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The Subchondral Bone Plate of the Femoral Head in Adult Rabbits

I. Spontaneous Remodelling Studied by Microradiography and Tetracycline Labelling

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Summary. The mineralized structures and spontaneous remodelling of the subcondral bone-plate in the femoral head of adult rabbits of different ages were studied by microradiography and fluorescence microscopy after administration of Tetracycline between 8 months and 2 days before death. In animals over 1 year of age the subchondral bone-plate was constructed of evenly mineralized osteones, located mainly in the immediate vicinity of the calcified articular cartilage and lamellar bone bordering on the medullary cavity. In some small areas lamellar bone was the only bone tissue separating the medullary cavity from the calcified articular cartilage. In adult animals between 8 and 10 months of age the subchondral bone-plate was absent in minor areas, chiefly in the lateral parts of the femoral head. In the fluorescence microscopic study in animals older than 1 year (a) the osteones in the subchondral bone showed a very low labelling frequency, (b) fluorescence was observed mainly on the lamellar bone surfaces bordering on the medullary cavity and (c) the labelling in the cortical part of the subchondral bone was still present up to 8 months after administration of the Tetracycline. The cancellous bone of the epiphysis showed a low labelling frequency which was of the same order as that in the lamellar surfaces belonging morphologically to the subchondral bone plate.

From these observations it was concluded that (1) the cortical part of the subchondral bone-plate in rabbits over 1 year of age undergoes a low degree of spontaneous remodelling and (2) the cancellous bone of the epiphysis and the lamellar bone belonging morphologically to the subchondral bone-plate undergo low but similarly spontaneous remodelling. Duplicate fluorescence of the tidemark observed in a number of animals was considered to indicate that the mineralization of the articular cartilage with increasing age slowly progresses towards the articular surface.

Introduction

The present work is an investigation which will form the basis of a study of the reaction of the subchondral bone to damage to the articular cartilage of the femoral head in adult rabbits. Holmdahl and Ingelmark (1950) found, in histological studies of 10-months old albino rabbits, two different types of vascular contacts, dentritic and ampullar, between the calcified layer of the articular cartilage and the medullary cavity. These contacts were considered to be of possible importance for the nutrition of the articular cartilage. Mankin (1963) described the subchondral bone plate in the knee joint in adult rabbits (5 kg) as a well defined cortical shell. After intraarticular administration of ^{3}H-thymidine he found no labelled cells either in the articular cartilage or in the subchondral bone. From this he concluded that new bone formation could not be demonstrated in the

subchondral bone by this method. In growing rabbits Mankin (1962) found, by means of the same technique, that the deepest layer of articular cartilage constitutes the growth zone for the epiphyseal bone nucleus. At this age no cortical subchondral bone plate has been formed.

In vivo labelling with Tetracycline preparations was chosen as a method of investigation in the present study since these substances are permanently incorporated into bone tissue which is undergoing active mineralization at the time of their administration (fore references, see Ibsen and Urist, 1964). Calcifying cartilaginous callus has also been found to take up Tetracycline (Hulth and Olerud, 1964), but not normal articular cartilage (Milch *et al.*, 1958).

The aim of the present investigation was to study in the femoral head of adult rabbits of different ages, by means of microradiography and Tetracycline abelling, (a) the structure of the mineralized parts and the spontaneous remodelling of the subchondral bone and (b) the question of whether there is any further growth of the osseous part of the epiphysis after otherwise terminated longitudinal growth, as has been claimed by Johnson (1959). An attempt will be made, by means of these methods, to find a definition of the structure "subchondral bone plate," especially with respect to its delimitation from the remaining bone tissue in the epiphysis.

Material and Methods

Animal Material and in vivo *Labelling with Tetracyclines*

Group A. Albino rabbits of both sexes were used. Ten rabbits about 1 year old (3.7–4.3 kg), 4 about 2 years old and 6 of different ages between 4 and 10 months were given oxytetracycline in a dose of 25 mg/kg body weight intravenously and were killed 2 or 3 days later (Table 1).

Group B. Eleven rabbits with a nominal age of 8–9 months were given chlortetracycline 25 mg/kg) intravenously and were killed at different time points between 3 and 8 months later. Oxytetracycline (25 mg/kg) was given intravenously 2 or 3 days before death (Table 2).

Two rabbits about 1 year old were given no Tetracycline preparation and served as controls of possible autofluorescence.

Examination Methods

Microangiography with Indian ink or Micropaque, as described by Hulth and Olerud (1962) was performed in 6 animals. The femoral head with the neck and adjacent trochanteric regions were freed from soft tissues and divided in the coronal plane through the insertion of the ligamentum teres, dehydrated in absolute alcohol and embedded in methyl methacrylate. Consecutive sections parallel with the divisional surface in the coronal plane were sawn out and ground down to a thickness of 100 μ, and in some cases to 50, 30 and 20 μ. Microradiographic examination was performed in a Philips PW 1010/30 apparatus at 15 kW; the preparation lay in direct contact with Kodak spectroscopic plates 649-0. The ground sections were mounted in fluorescence-free balsam (Permount) and examined in a Zeiss fluorescence microscope. The filter combinations of exciter filters BG 38 and BG 11 with barrier filters 50 and 65, and BG 38 and UG 1 with barrier filter 41 were used. The fluorescence preparations were examined at a linear magnification of 20, 50 or 100 times and only in exceptional cases were higher mangifications used.

The articular surface of the femoral head was divided into 7 zones, as illustrated in Fig. 1. Zones I and VII are covered with articular cartilage to only a very small extent and are delimited medially by the last visible row of calcified articular cartilage. The size of the zones varied somewhat in each individual case. The delimitation of zone III was chosen in consideration of a study in which an articular cartilage defect was created in this area (Lemperg, 1971).

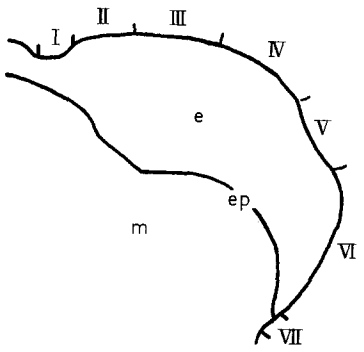


Fig. 1. Schematic division of the femoral head into different zones. *I* and *VII*: extends from the last visible row of calcified cartilage 1 mm laterally and lies to the greatest part outside the cartilagecovered surface of the femoral head. Zone *III* is about 3 mm in diameter and zones *II* and *IV* about 2 mm. *V* insertion of ligamentum teres. *VI* the medial articular surface. The measurements given in mm for zones *II* and *IV* are approximate, since the size of the femoral head varies somewhat. *e* epiphysis; *ep* epiphyseal plate; *m* metaphysis

Results

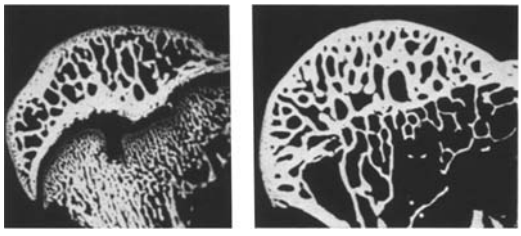
Unless otherwise stated, all observations reported refer to the central section in the coronal plane in which parts of the insertion of the ligamentum teres were visible.

Microradiographic Observations

The location of the epiphyseal plate and of the epiphyseal scare within the central part of the femoral head can be seen in Figs. 2a and b. The major part of the articular surface of the femoral head lay medial to the epiphyseal plate, with the exception of a small area in the lateral, ventral part lying on the metaphyseal side. In the peripheral segments of the femoral head (caudally and cranially) the whole of the cartilage covered surface of the femoral head lay medial to the epiphyseal plate.

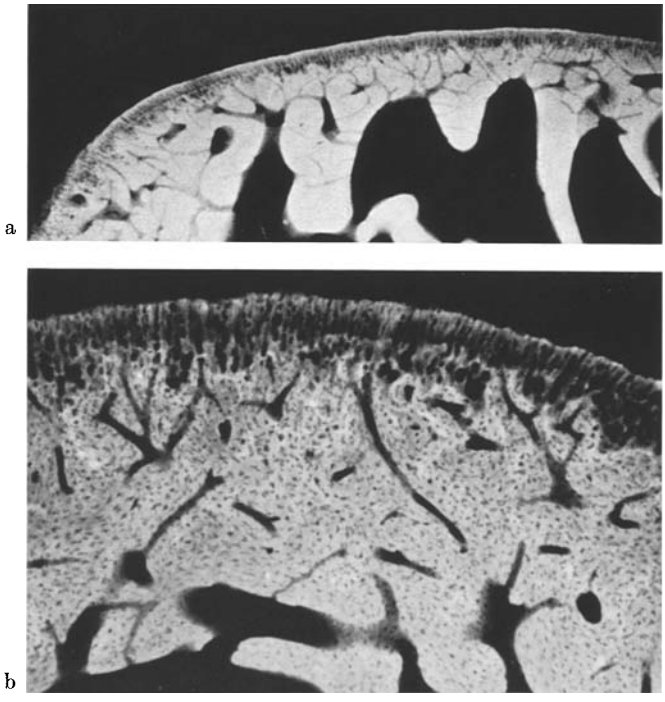
Observations in Animals with a Closed Epiphyseal Plate

The bone tissue between the deepest layer of calcified articular cartilage and the immediately adjacent medullary cavity was composed of osteones and lamellar bone. The thickness of this bone layer varied in different parts of the femoral head (Fig. 3a). The area of insertion of the ligamentum teres (zone V) consisted mainly of compact bone (Fig. 3a). In zones III, IV and VI the bone tissue adjacent to the calcified articular cartilage was composed mainly of osteones, while that part which lay against the medullary cavity consisted to the greatest part of lamellar bone (Figs. 3b and 4). In zone II, and in exceptional cases in other zones also, there were, however, areas in which only a thin layer of lamellar bone or a narrow band of highly mineralized tissue separated the calcified articular cartilage from the medullary cavity. The distribution, orientation and density of the vascular network between the medullary cavity and calcified cartilage are shown clearly by a tangen-



a b

Fig. 2. a Microradiogram of a coronal section through the proximal femur from a 4-month old rabbit, showing the location of the epiphyseal plate in the head and neck of the femur. b Similar section to that in (a), from a rabbit about 1 year old. Epiphyseal scar at the site of the epiphyseal plate



a

b

Fig. 3. a Microradiogram from a 9-month old rabbit. Dorsal articular surface of femoral head. To the left is the insertion of the ligamentum teres, consisting of compact bone; the bone tissue between the medullary cavity and calcified articular cartilage varies in thickness. Laterally (to the right) the medullary cavities lie closer to the articular cartilage than in the medial area. ×50. b Rabbit over 1 year of age. The bone tissue under the calcified articular cartilage consists of evenly mineralized osteones. Vascular canals traverse the bone tissue in different directions and connect the medullary cavities with osteones and with each other. ×115

tial section from the cranial part of the femoral head (Fig. 5a). Canals with a diameter of about 10–15 μ entered the calcified cartilage at regular intervals, but without penetrating the tidemark (calcified line).

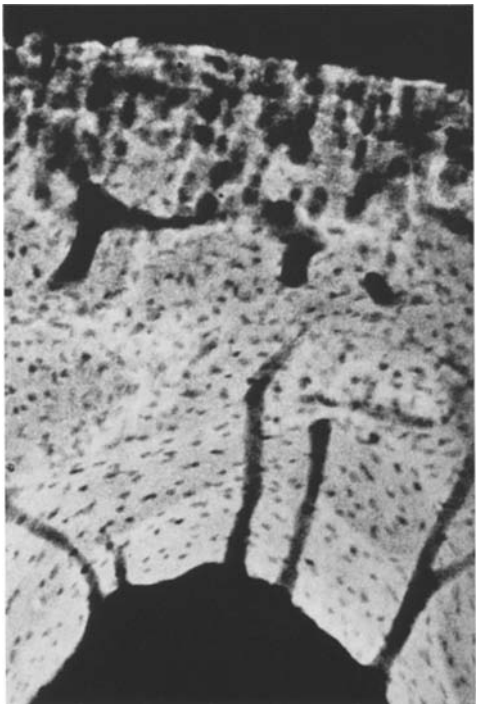


Fig. 4. Microradiogram from a rabbit over 1 year of age. Lamellar bone with high mineralization is delimiting the medullary cavity. ×365

The mineralization of the osteones was even, and resorption cavities were observed in only one animal over 1 year old—in a 2-year old animal. The lamellar bone lying against the medullary cavity sometimes showed a high density (Fig. 4), but signs of bone resorption were also found at identical locations in some cases. The frequent occurrence of oblique surfaces in these areas rendered evaluation of the mineralization difficult, however.

There were some differences between animals of ages 1 year and older and those below 1 year. In the younger animals areas in which only a thin layer of lamellar bone separated the calcified cartilage from the medullary cavity were more common and more extensive over the articular surface than in the older animals, and isolated canals with a diameter of 10–15 μ reached the tidemark. In small areas there was direct contact between the medullary cavity and the calcified articular cartilage.

Observations on Fluorescence Microscopy

Autofluorescence. With the filter combination BG 38 and BG 12 and barrier filters 50 and 65, some autofluorescence of the chondrocytes in the calcified cartilage and also of osteocytes was seen. With the filter combination BG 38 and UG 1 and barrier filter, autofluorescence was either not visible or very weak in these structures, and Tetracycline-inducde fluorescence was selectively visible.

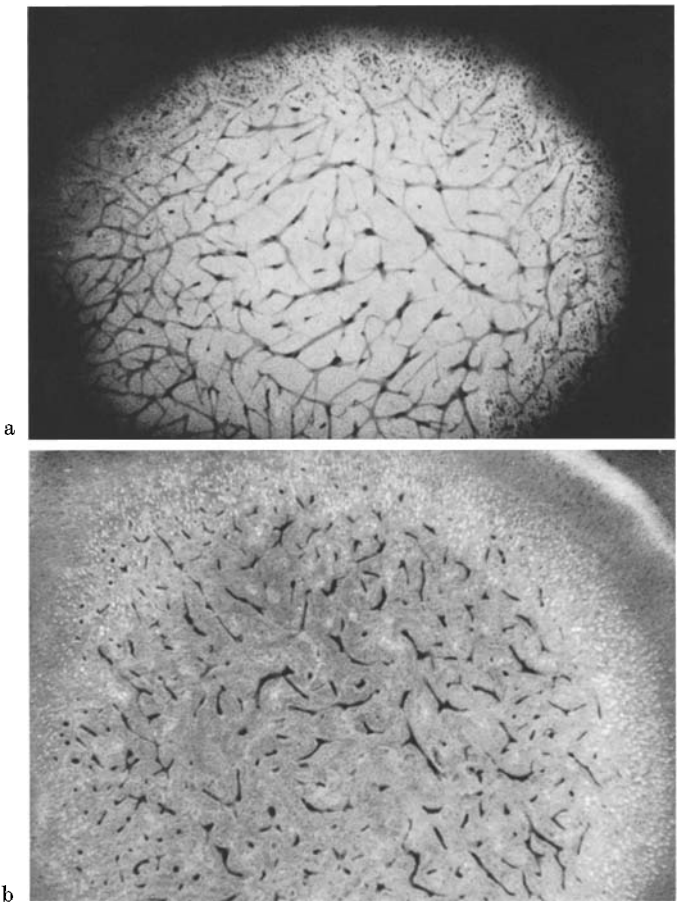


Fig. 5. a Microradiogram from a tagential section from the cranial part of the femoral head from a rabbit over 1 year of age. The section is taken from bone tissue lying directly under the articular cartilage. In the central part of the photograph vascular canals are seen in compact bone constructed of osteones. Calcified cartilage is seen peripherally. Vascular canals are lying partly within the calcified cartilage. 30 μ thick sections. $\times 40$. b Fluorescence photomicrograph from a similar section as in (a) Autofluorescence of the chondrocytes in the calcified articular cartilage. No Tetracycline-induced fluorescence. $\times 72$

Tetracycline-Induced Fluorescence, Group A

Tetracycline-induced fluorescence was recorded in the following structures of the subchondral bone and in the articular cartilage: a) tidemark, in Zones II–VI (Fig. 6); subperiosteal fluorescence in zones I and VII; b) osteones; c) intracartilaginous vascular canals; and d) lamellar bone tissue, delimiting the medullary cavity situated nearest to the articular cartilage; only the surface facing the articular surface was evaluated (Fig. 7). In the subchondral bone the number of labelled structures per zone was counted. The fluorescence of the tidemark and the subperiosteal fluorescence were recorded as present (+) or absent (−). In the

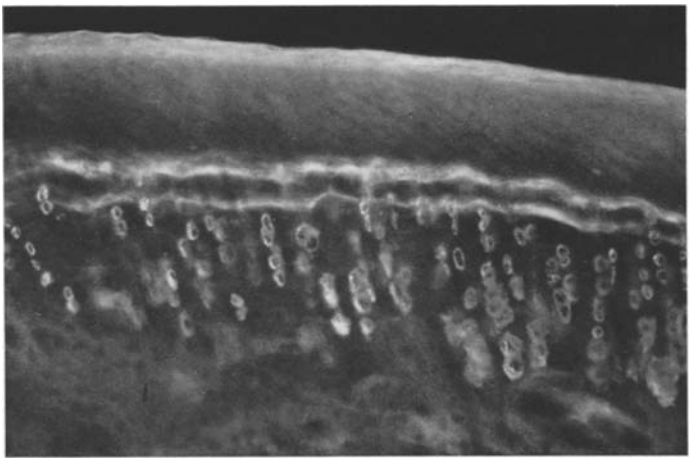


Fig. 6. Fluorescence photomicrograph from double-labelled animal (no. 2) with an interval of 7 months between the Tetracycline doses. The fluorescence in the tidemark close to the articular surface can be attributed to the second Tetracycline dose given 2 days before death. ×250

cancellous bone of the epiphysis and metaphysis the frequency of fluorescent surfaces was estimated in relation to the number of surfaces visible, which varied in different preparations.

Table 1 shows the observations on the animals in group A (one dose of oxytetracycline). The number of fluorescent structures is given for zones II–VI combined, since no difference between these zones was found in detailed studies. It is evident from the table that in zones II–VI in animals up to the age of 9 months fluorescence occurred regularly in osteones in the subchondral bone. Among animals exceeding 10 months of age in only 2 out of 15 cases was one osteone seen to show fluorescence. In Fig. 5b, a tangential section through the subchondral bone from a 1-year old animal, a large number of osteones are visible, none of which show fluorescence. The animals over 10 months of age exhibited fluorescence mainly on the lamellar bone surfaces delimiting the medullary cavity (structure d). Fluorescence in the tidemark occurred in only a limited number of animals and did not always cover the whole area of the femoral head. Fluorescence which could be attributed with certainty to intracartilaginous vascular canals was seldom observed. In the calcified layer of articular cartilage weakly fluorescent gracile bands were observed with higher magnifications.

Zones I and VII showed a greater number of fluorescent structures; when comparing these figures with those from zones II–VI it should be noted that the total bone surface in zones I and VII is only a fraction of that in zones II–VI.

The bone surfaces in the cancellous bone of the epiphysis showed a varying labelling frequency, but it was always distinctly higher than that in the subchondral bone. In the cancellous bone of the metaphysis the labelling frequency was considerably higher in most cases than in the epiphysis.

Table 1. *Distribution of Tetracycline-labelled structures in different parts of the femoral head in rabbits of different ages*

Approx. age of animal	Subchondral bone										Amount of fluorescence in cancellous bone of		Remarks
	zone I			zones II—VI				zone VII					
	a	b	d	a	b	c	d	a	b	d	epiphysis	metaphysis	
4 months	large number of labelled structures in all zones										+++	++++	growing epiphyseal plate
5 months	+	2	1	+	2	2	6	+	—	2–3	++	+++	epiphyseal plate remnants
6 months	—	1	1	—	2	2	2	—	—	—	++	+++	epiphyseal plate remnants
8 months	—	—	1	—	4	—	4	—	—	1	++	+++	
9 months	+	—	1	—	2	—	—	+	2	1	++	+++	
10 months	—	—	—	—	—	—	5	—	—	1	+	++	
1 year	—	—	—	—	—	1	—	—	—	—	++	+++	
1 year	+	—	—	—	—	—	—	+	—	—	+	++	
1 year	+	—	—	+	—	—	1	+	—	1	+	++	
1 year	—	—	1	—	—	—	3	—	—	1	++	++	
1 year	+	—	1	—	—	—	—	+	—	1	+	++	
1 year	—	—	—	—	—	1	2	+	—	—	+	++	
1 year	+	—	1	—	—	—	1	—	—	—	+	+++	
1 year	+	—	—	+	—	—	—	+	—	—	+	++	
1 year	—	—	—	—	—	—	—	—	—	—	++	++	
1 year	+	1	2	+	—	—	1	+	—	—	++	+++	
2 year	—	1	1	—	—	—	—	—	1	1	+	++	
2 year	—	1	—	—	—	—	—	+	—	—	+	+	
2 years	—	—	—	—	1	—	—	—	—	—	+	+	
2 years	—	—	—	+	1	—	—	—	—	—	+	+	

The observations reported in the table refer to the central coronal section from animals which had been given one dose of Tetracycline 2–3 days prior to death. The location of zones I–VII in the subchondral bone are given in Fig. 1. The values given in columns b–d indicate the number of the following defined structures showing fluorescence: a = tidemark; in zones I and VII subperiosteal fluorescence; was registered as present (+) or absent (—), b = osteone, c = canal in calcified cartilage, d = surface of lamellar bone confining the medullary cavity and facing the articular surface (for details see text). The number of bone surfaces showing labelling in the epiphysis and metaphysis was estimated.

Fig. 7

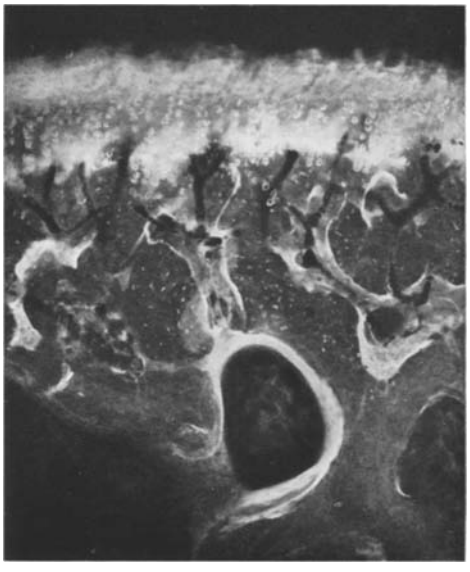


Fig. 8

Fig. 7. Fluorescence photomicrograph from a double-labelled animal. The fluorescence from the first labelling 4 months before death lies 3 scale divisions from the bone surface delimiting the medullary cavity. 30 μ thick section. ×365. 1 scale division = 8 μ

Fig. 8. Fluorescence photomicrograph from a double-labelled animal (no. 3) given Tetracycline both 7 months and 2 days before death. Part of the first labelling to the left lies in the middle of bone tissue and the type of the originally labelled structure can no longer be determined with certainty. Note that fluorescent bone tissue from the first labelling is seen immediately adjacent to the calcified articular cartilage, indicating that no longitudinal growth has taken place since the first labelling occasion. ×115

Tetracycline -Induced Fluorescence, Group B

Table 2 shows the observations on the animals from group B, which were given two doses of Tetracycline, one between 3 and 8 months and the other 2–3 days before death. Fluorescence observed directly on bone surfaces was ascribed to the second labelling, while that which did not lie directly on bone surfaces was ascribed to the first labelling (Fig. 8).

In the *subchondral bone* the number of fluorescent structures from the first labelling was greater than that from the second labelling. The labelling frequency from the first labelling agrees well in most animals with that given in Table 1 for animals 8–9 months old. Animals 3 and 6 showed a higher labelling frequency, probably on account of a lower skeletal age and animal no. 11 a lower labelling frequency because of a higher age on the first labelling occasion. The labelling frequency from the second labelling corresponds well with that reported in Table 1

Table 2. *Distribution of Tetracycline labelling in different parts of the femoral head of adult rabbits at different time points after administration of the Tetracycline*

Animal No.	Times between administration of Tetracycline and death		Subchondral bone, zones II–VI								Epiphysis		Metaphysis	
			A				B				A	B	A	B
			a	b	c	d	a	b	c	d				
1	8 months	2 days	–	2–3	–	×	–	–	–	–	++	–	–	++
2	7 months	2 days	+	2	1	2–3	+	–	–	–	+	+	+	++
3	7 months	2 days	–	×	×	×	–	–	1	4	+	+++	–	+++
4	5 months	2 days	–	1	1	2	(+)	1	–	1	+	+	+	++
5	5 months	2 days	–	2–3	–	3–4	(+)	–	–	–	(+)	(+)	+	+++
6	5 months	3 days	–	×	×	×	–	–	–	1	++	+	+	+++
7	4 months	2 days	+	2	–	1	+	–	–	–	(+)	(+)	+	+++
8	4 months	2 days	–	1–2	–	3	+	1	–	–	+	+	++	+++
9	4 months	2 days	–	3	–	1	–	–	–	1	+	+	+	+
10	4 months	3 days	+	1	–	–	+	–	–	–	(+)	(+)	+	+++
11	3 months	2 days	–	–	–	–	–	–	–	–	+	(+)	++	++

A = First labelling. B = Second labelling before death. The letters a–d stand for the same structures as in Table 1. Parenthese indicate a low labelling intensity. × = In columns b–d in subchondral bone indicates that exact identification of the labelled structures and calculation of their exact number was not possible (for details see text).

for animals over 1 year old. In animals 3 and 6 the exact number and identity of labelled structures could not be determined with an adequate degree of certainty (Fig. 8), and no figures have therefore been given in the respective columns for these animals. The labelling of lamellar bone delimiting the medullary cavity (structure d) gave a picture compatible with appositional growth in the direction towards the medullary cavity. In animals 3 and 6, with observation times of 5 and 7 months, lamellar bone had been replaced by osteones in some areas, which was evident from the localization and appearance of the first labelling in relation to existing osteones. Fluorescent bone tissue attributable to the first labelling was observed after 7 months close to the calcified articular cartilage (Fig. 8), indicating that no active longitudinal growth had taken place since the first labelling.

The *tidemark* showed duplicate labelling in 3 animals; the fluorescence nearer to the articular surface can be attributed to the second labelling (Fig. 6). In isolated cases fluorescence was observed around vascular canals in the calcified articular cartilage but could not be attributed with certainty to either the first or the second labelling.

In the *epiphysis* the number of labelled bone surfaces from the first and second labelling were of the same order of size. Exceptions were animals no. 6 (5 months) and no. 1 (8 months), where more fluorescent surfaces were observed from the first labelling than from the second. In animal no. 3 the opposite was found, indicating that the greater part of the labelled bone had become resorbed since the first labelling occasion. When interpreting this table it should be noted that at the time of the second labelling all animals were over 1 year of age and that the bone surfaces of the epiphysis in these animals usually showed a low labelling frequency, as can be seen in Table 1.

In the *metaphysis* the labelling frequency from the first labelling was distinctly lower than that from the second labelling at all observation times exceeding 4 months. Of the animals examinated at observation times of 7 and 8 months, only one showed residual labelling from the first administration of Tetracycline.

Microangiographic Observations

The blood vessels of the periosteum and medullary cavity and vessels of larger calibre in the cortical layer of subchondral bone showed good filling with contrast medium. On the other hand, filling of the smaller vessels, especially the intracartilaginous vascular canals, was often incomplete. Areas with good filling of all vessels and closely adjacent areas with no filling were observed regardless of whether Indian ink or Micropaque was used as the contrast medium.

Discussion

The femoral head of adult rabbits of different ages was studied by a combination of microradiography and fluorescence microscopy after administration of Tetracycline. Attention was devoted to the bone tissue between the articular cartilage and the most closely situated medullary cavity, the "subchondral bone plate", and its spontaneous remodelling.

The microradiograms showed that the subchondral bone plate is not uniformly constructed and that its structure is not the same over the whole surface of the femoral head. In rabbits over 1 year of age the major part of the bone tissue adjacent to the calcified articular cartilage is composed of fully mineralized osteones and is often demarcated from the nearest part of the medullary cavity by a layer of lamellar bone. In some small areas, however, mainly in lateral parts of the femoral head, this layer of cortical bone is absent and only a thin layer of lamellar bone divides the medullary cavity from the calcified articular cartilage. These findings differ somewhat from the observations made on the femoral head in 18-month old rabbits by Greenwald and Haynes (1969) and on the knee joint in adult rabbits by Mankin (1963), who described the subchondral bone as a well defined cortical shell.

The number of Tetracycline-labelled osteones in the layer of cortical bone was very low in animals older than 1 year, indicating that spontaneous remodelling does take place but that it is a limited process. Mankin (1963), after administration of ^{3}H-thymidine, found no signs of cell proliferation and new bone formation in the subchondral bone of adult rabbits. This difference in results may be due to the fact that Tetracycline labelling is a more sensitive method for studies of new bone formation. Remodelling of osteones in the subchondral bone seems to take place very slowly after termination of longitudinal growth, which is supported by the finding that the greater part of the expected labelling still remained up to 8 months after administration of the Tetracycline.

Vascular canals within the boundary of the calcified articular cartilage, described as "dendritic contacts" by Holmdahl and Ingelmark (1950), were seen even in animals ober 1 year old, and to some extent they belonged to very small osteones. Tetracycline fluorescence localizable to these intracartilaginous canals has seldom been observed in animals older than 8 months. The thickness of the ground sections, however, obviously rendered difficult localization of the fluorescence in these structures. With higher magnifications gracile fluorescent bands were visible in the calcified cartilage, possibly corresponding to parts of intracartilaginous canals. A falsely low estimate of the mineralization activity may, therefore, have been made systematically in this structure.

In a number of animals sharply demarcated Tetracycline-induced fluorescence was observed in the tidemark, somtimes comprising the whole area of the femoral head but in other cases limited to certain parts. This finding indicates that the tidemark is periodically but not continously the seat of active mineralization. The duplicate labelling of the tidemark supports the view that mineralization of the articular cartilage progresses very slowly towards the articular surface with increasing age, which has been concluded from histological sections described previously by Fawns and Landells (1953), among others.

The only part of the subchondral bone plate which showed signs of new bone formation to any large extent in animals over one year of age were the lamellar bone surfaces bordering on the medullary cavity. The bone metabolism on these surfaces seemed to correspond with that observed in the rest of the epiphyseal bone tissue. The observations made on the animals with long-term labelling point to a slow appositional growth of these lamellar bone surfaces in the direction towards the medullary cavity.

The definition of the subchondral bone plate which has been given on the basis of its localization between the articular cartilage and medullary cavity needs to be complemented. In rabbits over one year of age that part constructed of osteones shows a very low degree of spontaneous remodelling, while that consisting of lamellar bone shows a higher degree and belongs in this respect to the cancellous bone of the epiphysis.

The duplicate labelling of the tidemark cannot be interpreted as a definite sign of active longitudinal growth of the epiphyseal bone in normal adult rabbits, since Tetracycline labelling was still visible in the borderline between bone and calcified articular cartilage after many months. This study in the rabbit has therefore provided no support for the hypothesis presented by Johnson (1959), among others, that active longitudinal growth in the epiphysis continues after otherwise terminated longitudinal growth. A reservation must be made, however, for the fact that the observation time in the present study was only 8 months, but this is a considerable proportion of the total lifetime of the rabbit.

The findings in the present investigation can be of importance for studying and interpreting the effect of experimentally created articular cartilage defects of different depths in different parts of an articular surface in animals of various ages. Of interest in this respect is the fact that the structure and the spontaneous remodelling of the bone tissue between the articular cartilage and medullary cavity have been found to vary in different parts of an articular surface.

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