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An autoradiographic study with ^{35}S-sulphate on the growth in diameter of epiphyseal cartilage in rabbits

A. LANGENSKIÖLD
T. RYTÖMAA &
T. VIDEMAN

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INTRODUCTION

There is no unanimity of opinion in the literature regarding the manner in which the epiphyseal cartilage grows in diameter. It seems to be a widespread opinion that the diametric growth of the epiphyseal plate takes place by apposition of cells from a perichondrium on the peripheral surface and above all from the border zone between the cartilage and the diaphyseal periosteum, the so-called »ossification groove» of Ranvier. This opinion has been put forward by KOLLATH and LACROIX, among others. The contrary opinion that the cells of the »ossification groove» derive their origin from the epiphyseal cartilage and that the latter grows in diameter by interstitial growth, was adopted by several classic authors (RANVIER, POLICARD, CAREY, DAHL).

Recent experiments presented in support of the view that the diametric growth of the epiphyseal plate occurs by apposition of cells from the periphery, include those of TONNA (1961) and TONNA & CRONKITE (1963). In the light of autoradiographic studies in mice with ^{3}H-thymidine, the conclusion was reached that the periosteum acts as a chondro-osteogenic progenitor-cell pool by supplying cells for the expansion of the epiphyseal cartilage. RIGAL (1962), however, arrived at the contrary opinion on the basis of his extensive studies with rats and rabbits, employing cell-labelling with ^{3}H-thymidine both *in vivo* and *in vitro*. His conclusion was that endochondral proliferation of cells was responsible for the diametric growth of the epiphyseal cartilage.

Other approaches to the problem have been made in many ways and by many authors, but opinions have remained divided. For instance, FRIEDENBERG (1956, 1957) resected a part of the epiphyseal plate surgically and interpreted the narrowed transverse diameter of the newly-formed bone as an indication against the idea of enchondral growth. LEBLOND & GREULICH (1956) suggested that transformation of fibroblasts into chondrocytes takes place in the periphery of the epiphyseal cartilage and that this apposition is responsible for the diametric growth of the epiphyseal plate.

On the basis of radiological findings in Ollier's disease, one of the present writers (LANGENSKIÖLD, 1947) arrived at the conclusion that the epiphyseal cartilage grows interstitially in width. In a subsequent experimental study of localized injury to the cartilage by roentgen rays, further strong support for the interstitial growth mechanism was obtained (LANGENSKIÖLD & EDGREN 1949). It was demonstrated that regeneration of the injured portions of the epiphyseal plate occurred by displacement of cells from the central parts of the epiphyseal cartilage to its periphery. These results were not in accordance with the idea that the diametric growth of the epiphyseal plate is caused by apposition of cartilage from the perichondrial ring.

In the search for information on dynamic processes, static observations are clearly of little value. The introduction of radio-actively labelled precursor substances for biological studies has provided many new methods of experimental attack on dynamic phenomena. As far as population kinetics are concerned, cell proliferation, migration and death are often, but not always, best followed by means of DNA labels, such as ^{3}H-thymidine. As already indicated, the growth of the epiphyseal cartilage has been studied with ^{3}H-thymidine, employing autoradiographic methods (TONNA 1961, RIGAL 1962). Despite the undeniable value of these experiments, it is not surprising that the conclusions based on the results obtained have been equivocal, at least as far as the problem of interstitial versus appositional growth is concerned. *Direct* evidence of cellular migration in or out of the epiphyseal cartilage in the *transverse direction* is virtually impossible to achieve from the data available, because cell-labelling occurs and persists for the same length of time in both 'organs' (TONNA 1961, RIGAL 1962).

In addition to cell proliferation, an essential feature of cartilage growth is the formation of ground substance. Thus there are possibilities to approach the problem of diametric growth of the epiphyseal cartilage by studying the formation of the organic matrix laid down through the activity of the cells. In fact, the increase in the amount of the extracellular substance is intimately related, not only to the cartilage growth in general, but directly to the intracellular activity.

^{35}S-labelled sulphate has been stated to be a unique and an almost ideal medium in the study of the formation and metabolism of the ground substance of cartilage tissue (AMPRINO 1956). It was demonstrated by AMPRINO that the increase in the cartilage matrix runs parallel to an increase in the sulphate incorporated. Shortly (1—2 hrs) after the administration of ^{35}S-sulphate to an experimental animal, the label can be detected autoradiographically within the cells; at this time the extracellular matrix gives a

much weaker reaction. The initial labelling is most intense in the cells of the growth zone of the epiphyseal cartilage (BÉLANGER 1956, ENGFELDT & WESTERBORN 1960, DZIEWIATKOWSKI 1962b).

It has been well demonstrated that cartilage is permeable to inorganic sulphate, both *in vivo* and *in vitro* (CAMPO & DZIEWIATKOWSKI 1961). Almost as rapidly as sulphate enters the tissue, the inorganic ion is utilized by the cells in the synthesis of chondroitin sulphate (DZIEWIATKOWSKI 1962a). After 4-hours labelling *in vitro*, only about 15 % of the radioactivity is seen in the matrix; on the other hand, 64 to 83 % of the total ^{35}S could be isolated as chondroitin sulphate (DZIEWIATKOWSKI 1962a). A chromatographic separation of ^{35}S-labelled material in the metaphyses of 7-day-old rats has shown that 24 hours after the administration of ^{35}S-sulphate the inorganic sulphate could account for no more than 20 % of the ^{35}S (DZIEWIATKOWSKI 1962b).

Further chemical analyses have demonstrated that the ^{35}S-labelled primarily intracellular and subsequently extracellular material consists of a mixture of chondroitin sulphuric acids A and C (THORP & DORFMAN 1963). Possible intermediates in the synthesis are sulphated nucleotides reaching maximum activity in the rat after 6—8 hours; chondroitin sulphate itself reaches maximum specific activity after 24 hours (PICARD ET AL. 1964), i.e., at the time of peak labelling as seen in autoradiography (BÉLANGER 1956, ENGFELDT & WESTERBORN 1960, DZIEWIATKOWSKI 1962b). The Golgi zone plays a considerable role in the intracellular synthesis of mucopolysaccharides. In four hours the newly synthesized material has been transported into the cartilage matrix (ROHR & WALTER 1966).

One or two days after the administration of ^{35}S-sulphate a considerable amount of ^{35}S is still retained in the cells, although a significant activity is also seen in the extracellular matrix. Six to seven days after labelling, the radioactivity has concentrated almost exclusively in the organic matrix (BÉLANGER 1956, ENGFELDT & WESTERBORN 1960, DZIEWIATKOWSKI 1962b). After 2—3 weeks, a weak reaction is detectable in the cartilage matrix and in the trabeculae of the metaphysis (ENGFELDT & WESTERBORN 1960).

In the light of the above-mentioned findings, ^{35}S- labelled sulphate seems to provide a new medium for an experimental approach to the problem of transverse growth in the epiphyseal cartilage. Such experiments have recently been reported by SCLOMON (1966), who carried out an autoradiographic study of the diametric growth of the epiphyseal plate, using ^{35}S-sulphate in growing rabbits.

The results reported by SOLOMON indicated that 6 days after injection of ^{35}S-sulphate the epiphyseal cartilage showed uniform labelling throughout the central part of the plate, but at the periphery, immediately beneath the perichondrium and extending slightly towards the previously labelled articular cartilage, there were clumps of cells much more faintly labelled. From this observation and from the availability time of ^{35}S-sulphate (extending over 2 or 3 days according to SOLOMON) he concluded that the faintly-labelled cells at the periphery of the cartilage plate shown in the Figures published, had been added since the injection of the radio-isotope. He considered it an evidence of appositional growth from the perichondrium.

The conclusions drawn by SOLOMON as to the growth in diameter of the epiphyseal plate are not consistent with several phenomena occurring in pathological bone growth (LANGENSKIÖLD & EDGREN 1949). Therefore, the present authors considered it desirable to re-evaluate the ^{35}S-labelling patterns in the epiphyseal cartilage with the particular purpose of elucidating its mechanism of diametric growth.

MATERIAL AND METHODS

The experimental series consisted of 34 rabbits varying in age from 9 to 28 days at the time of administration of the label. Each animal received 3μc/g of ^{35}S-sodium sulphate (carrier free; The Radiochemical Centre, Amersham) in a single intraperitoneal injection. The rabbits were killed at intervals ranging from 1 hour to 44 days after the sulphate injection. A total of 350 autoradiograms were analysed from these animals.

Immediately after death, the bones to be studied were dissected out and specimens were prepared from the proximal and distal epiphyses of the tibia and the femur, from the proximal epiphysis of the humerus, and from the distal epiphysis of the radius. After fixation in 10 % neutral formalin and decalcification in 5 % nitric acid, the tissues were embedded in paraffin, sectioned at 6 μ, and stained with Weigert's haematoxylin van Gieson.

It was essential for a successful realization of the purpose of this study that the autoradiographic technique adopted should allow estimation of the relative ^{35}S-activities in the different regions within each individual epiphysis. Comparison of labelling intensities from one specimen to another was only of secondary importance. Autoradiography was therefore used mainly as an instrument capable of recording the *distribution patterns* of the labelled material at different intervals after the injection of ^{35}S-sulphate.

The objects analyzed in the autoradiograms consisted of fairly large pieces and were clearly visible to the naked eye. It was thus apparent that high-resolution autoradiography, suitable for grain counting at the cellular level, did not seem suitable for this study. Consequently, contact or 'macroscopic' autoradiography was adopted, employing X-ray film (Dental X-ray Film, No 3P, Ilford) for autoradiography.

Specific adjustments of the exposure times seemed necessary for the different preparations because over-exposure could cause blurring of possibly important contrasts in the autoradiographic image.

It was considered advisable to aim always at a similar blackening of the 'hottest' area wherever it happened to be. This approach did not exclude the possibilities of comparing the same area in different preparations because

the density of the autoradiographic image would be proportional to the exposure time within certain limits. In practice, exposure times were based on an empirical basis. The times applied varied from 4 to 60 days.

Another essential requirement for the autoradiographic technique adopted was a consistent method of maintaining close and even contact between radioactive sample and recording emulsion. The technique with which good contact was achieved deserves some emphasis. Heavy and probably even variable pressure on the emulsion may lead to the artificial formation of silver halides. Among these mechanisms are, such factors as the pressure itself, the bending of the film, especially at the border areas of the preparation, and chemical interaction. The technique evolved for this study is illustrated in Figure 1.

Apart from the 34 animals used for the main experimental series of this study, ten additional rabbits of the same ages were followed roentgenologically. From these animals the longitudinal and transverse growth of the bones was measured during the experimental period.

The possibility that a part of the autoradiographic image could have been due to artificial interaction of the tissue with the X-ray film was checked in 3 rabbits which had not been injected with ^{35}S-sulphate.

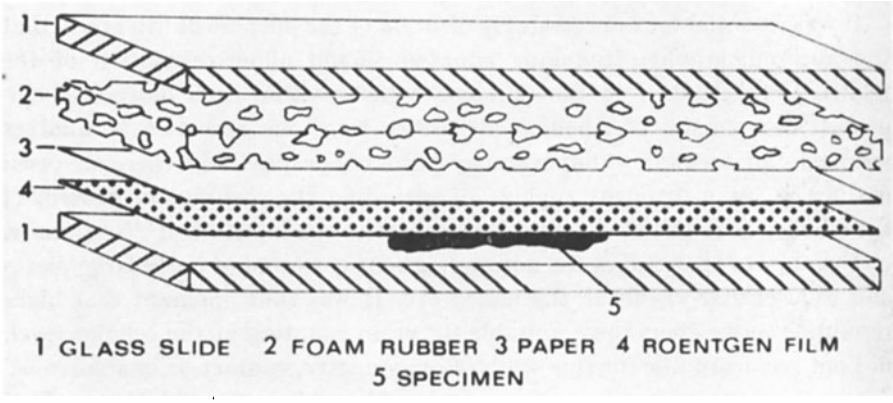

Figure 1. Arrangement for exposure of autoradiograms. Contact between the sample and the film is maintained by a rubber string around the block. Cutting of the string on sharp glass edges is prevented by paper wrapping.

RESULTS

The results are illustrated in Figures 2—20. In each figure a pair of photographs is seen: the lower one is an ordinary histological preparation which has produced the matching autoradiograms in Figures 2-18. The interval between the injection of the ^{35}S-sulphate and the killing of the rabbit is indicated below each figure (= T); further details, such as the age (= A) of the animals and the exposure time (= E) of the autoradiograms, are given in the legends.

Judging from the experimental results, the distribution of the label at different times after ^{35}S-sulphate injection is as follows:

1. Within 2 hours after the administration of the sulphate, considerable activity has been incorporated in organic matter (fixed and demineralized section; see also the data referred to in the introduction). The highest activity occurs in the zone of cartilage cell columns of the epiphyseal cartilage. It is worth emphasizing that a slight but distinct difference between the central and peripheral areas of the epiphyseal plate can be seen: in the former the density is more intense than in the latter. Another point of interest is the fact that on both sides of the intensely-labelled zone of cell columns there are narrow layers of cartilage with very little, if any, activity. The upper one of these consists of the layer of the reserve cells.

It may be noted further that the perichondrial ring does not exhibit much activity.

2. After 24 hours the labelling has obviously reached its maximum, judging from the appearance of the autoradiogram and from the exposure time used. With respect to the distribution of the label, there is not much deviation from the earlier autoradiograms. A similar difference between the central and peripheral parts of the epiphyseal cartilage is still visible.

3. During the next 3 days the labelling patterns have clearly changed. In comparison to the 1-day autoradiogram, ^{35}S has been included in the metaphysis due to a longitudinal growth of the bone. Another obvious change involves a decrease in the labelling intensity in the bony epiphysis (cf. the respective exposure times of the autoradiograms).

Probably the most obvious change, however, involves the radioactivity in the layer of the reserve cells. The labelling intensity in this layer now appears similar to that seen in the zone of cartilage cell columns; in absolute terms, however, the activity may not differ significantly from the 1-day level. With respect to the relationship between the labelling of the peripheral and the central parts of the epiphyseal plate the labelling patterns appear unchanged.

4. After one week, the radioactive material has 'spread' quite evenly over a wide area. However, it can be seen that some parts of the articular cartilage exhibit higher activities than the rest. The layer of the reserve cells is beginning to turn into the most active layer of the epiphyseal plate.

5. During the second week the labelling of the layer of the reserve cells has become distinct in the autoradiograms.

6. Three weeks after the injection of sulphate the picture has changed once again: the peripheral areas of the epiphyseal plate and the adjacent articular cartilage do not show the same high (relative) activity as a week before. Even the reserve layer, which is now much more active than any other region in the epiphyseal cartilage, exhibits labelling which is lower in the peripheral than in the central areas.

The transverse growth of the epiphyseal plates of the tibia in a rabbit has been measured (X-ray pictures) as being about 70 % from the age of fourteen days to the age of thirty-five days (Figure 21).

DISCUSSION

Autoradiograms produced one, two and twenty-four hours after the injection of ^{35}S-sulphate give the impression that the peripheral areas of the epiphyseal plate are less active than the central ones. Thus far, the observations seem to agree with the predictions based on the assumption that the epiphyseal plate grows in diameter by apposition from the periphery. It follows from the very low labelling of the perichondrial ring (see Figures 2 and 3) that a supply of cells from it for the transverse expansion of the epiphyseal cartilage would rapidly decrease the peripheral labelling intensity. After four days a further reduction in the peripheral activity due to continuous apposition of lightly labelled cells could be expected. Further reduction in the activity would occur through the synthesis of unlabelled chondroitin sulphate by the transformed cells. The autoradiograms seen in Figures 5, 11 and 18 are not consistent with such a prediction.

Between the fourth and the seventh day a radical change in the labelling patterns occurred. In the peripheral parts of the epiphyseal cartilage, radioactivity had not decreased at all in comparison to the central areas. Autoradiograms obtained at twelve and fourteen days after the ^{35}S-injection (Figures 11 and 12) provide the final proof that the patterns seen at seven days were not due to any artefact. After considerable diametric growth of the epiphyseal plate, the peripheral parts of it had retained a marked content of ^{35}S (Figure 22).

The idea that the epiphyseal plate grows in diameter by appositional growth from a perichondrium at the periphery is in conflict with these experimental data.

After three weeks, the activity still distinct in the layer of the reserve cells is seen to decrease at different rates in the different parts of the layer. The rate of loss is more rapid at the periphery (Figures 7 and 16).

The autoradiographic findings can be explained as follows: after the sulphate injection, the initial incorporation is proportional to the rate of formation of organic complexes. With respect to the layer of the reserve cells, the label is taken up at a faster specific rate in the peripheral than in

the central regions of the layer. However, due to the excessive rate of incorporation in the zone of the cartilage cell columns, activity differences within the layer of the reserve cells are not recorded in autoradiograms obtained with a relatively short exposure time (Figure 23).

Because of the fast sulphate metabolism in the zone of the cartilage cell columns, both the incorporation and the loss must proceed at a fast rate in comparison to other areas. The rate of growth in the layer of the reserve cells is much slower. This is demonstrated both by the data presented here and by the results obtained with ^{3}H-thymidine (RIGAL 1962). Consequently, after a time determined by the relative metabolic rates in the different layers of the epiphyseal plate, the opposite to the initial labelling intensities is seen (compare Figure 8 with Figure 11). This also applies to the different regions of the layer of the reserve cells.

A conclusion drawn from the last-mentioned findings is that the expansion of the layer of the reserve cells occurs faster at the lateral parts of the epiphyseal cartilage. This would seem to be the most plausible explanation of the regional differences in the sulphate metabolism in the layer of the reserve cells. It is also consistent with the conclusion reached by RIGAL (1962) on the basis of ^{3}H-thymidine labelling.

The experiments have shown that ^{35}S taken up by the layer of the reserve cells in the epiphyseal plate within a period of a few days is retained in the ground substance *throughout* this layer after its growth in diameter of 70 per cent or more in three weeks. This phenomenon can hardly be explained without the assumption of the occurrence of interstitial growth and expansion transversely to the long axis of the bone in the layer of the reserve cells of the epiphyseal plate.

SUMMARY

In order to elucidate the question whether the epiphyseal plate grows in diameter by apposition of cells from a perichondrium at the periphery or by interstitial growth ^{35}S-sulphate was injected into thirty-four young rabbits and labelling of epiphyseal cartilages was followed by autoradiography. The ^{35}S-sulphate was injected one hour to forty-four days prior to the killing of the animal. Labelling patterns in the tibia, the femur, the humerus and the radius were followed by means of contact autoradiography, using a technique specially developed for the present purpose.

Initial labelling was strong in the layer of the cartilage cell columns (maximum after about twenty-four hours), intermediate in the periosteum of the diaphysis and in a narrow zone surrounding the bony epiphysis. Initial labelling was weak in the articular cartilage, in the perichondrial ring and also in the layer of the reserve cells. It seemed to be very weak in the bony epiphysis, the metaphysis and the diaphysis.

During the first three weeks the labelling patterns changed in a constant and characteristic manner in all bones studied. In about one to two weeks the layer of the reserve cells began to appear as the most intensely labelled area, especially in the peripheral parts of the layer (cf. Figures 5, 6, 11, 12, and 18).

After three weeks the labelling was most prominent in the entire layer of the reserve cells, being most intense in its central parts (cf. Figures 7 and 19). Radioactivity in the layer of the reserve cells was detectable even forty-four days after ^{35}S-sulphate administration.

The experiments have shown that ^{35}S taken up by the layer of the reserve cells in the epiphyseal plate within a period of a few days is retained throughout this layer after its growth in diameter of 70 per cent in three weeks (Figure 21 and Figure 20 B). This phenomenon is difficult to explain without the assumption of the occurrence of interstitial growth and expansion transversely to the long axis of the bone in the layer of the reserve cells of the epiphyseal plate.

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The experiments reported in this article were carried out by T. Videman.

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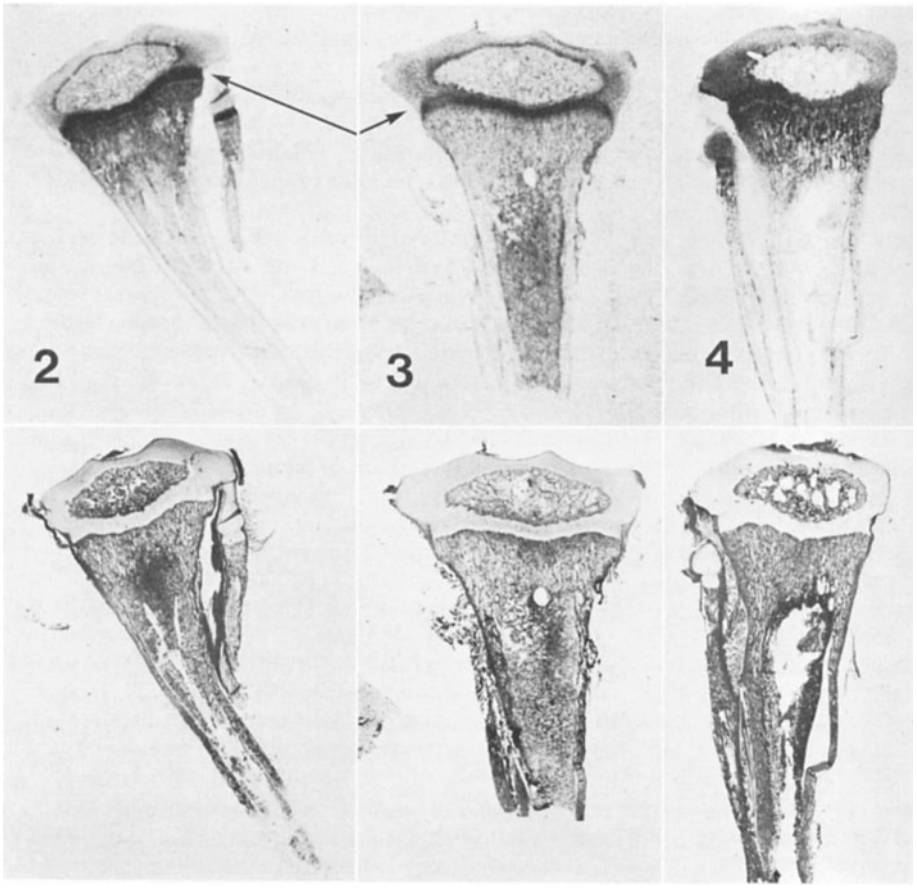


Glossary of symbols for figures 2 20
T = time of killing of the animal after administration of ^{35}S-sulphate (3 μc/g body weight).
E = exposure time of the autoradiogram.
A = age of the rabbit at time of sulphate injection.
Figures 2—20 are all reproduced in the same magnification.
Figure 2. T = 2 hrs, E = 14 days, A = 12 days. Tibia, prox.e. (e = epiphysis). Initial labelling has been most intensive in the layer of cartilage cell columns of the epiphyseal cartilage. In this figure, differences between central and peripheral areas of the cartilage are evindently masked due to relative over-exposure of the autoradiogram (cf.figs. 3, 8, and 9). Note that the perichondrial zone exhibits low labelling (arrow).
Figure 3. T = 1 day, E = 8 days, A = 11 days. Tibia, prox.e. The border area between the layer of cartilage cell columns and metaphyseal bone is less distinct than in Fig. 2; radioactivity is also seen in the uppermost parts of the metaphysis. Peripheral areas of the epiphyseal plate appear less active than central areas. Weak labelling of the perichondrial zone is also clearly seen (arrow)
Figure 4. T = 4 days, E = 14 days, A = 12 days. Tibia, prox.e. Note that the radioactivity now extends down into the metaphysis. The epiphyseal plate is fairly evenly labelled throughout its whole transverse diameter.

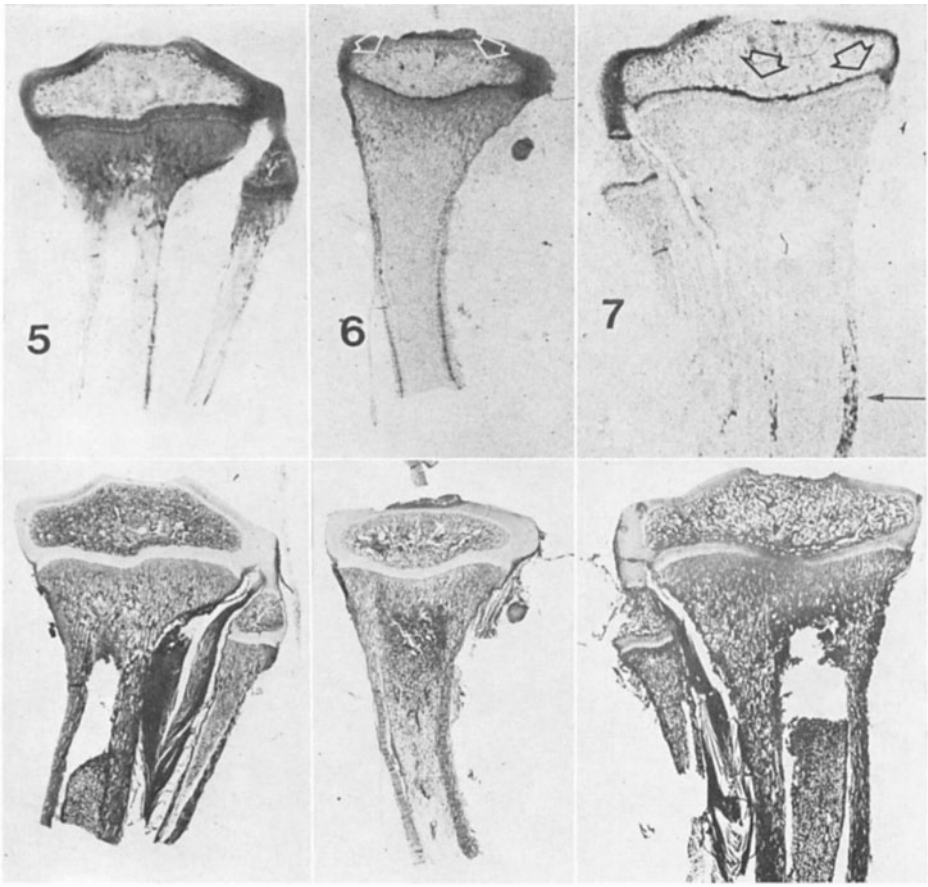


Figure 5. T = 7 days, E = 20 days, A = 12 days. Tibia, prox.e. Note the even distribution of radioactivity between the epiphyseal plate and the metaphysis. Peripheral areas of the epiphyseal plate (and the adjacent articular cartilage) appear more intensely labelled than central areas. First sign of the prominence of labelling of the layer of the reserve cells can be seen, especially in the periphery.

Figure 6. T = 9 days, E = 20 days, A = 12 days. Tibia, prox.e. Note that the layer of the reserve cells has become very distinct. It is clearly seen in this picture that the bony epiphysis has grown since the administration of ^{35}S-sulfate (arrows).

Figure 7. T = 21 days, E = 45 days, A = 9 days. Tibia, prox.e. (during the 3 weeks the weight of this rabbit increased from 130 g to 570 g). Note the distinct activity in the layer of the reserve cells and, in particular, the difference between its peripheral and its central parts (arrows). In the diaphysis, one can see the original areas of sulphate incorporation (arrow) indicating the radioactive substance accumulated in areas in which appositional growth of bone is taking place.

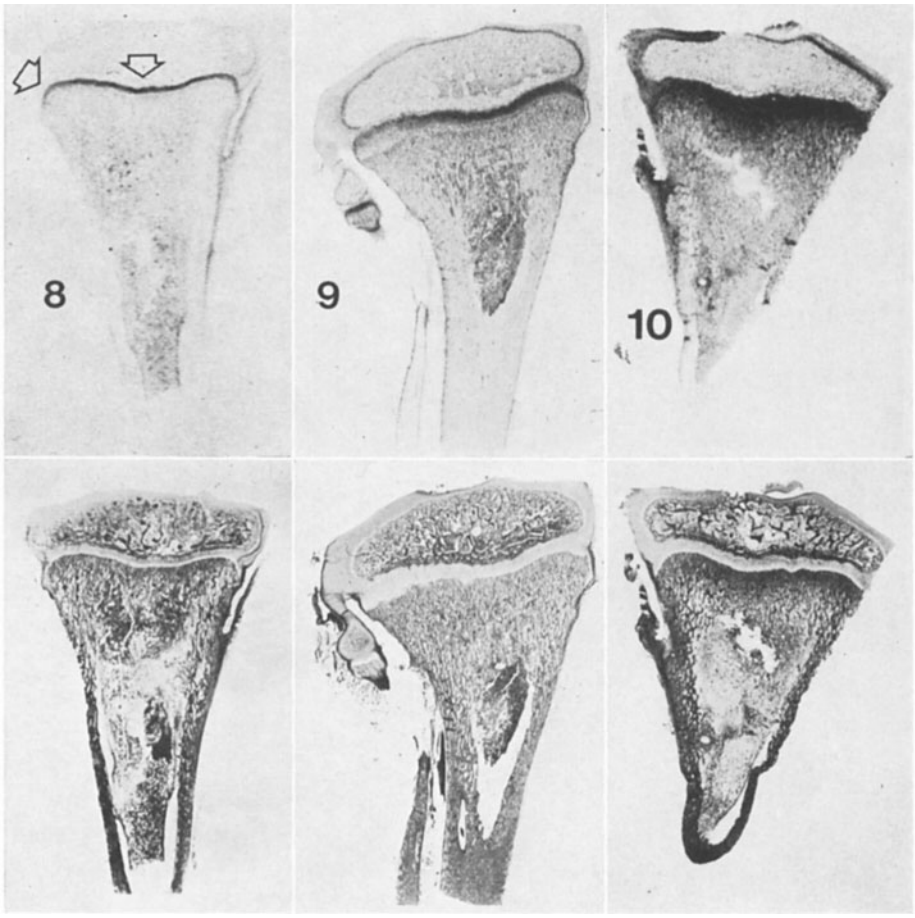


Figure 8. T = 2 hours, E = 7 days, A = 28 days. Tibia, prox.e. Principal incorporation of sulphate in the layer of cartilage cell columns in the epiphyseal plate is evident. Note that with a shorter exposure time than in Fig. 2, initial labelling appears to be less intense in the periphery than in central areas (arrows). Low activity in the perichondrial zone is also seen.

Figure 9. T = 1 day, E = 15 days, A = 30 days. Tibia, prox.e. Differences in sulphate incorporation between central and peripheral areas in the epiphyseal cartilage are particularly distinct.

Figure 10. T = 4 days, E = 7 days, A = 30 days. Tibia, prox.e. Note that labelling extends into the metaphyseal bone. Differences between central and peripheral areas in the epiphyseal cartilage are no longer distinct (cf. fig. 4).

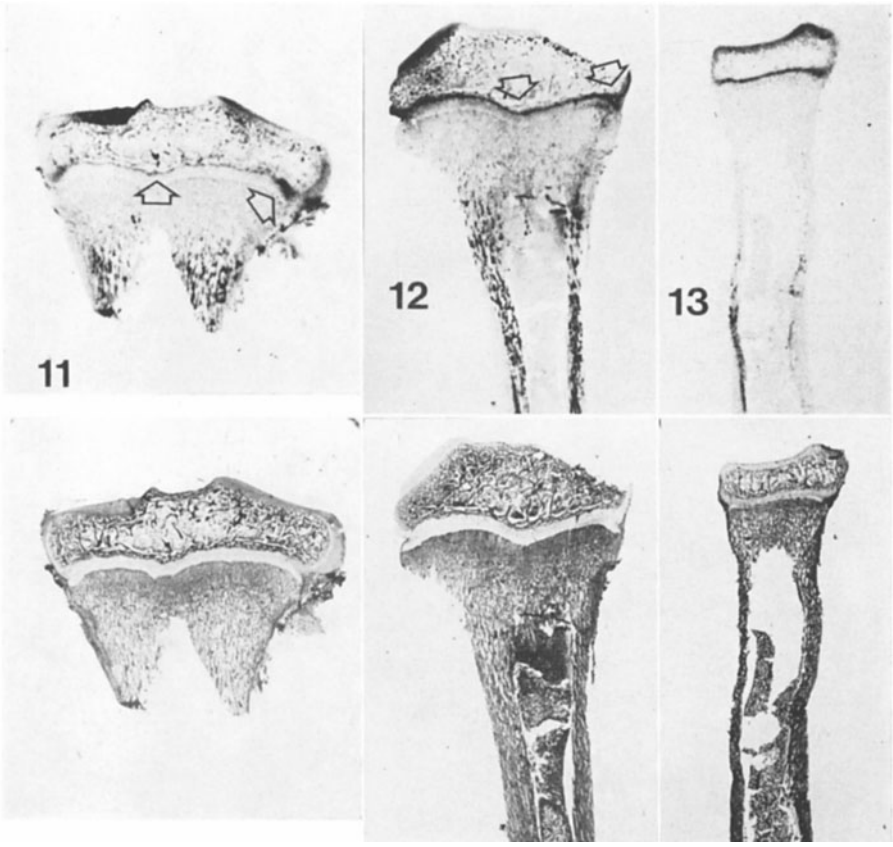


Figure 11. T = 12 days, E = 20 days, A = 34 days. Tibia, prox.e. The layer of the reserve cells has become the most active part of the epiphyseal cartilage, especially in its peripheral area (arrows). ^{35}S-sulphate incorporated in the metaphysis 12 days earlier is also distinct.

Figure 12. T = 14 days, E = 24 days, A = 30 days. Tibia, prox.e. Radioactivity in the reserve cell layer shows a further relative increase. Differences between peripheral and central areas are, however, still detectable (arrows). Note also the label in the diaphysis.

Figure 13. T = 21 days, E = 45 days, A = 14 days. Radius, distal e. The labelling pattern is basically similar to that seen in the tibia. The growth in length of the bone after administration of ^{35}S-sulphate can be estimated from the activity zone present in the diaphysis.

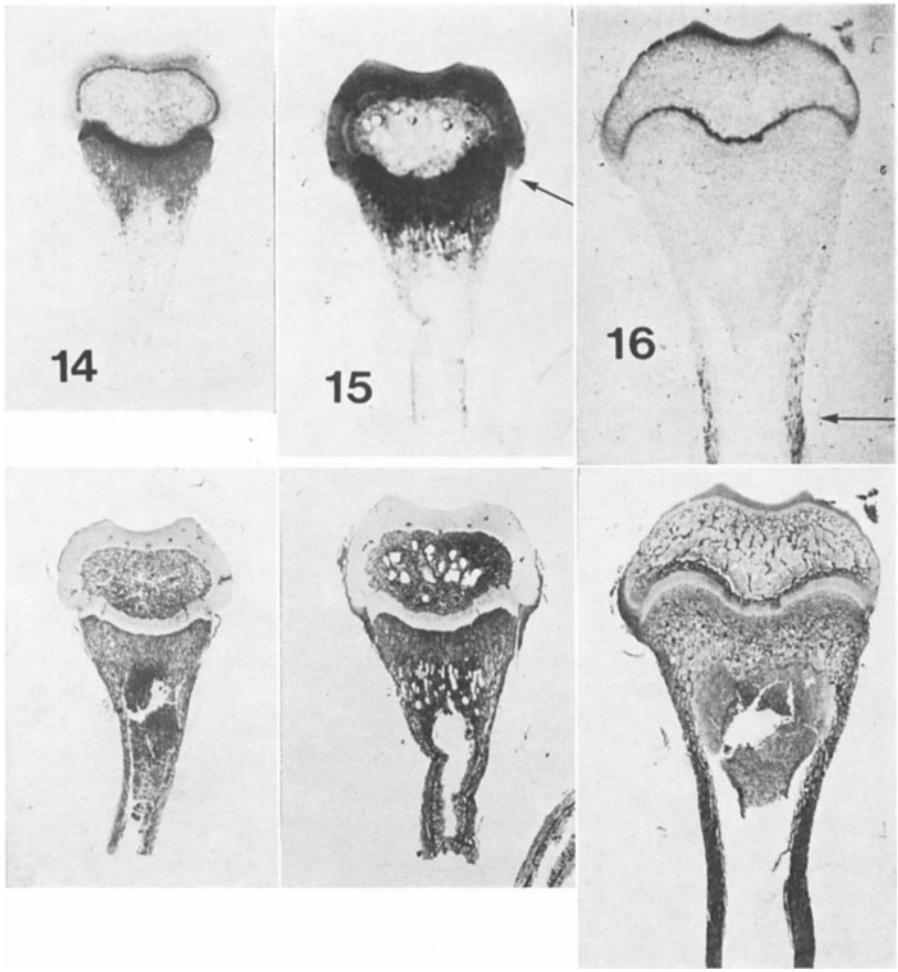


Figure 14. T = 2 hours, E = 14 days, A = 12 days. Femur, distal e. The initial labelling at the layer of cartilage cell columns by ^{35}S-sulphate does not differ from the pattern seen in other bones.

Figure 15. T = 4 days, E = 14 days, A = 12 days. Femur, distal e. Radioactivity is fairly evenly distributed between epiphyseal cartilage, metaphysis, and articular cartilage. Note that labelling in the perichondrial zone remains weak also in this figure (arrow).

Figure 16. T = 21 days, E = 45 days, A = 9 days. Femur, distal e. Radioactivity in the layer of the reserve cells is as distinct as in other epiphyses. Note the marked difference between the central area and the less intense peripheral areas (cf. fig. 7). Labelled sulphate in the diaphysis is also evident (arrow). ^{35}S is retained in the *ground substance throughout* the layer of the reserve cells even after considerable growth of the plate in diameter.

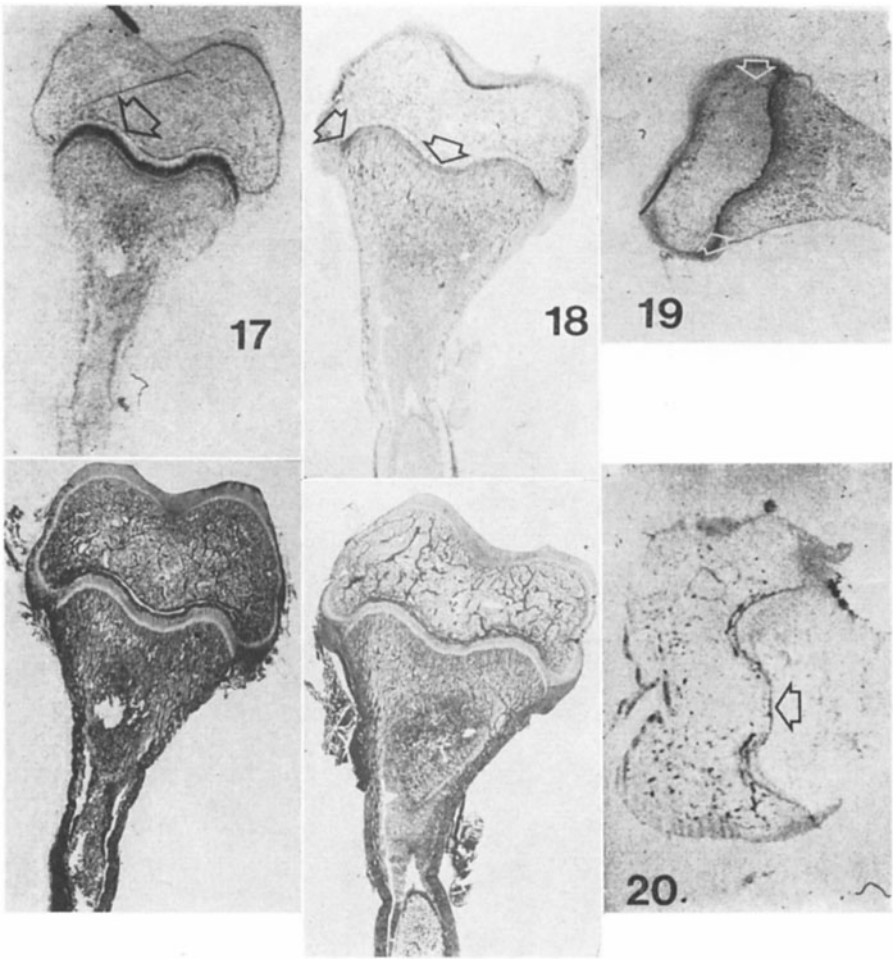
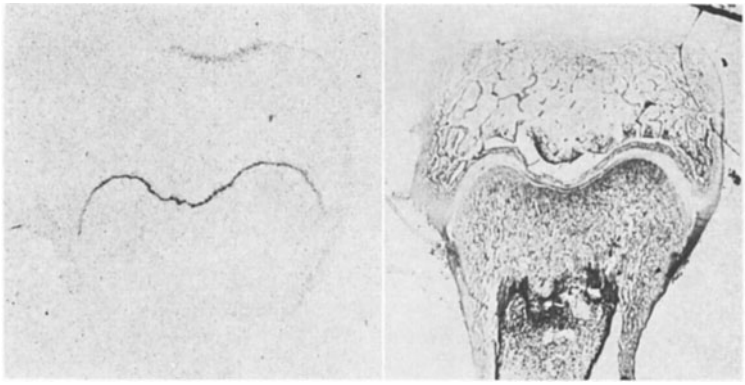


Figure 17. T = 1 hour, E = 7 days, A = 28 days. Femur, distal e. Initial uptake of ^{35}S-sulphate has taken place most intensely in the cartilage cell columns. Above them the reserve cells are seen as a light zone (arrow). Note that a narrow activity zone can been seen around the bony epiphysis. Above the epiphyseal plate it lies close to the layer of the reserve cells.

Figure 18. T = 8 days, E = 14 days, A = 21 days. Femur, distal e. The highest concentration of radioactive material begins to be seen in the layer of the reserve cells, especially in the periphery (arrows).

Figure 19. T = 9 days, E = 20 days, A = 12 days. Femur, distal e. The layer of the reserve cells exhibits the highest activity. The bony epiphysis contains a similar narrow zone of ^{35}S as seen in figure 17 (arrows) indicating the size of the bony nucleus at the time of labelling.

Figure 20 A. T = 44 days, E = 60 days, A = 20 days. Femur, distal e. It can be seen that even 1.5 months after ^{35}S-labelling, the entire layer of the reserve cells (arrow) yields a positive autoradiogram (note that physical half-life of ^{35}S is 87 days).



20 B 20 C

Figure 20 B. T=42 days, E=60 days, A=10 days. Femur, distal epiphysis, 42 days after injection. Distinct radioactivity extends throughout the layer of the reserve cells. *Figure 20 C.* Histological section corresponding to 20 B

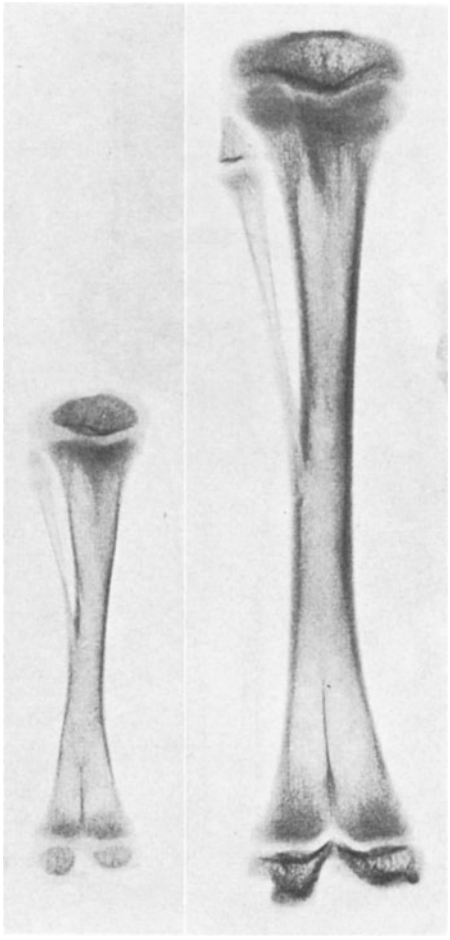


Figure 21. *A*. X-ray picture of the tibia in a 14-days-old rabbit. *B*. Tibia in a 35-days-old rabbit.
Both photographs are in the same proportion to the true size of the tibiae. Note that the diameter of the epiphyseal plate is about 70 per cent larger in the older animal. This proportion was checked in four other pairs of animals.

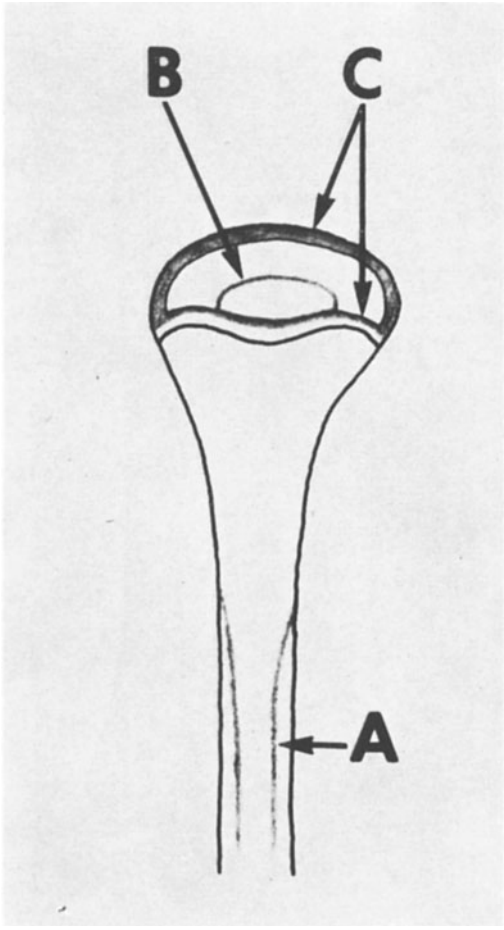


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Figure 22. Schematic representation of an autoradiogram about 3 weeks after ^{35}S-sulphate labelling.
A indicates radioactivity in the diaphysis, originally incorporated in the periosteum (cf. figs. 7,13 and 16);
B indicates label which has remained in the bony epiphysis (cf. figs. 6 and 19);
C indicates that the bulk of radioactivity is located in articular cartilage and, in particular, in the layer of the reserve cells of the epiphyseal plate (cf. figs. 6, 7, 13, and 16).
That ^{35}S is retained throughout the layer of the reserve cells after its growth in diameter of 70 per cent is only consistent with the assumption of the occurrence of interstitial growth and expansion transversely to the long axis of the bone in this layer of the cartilage.

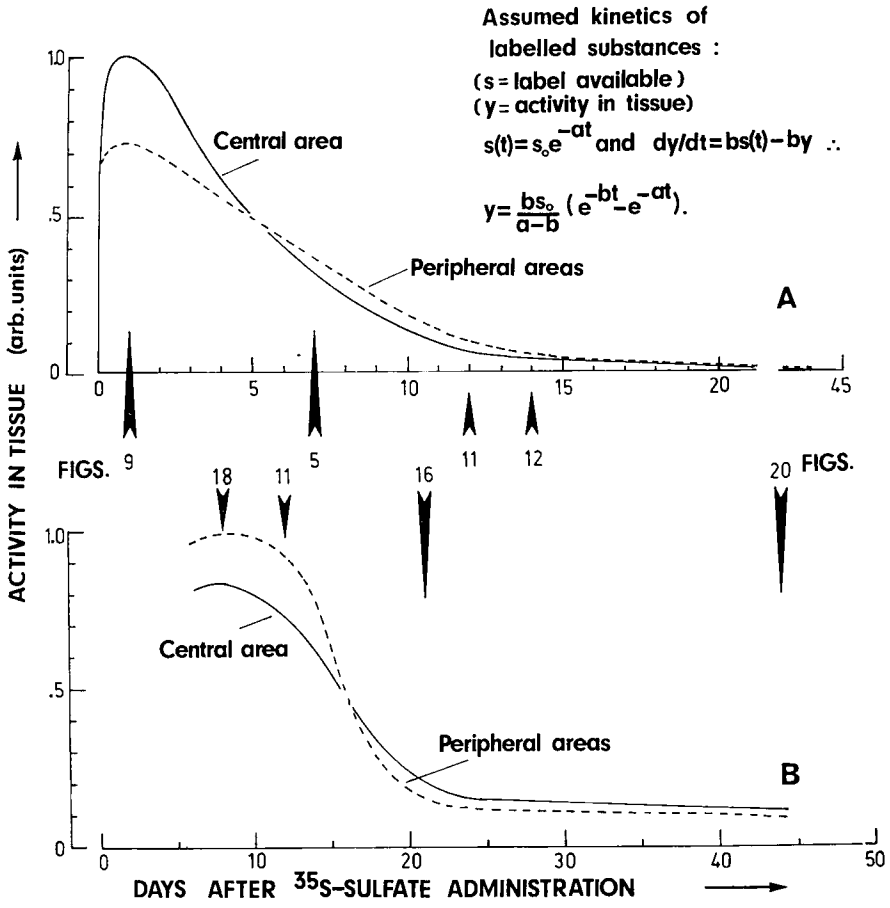

Figure 23. Graphic representation of time courses of radioactivity in different parts of the epiphyseal cartilage.
A: Layer of cartilage cell columns.
B: Layer of the reserve cells.
The curves drawn are based on rough visual estimates of the blackening in autoradiographic images, divided by the exposure time used. Curves thus obtained seem to differ little from the kinetic equations indicated in the figure (these, in turn, may be considered reasonable as a first approximation). Figures mentioned in the graph illustrate the changes in the labelling patterns.