The difference during this period was at most 19 ± 17 μ or 4.2 %. During the

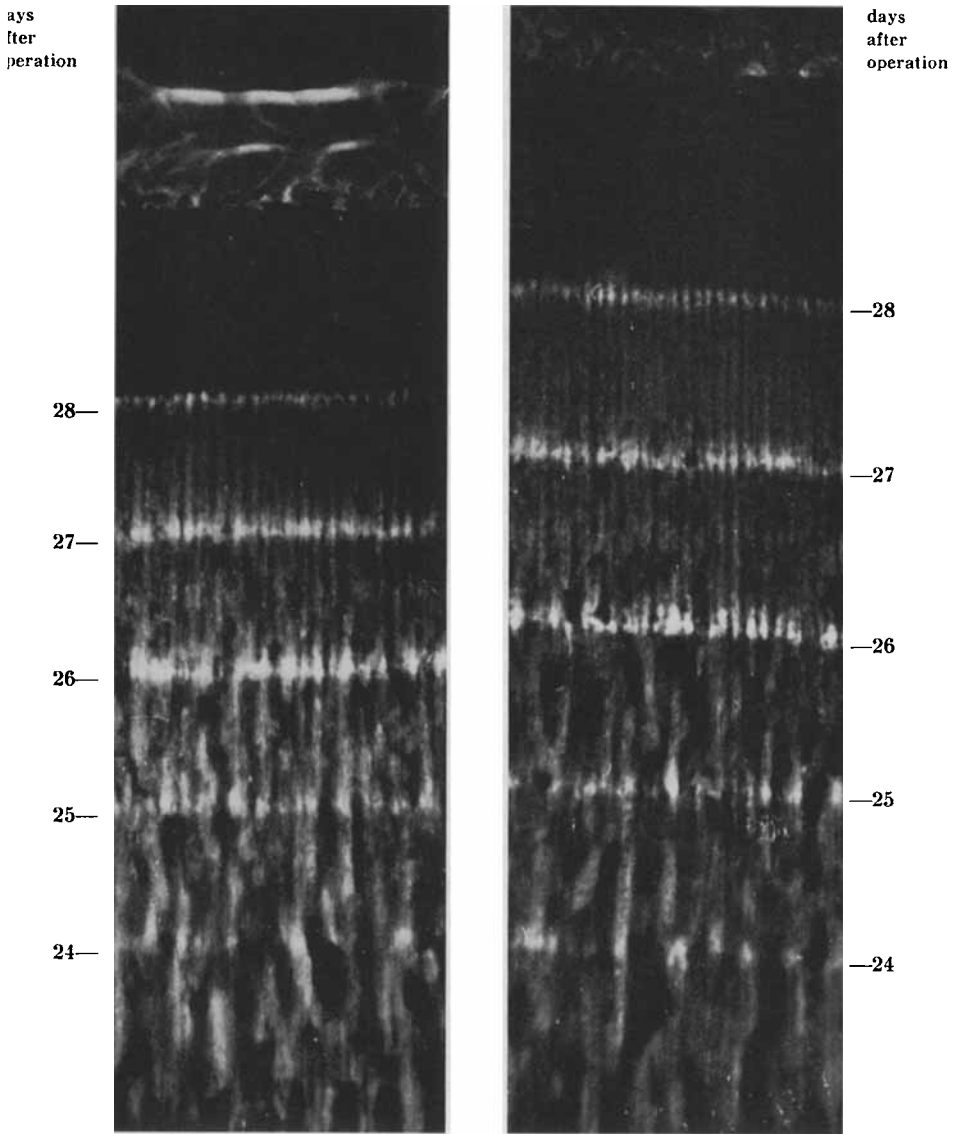

Fig. 23. Growth from distal growth plate of fibula on right and left side during 25th, 26th, 27th and 28th day after medullary plugging (50×).

3rd week the ratio changed and the growth was then usually some tens of microns higher on the operated side than on the control side during the following weeks (Fig. 23).

The total difference in postoperative growth increased in the distal fibula to reach a maximum after the 6th day when the difference was about 317 μ, *i.e.* the growth had all together been higher on the operated side than on the control side. During a short period this difference decreased to about 240 μ. Then the total postoperative difference again increased and at 40 days after the operation it was about 793 μ.

Tibial Diaphysis (Table 12)

Since it was possible to determine the growth per day from the proximal and distal growth plates, it was also possible to calculate the total growth of the tibial diaphysis per day.

During the first 6 days after the operation the rate of growth was much higher on the operated side. The difference was greatest on the 3rd postoperative day when it was 76 ± 23 μ. On the 7th day the ratio reversed, and during the following 10 days the growth per day was higher on the control side. During the subsequent period, until 40 days after the operation, the growth per day was often higher on the operated side. These differences were, however, not always significant. During the rest of the observation period there was no statistically significant difference.

The total difference in growth between the two sides varied with the duration of the postoperative period. After 6 days this difference was about 383 μ and during the following 10 days it decreased to about 200 μ. Then followed a slight increase in the difference from day to day with the result that the total growth after 40 days was about 687 μ higher on the operated side.

Fibular Diaphysis (Table 13)

The rate of growth of the fibular diaphysis during the first 6 days after the operation was 128 ± 27 μ higher on the operated side. During the following 10 days the rate of growth was significantly lower on the operated side. During the rest of the observation period no significant differences were found, though the growth per day during the first part was, as a rule, some tens of microns higher on the operated side.

The total increase in the length of the fibular diaphysis was about 641 μ on the 6th day after which the difference diminished to about 391 μ. It then gradually increased during the following period and on the 40th day after the operation it was about 675 μ.

MORPHOLOGY OF GROWTH REGIONS

General

Already during the first postoperative days a tongue of cartilage cells extending far into the metaphysis from the central part of the growth plate was observed in the proximal growth region of the tibia on the operated side.

Two days after the operation this tongue of cartilage cells (Fig. 24) was almost rectangular in frontal sections. At this time the most metaphyseally situated part extended to the fluorescent band, which marked the position of the calcification process at the time of the operation.

The tongue of cartilage cells consisted of hypertrophic cells which were smaller in size than normally, especially in the middle and epiphyseal part of the tongue. In the most metaphyseal part of the tongue the hypertrophic cells were somewhat larger and near the line of erosion single degenerative cells were also seen. The cartilage cells were arranged in columns, which could often be traced through the tongue and through that part of the growth plate epiphyseally thereto. The centrally situated cell columns usually ran straight in the longitudinal direction of the long bone, while the peripheral ones were curved with the convexity facing the central part of the tongue.

This picture became less and less marked in direction towards the perichondral lamella so that the cell columns in the measuring area ran practically straight in the longitudinal direction of the long bone and perpendicular to the line of erosion.

The cell columns in the area measured were not during the first postoperative days of such regular appearance as normally. The length of the cell columns varied more than normally, *i.e.* fewer cell columns extended from the zone of germinative cells to the line of erosion. The border between the zone of germinative cells and the zone of proliferative cells, like the line of erosion, ran more irregularly than normally.

The metaphyseal trabeculae, which were situated diaphyseally to the tongue of cartilage cells, appeared delicate and extended further in diaphyseal direction than normally. Between these trabeculae there was usually tissue poor in cells with few osteoblasts and no normal vessels.

Changes were also seen in those parts of the metaphysis bordering the tongue peripherally. The metaphyseal trabeculae in these parts also curved in varying directions. This was most marked nearest the tongue. The operation also appeared to affect the perichondral lamella

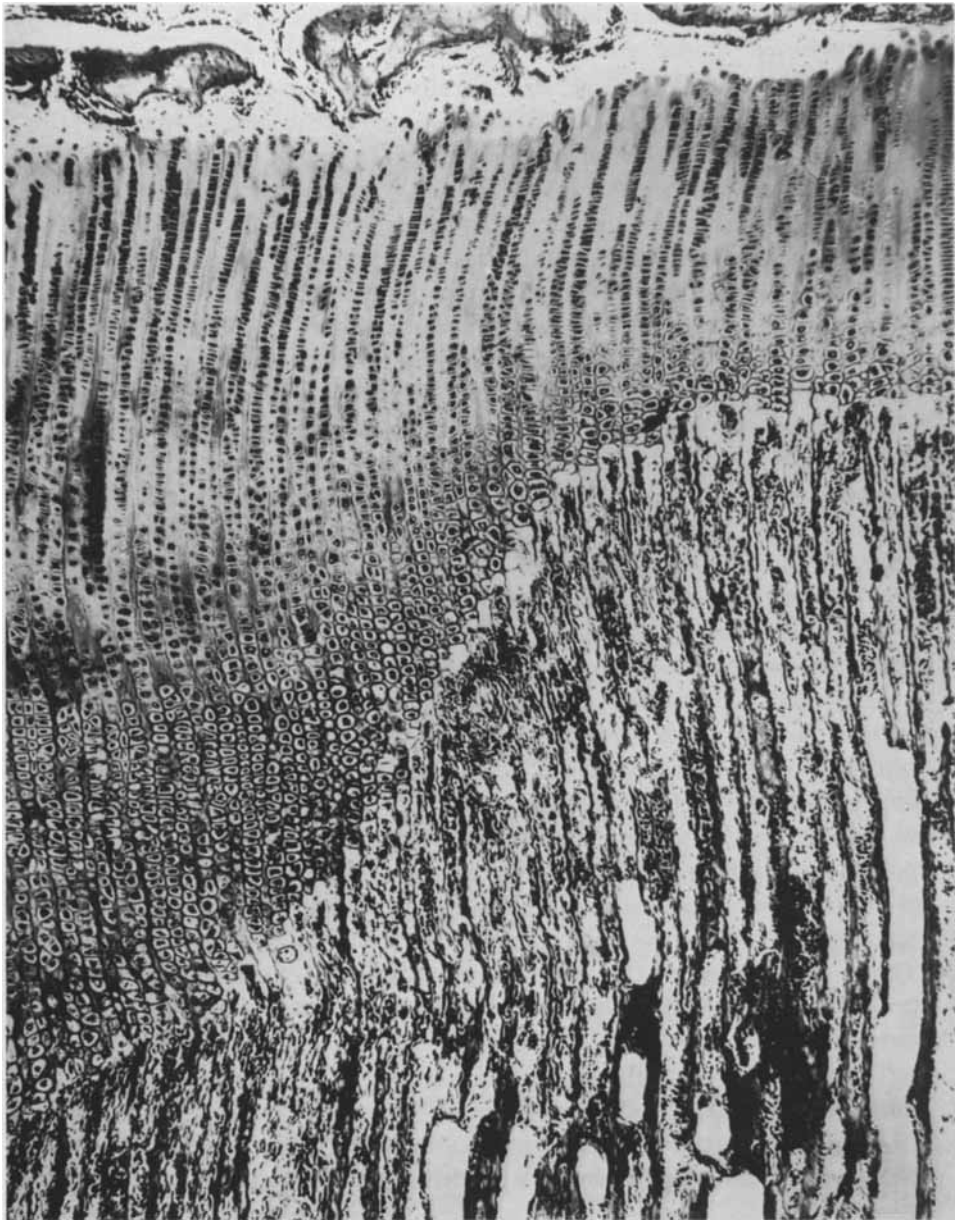

Fig. 24. Part of proximal growth region of left tibia 2 days after medullary plugging. To right, growth plate, and to left, tongue of cartilage cells extending from growth plate into metaphysis (75×).

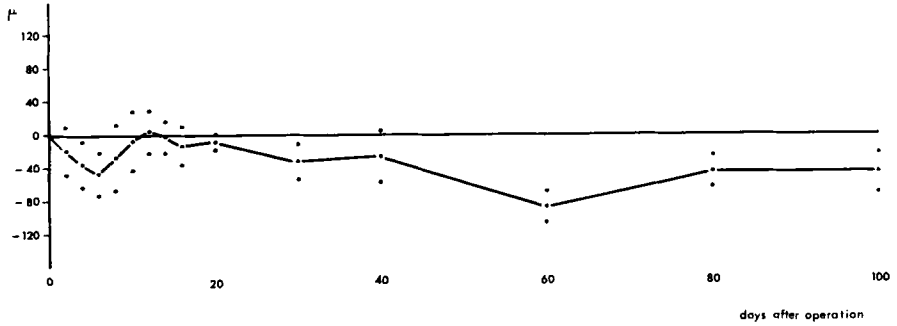

Fig. 25. Difference in length of cell columns between left and right proximal growth plate of tibia after medullary plugging (Table 14).

since here, too, there was a considerable bending at the level of the tongue of cartilage cells.

After 4 days the tongue extended almost twice as far into the metaphysis as 2 days previously, which was because the growth plate had in the meantime shifted in epiphyseal direction. The metaphyseal border of the tongue was very irregular. At this time and also during the following days isolated remnants of the cartilage cell tongue consisting of single hypertrophic and degenerative cartilage cells were observed in the metaphysis, at the same as the metaphyseal vessels metaphyseally to the tongue became normal and the trabeculae were covered by osteoblasts.

During the following days the metaphyseal and the peripheral parts of the tongue disappeared. The last remnants of the cartilage cell tongue disappeared, as a rule, between 6 and 10 days after the operation. From then on the new-formed metaphyseal trabeculae were again oriented in the longitudinal direction of the shaft bone.

Length of Cell Columns

Besides the above-mentioned changes in the morphology of the growth region the operation also affected the length of the cell columns. The differences between the growth plates on the operated and unoperated sides are described below.

Proximal Tibia (Table 14 and Fig. 25)

Two days after the operation no significant difference was found between the operated and the unoperated side, but a considerable variation was found between the animals.

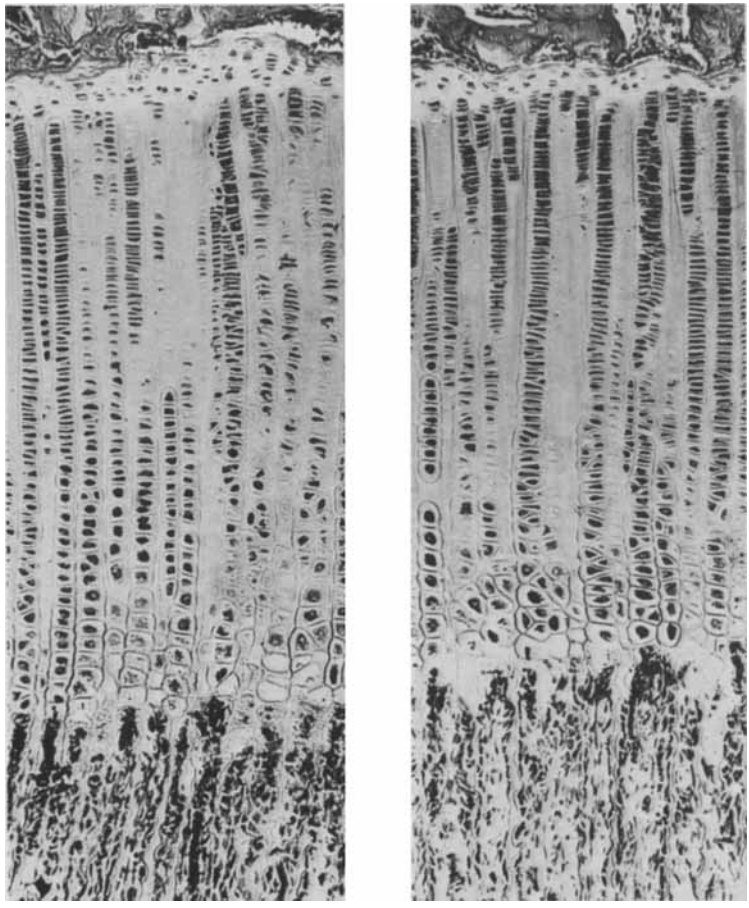

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Fig. 26. Right and left proximal growth plate of tibia 6 days after medullary plugging (115×).

During the following days, on the other hand, there was some difference between the sides. Four days after the operation the cell columns were 36 ± 28 μ shorter on the operated side. This difference was 5.6 % of the length of the cell columns on the control side. Two days later the difference was still larger (Fig. 26). Eight days after the operation no significant difference was found, which also appeared to be the case during the subsequent period.

At 60 days after the operation a significant difference of 87 ± 19 μ was found between the sides, which corresponds to 14.9 % of the

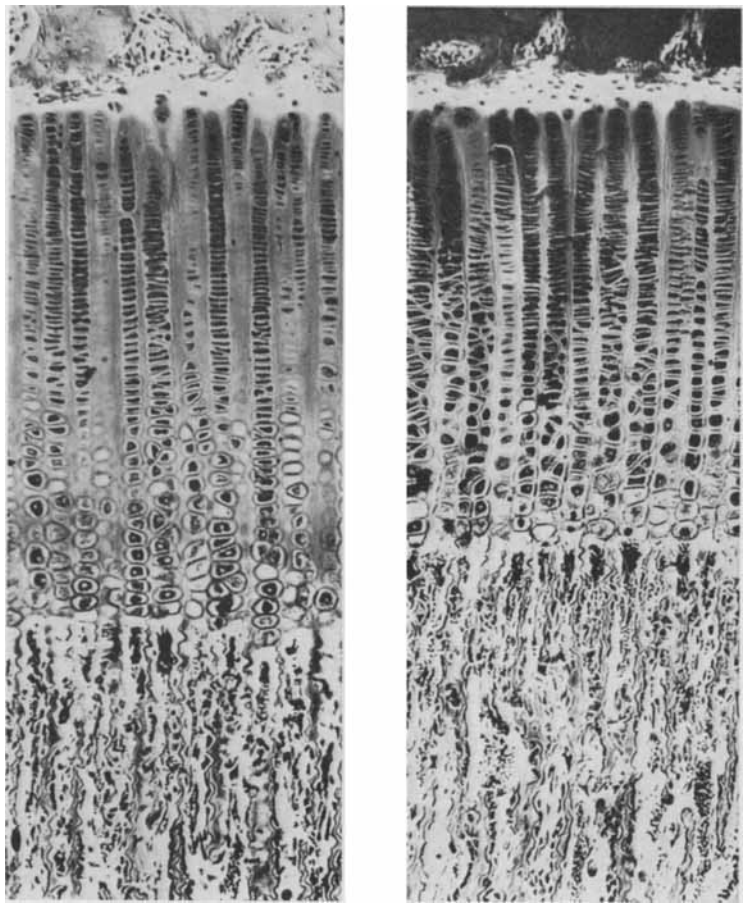

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Fig. 27. Right and left proximal growth plate of tibia 60 days after medullary plugging (115×).

length of the cell columns on the control side (Fig. 27). No significant difference was, however, found 80 and 100 days after the operation, though the difference was about 40—50 μ or 10—12 %.

Distal Tibia (Table 15 and Fig. 28)

Already 2 days after the operation a difference was found between the length of the cell columns on the two sides (Fig. 29). At this time the columns were 53±33 μ or 9.8 % longer on the operated side. During the following days the difference persisted practically unchanged.

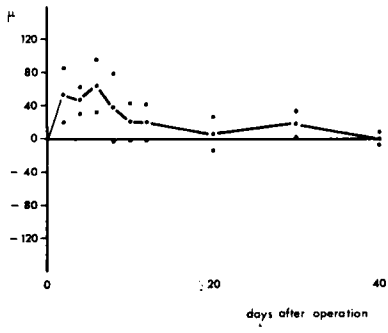

Fig. 28. Difference in length of cell columns between left and right distal growth plate of tibia after medullary plugging (Table 15).

Eight days after the operation there was still an almost significant difference. On that occasion the difference was, on the average, 38 ± 41 μ or 6.8 % of the length of the cell columns on the control side.

During the rest of the observation period until 40 days after the operation, no significant difference was found between the sides.

b. *Effect of Plugging of Metaphyseal Marrow Cavity and of Different Steps of Operation on Normal Growth Process*

In the previous section it was shown that plugging of the metaphyseal marrow cavity of the proximal tibia resulted in a difference in rate of growth and morphology between the two sides. On the other hand, it did not appear possible in that series (series A) to study the way in which the operation affected normal growth. It was therefore thought worth while to study the effect of the operation on the growth in members of the same litter rather than to compare the normal series (page 93) and the material from series A. In addition, it appeared to be of interest to find out what steps of the plugging of the metaphyseal marrow cavity influenced the endochondral growth process.

For this purpose the rate of growth and the length of the cell columns were measured in litter mates not subjected to any operation and in litter mates in which the metaphyseal part of the marrow cavity had been plugged or in which only certain steps of the operation had been performed (series B).

As before, the previously mentioned differences in growth rate between the sides occurred already during the first postoperative days. The growth per day was noted during the day before operation and during the first 4 postoperative days. The length of the cell columns was measured at the end of the experimental period.

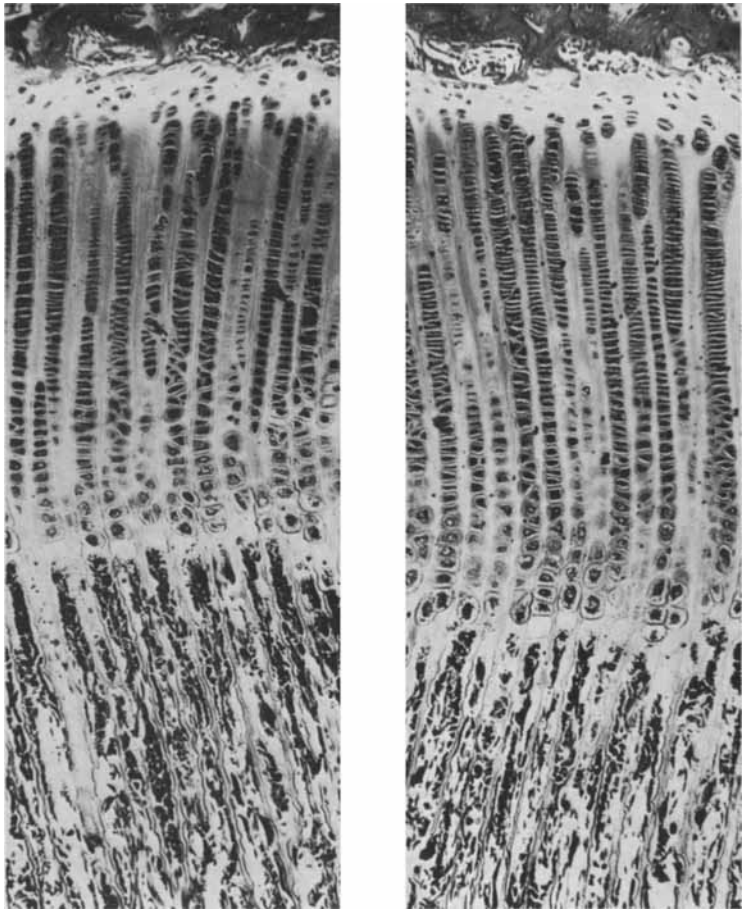

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Fig. 29. Right and left distal growth plate of tibia 2 days after medullary plugging (115×).

The normal rate of growth was calculated from the values found in the unoperated animals (Table 16) and on the day before the operation in the operated animals (Tables 17--22). This provided a possibility of calculation the effect of the operation on the normal rate of growth in different growth regions in animals subjected to operations.

But this could not be done regarding the length of the cell columns and therefore the length of the cell columns was measured at the end of the experimental period in operated animals and compared with the length found at the same time in the normal material (Tables 23 and 24).

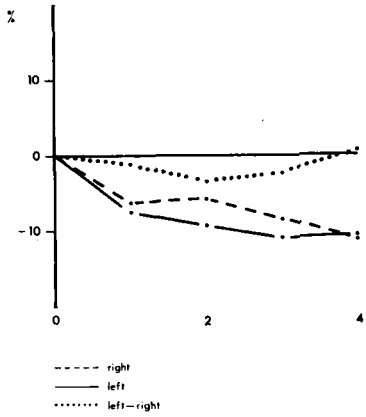

Fig. 30. Effect of plugging of marrow cavity on normal growth per day from proximal growth plate of tibia (Table 17).

PLUGGING OF METAPHYSEAL MARROW CAVITY

Rate of Growth (Tables 17 A and B)

Proximal Tibia (Fig. 30)

Control side: The rate of growth in this growth region was definitely affected by the operation. During the first day after the operation growth was, on the average, 36 μ, or 6.3 %, less than normal. During the following day the rate of growth was roughly the same as that on the first day. During the rest of the experimental period growth was more retarded. The retardation was about 63 μ (11.3 %) on the fourth day after the operation.

Operated side: In this growth region daily growth was also markedly retarded during the postoperative observation period. The reduction was about 40—60 μ per day, or 7—11 % of the normal growth per day.

On comparison between the control side and the operated side the growth per day was reduced by practically the same amount, except for the second day when the reduction was greater on the operated side. On that day this difference between the sides was only 3.5 % of the normal growth.

Proximal Fibula (Fig. 31)

Control side: The growth per day from the growth plate on the control side was definitely reduced during the postoperative period. This reduction was least on the second day, when it was about 34 μ, or 7.1 %, while on the fourth day it was about 66 μ or 13.9 %, of normal for that day.

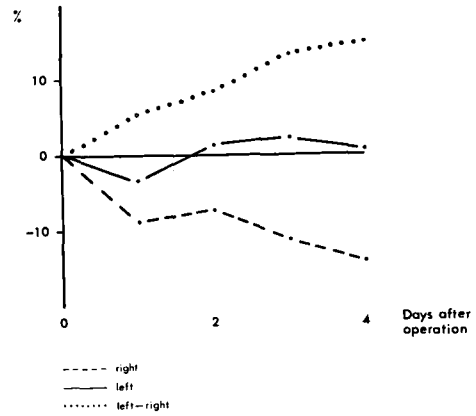

Fig. 31. Effect of plugging of marrow cavity on normal growth per day from proximal growth plate of fibula (Table 17).

Operated side: During the first postoperative day the reduction in this growth area was, on the average, 17 μ (3.4 %). During the remaining days growth was normal.

The above-mentioned varying growth resulted in a difference between the sides, increasing from about 27 μ (5.5 %) during the first day to about 70 μ (14.8 %) on the last day.

Distal Fibula (Fig. 32)

Control side: The operation caused a marked reduction in the growth per day in this growth region. The reduction was, on the average, 51—71 μ per day, and was least marked during the second day and most marked on the fourth when it was 14.6 % of normal.

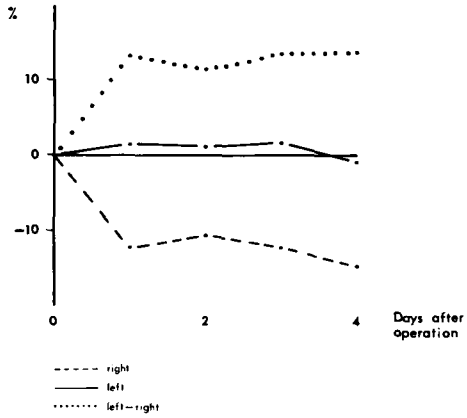

Fig. 32. Effect of plugging of marrow cavity on normal growth per day from distal growth plate of fibula (Table 17).

Operated side: In this growth region there was no definite change in the growth per day during the postoperative period.

On comparison between the control side and the operated side the growth per day was found to be about 57—67 μ higher on the operated side. This difference was due to the retarded growth on the control side and the practically unchanged rate of growth on the operated side.

Distal Radius

During the first postoperative day the growth was in average 26 μ (5.3 %) less than normal. On the following days retardation was somewhat more marked and was about 60 μ (12.5 %) on the fourth day after the operation.

Length of Cell Columns

Proximal Tibia (Table 23)

At the end of the experimental period the length of the cell columns in the growth plate on the control side in the operated animals was 590 ± 59 μ, while the corresponding value in a normal series at that time was 687 ± 21 μ. The difference, which was 14.2 % of the normal length of the columns, was statistically significant.

Also the length of the cell columns on the operated side was reduced. They were about 109 μ or 15.8 % shorter than in the normal material.

In the operated animals no significant difference was found in the length of the columns between the control and the operated side.

Distal Radius (Table 24)

Also in the growth plate of the distal radius the cell columns in the operated animals were shorter than in the normal series. The difference, about 53 μ (8.2 %), was almost statistically significant.

DESTRUCTION OF METAPHYSEAL MARROW CAVITY

Rate of Growth (Table 18 A and B)

Proximal Tibia (Fig. 33)

Control side: This type of operation also caused a reduction in the rate of growth from the growth plate on the control side. During the

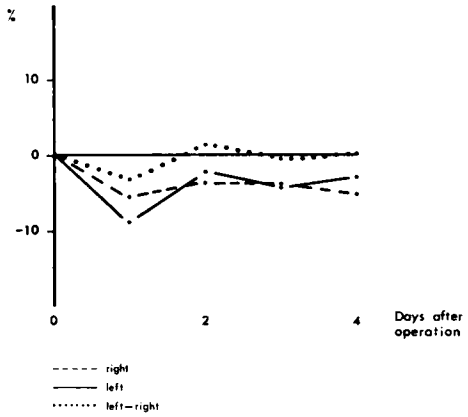

Fig. 33. Effect of destruction of marrow cavity on normal growth per day from proximal growth plate of tibia (Table 18).

first postoperative day the growth decreased about 30 μ more than normally. This reduction corresponded to 5.5 % of the normal rate growth. During the following days the reduction varied between about 19—27 μ (3.5—5.1 %).

Operated side: During the first postoperative day the growth was, on the average, 47 μ (8.8 %) less than normal. On the following days the retardation was less marked and varied between about 11—23 μ (2.1—4.3 %), of which only the latter value was almost significant.

On comparison between the control side and the operated side it was found that the operation had not caused any significant difference in the rate of growth.

Proximal Fibula

Control side: The rate of growth from this growth plate was also reduced after the operation. The reduction was about 18—28 μ per day, which corresponded to 4.1—6.4 % of normal.

Operated side: In this region the rate of growth was higher than under normal conditions. During the first day the average growth was only 12 μ (2.7 %) more than normal, but the difference increased during the following days and on the fourth day it was about 42 μ (9.6 %).

The difference in rate of growth between the two sides was marked and increased during the observation period. This difference was, on the average, 70 μ on the fourth day which corresponded to 16.0 % of the normal growth for that day.

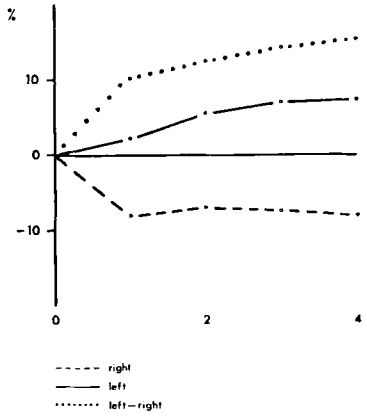

Fig. 34. Effect of destruction of marrow cavity on normal growth per day from distal growth plate of fibula (Table 18).

Distal Fibula (Fig. 34)

Control side: In this growth region the rate of growth was reduced during the postoperative period. It was, on the average, 30—40 μ per day and was practically the same on all of the days.

Operated side: The rate of growth from the growth plate on the operated side was reversed to that on the control side, *i.e.* it was higher than normal. During the first day growth was only about 10 μ (2.2 %) higher than normal, but this difference became more and more marked during the following days. On the fourth day it was thus, on the average, 33 μ which corresponded to an increase of the normal growth on that day by 7.3 %.

The rate of growth per day on the operated side was thus much higher than on the control side. The difference between the sides on the first day after the operation was, on the average, 48 μ, which corresponded to 10.2 % of the normal growth. During the following days the differences between the sides increased and on the fourth day it was about 70 μ (15.5 %).

Distal Radius

The operation was also followed by a distinct reduction in the rate of growth from this growth plate. This reduction was, on the average, 29—36 μ per day, which corresponded to 6.1—7.7 % of normal.

Length of Cell Columns

Proximal Tibia (Table 23)

The operation affected not only the rate of growth, but also the length of the cell columns on the control side. In this growth region

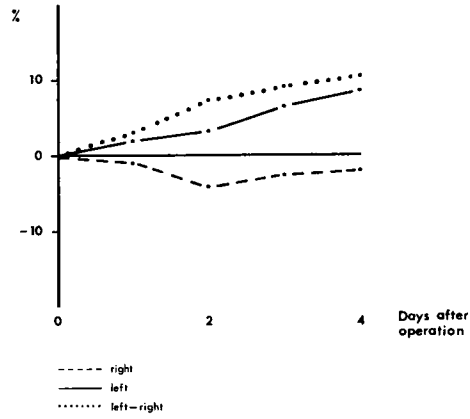

Fig. 35. Effect of drilling of corticalis on normal growth per day from proximal growth plate of tibia (Table 19).

the cell columns were in average 80 μ (11.6 %) shorter than normal. On the operated side there was a reduction in the length of the cell columns by about 87 μ (12.6 %), and there was no significant difference between the operated and the control sides.

Distal Radius (Table 24)

In this growth region the length of the cell columns was significantly reduced. The difference in this case was about 69 μ, which corresponds to 10.7 % of the normal length of the cell columns.

DRILLING OF METAPHYSEAL CORTICAL BONE

Rate of Growth (Tables 19 A and B)

Proximal Tibia (Fig. 35)

Control side: This operation affected the rate of growth in this growth region to a less extent than the previous types of intervention. During the postoperative observation period the rate of growth was significantly reduced only on the second day. The reduction was about 21 μ or 4.2 % of the normal for that day.

Operated side: The rate of growth in this growth region was higher than normal. On the first day the difference was insignificant but on the last day the increase was about 43 μ (8.6 %).

This type of operation was followed by an increasing difference between the sides, because the rate of growth on the control side was retarded, while that on the operated side was increased. The above-

mentioned difference between the sides increased during the observation period and on the fourth day it was, on the average, 53 μ, which corresponded to 10.5 % of the normal rate for that day.

Proximal Fibula

Control side: In this growth region the rate of growth was slightly reduced after the operation.

Operated side: The operation affected the rate of growth also in this region. On the first day the rate of growth was practically normal. On the fourth day the rate of growth was, on the average, 52 μ higher than normal.

For all days a significantly higher rate of growth per day was found from the growth plate on the operated side. The difference increased from about 30 μ on the first day to about 68 μ on the fourth.

Distal Fibula (Fig. 36)

Control side: During the first three postoperative days the rate of growth per day was significantly lower in this region. During this period the rate of growth was retarded by about 25 μ per day, which corresponded to about 6 % of the normal rate. On the fourth day the rate of growth was not significantly reduced.

Operated side: In this growth region the rate of growth per day was higher than normal on the second day of observation. The difference was most marked on the fourth day when it averaged 40 μ or 9.5 % of the normal rate for that day.

These changes gave rise to an increasing difference in rate of growth between the sides in the operated animals. On the first day it was, on the average, 33 μ higher on the operated side, while on the fourth it was about 58 μ, which corresponded to 13.5 % of the normal for that day.

Distal Radius

The operation also affected the rate of growth of the anterior limbs of the animals to a certain extent. The rate of growth in the distal radius was lower than normal on the first postoperative days.

Length of Cell Columns

Proximal Tibia (Table 23)

The growth plate on the control side after this operation showed a reduction in length of the cell columns by about 44 μ or 6.4 %, but this difference was not significant.

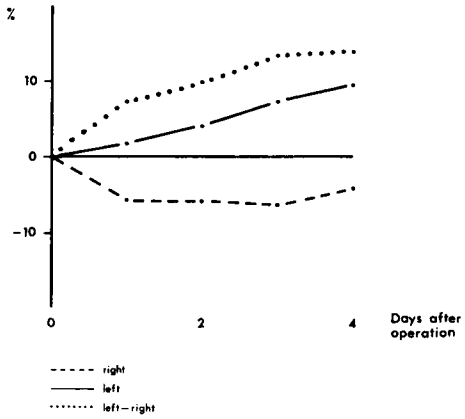

Fig. 36. Effect of drilling of corticalis on normal growth per day from distal growth plate of fibula (Table 19).

In contrast to what was seen after the previously mentioned operations this type of operation was not followed by any disturbance of the endochondral calcification and ossification process in the central part of the growth region on the operated side. The cell columns on the operated side appeared to be somewhat shorter than normal, but this difference was not significant.

Neither was any significant difference found between the control side and the operated side, though the cell columns were some tens of microns longer in the latter growth region.

Distal Radius (Table 24)

In the operated animals the cell columns at the end of the observation period were, on the average, 43 μ (6.8 %) shorter than normal. This difference was almost significant.

INCISION OF METAPHYSEAL PERIOSTEUM

Rate of Growth (Tables 20 A and B)

Proximal Tibia (Fig. 37)

Control side: In this growth region the rate of growth on the first day after the operation was significantly lower than normal. On the following days it was practically normal.

Operated side: Also in this growth region the rate of growth was significantly reduced on the first day after the operation. During the following days it became normal and was probably somewhat higher than normal on the third postoperative day.

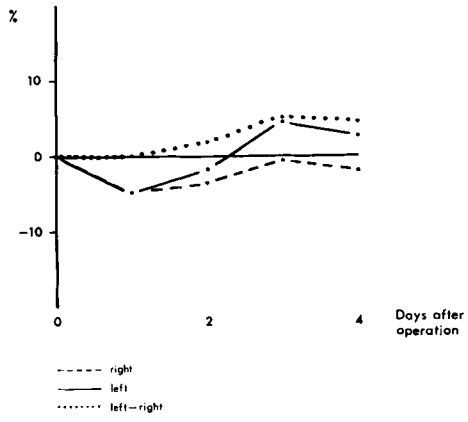

Fig. 37. Effect of periosteal incision on normal growth per day from proximal growth plate of tibia (Table 20).

Though the difference was, on the average, 25 μ (5 %) per day on the last two days of the observation period, the difference was not statistically significant.

Proximal Fibula

Control side: During the first postoperative day the rate of growth in this growth region was lower than normal. The reduction on that day was about 22 μ or 5.0 %. During the following day no significant change in the rate of growth was observed.

Operated side: The rate of growth from the growth plate on the operated side varied considerably, and during the first day it was somewhat lower than normal and on the following days significantly higher.

On comparison between the control side and the operated side it was found that the rate of growth during the postoperative period was probably higher in the latter growth region than in the former. The largest difference between the sides per day was, on the average, 27 μ or 6.1 % of normal which occurred on the last two days.

Distal Fibula (Fig. 38)

Control side: The rate of growth in this growth region was some tens of microns less than normal on the first two postoperative days. During the rest of the observation period, however, no reduction was seen.

Operated side: In contrast to what was seen on the control side, in this growth region it was not possible to demonstrate any significant change in the rate of growth.

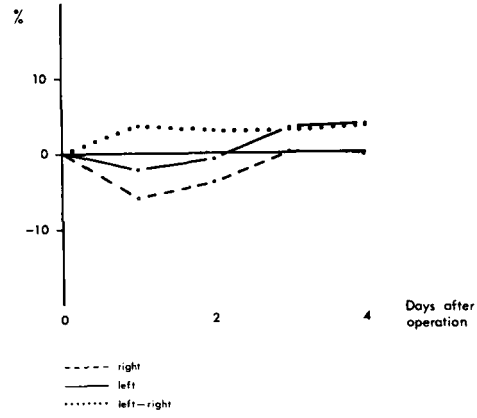

Fig. 38. Effect of periosteal incision on normal growth per day from distal growth plate of fibula (Table 20).

Therefore, during the postoperative period the rate of growth was somewhat higher on the operated side than on the control side, but the difference was statistically significant only for the first day after the operation.

Distal Radius

In this growth region the rate of growth was affected by the operation in largely the same way as in the growth regions of the tibia and fibula on the control side. The rate of growth on the first days was thus significantly lower than normal, after which it was largely normal.

Length of Cell Columns

Proximal Tibia (Table 23)

The length of the cell columns in the growth plates in the operated and unoperated side was the same as in the normal material.

Distal Radius (Table 24)

The cell columns in the growth plate of the distal radius were practically of the same length as normally.

INCISION OF SKIN AND ANAESTHESIA

Rate of Growth (Tables 21 A and B, 22 A and B)

Proximal Tibia, Proximal Fibula, Distal Fibula and Distal Radius

During the postoperative observation period no differences were found in the rate of growth from the above-mentioned growth regions

after skin incision. The anaesthesia during the operation had no effect on the rate of growth either.

Length of Cell Columns

Proximal Tibia and Distal Radius (Tables 23 and 24)

The length of the cell columns in the two above-mentioned growth regions in normals was found to be 687 ± 21 and 640 ± 28 μ, respectively, at the end of the observation period. The corresponding values in the two groups of experimental animals after skin incision and after anaesthesia only did not differ significantly from those found in the normals.

3. DISCUSSION

The investigation clearly showed that plugging of the marrow cavity influences endochondral growth, an observation in agreement with previous investigations (Trueta 1953, 1958, Ståhl 1957, Langenskiöld 1957, Contessa 1959, Taillard and Morscher 1965).

Investigations of the effect of periosteal stripping of the proximal part of the tibia (Brodin 1955) or the same operation combined with subperiosteal implantation of a skin graft (Elo 1960), showed practically the same effect of the operation on the growth regions of the proximal tibia and the distal tibia as that observed in the present investigation. In the above-mentioned investigations, however, the roentgenological method of determination did not allow close analysis of the growth process.

In the present investigation medullary plugging in the proximal tibia increased the rate of growth of the tibial diaphysis during the first week after the operation on the operated side, compared with that on the control side. During the rest of the observation period the difference was much less. The total postoperative difference between the sides in growth thus varied with the duration of the observation period. The difference between the sides was about 0.4 mm already after the first postoperative week and then varied during the rest of the observation period between 0.2—0.7 mm.

This in agreement with what has been reported after periosteal stripping (Lacroix 1947, 1951, Brodin 1955, Langenskiöld 1957, Elo 1960) and traumatisation of the marrow cavity (Langenskiöld 1957, Elo 1960) in the proximal part of the tibial diaphysis.

Previous investigations of the effect of implantation of different sub-

stances into the diaphyseal marrow cavity have, as mentioned in the survey of the literature, given contradictory results. These discrepancies between the results can probably be ascribed to different factors.

Thus the error of the methods used previously in the determination of growth is more or less equal to the difference recorded between the sides. Therefore the results are uncertain.

In a large number of investigations (Silfverskiöld 1934, Bisgard and Bisgard 1935, Gill and Abbott 1942, Sissons 1953, Brodin 1955, Heikel 1960, Elo 1960, Ryöppy 1965) of both normal growth and growth after different types of operations, growth from different regions of shaft bones has been calculated with the aid of radiopaque indicators implanted into the cortical bone of the diaphysis. Such implantation is believed usually not to influence the growth process to any appreciable extent (Bisgard and Bisgard 1935, Brodin 1955, Elo 1960, Ryöppy 1965). However, several investigators have observed that the implanted radiopaque marker was inclined to migrate (Silfverskiöld 1934, Bisgard and Bisgard 1935, Ryöppy 1965). In the present investigation it was found that traumatisation of the periosteum and of the cortical bone influenced the rate of growth both locally and generally. It is therefore probable that the use of radiopaque indicators for determining growth usually affected the rate of growth and possibly also contributed to the difference between the sides observed after so-called growth stimulating operations. It should, however, be mentioned that in these last-mentioned investigations radiopaque indicators were usually implanted both on the control side and the operated side, so that the effect on growth was probably the same on both sides.

The discrepancies between the results may be ascribed to differences in the intervals between the operation and examination of the effect of the operation. In the present investigation it was found that the difference between the control side and the operated side varied considerably with the interval after the operation, an observation made also by Brodin (1955) and Elo (1960) after periosteal stripping as well as by Blount (1955) and Goff (1960) after diaphyseal fractures in infants.

Previous investigations have been carried out on human beings and experimental animals of different ages. These differences may also have contributed to the discrepancies between the results. According to several investigations (Compere and Adams 1937, Goff 1960, Elo 1960, Tupman 1960, Taillard and Morscher 1965) the effect of the

operation is probably more marked in young than in older growing material.

The extent of the operation as well as its site has also varied from one investigation to another, and according to Elo (1960), influences the effect of the operation.

In many cases where a higher rate of growth has been noted on the operated side than on the control side after implantation of different substances into the marrow cavity, it is not possible to exclude subsequent chronic inflammation in the operative region owing to implantation of bacteria or irritating substances into the marrow cavity.

Analysis of the literature also revealed that the effect of a so-called growth stimulating operation as seen in experimental animals is not always the same as that seen in man. This is probably because in most cases the growth rate in the experimental animals is normal on both the operated and control side before the operation, while in human beings the operation is usually made in a bone already impaired.

As mentioned in the survey of the literature, it has been observed that plugging of the marrow cavity at the level of the nutrient canal with simultaneous destruction of the nutrient artery results in an increase in the rate of growth on the operated side compared with that on the other, while destruction of the nutrient artery only produces no such effect (Ollier 1867, Wu and Miltner 1937, Trueta 1953, 1958, Brookes 1957). The cause of the increase in growth in the former case was believed to be due to the blood supply to the marrow cavity being forced to flow via the metaphyseal perforating arteries for a long time with stimulation of growth as a result (Trueta 1953, 1958, Chigot 1958, Taillard and Morscher 1965).

In the light of the results obtained in the present investigation and in previous studies by Brodin (1955) and Elo (1960) the above-mentioned explanation of the development of the difference in rate of growth appears improbable. It was found in the present investigation that destruction of the vessels in the marrow cavity in the proximal part of the tibial diaphysis with simultaneous plugging produced no increase in the rate of growth from the proximal growth plate of the tibia on the operated side compared with that on the control side except during the latter part of the first week. The situation during the following period was the opposite, with the result that the total postoperative growth at the end of the observation period, was about some hundreds of microns less on the operated side. In the growth region of the distal tibia the situation was reversed and the difference

between the sides more pronounced. A similar reaction in the proximal and distal growth regions of tibia was observed also after destruction only of the vessels of the marrow cavity in the same part of the diaphysis.

When the operation comprised only traumatisation of the cortical bone and the vessels in the marrow cavity were left intact, the rate of growth from the distal and proximal growth plates of the tibia was increased, which suggests that the vessels must be intact if an operation is to produce a higher rate of growth from the growth plate on the operated side compared with that on the other side. This agrees well with the results of previous investigations by Brodin (1955) and Elo (1960).

The present investigation was not intended to elucidate the fate of the implanted homologous bone tissue, which will therefore not be discussed here. It appears probable, however, that there is no correlation between the effect on the rate of growth and the resorption of the transplant because the transplant is resorbed slowly and not eliminated until after about a month. This has recently been demonstrated by Puranen (1966) who used the tetracycline method in his investigation.

Also the growth from the proximal growth plate of the fibula is influenced by plugging of the marrow cavity in the proximal metaphysis of the tibia. In this growth region the rate of growth during the first postoperative week was higher on the operated side than on the control side. It was evident that the difference in growth per day between the two sides reached its maximum later than in the distal tibio-fibula, though the maximal difference between the sides appeared to be about the same. During the rest of the observation period the difference was insignificant and the rate of growth was usually somewhat higher on the control side.

The operation on the tibia results in the diaphyseal part of the fibula increasing about 0.6 mm more in length on the operated side than on the control side during the first postoperative week. During the following period this difference is probably reduced at first, and later slightly increases, since the gain in growth distally is probably not compensated entirely by the loss proximally.

The literature contains no investigation of the mode of action of a tibial operation on the growth of the fibula. On the other hand, it has been observed (Levander 1929, Compere and Adams 1937, Pease 1952, Blount 1955, Wray and Goodman 1961, Guldhammer 1963) that trau-

matisation of the tibia or femur usually results in an increased rate of growth of the femur and tibia, respectively, of the traumatised limb.

Elo (1960) found a distinct correlation between the degree of traumatisation and the difference in growth between the two sides, which was also found in the present investigation. In those cases where the vessels of the growth region were intact the difference in rate of growth during the days following after a more traumatising operation is greater and lasts longer than after a less traumatising one. In addition, it also appears that the difference in rate of growth during the first day is more pronounced in the distal tibio-fibula than in the proximal fibula.

The operations resulting in a higher rate of growth on the operated side than on the control side are known in the literature as growth stimulating and it has been assumed that these operations increase the rate of growth on the operated side without affecting other growth regions. It has previously, however, been observed that severe trauma such as a diaphyseal fracture influences the rate of growth both in the traumatised and the opposite limb (Wray and Goodman 1961, Guldhammer 1963, Lacroix 1964). No such observations have been reported in the literature after so-called growth stimulating operations.

In the present investigation it was found that unilateral plugging of the marrow cavity of tibia caused a reduction in the rate of growth during the first days after the operation, not only in the control side but also in the distal radius. This retarding effect appeared to be almost equally large in all growth regions and reached 10—15 % of the normal rate of growth.

The retarding effect is somewhat less pronounced in those cases where the operation consisted only of destruction of the marrow cavity, probably because this operation is less traumatising.

Also drilling of the cortical bone and, though to a less extent, incision of the periosteum cause retardation of growth, which, however, is less pronounced than after the previously mentioned operations. On the other hand, incision of the skin or anaesthesia had no effect on the rate of growth.

Wray and Goodman (1961) observed that a closed fracture of the tibia caused a retardation of the rate of growth of the femur on the opposite side during the first day after the trauma and that the rate of growth during the 2nd and 3rd week was higher than normal. The last-mentioned effect was also found in the present investigation on comparison with normal material (see page 93) and series A. This

observation appears to be uncertain, however, because the two materials are probably not strictly comparable (see page 100).

On the operated side the effect varied more widely with the type of operation and with the site of the growth region in relation to that of the operation area.

Thus, it was observed that the rate of growth from the growth plate of the proximal tibia was retarded, like that on the opposite side the first few days after plugging of the marrow cavity and after destruction of the marrow cavity. On the other hand, drilling of the cortical bone and, to a less extent, incision of the periosteum resulted in a postoperative increase in the rate of growth except during the first day.

The rate of growth from the growth region of the distal tibio-fibula, unlike that in the proximal fibula, was not retarded during the first postoperative day but was normal or even higher than normal. This explains why the difference in growth per day between the sides the first few days after the operation was more marked for the distal tibiofibula than for the proximal fibula.

The morphology of the growth regions was also influenced by the operation on the shaft bone in largely the same way as the rate of growth. The cell columns in the proximal growth plate of the tibia during the first few days after marrow plugging as well as after destruction of the marrow cavity in the proximal part of the tibial diaphysis were somewhat shorter on the operated side than on the control side at the same time as the rate of growth was the same or even lower on the operated side. This suggests that mitotic activity was lower on the operated side than on the control side the first few days after operation.

The difference between the sides disappeared after the first week, which suggests that the mitotic activity during the latter part of the first week was higher on the operated side. In addition, the length of the cell columns as well as the rate of growth was reduced on the operated side compared with the contralateral side 60 days after the operation, so that cell production was probably lower in the former growth region.

In the distal tibia the situation after marrow plugging was somewhat different. In this growth region the mitotic activity was probably higher on the operated side since the rate of growth during the first postoperative week was higher and the cell columns were longer. During the following period up to the 40th day after the operation no difference between the sides was found. This was probably because the

effect of the operation on the growth process during this period was small.

Comparison with a normal material (Group 1) revealed that marrow plugging caused a considerable reduction in the length of the cell columns both in the proximal tibia on the unoperated side and in the distal radius during the first few days after the operation. Since the rate of growth was at the same time significantly reduced this must mean a marked reduction of cell production. The situation was similar also in the proximal growth plate of the tibia on the operated side. A corresponding reaction probably also occurred after only destruction of the marrow cavity.

The effect of the operation on the length of the cell columns was insignificant after drilling of the cortical bone as well as after incision of the periosteum. This was, however, examined 4 days after the operation; therefore it cannot be excluded that it had some effect on the length of the cell columns the first days after the operation.

Only few investigations have previously reported the effect of so-called growth stimulating operations on the morphology of the growth regions. Elo (1960) appears to be the only one who measured growth from different growth regions and examined the regions histologically. But his investigation was, however, not done until 10 weeks after periosteal stripping with simultaneous implantation of a skin graft subperiosteally in the proximal part of the tibial diaphysis. He could not show any significant difference between the sides regarding morphology of the growth plates in the proximal and distal tibia, which may be explained by the fact that the difference in the rate of growth between the sides after this interval after the above-mentioned operation was insignificant.

Kishikawa (1936) found that the proximal growth plate of the tibia to be thinner on the operated side than on the control side after drilling of the proximal part of the tibial diaphysis, an observation also made by later investigators (Hansson and Wiberg 1963, Wiberg 1964). According to Kishikawa this thinning was due to "Verdünnung der hydropischen Zone und primären Balkenschicht" and was observed also after other so-called growth stimulating operations. On closer analysis of Kishikawa's report it was found that the differences described between the morphology of the growth regions on either side as well as the differences in growth were slight and were noted at widely varying intervals after the operation.

A similar reduction of the thickness of the growth plate has, as men-

tioned, also been described in previous investigations (Hansson and Wiberg 1963, Wiberg 1964), but this reduction was confined mainly to the zone of proliferative cells, while the more metaphyseal part did not appear to be appreciably reduced. The difference between the sides was most marked during the first few days after drilling and was usually seen during the first week. It was, however, not possible to decide whether the difference between the sides was due to an increased or reduced rate of growth on the operated side, since only the total postoperative difference in length between the sides was recorded.

The results obtained in the last-mentioned investigations on the effect of drilling agree well with those found in the present investigation after plugging or destruction of the marrow cavity in the proximal part of tibia.

There thus appears to be a direct correlation between the morphology of the growth region and the rate of growth also after so-called growth stimulating operations, which also has been shown previously under other conditions (Harris 1933, Asling et al. 1950, Sissons 1953, Johnson 1964).

A cartilaginous growth zone subjected to a so-called growth stimulating operation or some other form of trauma is claimed to close earlier than that on the opposite side (Blount 1955, Goff 1960). This is probably not because of an increased rate of growth in this growth region, since the rate of growth from the proximal growth plate of the tibia after marrow plugging in the adjacent metaphysis proved to be lower on the operated side than on the control side 100 days after the operation at the same time as the cell columns on the operated side were somewhat shorter. At this time, *i.e.* when the animals were 130 days old, the major part of the growth period had elapsed.

In previous investigations (Trueta 1958, Trueta and Amato 1960, Trueta 1963, Hansson and Wiberg 1963, Wiberg 1964), as in the present one, a tongue of cartilage was seen in the metaphysis after traumatisation of the metaphyseal vessels. This tongue developed because the destruction of the degenerative cartilage cells did not proceed at a normal rate. Owing to the simultaneous new-formation of cells further epiphyseally in the growth plate the cell columns increased in length. During the following days the destruction of the degenerative cells again increased as the destroyed metaphyseal vessels regenerated. The resorption of the cartilage cells proceeded quicker than the new-formation of the cartilage cells and the tongue of cartilage disappeared 6—10 days after the operation.

The variation in the orientation of the cell columns and the metaphyseal trabeculae was due to the cells of the tongue developing slower than the corresponding cartilage cells in the more peripheral parts of the growth plate.

Opinions differ on the cause of the difference in growth between the sides after a so-called growth stimulating operation. The difference is usually ascribed to interruption of the metaphyseal blood flow (Ferguson 1933), active hyperemia (Levander 1929, Kishikawa 1936, Bisgard 1936, Bertrand and Trillat 1948, Janes and Musgrove 1950, Trueta 1953, 1958, Brodin 1955, Compere 1958, Hiertonn 1961, Solá et al. 1963, Woodhouse 1963) or passive hyperemia (Hutchinson and Burdeaux 1954, Hiertonn 1961, Vanderhoeft and Kelly 1964, Keck and Kelly 1965). Other causes have also been suggested (Lacroix 1947, 1951, Ring and Ward 1958, Taillard 1959, Ring 1961, Hiertonn 1961, Wray and Goodman 1961).

It appears most probable that operations on shaft bones result in an increased blood flow in the epiphyseal, metaphyseal and perichondral vessels, with a higher postoperative rate of growth on the operated side as a result. Sundén (1967) thus observed a direct correlation between the difference between the sides in rate of growth and in blood flow. The same correlation has been observed previously by less refined and less reliable methods (Levander 1929, Brodin 1955, Wray and Lynch 1959, Hulth and Olerud 1960, 1961 a and b, Wray and Spencer 1960, Wray 1961, Wray and Goodman 1961, Taillard and Morscher 1965, Yabsley and Harris 1965).

Thus much suggests that the blood flow on the operated side is larger than on the control side the first week after marrow plugging, destruction of the marrow cavity as well as after drilling of the cortical bone in growth regions with intact vessels. The difference between the sides after periosteal incision is probably shorter and less marked.

It therefore appears less likely that plugging of the marrow cavity in the proximal tibia increases the blood flow in proximal tibia through the metaphyseal perforating arteries — in contrast to what has been claimed in previous investigations (Trueta 1953, 1958, Chigot 1958, Taillard and Morscher 1965) — because the initial difference in rate of growth between the sides was insignificant in the growth region whose metaphyseal vessels were destroyed while the rate of growth from the other growth regions with intact vessels on the operated side was much higher than on the control side. The situation is probably similar also after periosteal stripping, with injury of the metaphyseal

perforating arteries but not of the metaphyseal vessels from the nutrient artery, unless this vessel has been damaged in association with the operation.

Wray and Goodman (1961) appear to be the only ones who have previously tried to find out whether traumatisation also influences the growth regions on the control side. In their investigation they found that operative traumatisation of a shaft bone not only retarded growth from the growth region on the fractured side but also on the contralateral side. This can probably be explained by the assumption that an operation on a shaft bone causes a general impairment of the blood flow, which has been shown previously during the first week after severe traumatisation of a shaft bone (Gelin 1956, Bergentz 1961). This effect probably occurs also in the operated limb, but it is compensated there, entirely or partly, by local hyperemia.

It is thus clear from the observations in this investigation that it is questionable whether the so-called growth stimulating operations which cause a higher rate of growth on the operated side than on the control side can really be regarded as growth stimulating, because the difference between the sides after these operations is due to a larger extent to retardation of growth on the unoperated side than to stimulation on the operated side. In addition, the rate of growth during certain periods after these operations is higher on the operated side and during other periods on the control side.

4. SUMMARY

This part of the investigation was concerned with the effect of a so-called growth stimulating operation on the growth from different growth plates and on their morphology. The experimental animals, *i.e.* young rabbits, were divided into two series (A and B). The animals belonging to series A were operated upon at 30 days of age, the operation comprising plugging of the marrow cavity in the proximal metaphysis of the tibia with homologous bone tissue.

The animals belonging to series B were distributed among seven groups, one of which served as a control group and was not subjected to any intervention, while other groups were subjected to different types of treatment at 30 days of age. The treatment in each group consisted of one or more steps of the operation and ranged from anaesthesia alone to plugging of the marrow cavity.

The growth per day from different growth plates was determined

with the aid of OTC in the way described previously during a four (Series A) or five (Series B) day period, and the length of the cell columns in the growth plates was studied in the way described previously. The observation period for series A ranged from 2 days before to 100 days after the operation and for series B from 1 day before to 4 days after the operation.

It was found that medullary plugging of the proximal metaphysis of the tibia had a marked and regular effect on the rate of growth and on the length of the cell columns, and that this effect varied with growth region and with the interval after the operation.

The rate of growth from the proximal growth plate of the tibia during the first few days was practically the same on the operated side as on the control side, after which there was a temporary increase in the rate of growth on the operated side. During the subsequent period the rate of growth was either higher on the control side or equal on both sides.

During the first week the rate of growth from the proximal growth plate of the fibula was higher on the operated side, while during the rest of the observation period it was equal on both sides or even lower on the operated side during short periods.

The rate of growth during the first week after the operation from the distal tibio-fibula was much higher on the operated side, after which it was higher on the unoperated side. During the latter part of the observation period the rate of growth was lower on the unoperated side.

During the first week the cell columns in the proximal growth plate of the tibia were somewhat shorter on the operated side, which was also the case during the latter part of the observation period. In the growth plate of the distal tibia the situation was different. During the first week the cell columns were longer on the operated side, after which (up to 40 days) they were equally long on both sides.

In the second part of this study (Series B) attempts were made to find out the way in which these differences in rate of growth and length of the columns between the sides developed after marrow plugging.

It was found that medullary plugging of the proximal metaphysis of the tibia caused a marked retardation of the rate of growth from the proximal growth plate of the tibia on the operated side during the first few days after the operation. The rate of growth from the proximal growth plate of the fibula was also retarded the first day, but then became normal. During this period the rate of growth from the growth

plates of the distal tibio-fibula was normal. The rate of growth from other growth plates outside the operated limb was retarded the first days after the operation.

The same reaction, but less pronounced, was found also after destruction of the marrow cavity. The rate of growth from the growth plates of the proximal fibula and distal tibio-fibula on the operated side was then higher than normal except during the first day.

Drilling of the cortical bone was followed by an increase in the rate of growth from the growth plates on the operated side for the first few days after the operation, while the rate of growth from other growth plates was somewhat retarded during this period. A similar, but less pronounced and shorter reaction was observed after periosteal incision, while the incision of the skin, like anaesthesia, produced no demonstrable effect on the rate of growth.

The length of the cell columns varied in practically the same way as the rate of growth.

This part of the investigation thus suggests:

that a so-called growth stimulating operation on shaft bones such as medullary plugging has a generalised effect on the rate of growth from different growth plates, which is seen initially as a retardation of the rate of growth,

that the severity and the duration of the general effect vary with the severity of the trauma,

that the generalised effect is partly or entirely compensated on the operated side,

that the effect on the different growth plates on the operated side depends on the severity of trauma, the sites of the growth regions in relation to the operated area as well as the site of the trauma in relation to the vessels in the different growth regions. The length of the cell columns in different growth plates varies in practically the same way as the rate of growth.

GENERAL SUMMARY

The present investigation is concerned with intravital marking of the endochondral calcification process with oxytetracycline (Terramycin®) for determining the growth per day from different growth plates normally and after a so-called growth stimulating operation.

Chapter II reports an anlysis of the deposition of oxytetracycline in association with the endochondral calcification process in young rabbits, which were given 0.5—25 mg oxytretracycline per kg bodyweight intravenously and killed 1 minute—8 days after the injection. The deposition was studied under the fluorescence microscope in sections from material fixed in ethyl alcohol. A method was developed for the preparation of sections and care being taken to minimise the sources of error.

It was found that oxytetracycline is deposited immediately after injection in association with endochondral calcification in the zone of cartilage undergoing calcification. A fluorescent band developed which shifted further into the metaphysis and gradually lost contact with the growth plate because the latter simultaneously shifted in epiphyseal direction. The width of the fluorescent band varied with the dose given and with the rate of growth, while the intensity of the fluorescence varied not only with the dose but also with the age of the animal.

It was found that intravenous doses of 10 and 25 mg oxytetracycline per kg bodyweight often caused pathological changes in the growth regions. The frequency, extent and severity of the changes varied with the dose, rate of growth and probably also with the extent to which the growth regions were loaded.

It proved possible with the use of repeated intravenous injections of 1.0 mg oxytetracycline per kg bodyweight to determine the rate of

growth per day from different growth plates with a precision of 2—4 % of the normal rate of growth per day without any noticeable toxic effect on the rate of growth.

Chapter III is concerned with the normal rate of growth in relation to morphology in different growth regions in animals ranging in age from 20—70 days. It was found that the rate of growth from different growth plates as well as the length of the cell columns varies for different growth plates and with the age of the animals. A close correlation was found between the rate of growth and the length of the cell columns, the correlation varying with age and with growth region.

Chapter IV describes the effect of a so-called growth stimulating operation on the rate of growth and morphology of the growth regions. Most of the animals were subjected to plugging of the marrow cavity with homologous bone tissue in the proximal metaphysis of the tibia. Other experimental animals were divided into groups and subjected to only one or more steps of the operation and one group was left intact and served as controls. The growth per day from different growth plates was determined with oxytetracycline and determinations were made of the length of the cell columns.

It was found that plugging of the marrow cavity had marked and regular effect on the rate of growth and on the length of the cell columns, the effect varying with growth plate and interval after the operation.

CONCLUSIONS:

1. Oxytetracycline is deposited in association with the endochondral calcification process of shaft bones and when given in large doses it causes pathological changes.

2. Repeated marking of the endochondral calcification process with small doses of oxytetracycline provides a simple, quick and at the same time exact method for determining the rate of growth in length from different growth plates.

3. Normally, there appears to be a close correlation between the rate of growth per day and the length of the cell columns.

4. So-called growth stimulating operations on shaft bones cause both a generalised and a local effect on the rate of growth and on the length of the cell columns. The difference in growth between the sides is the result of both stimulated and retarded growth.

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TABLES

Table 1. *Methods for Measuring Growth in Length of Shaft Bone*

Type of marker	Technique	Author	Material	Error of method mm
1. no marker	x-ray	1957 Langenskiöld	rabbit	±1
„ „	„	1960 Elo	„	±0.1
„ „	„	1965 Taillard and Morscher	homo	±1
„ „	„	1966 Tapp	rat	±0.13
„ „	direct measurement	1957 Greville and Janes	dog	±1
„ „	„ „	1958 Ring and Lee	rabbit	±0.5
„ „	„ „	1961 Troupp	„	±0.5
2. anatomical landmarks	x-ray	1945 Hendryson	homo	—
„ „	„	1959 Heikel	rabbit	±0.5
„ „	direct measurement	1916 Digby	homo	—
„ „	„ „	1934 Payton	pig	—
„ „	„ „	1935 Bisgard and Bisgard	goat	—
3. transverse lines	x-ray	1933 Harris	homo	—
„ „	„	1963 Anderson et al.	„	—
„ „	„	1963 Guldhammer	„	—
4. radiopaque indicator	x-ray	1955 Brodin	rabbit	±0.3
„ „	„	1960 Goff	homo	±1.8—10
5. chemically fixed substances:				
bismuth	x-ray	1937 Caffey	homo	—
„	„	1942 Gill and Abbott	„	—
lead	x-ray	1943 Vahlquist	homo	—
„	light microscopy	1957 Amako and Honda	rabbit	—
phosphorus	x-ray	1935 Bisgard and Bisgard	goat	—
„	„	1952 Pease	homo	—
„	autoradiography	1950 Leblond et al.	rat	—
alizarin (madder)	direct measurement	1932 Payton	pig	—
„	light microscopy	1941 Aries	rat	—
tetracyclines	fluorescence microscopy	1963 Holmes	calf	—
„	„ „	1964 Hansson [1]	rabbit	±0.01—0.03
„	„ „	1966 Tapp	rat	±0.01
H^3-thymidine	autoradiography	1960 Kember	rat	—
6. destruction of metaphyseal vessels	light microscopy	1960 Trueta and Amato	rabbit	—

[1] Preliminary report of this investigation.

Table 2. *Error of Determination of Rate of Growth from Proximal Growth Plate of Tibia*
Analysis of Variance

Cause of variation	df	SS	MS	s	F
Between animals	2	38,600	—	—	—
Between right and left growth region	3	100	33.33	5.7	<1(-)
Between sections from same growth region..	24	4,300	179.17	13.4	3.25**
Between areas measured in same section ..	30	1,650	55.00	7.4	1.08(-)
Between repeated measurements in same area	60	3,050	50.83	7.1	—
Total	119	47,700	—	—	—

Mean growth rate 526 μ per day.

Table 3. *Error of Determination of Rate of Growth from Proximal Growth Plate of Fibula*
Analysis of Variance

Cause of variation	df	SS	MS	s	F
Between animals	2	32,830	—	—	—
Between right and left growth region	3	910	303.33	17.4	4.73**
Between sections from same growth region..	24	1,240	51.67	7.2	<1(-)
Between areas measured in same section ..	30	1,070	35.67	6.0	<1(-)
Between repeated measurements in same area	60	3,580	64.17	8.0	—
Total	119	39,000	—	—	—

Mean growth rate 454 μ per day.

Table 4. *Error of Determination of Rate of Growth from Distal Growth Plate of Fibula*
Analysis of Variance

Cause of variation	df	SS	MS	s	F
Between animals	2	44,650	—	—	—
Between right and left growth region	3	690	230.00	15.2	2.09(-)
Between sections from same growth region..	24	2,640	110.00	10.5	2.69**
Between areas measured in same section ..	30	1,070	35.67	6.0	<1(-)
Between repeated measurements in same area	60	2,450	40.83	6.8	—
Total	119	51,500	—	—	—

Mean growth rate 453 μ per day.

Table 5. *Rate of Growth per Day from Different Growth Plates*

Day after birth	Rate of growth from proximal growth plate (μ) M ± s		Rate of growth from distal growth plate (μ) M ± s		Rate of growth of diaphysis (μ) M ± s		Rate of growth from proximal growth plate in per cent of rate of growth of diaphysis M ± s	
Tibia								
20th	554	36	480	41	1,034	76	53.6	0.7
30th	561	30	489	43	1,050	73	53.5	0.9
40th	518	32	436	41	954	74	54.4	0.9
50th	426	28	334	19	760	46	56.1	0.7
60th	378	29	268	26	646	53	58.5	1.1
70th	362	26	234	27	596	49	60.8	1.9
Fibula								
20th	448	37	486	38	934	73	48.0	0.8
30th	479	34	489	41	968	74	49.5	0.7
40th	440	22	436	41	876	62	50.3	1.3
50th	368	22	336	26	704	47	52.3	0.7
60th	330	17	272	26	602	42	54.9	1.4
70th	332	22	234	22	566	45	58.7	1.0
Radius								
20th	170	32	444	40	614	57	27.6	3.9
30th	145	14	486	30	631	42	23.0	1.1
40th	96	15	470	39	566	39	17.0	2.7
50th	70	14	402	11	472	18	14.7	2.6
60th	34	17	358	40	392	41	8.7	4.1
70th	30	16	352	19	382	32	7.7	3.5

Table 6. *Error of Determination of Length of Cartilage Cell Columns in Proximal Growth Plate of Tibia*

Analysis of Variance

Cause of variation	df	SS	MS	s	F
a. Between animals	2	212,090	—	—	—
b. Between right and left growth region	1	120	120	11.0	$<1^{(-)}$
c. Between repeated measurements	1	≈0	≈0	≈0	$\approx 0^{(-)}$
d. Animal × side	2	7,470	3,735	61.2	11.75***
e. Animal × occasion of measurement	2	1,110	555	23.6	$1.75^{(-)}$
f. Occasion of measurement × side	1	20	20	4.5	$<1^{(-)}$
g. Animal × side × occasion of measurement	2	970	485	22.0	$1.53^{(-)}$
h. Between sections from same animal, side and occasion of measurement	48	17,440	363.33	19.1	$1.14^{(-)}$
i. Residual: between measurements in same section, animal, side and occasion of measurement	180	57,230	317.94	17.8	—
Total	239	296,450	—	—	—

Error of method calculated from b, c, d, e, f, g, h and i=18.9 μ.
Error of measurement calculated from i=17.8 μ.
Mean length of cartilage cell columns=680 μ.

Table 7. *Length of Cartilage Cell Columns in Different Growth Plates*

Age in days	Proximal growth plate (μ) M±s		Distal growth plate (μ) M±s	
Tibia				
20	670	92	—	—
30	753	66	621	18
40	709	53	—	—
50	671	52	524	43
60	612	43	—	—
70	591	35	460	10
Radius				
20	242	16	606	63
30	242	17	660	39
40	234	27	634	58
50	204	56	621	31
60	177	26	584	5
70	171	50	542	45

Table 8. *Effect of Various Factors on Normal Rate of Growth from Proximal Growth Plate of Tibia*
Analysis of Variance

Cause of variation	df	SS	MS	s	F
Between male and female animals ..	1	107.7	288.31	17.0	1.82(-)
Between litters	5	4,189.7	2,242.20	47.4	14.16***
Interaction	5	477.3	255.55	50.6	1.61(-)
Residual	24	3,800	158.33	12.6	—

MS-values corrected because experimental design was not symmetric (see Snedecor 1962).

Table 9. *Growth in Length from Proximal Growth Plate of Tibia in Series A*

Observation day	Number of animals	Growth per day in microns		Growth per day in per cent of growth of tibial diaphysis per day		Difference in growth per day between left and right		Total post-operative difference in growth in length between left and right sides
		right M±s	left M±s	right M±s	left M±s	μ M±s	% of right	μ
Day before operation								
penultimate	15	570±23	568±20	53.5±1.2	53.3±1.2	−2± 4(−)	−0.3	—
ultimate	15	561±19	563±19	53.6±1.2	53.6±1.2	2± 4(−)	0.4	—
Day after operation								
1st	15	531±24	536±21	54.7±1.2	51.4±1.2	5±12(−)	0.9	5
2nd	15	527±22	525±22	54.3±1.2	51.1±1.2	−2±12(−)	−0.4	3
3rd	15	520±28	531±33	54.0±1.6	51.1±1.5	11±16**	2.1	13
4th	15	511±30	527±31	54.1±1.7	51.6±1.7	16±14***	3.1	29
5th	15	516±27	541±36	53.1±1.1	51.8±1.5	25±25**	4.9	55
6th	15	513±28	525±30	52.7±0.8	52.2±1.4	11±14**	2.2	66
7th	15	507±37	505±36	53.0±1.2	53.2±1.3	−3±15(−)	−0.5	63
8th	15	507±39	497±41	52.9±1.4	53.4±1.3	−10± 9***	−2.0	53
9th	12	518±40	501±50	53.4±1.4	53.7±1.4	−17±14**	−3.2	37
10th	12	521±33	503±45	53.5±1.3	53.6±1.3	−18±17**	−3.5	18
11th	11	531±23	522±31	53.6±1.0	53.4±0.6	−9±14(−)	−1.7	9
12th	11	519±26	512±32	53.6±0.9	53.4±0.7	−7±13(−)	−1.4	2
13th	13	520±37	508±44	54.1±1.3	53.7±1.0	−12±15**	−2.2	−10
14th	13	515±26	506±29	54.2±1.3	53.9±0.9	−9±12**	−1.8	−19
15th	10	502±28	492±32	54.6±1.0	54.3±0.8	−10±12*	−2.0	−29
16th	10	491±17	481±19	54.6±1.0	54.2±1.1	−10±11*	−1.2	−39
17th	5	472±55	472±58	55.4±1.2	54.5±1.4	0± 7(−)	0.0	−39
18th	5	468±48	472±51	55.9±1.5	54.7±1.4	4± 5(−)	0.9	−35
19th	5	464±36	468±40	56.8±0.7	55.7±1.3	4± 5(−)	0.9	−31
20th	5	448±53	450±55	57.0±0.4	55.7±1.0	2± 4(−)	0.4	−29
21st	4	475±31	478±30	56.4±1.0	55.6±1.0	3± 5(−)	0.5	−26
22nd	4	468±36	465±39	56.4±0.7	55.2±0.6	−3± 5(−)	−0.5	−29
23rd	4	463±39	460±41	55.9±0.9	54.4±0.5	−3± 5(−)	−0.5	−31
24th	4	460±34	458±36	56.3±1.1	54.6±0.7	−3± 5(−)	−0.5	−34
25th	4	428±19	433±29	56.5±1.6	53.9±0.9	5±17(−)	1.2	−29
26th	4	430±22	430±29	57.2±1.6	54.3±1.0	0±16(−)	0.0	−29
27th	5	454±36	458±37	56.2±0.4	54.6±0.8	4±15(−)	0.9	−25
28th	5	450±34	452±31	56.6±0.8	54.9±1.5	2±11(−)	0.4	−23
29th	5	452±31	448±35	56.6±1.1	55.0±1.4	−4± 5(−)	−0.9	−27
30th	5	446±27	440±28	56.8±0.7	54.9±1.1	−6± 5(−)	−1.3	−33
31st	4	433±74	428±74	57.0±0.9	54.9±0.6	−5± 6(−)	−1.2	−38
32nd	4	425±68	418±69	57.1±1.1	55.0±1.3	−8±10(−)	−1.8	−45
33rd	4	423±46	418±52	57.9±2.3	55.7±2.4	−5± 6(−)	−1.2	−50
34th	4	425±48	420±54	57.9±2.4	56.0±2.4	−5± 6(−)	−1.2	−55
35th	4	408±39	400±47	57.8±3.5	55.3±2.3	−8±10(−)	−1.8	−63
36th	4	400±24	393±33	57.9±3.2	55.7±1.9	−8±10(−)	−1.9	−70
37th	6	402±22	395±27	57.8±1.4	55.5±1.2	−7±10(−)	−1.7	−77
38th	6	390±24	382±30	57.9±1.0	55.5±1.3	−8±10(−)	−2.1	−85
39th	4	390±32	380±32	59.4±0.8	56.4±1.2	−10±12(−)	−2.6	−95
40th	4	385±26	375±27	58.8±1.2	55.9±1.4	−10±12(−)	−2.6	−105
57th–60th incl.	3	308±12	274±18	64.1±0.9	59.6±1.7	−33±12*	−10.8	—
77th–80th incl.	3	223±22	201±25	69.0±2.4	63.5±2.6	−22± 5*	−9.8	—
97th–100th incl.	3	132±40	124±43	81.4±5.9	76.1±9.0	−8± 9(−)	−5.7	—

Table 10. *Growth in Length from Proximal Growth Plate of Fibula in Series A*

Observation day	Number of animals	Growth per day in microns		Growth per day in per cent of growth of tibial diaphysis per day		Difference in growth per day between left and right		Total post-operative difference in growth in length between left and right sides
		right M±s	left M±s	right M±s	left M±s	μ M±s	% of right	μ
Day before operation								
penultimate	15	463±16	465±16	48.3±1.3	48.3±1.1	2± 4(-)	0.4	—
ultimate	15	463±24	462±25	48.8±1.2	48.6±1.4	−1± 3(-)	−0.2	—
Day after operation								
1st	15	438±23	471±22	49.9±1.2	48.2±1.0	33±11***	7.6	33
2nd	15	443±30	499±32	49.9±1.4	49.8±0.9	56±21***	12.6	89
3rd	15	437±25	499±26	49.7±1.4	49.6±1.0	63±15***	14.4	152
4th	15	429±25	496±31	49.6±1.4	50.1±1.3	67±19***	15.7	219
5th	15	427±21	495±36	48.4±1.1	49.6±1.2	67±19***	15.8	286
6th	15	433±27	471±21	48.4±0.8	49.5±1.3	38±13***	8.8	325
7th	15	427±36	433±36	48.7±1.0	49.4±1.0	6±11*	1.4	331
8th	15	431±34	423±37	48.8±1.2	49.3±1.2	−8±13*	−1.9	323
9th	12	440±36	425±46	49.4±1.4	49.6±1.2	−15±15**	−3.4	308
10th	12	451±32	428±40	49.9±1.0	49.6±1.1	−23±15***	−5.0	285
11th	11	460±18	444±19	50.0±1.4	49.2±1.1	−16±13***	−3.6	269
12th	11	454±20	435±24	50.3±1.3	49.4±1.1	−18±15**	−4.0	251
13th	13	448±39	425±42	50.4±1.2	49.2±0.8	−24±14***	−5.3	227
14th	13	443±30	422±33	50.4±1.2	49.3±1.0	−22±16***	−4.9	205
15th	10	429±31	404±29	50.6±1.1	49.3±1.2	−25±14***	−5.8	180
16th	10	426±21	403±21	51.1±1.1	49.7±1.0	−23±14***	−5.4	157
17th	5	418±41	398±43	52.5±1.5	50.3±1.4	−20±10*	−4.8	137
18th	5	410±37	398±43	52.7±2.0	50.5±1.4	−12± 8*	−2.9	125
19th	5	414±29	398±37	54.0±0.8	51.7±0.9	−16±11*	−3.9	109
20th	5	396±46	384±48	54.0±0.5	51.7±1.1	−12± 8*	−3.0	97
21st	4	423±40	410±36	53.5±0.7	51.8±0.6	−13±10(-)	−3.0	85
22nd	4	415±38	405±31	53.4±1.0	51.8±1.1	−10± 8(-)	−2.4	75
23rd	4	408±42	393±40	52.8±1.3	50.5±1.3	−15±13(-)	−3.7	60
24th	4	405±38	390±35	53.1±1.0	50.7±1.8	−15±19(-)	−3.7	45
25th	4	383±25	363±17	53.7±1.1	49.5±2.0	−20±15(-)	−5.2	25
26th	4	380±26	363±17	54.1±0.8	50.1±1.7	−18±15(-)	−4.6	7
27th	5	402±27	392±31	53.2±1.3	50.7±1.3	−10±10(-)	−2.5	−3
28th	5	394±34	386±34	53.2±1.0	51.0±1.4	−8± 4*	−2.0	−11
29th	5	400±23	392±27	53.6±1.2	51.7±1.3	−8± 8(-)	−2.0	−19
30th	5	400±26	392±30	54.1±1.0	52.0±1.0	−8± 8(-)	−2.0	−27
31st	4	395±63	380±63	54.8±1.0	51.9±1.0	−15±13*	−3.8	−42
32nd	4	393±57	378±56	55.2±1.1	52.6±1.5	−15± 6*	−3.8	−57
33rd	4	378±52	368±50	55.1±1.8	52.5±1.9	−10± 8(-)	−2.6	−67
34th	4	375±47	365±52	54.8±1.9	52.6±1.9	−10± 8(-)	−2.7	−77
35th	4	363±36	353±43	54.9±1.1	52.1±1.6	−10± 8(-)	−2.8	−87
36th	4	358±35	353±36	55.1±1.1	53.0±1.5	−5± 6(-)	−1.4	−92
37th	6	358±21	353±18	55.0±1.8	52.7±1.1	−5± 8(-)	−1.4	−97
38th	6	348±25	342±21	55.1±1.5	52.8±1.1	−7±12(-)	−1.9	−104
39th	4	345±24	335±17	56.4±1.7	53.4±1.3	−10±14(-)	−2.9	−114
40th	4	343±29	338±25	56.0±1.5	53.4±1.6	−5± 6(-)	−1.5	−119
57th–60th incl.	3	276±13	257±18	61.5±1.1	58.0±1.8	−19±12(-)	−7.0	—
77th–80th incl.	3	209±21	203±25	67.6±2.4	63.7±2.9	−7± 8(-)	−3.2	—
97th–100th incl.	3	136±47	127±47	81.8±5.3	76.4±8.7	−9± 9(-)	−6.8	—

Table 11. *Growth in Length from Distal Growth Plate of Fibula in Series A*

Observation day	Number of animals	Growth per day in microns		Difference in growth per day between left and right		Total post-operative difference in growth in length between left and right sides
		right M ± s	left M ± s	μ M ± s	% of right	μ
Day before operation						
penultimate	15	497 ± 23	498 ± 23	2 ± 4(−)	0.3	—
ultimate	15	487 ± 23	489 ± 24	2 ± 4(−)	0.5	—
Day after operation						
1st	15	440 ± 15	506 ± 29	66 ± 22***	15.0	66
2nd	15	444 ± 23	503 ± 28	59 ± 16***	13.4	125
3rd	15	443 ± 33	508 ± 31	65 ± 16***	14.8	191
4th	15	435 ± 29	494 ± 34	59 ± 20***	13.5	249
5th	15	457 ± 28	503 ± 44	47 ± 23***	10.2	296
6th	15	461 ± 29	481 ± 38	21 ± 17***	4.5	317
7th	15	451 ± 49	445 ± 49	−6 ± 13(−)	−1.3	311
8th	15	453 ± 54	436 ± 53	−17 ± 13***	−3.8	293
9th	12	452 ± 46	433 ± 55	−19 ± 17**	−4.2	274
10th	12	453 ± 45	437 ± 55	−17 ± 17**	−3.7	258
11th	11	460 ± 32	456 ± 31	−4 ± 8(−)	−0.8	254
12th	11	449 ± 32	446 ± 33	−3 ± 5(−)	−0.6	251
13th	13	442 ± 45	439 ± 49	−3 ± 6(−)	−0.7	248
14th	13	437 ± 33	434 ± 31	−3 ± 8(−)	−0.7	245
15th	10	418 ± 29	414 ± 27	−4 ± 7(−)	−1.0	241
16th	10	408 ± 21	407 ± 20	−1 ± 6(−)	−0.1	240
17th	5	380 ± 48	394 ± 51	14 ± 5**	3.7	254
18th	5	370 ± 52	390 ± 42	20 ± 12*	5.4	274
19th	5	354 ± 35	372 ± 29	18 ± 13*	5.1	292
20th	5	338 ± 53	358 ± 37	20 ± 10**	5.9	312
21st	4	368 ± 38	383 ± 36	15 ± 13(−)	4.1	327
22nd	4	363 ± 34	378 ± 39	15 ± 13(−)	4.1	342
23rd	4	365 ± 33	385 ± 31	20 ± 16(−)	5.5	362
24th	4	358 ± 33	380 ± 32	23 ± 10*	6.3	385
25th	4	330 ± 23	370 ± 32	40 ± 14*	12.1	425
26th	4	323 ± 26	363 ± 38	40 ± 18*	12.4	465
27th	5	354 ± 32	382 ± 39	28 ± 19*	7.9	493
28th	5	346 ± 32	372 ± 39	26 ± 19*	7.5	519
29th	5	346 ± 24	366 ± 27	20 ± 7**	5.8	539
30th	5	340 ± 27	362 ± 30	22 ± 4***	6.5	561
31st	4	328 ± 63	353 ± 66	25 ± 10*	7.6	586
32nd	4	320 ± 57	343 ± 68	23 ± 13*	7.0	608
33rd	4	310 ± 58	335 ± 66	25 ± 10*	8.1	633
34th	4	313 ± 61	333 ± 68	20 ± 8*	6.4	653
35th	4	300 ± 57	325 ± 52	25 ± 24(−)	8.3	678
36th	4	293 ± 43	313 ± 40	20 ± 22(−)	6.8	698
37th	6	293 ± 19	317 ± 14	23 ± 16*	8.0	721
38th	6	283 ± 19	305 ± 10	22 ± 15*	7.6	743
39th	4	268 ± 22	293 ± 15	25 ± 13*	9.3	768
40th	4	270 ± 29	295 ± 24	25 ± 13*	9.3	793
57th–60th incl.	3	173 ± 7	186 ± 5	13 ± 6(−)	7.7	—
77th–80th incl.	3	101 ± 21	117 ± 27	16 ± 6*	15.7	—
97th–100th incl	3	33 ± 20	43 ± 33	11 ± 12(−)	33.2	—

Table 12. *Growth of Tibial Diaphysis in Series A*

Observation day	Number of animals	Growth per day in microns		Difference in growth per day between left and right		Total post-operative difference in growth in length between left and right sides
		right M±s	left M±s	μ M±s	% of right	μ
Day before operation						
penultimate	15	1,067±38	1,067±36	0± 6(-)	0.0	—
ultimate	15	1,048±36	1,052±36	4± 7(-)	0.4	—
Day after operation						
1st	15	971±31	1,042±44	71±24***	7.3	71
2nd	15	971±38	1,028±44	57±17***	5.9	128
3rd	15	963±53	1,039±57	76±23***	7.9	204
4th	15	946±51	1,021±55	75±23***	7.9	279
5th	15	973±51	1,045±75	72±42***	7.4	351
6th	15	974±58	1,006±67	32±24***	3.3	383
7th	15	958±89	949±88	−9±25(-)	−0.9	374
8th	15	961±96	933±98	−27±21***	−2.8	347
9th	12	969±83	933±102	−36±27***	−3.7	311
10th	12	974±77	939±98	−35±30**	−3.6	276
11th	11	991±54	979±61	−12±16*	−1.2	264
12th	11	968±57	958±64	−10±13*	−1.0	254
13th	13	962±80	948±86	−15±19*	−1.5	240
14th	13	952±54	940±57	−12±15*	−1.3	227
15th	10	920±51	906±57	−16±14**	−1.7	211
16th	10	899±33	888±34	−11±12*	−1.2	200
17th	5	852±102	866±105	14± 9*	1.6	214
18th	5	838±98	862±90	24± 9**	2.9	238
19th	5	818±71	840±66	22± 8**	2.7	260
20th	5	786±95	808±91	22± 8**	2.8	282
21st	4	843±68	860±65	18±10*	2.1	300
22nd	4	830±69	843±77	13±15(-)	1.5	312
23rd	4	828±70	845±71	18±13(-)	2.1	330
24th	4	818±65	838±67	20± 8**	2.4	350
25th	4	758±36	803±59	45±30(-)	5.9	395
26th	4	753±43	793±67	40±27(-)	5.3	435
27th	5	808±68	840±75	32±33(-)	4.0	467
28th	5	796±65	824±67	28±29(-)	3.5	495
29th	5	798±52	814±58	16±11*	2.0	511
30th	5	786±54	802±56	16± 5**	2.0	527
31st	4	760±137	780±140	20±14(-)	2.6	547
32nd	4	745±124	760±136	15±19(-)	2.0	562
33rd	4	733±103	753±116	20±14(-)	2.7	582
34th	4	738±108	753±120	15±13(-)	2.0	597
35th	4	708±88	725±94	18±31(-)	2.5	614
36th	4	693±60	705±69	13±30(-)	1.8	627
37th	6	695±36	712±37	17±24(-)	2.4	643
38th	6	673±40	687±39	13±21(-)	2.0	657
39th	4	658±43	673±45	15±13(-)	2.3	672
40th	4	655±55	670±58	15±13(-)	2.3	687
57th–60th incl.	3	480±15	460±19	−20±10(-)	−4.2	—
77th–80th incl.	3	323±43	318±51	−6±10(-)	−1.8	—
97th–100th incl.	3	164±60	168±74	3±16(-)	2.0	—

Table 13. *Growth of Fibular Diaphysis in Series A*

Observation day	Number of animals	Growth per day in microns		Difference in growth per day between left and right		Total post-operative difference in growth in length between left and right sides
		right M±s	left M±s	μ M±s	% of right	μ
Day before operation						
penultimate	15	960±31	963±34	3± 5(-)	0.3	—
ultimate	15	950±42	951±41	1± 3(-)	0.1	—
Day after operation						
1st	15	878±33	977±47	99±27***	11.3	99
2nd	15	887±48	1,003±57	115±31***	13.0	215
3rd	15	879±53	1,007±54	128±27***	14.6	343
4th	15	864±49	990±59	126±33***	14.6	469
5th	15	884±49	998±77	114±40***	12.9	583
6th	15	893±54	952±56	59±20***	6.6	641
7th	15	878±83	878±84	0±23(-)	0.0	641
8th	15	884±87	859±89	−25±18***	−2.9	616
9th	12	892±78	858±99	−34±28**	−3.8	582
10th	12	904±76	865±94	−39±30***	−4.3	543
11th	11	920±46	900±46	−20±17**	−2.2	523
12th	11	903±48	882±54	−21±16**	−2.3	502
13th	13	891±81	864±85	−27±15***	−3.0	475
14th	13	880±59	855±61	−25±18***	−2.8	450
15th	10	847±57	818±53	−29±15***	−3.4	421
16th	10	834±38	810±37	−24±17**	−2.9	397
17th	5	798±86	792±92	−6±15(-)	−0.8	391
18th	5	780±84	788±82	8±11(-)	1.0	399
19th	5	768±64	770±65	2±13(-)	0.3	401
20th	5	734±85	742±84	8± 8(-)	1.1	409
21st	4	790±77	793±71	3±21(-)	0.3	412
22nd	4	778±70	783±69	5±13(-)	0.6	417
23rd	4	773±73	778±68	5±17(-)	0.6	422
24th	4	763±70	770±61	8±17(-)	1.0	429
25th	4	713±46	733±41	20±16(-)	2.8	449
26th	4	703±51	725±53	23±22(-)	3.2	472
27th	5	756±56	774±67	18±15(-)	2.4	490
28th	5	740±64	758±70	18±18(-)	2.4	508
29th	5	746±44	758±50	12±13(-)	1.6	520
30th	5	740±51	754±59	14±11(-)	1.9	534
31st	4	723±127	733±129	10±22(-)	1.4	544
32nd	4	713±114	720±123	8±13(-)	1.1	551
33rd	4	688±109	703±115	15±13(-)	2.2	566
34th	4	688±108	698±119	10±14(-)	1.5	576
35th	4	663±85	678±93	15±26(-)	2.3	591
36th	4	650±67	665±73	15±19(-)	2.3	606
37th	6	652±33	670±28	18±20(-)	2.8	625
38th	6	632±39	647±29	15±21(-)	2.4	640
39th	4	613±41	628±28	15±24(-)	2.4	655
40th	4	613±56	633±44	20±18(-)	3.3	675
57th–60th incl.	3	448±16	443±19	−6±11(-)	−1.3	—
77th–80th inc.	3	310±41	319±50	9±12(-)	3.0	—
97th–100th incl.	3	168±67	170±78	2±14(-)	1.0	—

Table 14. *Length of Cell Columns in Proximal Growth Plate of Tibia in Series A*

Interval between operation and sacrifice (days)	Number of animals	Length of cell columns (μ)		Difference between left and right	
		right M±s	left M±s	μ M±s	% of right
2	6	654±42	634±61	−19±30(−)	−4.9
4	9	650±59	613±43	−36±28**	−5.6
6	6	670±31	622±33	−47±26**	−7.1
8	9	716±55	689±62	−27±40(−)	−3.8
10	6	687±59	680±62	−7±36(−)	−1.1
12	6	722±85	726±94	4±26(−)	0.5
14	5	707±53	703±56	−3±19(−)	−0.5
16	8	681±48	668±38	−13±24(−)	−1.9
20	3	650±104	641±101	−8± 9(−)	−1.3
30	3	663±30	631±37	−32±21(−)	−4.8
40	4	619±31	593±45	−26±31(−)	−4.1
60	3	584±42	497±25	−87±19*	−14.9
80	3	452±51	408±44	−44±19(−)	−9.8
100	3	366±43	320±20	−45±24(−)	−12.4

Table 15. *Length of Cell Columns in Distal Growth Plate of Tibia in Series A*

Interval between operation and sacrifice (days)	Number of animals	Length of cell columns (μ)		Difference between left and right	
		right M±s	left M±s	μ M±s	% of right
2	4	537±30	590±16	53±33*	9.8
4	7	538±56	585±54	47±16***	8.7
6	4	547±57	611±40	64±32*	11.7
8	7	562±42	600±35	38±41*	6.8
10	5	569±43	590±41	21±22(−)	3.6
12	3	630±69	650±74	20±22(−)	3.2
20	3	515±79	520±75	6±21(−)	1.1
30	3	525±31	543±16	18±16(−)	3.5
40	4	495±16	495±15	0± 8(−)	0.1

Table 16. *Growth per Day from Different Growth Plates in Group 1 (Controls) in Series B*
Number of Animals: 9

Day after birth	Proximal tibia (μ)				Proximal fibula (μ)				Distal fibula (μ)				Distal radius (μ)	
	Growth per day		Change in relation to that on 30th day		Growth per day		Change in relation to that on 30th day		Growth per day		Change in relation to that on 30th day		Growth per day	Change in relation to that on 30th day
	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	left M±s	left M±s
30th	553±39	551±38	--	—	463±31	467±31	—	—	477±38	476±37	—	—	479±33	—
31st	544±42	544±41	−9± 6	−7± 7	459±31	462±31	−4± 5	−4± 5	476±38	477±39	−1± 3	1± 8	474±32	−4± 7
32nd	541±35	541±38	−12± 8	−10± 7	453±27	458±28	−9± 9	−10±10	472±38	474±37	−4± 5	−1± 6	480±30	1± 9
33rd	532±34	532±35	−21±12	−19±12	450±23	452±25	−13±12	−14±10	464±35	466±35	−12±13	−10±14	479±23	0±14
34th	531±27	532±29	−22±17	−19±15	449±21	452±22	−14±16	−14±14	463±31	462±30	−13±16	−12±20	472±24	−7±15

Table 17 A. *Growth per Day from Different Growth Plates in Group 2 (Plugging of Marrow Cavity) in Series B.*
Number of Animals: 3

Day after birth	Proximal tibia (μ)				Proximal fibula (μ)				Distal fibula (μ)				Distal radius (μ)	
	Growth per day		Change in relation to that on 30th day		Growth per day		Change in relation to that on 30th day		Growth per day		Change in relation to that on 30th day		Growth per day	Change in relation to that on 30th day
	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	left M±s	left M±s
30th	573±15	577±15	—	—	487±25	487±25	—	—	497±25	497±25	—	—	487±25	—
31st	530±20	527±15	−43± 6	−50±10	440±36	467±23	−47±12	−20±10	437±15	503±30	−60±10	7± 6	457±25	−30± 0
32nd	530±26	513±15	−43±12	−63± 6	443±40	483±30	−43±15	−3± 6	443±25	500±30	−53± 6	3± 6	460±20	−27± 6
33rd	507±21	497±11	−67± 6	−80±10	420±26	483±15	−67±12	−3±12	427±21	493±23	−70±20	−3±15	440±10	−47±31
34th	490±26	497±29	−83±12	−80±20	407±21	477±21	−80±10	−10±10	413±25	480±17	−83±25	−17±15	420±10	− 67±31

Table 17 B. *Effect of Plugging of Marrow Cavity on Normal Growth per Day (Group 1 and 2)*

Day after operation	Proximal tibia						Proximal fibula					
	right		left		left-right		right		left		left-right	
	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%
1st	−36***	−6.3	−42***	−7.4	−7(−)	−1.2	−42***	−8.8	−17***	−3.4	27***	5.5
2nd	−32***	−5.7	−52***	−9.3	−20**	−3.5	−34***	−7.1	6(−)	1.3	40***	8.4
3rd	−47***	−8.4	−60***	−10.8	−13(−)	−2.4	−53***	−11.2	11(−)	2.2	63***	13.4
4th	−63***	−11.3	−59***	−10.7	3(−)	0.6	−66***	−13.9	4(−)	0.9	70***	14.8

Day after operation	Distal fibula						Distal radius	
	right		left		left-right		left	
	μ	%	μ	%	μ	%	μ	%
1st	−60***	−12.1	7(−)	1.3	67***	13.4	−26***	−5.3
2nd	−51***	−10.2	6(−)	1.2	57***	11.5	−28***	−5.7
3rd	−59***	−12.1	8(−)	1.6	67***	13.7	−47**	−9.6
4th	−71***	−14.6	−4(−)	−0.8	67***	13.8	−60***	−12.5

Table 18 A. *Growth per Day from Different Growth Plates in Group 3 (Destruction of Marrow Cavity) in Series B.*
Number of Animals: 4

Day after birth	Proximal tibia (μ)				Proximal fibula (μ)				Distal fibula (μ)				Distal radius (μ)	
	Growth per day		Change in relation to that on 30th day		Growth per day		Change in relation to that on 30th day		Growth per day		Change in relation to that on 30th day		Growth per day	Change in relation to that on 30th day
	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	left M±s	left M±s
30th	545± 6	543± 5	—	—	453±15	453±15	—	—	465±17	465±17	—	—	470±29	—
31st	508±26	488±54	−38±26	−55±52	423±17	460±27	−30± 8	8±17	428±13	475±13	−38±15	10±12	435±29	−35±10
32nd	515±24	520±29	−30±27	−23±30	425±26	480±16	−28±13	28±10	430±20	488±17	−35±24	23±22	443±26	−28±28
33rd	505±24	500±18	−40±27	−43±22	413±22	470±12	−40± 8	18±17	420±22	485±17	−45±30	20±26	435±33	−35±37
34th	498±24	508±21	−48±29	−35±25	410±35	480±16	−43±21	28±15	415±27	485±17	−50±39	20±32	428±43	−43±48

Table 18 B. *Effect of Destruction of Marrow Cavity on Normal Growth per Day (Group 1 and 3)*

Day after operation	Proximal tibia						Proximal fibula					
	right		left		left-right		right		left		left-right	
	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%
1st	−30*	−5.5	−47***	−8.8	−18(−)	−3.3	−26***	−5.7	12*	2.7	38***	8.4
2nd	−19*	−3.5	−11(−)	−2.1	8(−)	1.4	−18**	−4.1	37***	8.3	55***	12.4
3rd	−20*	−3.8	−23*	−4.3	−3(−)	−0.5	−26***	−6.0	31***	7.2	58***	13.1
4th	−27*	−5.1	−14(−)	−2.8	13(−)	0.2	−28**	−6.4	42***	9.6	70***	16.0

Day after operation	Distal fibula						Distal radius	
	right		left		left-right		left	
	μ	%	μ	%	μ	%	μ	%
1st	−38***	−8.1	10*	2.2	48***	10.2	−31***	−6.6
2nd	−32***	−7.0	25**	5.5	58***	12.4	−29*	−6.1
3rd	−34**	−7.5	31**	6.9	65***	14.3	−35*	−7.4
4th	−37*	−8.2	33*	7.3	70***	15.4	−36(−)	−7.7

Table 19 A. *Growth per Day from Different Growth Plates in Group 4 (Drilling of Corticalis) in Series B.*
Number of Animals: 4

Day after birth	Proximal tibia (μ)				Proximal fibula (μ)				Distal fibula (μ)				Distal radius (μ)	
	Growth per day		Change in relation to that on 30th day		Growth per day		Change in relation to that on 30th day		Growth per day		Change in relation to that on 30th day		Growth per day	Change in relation to that on 30th day
	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	left M±s	left M±s
30th	520±16	523±13	—	—	433±19	433±19	—	—	438±31	438±31	—	—	450±20	—
31st	508±13	525±24	−13±21	3±32	410± 8	440±18	−23±22	8±26	413±17	445±29	−25±26	8±33	433±15	−18±13
32nd	488±15	528±17	−33±22	5±24	405± 6	450±14	−28±22	18±24	410±22	453±22	−28±22	15±26	438±17	−13±10
33rd	488±21	535±37	−33±31	13±42	398±10	455±21	−35±26	23±33	400±22	458±21	−38±26	20±36	435±19	−15±19
34th	490±24	545±37	−30±36	23±43	403±10	470±32	−30±24	38±38	408± 5	465±17	−30±32	28±39	438±19	−13±26

Table 19 B. *Effect of Drilling of Corticalis on Normal Growth per Day (Group 1 and 4)*

Day after operation	Proximal tibia						Proximal fibula					
	right		left		left-right		right		left		left-right	
	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%
1st	−5(−)	−0.9	10(−)	2.0	15(−)	2.9	−18*	−4.2	12(−)	2.8	30**	7.0
2nd	−21**	−4.2	16*	3.2	38***	7.4	−18*	−4.3	27**	6.4	45***	10.6
3rd	−13(−)	−2.5	33*	6.5	45**	9.0	−21*	−5.0	36***	8.7	58***	13.7
4th	−9(−)	−1.9	43**	8.6	53**	10.5	−16(−)	−3.7	52***	12.4	68***	16.1

Day after operation	Distal fibula						Distal radius	
	right		left		left-right		left	
	μ	%	μ	%	μ	%	μ	%
1st	−25*	−5.7	8(−)	1.7	33*	7.4	−13*	−2.9
2nd	−25**	−5.7	18*	4.1	43***	9.8	−14*	−3.0
3rd	−26*	−6.2	31**	7.3	58***	13.5	−15(−)	−3.3
4th	−17(−)	−4.0	40**	9.5	58**	13.5	−6(−)	−1.3

Table 20 A. *Growth per Day from Different Growth Plates in Group 5 (Periosteal Incision) in Series B.*
Number of Animals: 3

Day after birth	Proximal tibia (μ)				Proximal fibula (μ)				Distal fibula (μ)				Distal radius (μ)	
	Growth per day		Change in relation to that on 30th day		Growth per day		Change in relation to that on 30th day		Growth per day		Change in relation to that on 30th day		Growth per day	Change in relation to that on 30th day
	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	left M±s	left M±s
30th	540±10	543± 6	—	—	450±10	450±10	—	—	453± 6	453± 6	—	—	457±25	—
31st	507±25	510±26	−33±23	−33±23	423± 6	437±12	−27± 6	−13± 6	427±15	443±21	−27±15	−10±17	430± 6	−27±15
32nd	510±36	523±40	−30±40	−20±40	437±15	457±23	−13±23	7±10	433±21	447±35	−20±20	−7±31	433±21	−23±15
33rd	517±25	547±25	−23±31	3±25	430± 0	457±15	−20±10	7±21	443±25	457±38	−10±20	3±32	440±26	−17±15
34th	510±28	537±35	−30±46	−7±35	427±15	453±15	−23±21	3±25	440±36	457±46	−13±31	3±40	440±30	−17±25

Table 20 B. *Effect of Periosteal Incision on Normal Growth per Day (Group 1 and 5)*

Day after operation	Proximal tibia						Proximal fibula					
	right		left		left-right		right		left		left-right	
	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%
1st	−26***	−4.8	−26***	−4.8	0(−)	0.0	−22***	−5.0	−9*	−2.0	13**	3.0
2nd	−19(−)	−3.6	−9(−)	−1.7	10(−)	1.9	−4(−)	−0.9	16*	3.7	20(−)	4.5
3rd	−3(−)	−0.6	23*	4.5	27(−)	5.1	−6(−)	−1.4	21*	4.7	27*	6.1
4th	−9(−)	−1.8	14(−)	2.7	23(−)	4.5	−9(−)	−2.0	17(−)	4.1	27(−)	6.1

Day after operation	Distal fibula						Distal radius	
	right		left		left-right		left	
	μ	%	μ	%	μ	%	μ	%
1st	−27***	−5.9	−10(−)	−2.2	17*	3.7	−22**	−4.9
2nd	−17*	−3.8	−4(−)	−0.9	13(−)	3.0	−24**	−5.3
3rd	1(−)	0.2	14(−)	3.3	13(−)	3.0	−17(−)	−3.7
4th	−1(−)	−0.1	16(−)	3.7	17(−)	3.8	−10(−)	−2.2

Table 21 A. *Growth per Day from Different Growth Plates in Group 6 (Skin Incision) in Series B*
Number of Animals: 3

Day after birth	Proximal tibia (μ)				Proximal fibula (μ)				Distal fibula (μ)				Distal radius (μ)	
	Growth per day		Change in relation to that on 30th day		Growth per day		Change in relation to that on 30th day		Growth per day		Change in relation to that on 30th day		Growth per day	Change in relation to that on 30th day
	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	left M±s	left M±s
30th	537±12	537±12	—	—	440±20	440±20	—	—	450±10	450±10	—	—	460± 0	—
31st	527±25	523±25	−10±17	−13±15	437±25	440±10	−3± 6	0±10	443±31	447±15	−7±21	−3± 6	450±17	−10±17
32nd	527±23	520±20	−10±20	−17±12	437±25	440±10	−3± 6	0±10	453±29	453±25	3±21	3±23	450±17	−10±17
33rd	523±15	520±10	−13±21	−17±15	437±31	433±15	−3±12	−7± 6	440±20	440±10	−10±10	−10± 0	447±21	−13±21
34th	520±15	520±15	−17±15	−17± 6	437±21	437±16	−3± 6	−3±15	440±20	433±15	−10±10	−17± 6	443±15	−17±15

Table 21 B. *Effect of Skin Incision on Normal Growth per Day (Group 1 and 6)*

Day after operation	Proximal tibia						Proximal fibula					
	right		left		left-right		right		left		left-right	
	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%
1st	−2(−)	−0.4	−6(−)	−1.0	−3(−)	−0.6	1(−)	0.3	4(−)	1.0	3(−)	0.8
2nd	1(−)	0.2	−6(−)	−1.1	−7(−)	−1.3	6(−)	1.4	9(−)	2.2	3(−)	0.8
3rd	7(−)	1.3	3(−)	0.6	−3(−)	−0.7	11(−)	2.5	7(−)	1.7	−3(−)	−0.8
4th	4(−)	0.8	4(−)	0.8	0(−)	0.0	11(−)	2.6	11(−)	2.6	0(−)	0.0

Day after operation	Distal fibula						Distal radius	
	right		left		left-right		left	
	μ	%	μ	%	μ	%	μ	%
1st	−7(−)	−1.5	−3(−)	−0.7	3(−)	0.8	−6(−)	−1.2
2nd	6(−)	1.4	6(−)	1.4	0(−)	0.0	−11(−)	−2.4
3rd	1(−)	0.3	1(−)	0.3	0(−)	0.0	−13(−)	−2.9
4th	3(−)	0.6	−4(−)	−0.9	−7(−)	−1.5	−10(−)	−2.2

Table 22 A. *Growth per Day from Different Growth Plates in Group 7 (Anaesthesia) in Series B*
Number of Animals: 4

Day after birth	Proximal tibia (μ)				Proximal fibula (μ)				Distal fibula (μ)				Distal radius (μ)	
	Growth per day		Change in relation to that on 30th day		Growth per day		Change in relation to that on 30th day		Growth per day		Change in relation to that on 30th day		Growth per day	Change in relation to that on 30th day
	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	right M±s	left M±s	left M±s	left M±s
30th	538±19	533±17	—	—	448±26	443±22	—	—	453±21	450±18	—	—	455±21	—
31st	530±22	528±19	−8± 5	−5± 6	443±30	438±26	−5± 6	−5± 6	450±18	448±21	−3± 5	−3± 5	455±21	0± 0
32nd	523±25	523±22	−15±13	−10± 8	443±30	435±25	−5± 6	−8± 5	448±21	445±17	−5± 6	−5± 6	453±22	−3± 5
33rd	523±17	525±21	−15±10	−8± 5	440±29	435±25	−8± 5	−8± 5	450±24	448±21	−3±10	−3±10	448±24	−8±10
34th	518±25	520±24	−20±16	−13±10	440±33	433±29	−8±13	−10±14	443±29	443±22	−10±14	−8±13	448±31	−8±13

Table 22 B. *Effect of Anaesthesia on Normal Growth per Day (Group 1 and 7)*

Day after operation	Proximal tibia		Proximal fibula		Distal fibula		Distal radius	
	right and left		right and left		right and left		left	
	μ	%	μ	%	μ	%	μ	%
1st	2(−)	0.3	−1(−)	−0.1	−3(−)	−0.6	4(−)	1.0
2nd	−1(−)	−0.3	3(−)	0.7	−2(−)	−0.5	−4(−)	−0.8
3rd	9(−)	1.7	6(−)	1.5	9(−)	1.9	−8(−)	−1.6
4th	4(−)	0.8	6(−)	1.3	4(−)	0.9	−1(−)	−0.2

Table 23. *Length of Cell Columns in Proximal Growth Plate of Tibia in Series B*

Material	Number of animals	Length of cell columns (μ)		Length of cell columns in relation to controls (group 1)					
		right	left	right		left		left-right	
		M ± s	M ± s	μ	%	μ	%	μ	%
Controls (group 1)	9	687 ± 21	—	—	—	—	—	—	—
Plugging of marrow cavity (group 2)	3	590 ± 59	578 ± 44	−97**	−14.2	−109***	−15.8	−12(−)	−1.7
Destruction of marrow cavity (group 3)	4	607 ± 35	600 ± 32	−80***	−11.6	−87***	−12.6	−7(−)	−1.0
Drilling of corticalis (group 4)	4	643 ± 55	665 ± 62	−44(−)	−6.4	−23(−)	−3.3	21(−)	3.1
Periosteal incision (group 5)	3	683 ± 16	689 ± 14	−4(−)	−0.6	2(−)	0.2	6(−)	0.8
Skin incision (group 6)	3	676 ± 26	668 ± 25	−11(−)	−1.6	−19(−)	−2.8	−8(−)	−1.2
Anaesthesia (group 7)	4	684 ± 10	—	−3(−)	−0.5	—	—	—	—

Table 24. *Length of Cell Columns in Distal Growth Plate of Radius in Series B*

Material	Number of animals	Length of cell columns (μ)	Length of cell columns in relation to controls (group 1)	
		right	right	
		M ± s	μ	%
Controls (group 1)	9	640 ± 28	—	—
Plugging of marrow cavity (group 2)	3	587 ± 6	−53*	−8.2
Destruction of marrow cavity (group 3)	4	571 ± 12	−69***	−10.7
Drilling of corticalis (group 4)	4	597 ± 26	−43*	−6.8
Periosteal incision (group 5)	3	600 ± 41	−40(-)	−6.2
Skin incision (group 6)	3	618 ± 22	−22(-)	−3.4
Anaesthesia (group 7)	4	629 ± 21	−11(-)	−1.7