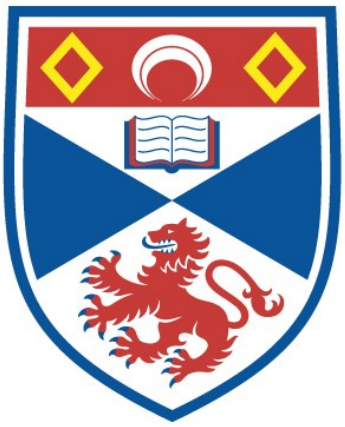
THE RELATION OF CARTILAGE CANALS TO THE PROCESS OF OSSIFICATION

Maung Tin Maung

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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A THESIS

PRESENTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

OF

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BY

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DECLARATION

I hereby declare that the following thesis is my own composition, that the work of which it is a record has been carried out by me, and that it has not previously been presented for a higher degree.

UNIVERSITY AND RESEARCH TRAINING

In September, 1949, I graduated M.B., B.S. from the University of Rangoon. I worked as Demonstrator for two years and as Assistant Lecturer for one year in the Anatomy Department of the same University.

I was admitted as a research student in October, 1952, and under the supervision of Professor Walmsley, I have performed the research which forms the subject of this thesis.

CERTIFICATE

I certify that M. Tin Maung has spent nine terms in research work under my direction, that he has fulfilled the conditions of Ordinance No.16 (St.Andrews) and that he is qualified to submit the following Thesis in application for the Degree of Doctor of Philosophy.

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1. INTRODUCTION AND REVIEW OF LITERATURE

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Cartilage as it occurs in the human adult is generally regarded as an avascular tissue and the absence of blood vessels within it qualifies it to be grouped with those rare tissues in the body which can survive through a process of diffusion. It is, however, well known that cartilage in the foetus and the new born and adult may contain its own endochondral vascular system and that this invasion of cartilaginous matrix by blood vessels occurs in the cartilaginous ends of all typical long bones before the secondary centres of ossification appear in them. From a review of the literature on cartilage canals there would appear to be considerable dubiety as to the manner in which cartilage canals first appear and their subsequent develop: :ment and function. This thesis represents a study of cartilage canals in the epiphyses of the long bones of human foetuses and also the epiphyses of the long bones of other mammals.

The definition of a cartilage canal, as given by Stump (1925) is now generally accepted. Stump considered that a cartilage canal is a channel containing an arteriole which carries the blood into the cartilage, a vein bringing it out, and a capillary plexus between them, all lying in a matrix of connective tissue. The whole structure, including both the vessels and the connective tissue, is known as a cartilage canal.

It is often believed that Hunter described blood vessels within cartilage as long ago as 1742. Hunter was, however, most guarded in his account of the vessels within cartilage and he states that in adult subjects, he was not able by his injection methods to demonstrate the vessels in the cartilage substance. In describing the circulous articuli vasculosus in young subjects, however, he states that the branches of it divide into still smaller ones around the neck of a bone in their progress to the centre of the cartilage. He qualifies this assertion, however, by the statement, "We are very seldom able to trace them (vessels) into its substance (cartilage) because they terminate abruptly at the edge of the cartilage like the vessels on the albuginea oculi when they come to the cornea."

Langer (1876) mentioned and illustrated by drawings, the vessels in the cartilages of the epiphyses of long bones of human foetuses.

Parsons (1905) noted vessels passing from the meta: :physis of a long bone to a newly formed ossific centre in the adjacent epiphysis and thought they were the route by which osteoblasts reached the new centre. But he did not mention the development and nutritive value of the vascular canals in the cartilage of an epiphysis. Bardeen (1910) in Keibel and Mall, stated that "Blood vessels, which spring from the periosteum and from the bone marrow, penetrate into the epiphyseal cartilage long before ossification begins .... In some cartilages the blood

vessels appear in the third foetal. In the seventh all the larger cartilaginous areas show rich vascular plexuses."

Stump (1925) mentioned the vascular channels beginning at the periphery as a mesenchymal penetration from the perichondrial zone, and extending into the centre of the cartilage of an epiphysis, and demonstrated by photomicro: :graphs for the first time the form and structure of the canal and its contents.

Hintzsche (1927) described the vascular canals in the cartilaginous epiphyses of the long bones in man, and demonstrated their form by drawings and negative models.

Haines (1933) described cartilage canals in man by a series of reconstructions of short and long bones in various developmental stages. He described the first appearance of the canals as simple unbranched structures and traced their development to a complex branched form. In the course of his investigations he recognised certain different types of canals, some of which are schematically shown in figure 1.

(1) Simple canals, branched and unbranched, which arise from the perichondrium by a single root.

(2) Multiple rooted canals, arising by two or more separate roots which subsequently unite soon after entering the cartilage.

(3) Tunnel canals, which form a direct communication between one area on the surface of the cartilage and another.

(4) Divided canals, in which the canals break into two divisions which later reunite to form again a simple canal.

(5) Communicating canals which, entering epiphysis from the perichondrium, turn towards the shaft and become continuous with the marrow spaces of the metaphysis (Fig.1,b).

(6) Centrifugal canals extend from the epiphyseal ossific centre towards the epiphyseal surface. Haines suggested that they represented the distal ends of canals in which continuity had been severed by the formation of the ossific centre (Fig.1,d). The proximal segment of such an interrupted canal is described as a nutrient canal (Fig.1,c).

(7) Centrifugal communicating canals are communicating canals the distal end of which open into the epiphyseal ossific centre (Fig.1,e).

Haines evolved a celluloid method of making transparent models of cartilage masses, which gives accurate information about distribution and branching of canals, but not about their thickness at any length.

Hurrell (1934) in his investigation of the vascular canals in the cartilages of long bones and the tarsal bones of the human foetus,verified and amplified the findings of other workers.

Le Gros Clark (1939) mentioned that vascular canals are absent in the relatively slender laryngeal cartilages of man but well developed in the much larger laryngeal cartilages of the ox.

... costal cartilage of ox.

2. MATERIALS AND TECHNIQUES

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This investigation has been carried out mainly on human material with additional observations on material from young kittens. In addition, observations were made on the cartilage of the skate (Raja batis) and also on the limb bones of young chicks. The human material consisted of six foetuses having C.R. lengths of 55 mm.(3 months), 104 mm. (4 months), 140 mm.(5 months), 180 mm.(6 months), 220 mm. (7 months) and 350 mm.(9 months) respectively. The cartila: :ginous parts of most of the limb bones were cut in serial sections from paraffin blocks and stained with haematoxylin and eosin. The distribution of cartilage canals was studied mainly from transparent reconstructions and these were of two types. By tracing the outline of the canals on cellophane papers a two dimensional view was obtained: this method is comparatively quick and inexpensive and for certain purposes very useful. On other occasions the tracings were made on sheets of transparent "xylonite", which was obtained in sheets of $\frac{1}{2}$ mm. and 1 mm. thickness, and such reconstructions when viewed over an X-ray viewing box gave a clear three dimensional view of the canals. These observations were supplemented by studying cleared specimens of foetal cartilage which had the vessels injected with india ink.

3. THE TIME OF APPEARANCE OF CARTILAGE CANALS IN CERTAIN OF THE TEMPORARY CARTILAGES OF MAN

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The time of appearance of cartilage canals has been found to vary considerably in different masses of cartilage.

In the proximal and distal epiphyses of the femur the canals make an early appearance and can be readily distinguished in specimens at the third month of intra-uterine life (fig.2). Furthermore, they quickly develop and become more complex in form so that in a specimen from a four months' foetus (fig.3) they form a rich pattern in the peripheral part of the cartilage.

On the other hand in the proximal epiphyses of the fibula the canals appear at rather a later date. In the three months specimen the mass of cartilage is completely avascular and even at the fifth month of intra-uterine life the canals have not developed to that degree of complexity which is apparent in the femoral epiphyses at a similar age.

In the bones of the foot simple tubular cartilage canals make their appearance in the cartilaginous talus and calcaneus during the third month of intra-uterine life (fig.4). On the other hand, the smaller tarsal bones are still avascular at the fourth month (fig.5) and canals are seen first in the specimen taken from the five months old foetus. The same is true of the epiphyseal cartilages of the metatarsals and phalanges.

In the innominate bone canals are present during the fourth month of intra-uterine life, but they are restricted, at this stage, to the comparatively thick region of cartilage at the lower part of the body of the ischium (Fig.6). In the remainder of the body and ramus of the ischium and in the pubis and ilium where the cartilage is comparatively thin canals are absent and do not make their appearance until the fifth month.

It is apparent from this data that although the time of appearance of cartilage canals in a mass of cartilage shows some connection with the time of ossification - cartilage canals appearing earlier in those masses which become ossified earlier - this relationship does not hold for all bones. Thus canals appear in the proximal epiphysis of the tibia during the third month and in the innominate bone during the fourth month, whereas the onset of ossification occurs in the opposite order: ossification has commenced in all segments of the innominate bone by the fifth month of intra-uterine life but is delayed until the first year of life in the proximal tibial epiphysis. It seems probable therefore that the appearance of cartilage canals is not directly linked with the onset or process of ossification in the cartilage mass.

A closer relationship can be established between the appearance of cartilage canals and the size of the cartilage concerned. It appears probable, as has been suggested by

Haines (1953) that the formation of cartilage canals commences when the cartilage mass exceeds the maximum size which can be nourished by diffusion of fluid from the surface. It is important to note, however, that by size one does not necessarily mean volume of cartilage. The key factor is the distance of a given region of cartilage from a surface. Thus, as is demonstrated in fig.7, if the mass of cartilage were in the form of a sphere of radius "r", the volume would be $4.18r^3$ and no part of cartilage would be more than a distance "r" from the surface. On the other hand, if the cartilage were in the form of a flat plate, having a uniform thickness of 2r, all parts of the cartilage would again lie within a distance "r" of the surface, and the volume could be increased to infinity without disturbing that relationship. The influence of this factor must be considered in comparing the time of canalisation of a cartilaginous long bone epiphysis and the cartilaginous innominate bone. Thus canals appear in the distal femoral epiphysis in the third month and in the innominate bone at the fourth month. The total volume of an innominate bone is always greater than that of the femoral epiphysis: but its canalisation is comparatively delayed because, the cartilage being thin and plate-like, the distance of any part of the cartilage from the surface is relatively small.

It comes to this then, that canalisation of cartilage occurs in any situation when any part of the cartilage

exceeds a certain distance from the surface. and can no longer be nourished by diffusion from the surface. An estimate of this critical distance,can, it is believed, be estimated from a study of the cartilage canals in the distal femoral epiphysis. In the intercondylar segment of this epiphysis the cartilage adjacent to the patellar surface is nourished in part by diffusion from the surface and in part from canals which grow towards that surface from the region of the suprapatellar pouch and from the intercondylar fossa. The distance of the tips of these canals from the surface has been measured at different ages from the fourth month to full-term, as described later in section 7. The distance increases slowly during development from 0.5 mm. at the fourth month to just over 1.0 mm. at full-term. Haines (1937) demonstrated that in the development of the patella, the distance between the articular surface and the tips of the canals growing from the superficial surface varies in almost exactly the same way.

Now such regions of cartilage are nourished by diffusion in two directions, so that the maximum thickness of cartilage which can be supported by this method is half the above measurements. In other words, it seems probable that the maximum thickness of cartilage which can be nourished by diffusion varies with the age of the foetus being 0.25 mm. at the fourth month and gradually increasing to about 0.6 mm. at full-term. Thus if one imagined by hypothetical masses

of cartilage which were all spherical in form, the critical size at which canalisation would occur would be a radius of 0.25 mm. at the fourth month, a radius of 0.32 mm. at the fifth month and so on up to full-term as shown in Fig.8.

4. THE DISTRIBUTION OF CARTILAGE CANALS IN THE HUMAN LONG BONE EPIPHYSIS

4. THE DISTRIBUTION OF CARTILAGE CANALS IN THE HUMAN LONG BONE EPIPHYSIS

The distribution of cartilage canals in the human long bone epiphysis is greatly influenced in each instance by the character of the various cartilage surfaces. It is axiomatic that a canal can only arise from a cartilage surface which is clothed by vascular tissue. This excludes the origin of canals from those wide areas of the surface of most epiphyses which are articular in character. Furthermore, although the vascular bone marrow of the metaphysis comes into direct contact with the actively growing cartilage of the epiphyseal plate it is a matter of common experience amongst workers in this field that canals passing from the metaphysis into the cartilage of epiphysis are few in number. Thus practically the origin of cartilage canals is restricted to the non-articular free surfaces of each epiphysis. This fact is one of the major determinants in the distribution of cartilage canals. Because of it cartilage canals are seldom found to be arranged in a radial fashion in relationship to the whole epiphysis as visualised in the diagram in Fig.9,i. On the contrary, the canals are arranged in a series of groups and the canals in each group flare out radially from the area from which they arise as indicated in the schematic diagram in Fig.9,ii. This distribution is dependent on the restricted area of origin of the canals and the necessity of

nourishing a large mass of tissue which has a small critical distance for nourishment by diffusion. Because of this distribution certain features of the canals are only to be expected. First some canals will be found running at places horizontal to or inclined towards the surface instead of running radially away from it, e.g. "a" in Fig.9,ii . Secondly, those canals which arise close to the metaphysis will be expected to bend away from the centre of the epiphysis towards the metaphysis, eg. "b" in Fig.9,ii; indeed some of these canals extend right through the cartilage and become continuous with the medullary cavity. These communicating canals will be considered later. Thirdly, those canals arising close to the margin of an articular surface will also bend away from the centre of the epiphysis towards the articular surface.

This general thesis is illustrated by the distribution of canals in the lower end of the femur at full-term. Fig.10 is a drawing of a reconstruction of the intercondylar segment and it shows the origin of canals restricted to the region of the suprapatellar pouch anteriorly and the region of the intercondylar notch posteriorly. The branches of each group flare out from their area of origin to reach the metaphyseal region and the patellar articular surface.

During the development of an epiphysis some measure of constancy is found in the pattern of the cartilage canals at different ages but it is not an absolute constancy.

Thus one group of canals always arises from the same area and in that group the direction of the central and peripheral members always remains much the same throughout the develop: :ment. Furthermore, certain canals can be seen in specimens of different ages, supplying similar areas of cartilage and having similar though not identical forms. Thus if one considers the most distal canal arising from the supra: :patellar surface of the femur its position, distribution and form show a considerable degree of constancy as shown in the photographs of the transparent reconstructions in Figs.11, 12, 13 and 14. In each case the canal arises by a single stem and this subsequently divides into two sets of branches. One branch extends in a radial direction towards the centre of the cartilage mass while one or two branches extend downwards and forwards towards the patellar surface. Despite this similarity however, the canals are by no means identical.

In the early work on cartilage canals many workers and in particular Stump (1925) in his article on the histo: :genesis of bone, claimed that the cartilage canals within epiphyseal cartilages extended to the centre of these masses and there established a prolific anastomosis. This view is not now accepted and no evidence in favour of it has been noted in the present investigation. The only canal which might be regarded as forming anastomoses are those which collectively form what Haines described as a tunnel canal.

The incidence of such formations however, is restricted to projecting tongues of cartilage and according to Haines they are formed by the total inclusion of a single vessel within the enlarging cartilage and not by the anastomosis of original separate channels. Apart from these formations, cartilage canals are discrete channels forming no communica: :tion with adjacent canals. Furthermore, they do not penetrate, as Stump claimed, to the centre of the cartilage: rather they stop short of the central region, leaving an avascular central zone, the appearance and significance of which will be discussed later.

5. COMMUNICATING CANALS

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The group of canals designated "Communicating Canals" by Haines have frequently been the subject of controversy, first as regards their existence, secondly, as regards their formation and thirdly, as regards their function.

Harris (1929) was strongly of the opinion that such canals did not exist in any normal bone but despite the weight of his opinion it is now fairly generally agreed that they are common phenomena in relation to the epiphyses of long bones. Certainly, in the present investigation, they have been by no means rare and there has been no evidence suggesting abnormality in any of the specimens examined.

Parsons (1905) noted numerous communicating canals in the epiphyses of the deerlet (Tragulus) and regarded them as representing osteogenic buds growing up from the shaft to form the centre of the epiphysis.

Langer (1876) followed by Hintzsche (1927) and others, held that the connection of the canals coming from the perichondrium with the diaphysial marrow spaces was brought about by secondary outgrowths from the diaphysis growing out to meet and anastomose with the incurved perichondrial canals. Furthermore, they held that the apparently purposeful establishment of such communications indicated that the canals so formed served some special function.

Bidder, A.(1906) noted the presence of vascular canals

going through the growing end of the shaft from the diaphyseal marrow cavity into the epiphyseal cartilage. He maintained that these canals contained osteoblasts from the first, that is the fourth month of foetal life: that these were destined for the development of the epiphyseal centre of ossification, and that this was the only regular source of osteoblasts and blood upon which this site of ossification could draw. In the present investigation blood vessels from the diaphysis running downwards into the cartilage of the epiphysis of the lower end of the femur of a one day old fowl, have been seen by injection methods (fig.15).

Hurrell (1934) on the other hand, held the opinion that the connections between the epiphyseal canals and the medullary spaces of the metaphysis, were probably only accidental and functionless, and that they were brought about by the extension of ossification from the shaft of the bone into those regions of the cartilaginous epiphysis already occupied by canals from the perichondrium. He considered that such communications after remaining patent for a short time were flattened out and occluded by the pressure of the swelling up of the surrounding cartilage cell columns in advance of the extending shaft bone, and that their rela: :tively brief persistence indicated their accidental and functionless nature.

Haines concurred with Hurrell's theory regarding the formation of the communicating canals but considered that

occlusion of the communications did not occur until ossification occurred in the epiphyses and that even then it failed to occur in some instances. Failure of occlusion resulted in the formation of a centrifugal communicating canal, that is a canal passing directly from the bone marrow of the shaft to that of the epiphyseal centre. Haines presumed such an unusual occurrence might lead to the mis: :conception that osteogenic buds grow up from the shaft into the epiphysis, giving rise to the centre of ossifica: :tion, but he found no evidence to support this hypothesis.

To work out the development of the communicating canal, several serial sections of the ends of different long bones at the same age and at different ages have been carefully studied.

I have found that they can be seen to occur in man as early as the fourth month of foetal life. The clear, well-formed communicating canals have been seen in the upper end of the femur (fig.16,a) and the upper end of the tibia (fig.16,b) of a four months old foetus. In all these, it has been noticed that the cartilage masses have come to possess fairly rich vascular canals.

But in the case of the upper end of the fibula of a five months foetus in which the size of the cartilage mass is comparatively smaller and the number of the canals is still few, communicating canals have not been seen. In this, the canals are relatively very short, not penetrating

to more than one third of the thickness of the cartilage mass. Similarly, in the lower end of the femur of a three months old foetus in which the number of vascular canals is still few and the canals are relatively short, communicating canals have not been seen to occur.

So, it seems that communicating canals appear as the vascular canals are increased in number and length.

As development proceeds some of the canals appear to become obliterated in the region of proliferating cartilage adjacent to the metaphysis. The lumen becomes progress: :ively smaller (fig.17) until the walls come into contact with one another and the site of the original communication is marked by a streak of acellular hyaline matrix passing from the epiphysis to the diaphysis between the prolifera: :ting cell columns and showing an eosinophilic staining reaction which marks it off from the surrounding basophilic matrix (fig.18). It appears that this fate only befalls a few of the communicating canals between their formation and the onset of epiphyseal ossification.

After the onset of epiphyseal ossification obliteration of the communicating canals occurs more rapidly so that by the time ossification of the epiphysis is complete and the cartilage has been reduced to a frank epiphyseal plate no communications between the medullary spaces of the diaphysis and the epiphysis remain.

The various developmental stages of the communicating

canals can be seen in the same epiphysis of the bone.

Fig.19. A simple short canal, A, in the lower end of the femur of a full-term foetus, which has penetrated to one eighth of the thickness of the cartilage mass and just bent round towards the diaphyseal end, but the tip of the canal is still far away from the diaphyseal end. It may be taken as the earliest stage in the development of a communicating canal.

Fig.19. A simple canal, B, which after penetrating radially has bent round towards the diaphyseal end. The tip of the canal reaches to within a few cartilage cells from the diaphyseal end. It may be taken as an advanced stage in the develop: :ment of a communicating canal.

Fig.20. The canals A, B and C present various developmental stages of the communicating canal in the lower end of the femur of a four months old human foetus.

From the examination of several serial sections of the ends of different long bones in man at different ages, it is considered that there are two main directions in which the cartilage canals grow. Thus, the canals fall into two groups. One which is directed towards the deeper part of

the cartilage mass i.e. the canals growing in a radial direction from the surface of the cartilage, and another which is directed towards the diaphyseal end. The latter one consists of the canals which arise from the perichondrium near the end of the shaft and bend towards the end of the diaphysis.

The two main directions I have mentioned, suggest that there are some factors influencing the directions of the growth of the ends of the cartilage canals. It is agreed nutrition is poorer towards the deep part of the cartilage. Thus, lack of nutrition of the cells in the deeper parts of the cartilage and the accumulation of metabolic waste products may be exercising a chemio-taxic influence on the growth of the canals.

Thus, it is considered that probably the primary cause of the formation of the communicating canal is the chemio-taxic influence in the zone of actively growing cartilage in the region adjacent to the metaphysis, which directs the ends of the vascular canals arising from the perichondrium near the end of the shaft to bend towards the diaphyseal end.

It would appear that a secondary cause is that if the ends of the canals lie in the growing cartilage of the end of the bone they may persist, while the cartilage around them is calcified and then eroded, thus coming into secondary continuity with the young marrow of the shaft to form the communicating canals.

Histology of Communicating Canals

Generally the histology of the communicating canals is the same as that of the cartilage canals in the epiphysis, which have no communications with the shaft. They contain a packing of embryonic connective tissue cells, fibres and blood vessels. The histology of the communicating canal at an older age does not differ from that of an earlier age. At no age have they been seen to contain osteoblasts with the exception of one in the section of the lower end of the femur of a six months old foetus, which is seen close to the perichondrium and contains osteoblasts.

Communicating canals in the medial end of the clavicle

A special type of communicating canal has been observed in the cartilaginous medial end of the clavicle of a four months old human foetus. In this, the buds of mesenchymal connective tissue and blood vessels arising from the perichondrium of the sternal end of the bone (Fig.21), are seen to penetrate through the matrix of the cartilage cells and come into continuity with the marrow of the bone. Some of the buds reach only to within two or three cartilage cells from the young marrow of the bone. These might be compared to various developmental stages of the communicating canals in the epiphysis of a long bone. But, it is remarkable to find that these buds are absent in the other cartilaginous end of the bone, that is the acromial end of the bone.

The cellular contents of these buds are identical with that of a cartilage canal. The cells are round or oval and spindle-shapod. The blood vessels are also contained in these buds. The only difference from the cartilage canal is that the lumen or channel in which the cells are contained cannot be made out as that of the cartilage canals. However, in some sections the lumen of the blood vessels are well shown.

The findings of these vascular connective tissue buds might be interpreted that to meet the requirement of the cells in the actively growing end of the clavicle they have to grow in from the perichondrium to assist in the supply of nutriment, removal of metabolic waste products and also, possibly, in the process of ossification at the end of the diaphysis.

Presumably, less activity in the growth of the bone at the acromial end may account for the absence of such vascular connective tissue buds at this cartilaginous end.

Functions of the communicating canal

What is considered to be the function of the communica: :ting canal has been partly mentioned namely that they assist in the supply of nutrition to and in the removal of waste products from the cells in the active juxta-metaphyseal cartilage. These functions are carried out mainly by the blood vessels in the canals. Since it also contains embryonic

connective tissue cells and fibres which are indistinguish: :able from the marrow connective tissues with which they come into continuity, and from that of the canals ending in the centre of ossification in the epiphysis, the communicating canals might be taken to be partly responsible for taking part in the ossification of the epiphysis. However, the almost invariable absence of osteogenic elements in these canals which was noted above gives no support to this hypothesis and one is led to assume that the functions of these canals are the same as those of other canals of simpler form and that their connection with the shaft is an accidental and purposeless feature.

6. HISTOLOGY OF CARTILAGE CANALS

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The structure of cartilage canals and their contents

(a) Wall of cartilage canal

Cartilage canals in the larger cartilages of older foetuses are relatively large channels and in the lower end of the femur of the full-time foetus many attain a diameter of 1,500 μ or even more. The composition of the bounding wall of a canal shows great variations not only with the age of a foetus but also with the depth of the canal from the surface. The variation in structure of the canal wall with age is in accordance with the change in structure of the perichondrium as the foetus grows older. In foetuses of about 4 months the perichondrium of the cartilage of the lower end of the femur consists of an outer layer which is predominantly fibrous and an inner layer which is formed of looser connective tissue and becomes more cellular in its deeper part. This more cellular region gradually merges with the cartilage and there is a gradual transition from primitive undifferentiated cells through chondroblasts to chondrocytes (figs.22 and 23). This cellular zone is regarded as the chondrogenetic zone and to it Hurrell has given the term "transition zone". The classical description of the perichondrium as consisting of two distinct layers, does not therefore, appear to be truly warranted as there is in succession from superficial to deep surface, a dense

collagenous layer, a loose collagenous layer and a third cellular layer showing cellular differentiation and merging with the cartilage - none of these layers are sharply demarcated from each other.

A cartilage canal in a young foetus at its ingrowth from the surface perichondrium carries with it the cells of the transition zone and also a mass of loose embryonic connective tissue in which the blood vessels are embedded. This transition zone persists throughout the length of the canal in young foetuses, although it may become thinner, and even disappear as a canal is traced towards the central part of the cartilage mass (figs.24 and 25).

With the increase in age the transitional zone of the perichondrium becomes less distinct and the appearance of it in the neck of the femur in a full-time foetus is shown in fig.26. The main mass of the perichondrium is here formed of dense fibrous tissue which in some places abuts on the cartilage directly but in other regions is separated from it by loose connective tissue in which blood vessels lie. The canals in all such older foetuses reflect the nature of the perichondrium as they show even in their most superficial parts only a very thin transition zone (fig.27,a) which gradually disappears.

In summary therefore, it may be stated that the wall of the superficial part of a cartilage canal is usually formed of a transition zone of cartilage in young foetuses,

but in the deeper parts of the cartilage, the transition zone may become thinner; in older foetuses the wall of a cartilage canal in the greater part of its length is formed directly by cartilage, and only in its superficial part is there a transition zone.

(b) Blood vessels of cartilage canal

A single arteriole usually passes into the surface opening of the canal in a central position embedded in loose embryonic connective tissue (fig.28). During its course along the canal it retains an approximately central position except where the canal is curved when it tends to bridge the gap and become applied to the shorter wall of the curve. It is noticeable that the canals show local dilatations and the ratio of the diameter of the main arteriole to its canal varies between $\frac{1}{3}$ and $\frac{1}{8}$; the terminal part of the arteriole has, however, a smaller relative size compared with the diameter of the canal. The main arteriole gives off small branches at irregular intervals along its course and these branches pass into daughter canals (fig.29). Some branches bifurcate shortly after their origin and the resultant vessels may continue in the mother canal for a short distance before passing into their respective daughter canals; it is for this reason that occasionally two or even three arterioles may be seen within one cartilage canal when it is sectioned

transversely. The branches of the arteriole are usually relatively short and each of them appears in injected specimens to terminate in a small mass of capillaries which has an irregular clublike form (fig.29). The capillaries open into greatly dilated venous sacs or sinusoids and the walls of these are formed by a single layer of endothelial cells supported by the surrounding loose connective tissue (fig.30). Although no full injection of the veins has been obtained in the sections I have examined, the veins would appear to show numerous anastomoses with each other and at their exit from the cartilage into the veins of the perichondrium they are frequently seen to be three or four in number.

(c) Connective tissue content of cartilage canal

The cellular and fibrous content of a cartilage canal cannot be distinguished from that of the deeper layer of the perichondrium and these, as has been stated, are continuous with each other. The texture of the connective tissue of the canal is therefore of a loose nature. The nuclei of the cells are round or spindle-shaped (fig.30), and in haematoxylin and eosin stained sections they appear pale.

In order to study the connective tissue cells and fibres of the canal at different levels, a cartilage canal of a full-term foetus has been traced from the surface to

its deepest part which is near the secondary centre of ossification and the diaphysis; this canal differs from the majority in this specimen, as its lumen does not communicate with the alveoli of the bone of the secondary centre.

The mixed nature of the nuclei of the cells is seen to extend throughout the whole length of the canal and does not vary in different parts, as described by Hurrell; neither is there any change in the density of the connective tissue along the length of a canal (fig.27, a, b, c).

A comparative study of the connective tissue content of cartilage canals at different foetal ages has been made. It has been noted that in some canals the connective tissue appears to become increasingly dense with increase in age and an example of this is shown in fig.31, which illustrates the nature of the connective tissue in the canal of a full-time foetus.

7. GROWTH OF CARTILAGE CANALS

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The growth mechanism of cartilage canals has been in dispute for some years. There are two broad theories:

Haines (1933) maintained that the canals grew in length solely by a process of inclusion, and his view is widely accepted at the present time. In his view, it is held that all the vessels which eventually occupy any mass of cartilage are in existence at an earlier stage of develop: :ment as part of the vascular bed in the surrounding undifferentiated mesenchyme. Fig.32a shows in diagrammatic form an avascular mass of cartilage at an early stage of development surrounded by a vascular region of undifferentiated mesenchyme. As development proceeds the cartilage mass enlarges by surface accretion due to the differentiation of surrounding mesenchyme. During this process some of the vessels in the original vascular bed of the mesenchyme are obliterated but other vessels persist and occupy undifferen: :tiated tracts in the newly formed cartilage (Fig.32b). With continued growth of cartilage more and more of the mesenchymal vascular bed is included in cartilage and this process continues until the growth of the cartilage is complete or until it is completely ossified.

Hurrell (1934), on the other hand, dismisses Haines' views on the grounds that whereas the vessels of the peri: :chondrium are predominantly concentric in orientation with

the cartilage mass, the subsequently developed cartilage canals are arranged in large measure in radial directions. He consequently believed that the canals grew actively into the cartilage mass by a process of chondrolysis, the cells and fibres of the original cartilage being transformed into the connective tissue cells and fibres of the canal.

It is considered that some light is thrown on the growth of cartilage canals by a study of the intercondylar segment of the distal end of the human foetal femur.

The growth mechanism of the cartilaginous distal femoral epiphysis

Any theory of the method of growth of cartilage canals must be founded on a knowledge of the growth of the cartilage mass which contains the canals, and on this matter there is some controversy.

It is apparent that in the growth of any mass of cartilage, enlargement may be brought about theoretically by one or both of two methods, namely on the one hand, surface accretion by the differentiation of mesenchyme in the chondrogenetic zone adjacent to the cartilage surface, and on the other, interstitial growth by the multiplication of cartilage cells and the progressive production of ground substance. Both methods of growth are generally accepted, it is their relative importance which is in dispute.

Streeter (1949) in his essay on the early development of the skeleton, believed that in the growth of the

cartilaginous shafts of long bones, surface accretion was the predominant factor, and although this belief is not founded on the wide experimental evidence which points to a similar growth mechanism in bone itself, it is neverthe: :less widely accepted.

It seems possible, however, that in the growth of the cartilaginous epiphyses of long bones the mechanism may be somewhat different. There is a considerable body of opinion which believes that in this instance the growth is, in large measure, interstitial in nature, and this belief is founded on two facts.

Harris (1933) showed that mitotic figures in a growing cartilaginous epiphysis were largely restricted to a spherical zone which lay at about $\frac{3}{4}$ of the distance from the centre to the surface. He named this zone the "mitotic annulus" and considered that it was here that much of the growth of the cartilage occurred. More recently his view has been strongly supported by Davis and Young (1954). They demonstrated that if sulphate labelled with radio-active S^{35} was administered to immature rats, subsequent autoradiographs show a considerable epiphyseal accumulation of the sulphur in the region of Harris' mitotic annulus, an accumulation which is much more dense than that in the perichondrial and immediate subperichondrial regions.

It seems probable therefore that whereas in the enlargement of small masses of cartilage, surface accretion

is the predominant mechanism, in the growth of larger cartilage masses such as the epiphyses and the short bones, interstitial growth plays a more important - even a predominant part.

Another problem exists in relation to the growth of the larger cartilage masses namely, the relationship of surface accretion to articular surfaces. It has been assumed by Haines (1937) that no surface accretion occurred on the articular surface of the patella during its growth but this can hardly be so in a structure such as the lower end of the femur. Fig.33 is a sketch of the intercondylar segment of the lower end of the femur: the region marked A is in relation to the suprapatellar pouch of synovial membrane and is covered by a typical perichondrium. The region marked B is the anterior wall of the intercondylar notch and it is also covered by a typical perichondrial layer. The region marked C is the articular surface related to the patella.

Bearing in mind the continuity of the form of the distal end of the femur during development it is difficult to believe that surface accretion occurs in the regions A and B while none occurs in the region C. Furthermore, in the region C the cartilage is covered by a layer of tissue which although it differs in some respects from frank perichondrium, does bear a large measure of similarity to that tissue. Its cells are of similar form, though their

tangential orientation is more marked, and the intercellular ground substance is strongly eosinophilic and only slightly metachromatic on staining with toluidine blue.

On the basis of the evidence outlined above it is considered that the growth of the cartilaginous lower end of the femur is accomplished by two mechanisms: that the predominant growth mechanism is an interstitial growth, and that this is augmented by a surface accretion through differentiation of mesenchyme - a surface accretion which is common in nature and degree over all surfaces of the cartilage mass.

The pattern of cartilage canals in the intercondylar segment of the lower end of the human femur

A section through the intercondylar segment of the distal epiphysis of the human femur at seven months is shown in fig.34. The posterior surface which forms the anterior wall of the intercondylar notch is non-articular and is covered by vascular perichondrium. The distal surface and the lower part of the anterior surface is articular and is clothed by the thin avascular layer of mesenchyme, which, as stated in the previous section, is considered to be chondrogenetic. The upper part of the anterior surface is in relationship with the developing suprapatellar pouch of synovial membrane and is thus covered by vascular perichondrium.

Since cartilage canals can only arise from a surface

at which the cartilage is clothed by vascular perichondrium, the origin of canals in this segment of the distal epiphysis of the femur is restricted to the posterior surface and the upper part of the anterior surface. There are thus two distinct sets of canals, anterior and posterior.

Observations

The form and distribution of the anterior and posterior canals in the intercondylar segment of the distal epiphysis of the human femur have been studied in transparent reconstructions of specimens from foetuses at three, four, five, seven and nine months. In each case serial sections were prepared of the intercondylar segment, the sections being cut in a sagittal plane at a thickness of 10 μ. Reconstructions were made by tracing the outline of the cartilage and the cartilage canals, in every fourth section on to $\frac{1}{8}$ mm. thick sheets of celluloid at a magnification of $12\frac{1}{2}$. Placed on an X-ray viewing box such reconstructions are highly transparent and the course and distribution of the canals can be readily visualised. Fig.35 is a photo: :graph of the reconstructed intercondylar segment in the seven months specimen.

At all the stages of development which have been examined the anterior and posterior canals extend predomin: :antly towards the central part of the cartilage mass, but in addition the lower members of each group possess branches

which extend towards the articular antero-inferior aspect of the femur.

It is on the growth pattern shown by the lowest canal in both the anterior and posterior groups that the subsequent argument is based and these two canals will be referred to henceforth simply as the anterior canal and the posterior canal. Figs.36, 37, 38 and 39 show the reconstructions of these canals in the three months, four months, five months and nine months specimens, and in fig.35 the arrows A and B indicate the same canals in the 7 months specimen.

Both canals maintain a considerable similarity of form throughout their development. At three months the anterior canal is a simple straight canal extending towards the centre of the cartilage. At four months it has developed one or two descending branches which pass down:wards towards the antero-inferior surface of the femur and this pattern is duplicated in ever enlarging form in the five months, seven months and nine months specimens; in the latter specimen the main canal opens into the recently formed ossific centre.

The posterior canal is also present in the three months specimen. At four months it has assumed its definitive pattern possessing a stem which divides into two sets of branches: one or two ascending branches are directed upwards towards the centre of the cartilage and a

tuft of branches continues anteriorly towards the antero-inferior surface of the femur, just below the region supplied by the descending branches of the anterior canal.

The graphs in fig.40 express certain measurements which have been taken from the reconstructions and checked on the relevant slides. Line A shows the maximum antero-posterior width of the intercondylar segment of the femur from the third to the ninth months of the intra-uterine life. It will be noted that the increase during this period of growth is approximately linear. As the distal end of the femur enlarges the details of its shape and of its relationship to the shaft show little change. It therefore seems probable that little difference exists in the rate of growth along different radii and as a corollary it follows that the rate of growth along any radius is half the rate of growth along any diameter and can consequently be expressed by the inclination of line B.

Line C represents the length of the anterior canal between the three months and nine months stages. The line is almost parallel to line B which represents the rate of radial growth. It is therefore suggested that this canal has increased in length purely as a result of enlargement of the cartilage mass and that its elongation is consequently the result of two simultaneously operating mechanisms. The first mechanism is that accepted by some workers as a common method of growth, namely, the progressive inclusion

of vessels within the cartilage mass as a result of surface accretion. And the second is a progressive radial stretching of the canal as a result of interstitial cartilage growth.

This hypothesis is supported by a consideration of the changing relationship of the origin of the descending branch to the stem of the anterior canal during the stages of development which have been examined. If the whole canal elongated during this period purely by a process of inclusion, it would be expected that the origin of the descending branch would be found at a progressively increasing distance from the root of the canal on the cartilage surface, but at a constant distance from its central tip. In fact if the pattern in the five months specimen is regarded as an individual variation, the distance of the origin of the descending branch of the anterior canal from the central tip of the canal shows a considerable increase between the fourth and nine months of development and such an increase can only be explained on the basis of stretching of that segment by interstitial growth.

However, the development of this descending branch of the anterior canal suggests that still another mehhanism may be involved in the growth of cartilage canals. Line D represents the radial distance of the tip of this descending

branch from the antero-inferior surface of the cartilage at the observed stages during development. It will be noted that the rate of increase of this distance, as indicated by the inclination of line D, is slow and in particular, that it is considerably slower than either the rate of radial growth of the whole cartilage mass as indicated by the inclination of line B, or the rate of radial migration of the origin of the descending branch from the cartilage surface as indicated by the inclination of line C.

Haines in his study of cartilage canals of the patella noted that they grew from the superficial surface of that bone towards the deep surface and that the distance between this deep surface and the canal tips changed at almost exactly the same rate as did the distance between the tip of the descending canals and the femoral surface in the present investigation. He assumed that no surface accretion occurred on the deep surface of the patella and that no interstitial growth occurred within the patella, and postulated first that the canals grew entirely as a result of inclusion by surface accretion, and secondly, that the increase in the avascular zone adjacent to the deep surface of the patella was a result of degeneration of the deep parts of the canals.

Such a theory cannot explain the changing pattern of the anterior canal in the femur. If the anterior canal

grew only by inclusion then the tip of the descending branch would migrate from the surface at a rate which was at least equal to the rate of growth of the whole canal. Neither is the mechanism of interstitial stretching applicable in this instance for since the stretching must occur in a radial direction, one would expect that features which commenced at similar distances from the surface, such as the origin and tip of the descending branch (.75 mm. and .53 mm. respectively), would still be similar distances from the surface at later stages of development. In fact however, at nine months the tip is 1.08 mm. from the surface whereas the origin is 3.8 mm. from the surface.

The only theory which appears to explain the facts satisfactorily is that as the origin of the descending branch of the anterior canal is carried farther from the surface by a combination of inclusion and interstitial stretching so the tip of the canal grows actively towards the antero-inferior surface of the cartilage. During the fifth month of development, it would appear that the rate of this active growth equals the rate of growth of the cartilage so that the distance of the tip of the canal from the surface remains constant. Thereafter the rate of growth of the canal lags behind the rate of growth of the cartilage so that in the ensuing three months the distance from the canal tip to the surface is doubled.

The theories put forward regarding the growth of the anterior canal form a satisfactory explanation also for the growth of the posterior canal during its develop: :ment from the fourth to the ninth month stage. As already described this canal passes from the intercondylar notch towards the antero-inferior surface of the femur and gives off an ascending branch which ascends towards the central part of the cartilage. It will be noted that throughout development the tip of this canal maintains a similar relationship to the antero-inferior surface of the femur as does the descending branch of the anterior canal. It is therefore suggested that the canal grows by a combination of three methods: inclusion on the posterior surface of the femur, stretching due to interstitial growth of the cartilage and active invasion of the cartilage by the tip of the canal.

8. ORIGIN OF THE CONTENTS OF CARTILAGE CANALS

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Stump, in his observations, found cartilage cells in the lumen of cartilage canals. He interpreted them as cartilage cells freed from the surrounding cartilage by solution of the cartilage matrix apparently by the action of the chondroblasts. He considered that the freed cartilage cells merged with the invading mesenchymal connective tissue from the perichondrial zone and were eventually indistinguishable.

Similarly, Haines (1933) observed in a section from the navicular bone, a canal which contained nothing but ordinary cartilage cells similar to those outside it, except at one point where a few connective tissue cells were seen. He held that these few cartilage cells could hardly be responsible for the formation of the whole cartilage canal by solution of chondromucin. He suggested rather that the structure was the remains of a canal whose vessels had become obliterated during growth, the connective tissue matrix being transformed into cartilage in just the same way as the connective tissue of the perichondrium is transformed. Thus, he believed that the presence of cartilage cells within a canal was evidence of its retro: :gression.

In my examination of the sections of the ends of long bones in man I have not seen cartilage cells in the

lumen of the cartilage canals, but I have seen a few cartilage cells to occur amidst the cells of the transitional zone, occasionally.

Hurrell (1934) was of opinion that the connective tissue contained in the cartilage canals were partly formed from the cartilage cells and fibres surrounding the cartilage canals by back differentiation of the cartilage to an embryonic type of connective tissue. This view was based on his findings, in the sections of tarsal bones, of the cartilage cells around the cartilage canal showing gradual transition. That is, he noted that going towards the canal from the general body of the cartilage, the cell bodies became enlarged, the cartilage nuclei changed gradually to the large, pale-staining, active appearance of those of young connective tissues while the matrix of the cartilage was reduced, until the cavities containing the cells were opened and merged into the canal lumen. This gradual transition in the cartilage cells around the canal is not found to occur in the cartilages of the epiphyses of man. In man, as mentioned before, there is a transitional zone (chondrogenetic zone) containing mostly roundish cells between the contents of the cartilage canal and the cartilage around, which is exactly similar to the zone between the perichondrium and the cartilage. This finding leads me to hold a view different from Hurrell's, that is,

that the connective tissue content of the cartilage canals of the epiphysis has the potentiality of differentiating into cartilage cells and of thus adding new cartilage cells around the canal.

It may be mentioned here that in my study of the sections of the epiphyses of kittens in various developmental stages I have not observed the gradual transition in cartilage cells to connective tissue cells around the cartilage canal as Hurrell mentioned, nor the transitional zone (chondrogenetic zone) between the cartilage and the connective tissue content of the canal as I have mentioned. The clear boundary between the canal contents and the cartilage around is found to occur in all the canals and at any level of the canal (fig.41). But the cartilage canals contain the same embryonic connective tissues and vessels as in man.

This would suggest that there is no possibility of the connective tissue cells of the cartilage canal of the epiphysis being formed from the cartilage cells around the cartilage canal. The only possibility that could be deduced is that the connective tissue cells of the canal are derived from the perichondrium.

Thus, as in all the canals, both in man and kitten, with a few exceptional canals arising from the diaphysis, the content can be traced into continuity with that of the

deeper layer of perichondrium, and since the contents of the deeper layer of the perichondrium and the cartilage canal are indistinguishable, it may be concluded that the contents of cartilage canals are derived from the perichondrium.

9. THE SITE OF OSSIFICATION IN VASCULARISED CARTILAGES

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Parsons working on the epiphyses of man and animals, suggested that the centre of epiphyseal ossification begins as a degenerative process in the least vascular part of the cartilaginous end of the bone, that is to say, the centre of it, and that the larger the mass of the cartilage, the less well nourished would the centre of it be, and so more liable to the early deposit of lime salts.

Hintzsche (1928), however, in his reconstructions of the lower end of the femur, could find no difference between the nutrition of the site of ossification and that of many other parts of the cartilage, so that he could not consider lack of nutrition as a cause of degeneration. Nor did he find any causal connection between the position of cartilage canals and the site of the centre of ossification. He suggested that the site of the centre of ossification is determined by mechanical forces, either in each individual embryo or in the ancestors of the species. He gave an example of the mechanical effect of the patella as it glides over femur, working either directly on the foetus itself, or more probably, through its ancestors. Haines, on the other hand, working on the epiphyses of man and kittens, reverted to the older theory and held that the cartilage

canals determine by their distribution the position where the centre appears.

It has been found in the present investigation that in cartilages which contain cartilage canals before and at the time of their ossification, the special relationship between the site of ossification and the canals shows considerable variation.

As has already been noted the cartilage canals of a cartilage never grow from the whole of its surface. Rather they are restricted in their origin to certain non-articular regions of the surface so that they form well defined groups. It has also been noted that the canals of different groups never anastomose with one another. The consequence is that there is always within the cartilage an avascular zone or zones which lie between two or more groups of canals and are nourished by diffusion from either side.

In the epiphysis of long bone, this avascular zone always lies about the centre of the cartilage mass, and in the bones which have been examined, this has been an invariable association between this zone and the onset of epiphyseal ossification. In each specimen as development proceeds the cartilage of the avascular zone undergoes calcification and subsequently ossifies.

This relationship is illustrated in the succeeding illustrations. Fig.42 shows a coronal section through

the lower end of the femur in a ten days old kitten, that is, before the onset of epiphyseal ossification. The avascular zone (A.Z.) is seen situated about the centre of the cartilage mass, surrounded by vascular canals which enter the bone from the anterior and posterior aspects. Fig.43 is a coronal section of the same region in a fifteen days old kitten, that is just after the onset of epiphyseal ossification. The centre of ossification is seen to occupy the site of the original avascular zone, and the canals which originally surrounded the avascular zone now open into its periphery to become continuous with the marrow spaces. The same relationship is seen in the upper end of the humerus in the kitten. Fig.44 is a coronal section through the upper end of the humerus at four days and it shows a typical avascular zone situated about the centre of the cartilaginous head. Fig.45, on the other hand, shows the same part from a fifteen days old kitten and the site of the avascular zone is now seen to be occupied by the centre of ossification. The arrangement noted in the epiphyses of the kitten is repeated in the ossification of the epiphyses of human long bones. Fig.46, is a sagittal section through the lower end of the femur in the seven months foetus and it will be noted that the avascular zone occupies a more or less central position being limited anteriorly and posteriorly by the canals

arising from the corresponding aspects of the cartilage. Fig.47 shows the lower end of the human femur at full-term: the centre of ossification has formed in the site of the avascular zone, and the surrounding canals now open into its marrow space.

However, although this relationship between the site of ossification and the site of the avascular zone is apparently common to all the long bone epiphysis it is not met with in the ossification of certain other vascularised cartilage masses. Thus, the human ischium contains cartilage canals before the onset of ossification, but when ossification does occur it commences in close relation: :ship to the surface and is associated with deposit of periosteal bone (Fig.48).

A somewhat similar arrangement is seen in the ossification of the talus. In this bone the cartilage canals are restricted to the non-articular areas of its surface, and the largest canals form two groups arising from the sulcus tali and the dorsal surface of the head and neck respectively. The avascular zone between these two groups lies centrally at the junction between the head and neck, but the initial calcification which precedes ossifica: :tion occurs in immediate relationship to the sulcus tali. Moreover, it usually occurs as a collar around the stem of one of the large canals entering the cartilage from the surface, and is frequently associated with a periosteal

plaque of bone (Fig.49).

From this variable association between the centre of ossification and the avascular zone, it appears probable that there is no connection per se between the distance of cartilage from a vascular source and the onset of calcification and subsequent ossification. Calcification and ossification may occur in an avascular zone, but it may equally well, as in the talus, occur directly around the stem of the cartilage canal, that is as close as possible to a source of nourishment. It would follow from this that calcification is not in itself a symptom of poor nutrition of cartilage; an observation which is in keeping with the fact that the calcified cartilage which normally separates articular cartilage from subjacent bone in an adult bone is much closer to a source of nutrition in the form of medullary vessels than the uncalcified cartilage adjacent to it (fig.50).

Neither does it appear probable that calcification is a result of ageing in cartilage. In long bone epiphysis ossification certainly occurs close to the centre of the cartilage mass and consequently amongst what is probably the oldest part of the cartilage, in the case of the talus and the ischium there is no evidence to suggest an eccentric growth rate which would place the oldest cartilage eccentrically within the whole mass.

One is forced therefore to fall back on the view that the site of ossification in a vascularised mass of cartilage is due simply to the inherent growth pattern of the part and has no relation to the age of the cartilage or to its state of nutrition.

The earliest stages of ossification in long bone epiphyses occur with great rapidity and consequently they have not been observed in the present investigation. The earliest stage which has been observed is represented by the fig.51. Here the calcified cartilage has been eroded by communicating cavities and bone has begun to form on the cartilaginous remnants. Opening into the labyrinthine marrow space are several cartilage canals. One assumes that the process preceding this stage consisted of a calcification of cartilage in the avascular zone and that when this calcification extends out as far as the cartilage canals, buds of vascular connective tissue from the canal contents grow out and erode the calcified cartilage. It seems apparent furthermore, that the bone which has been laid down on the cartilaginous remnants is due to the activity of osteoblasts which have been formed from the multipotential mesenchymal cells in the cartilage canals. As stated this process of invasion of calcified cartilage from the cartilage canals has not been seen in the epiphyses of the long bones. It has, however, been observed in the talus and is illustrated in fig.52.

This figure shows the eccentric area of calcified cartilage, closely related to one side of a cartilage canal arising from the sulcus tali. The wall of the canal on this aspect has disappeared and capillary turfs are seen to be invading and destroying the neighbouring calcified cartilage.

However, although this close association between the process of ossification and cartilage canals exists in the human talus and probably also in the human long bone epiphyses, the association is not found in some types of animals. Thus in the head of the first metatarsal of the full-term human foetus the cartilage contains numerous cartilage canals (figs.53, 54 and 55), yet no secondary centre is formed in this region which, as is well known, is ossified by extension from the diaphysis. Again, in the cartilaginous fishes, such as the skate, cartilage canals are present in the vertebral bodies, although no ossification subsequently occurs (fig.56). And again, as Haines (1933) has pointed out, endochondral ossification occurs without the presence of cartilage canals in the greater trochanter of the rat.

10. SUMMARY AND CONCLUSIONS

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1. A review of the literature on cartilage canals is given.

2. The formation of cartilage canals commences when the cartilage mass exceeds the maximum thickness which can be nourished by diffusion of fluid from the surface. This maximum thickness or the critical size at which canalisation would occur, has been worked out in the distal femoral epiphysis at various developmental stages. It varies with the age of the foetus being 0.25 mm. at the fourth month and gradually increasing to about 0.6 mm. at full-term.

3. Because of the restricted area of origin of the canals in the cartilaginous epiphysis of long bones, the canals are seldom found to be arranged in a radial fashion in relationship to the whole epiphysis, but they are arranged so as to distribute the blood evenly through the whole mass.

4. (i) The clear, well-formed communicating canals have been seen in the epiphysis of human long bone as early as the fourth month of foetal life.

(ii) As development proceeds, some of the communicating canals appear to become obliterated in the region of proliferating cartilage adjacent to the metaphysis:

this obliteration of canals occurs more rapidly after the onset of epiphyseal ossification so that by the time ossification of the epiphysis is complete, no communications between the diaphysis and the epiphysis remain.

(iii) It is suggested that probably the primary cause of the formation of the communicating canal is the chemio-taxic influence in the zone of actively growing cartilage in the region adjacent to metaphysis, which directs the ends of the canals arising from the perichondrium near the end of the shaft to bend towards the diaphyseal end.

(iv) The probable function of the communicating canals is that they assist in the supply of nutrition to and in the removal of waste products from the cells in the active juxta-metaphyseal cartilage. The almost invariable absence of osteogenic elements in these canals gives no support to the hypothesis that they take part in the formation of the centre of epiphyseal ossification.

(v) The vascular connective tissue buds which are identical with the communicating canals in the epiphysis of long bone, have been observed in the cartilaginous sternal end of the clavicle of a human foetus.

5. It is suggested that the cartilage canals grow by a combination of three methods, that is by surface accretion, stretching due to interstitial growth and active invasion of the cartilage by the tip of the canal.

6. The cartilage canal connective tissue contents are of perichondrial origin, and are not formed by back differentiation of the cartilage to an embryonic type of connective tissue.

7. In the long bone of the human, the cartilage canals are probably responsible for the formation of the epiphyseal ossification centre.

REFERENCES

REFERENCES

BARDEEN, C.R. (1910). Keibel and Mall, Manual of Human Embryology, Philadelphia & London: J.B. Lippincott Company, 1, 292-311.

BIDDER, A. (1906). Arch. mikr. Anat., 68, 137-213.

DAVIES, D.V. and YOUNG, L. (1954). J. Anat., Lond., 88, 174--183.

HAINES, R.W. (1933). J. Anat., Lond., 68, 45-64.

HAINES, R.W. (1937). J. Anat., Lond., 71, 471-78.

HARRIS, H.A. (1929). J. Anat., Lond., 64, 3 - 4.

HARRIS, H.A. (1933). Bone Growth in Health and Disease. London: Humphrey Milford, 145-47.

HINTZSCHE, E. (1927). Zeitchr. F. mikr.-anat. Forschung, 12, 61-124.

HINTZSCHE, E. (1928). Zeitchr. F. mikr.-anat. Forschung, 14, 373-438.

HUNTER, W. (1742). "On the Structure and Diseases of Articular Cartilage." Philos. Trans., 42, 514-21

HURRELL, D.J. (1934). J. Anat., Lond., 69, 47-60.

LANGER, K. (1876). Denkschr. Akad. Wiss. Wien, Math.-nat. K 1, 63, 1 - 40.

Le GROS CLARK, W.E. (1945). The Tissues of the Body, 2nd Ed., Oxford: Geoffrey Cumberlege, 57 - 60.

PARSONS, F.G. (1905). J. Anat. and Phys., 39, 402-12.

STREETER, George L. (1949). Contributions to Embryology, Carnegie Institution. Developmental Horizons in Human Embryos, 4th Issue, 33, 149-167.

STUMP, C.W. (1925). J. Anat., Lond., 59, 136-152.

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