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A SERIAL AUTORADIOGRAPHIC ANALYSIS OF H^3 -GLYCINE
UTILIZATION AND DISTRIBUTION IN THE FEMORA OF GROWING MICE*

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Medical Research Center
Brookhaven National Laboratory
Upton, New York

(running headline)

"H³-Glycine Utilization by Bone"

A SERIAL AUTORADIOGRAPHIC ANALYSIS OF H^3 -GLYCINE
UTILIZATION AND DISTRIBUTION IN THE FEMORA OF GROWING MICE*

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Glycine is the predominant amino acid of collagen. The results of a variety of analytical analyses show that bone collagen consists of approximately 24 grams of glycine per 100 grams of dry, ash-free, protein and that its composition appears to be almost identical with skin collagen (5, 6). Collagen forms the predominant fibrous complement of bone matrix; making up 90-96% of the dry, fat-free weight of the organic matter of bone (11). Cells, namely fibroblasts (15) and osteoblasts (14), play a very important role in collagen formation. Excellent evidence is available for the intracellular origin of the building blocks (protofibrils) which are elaborated extracellularly and eventually polymerized to form collagen (15). Neuberger and Slack (13, 16), have shown that glycine is preferentially incorporated into collagen when administered to animals. Because of the abundance of glycine in collagen formation, the assessment of glycine localization, distribution, uptake and turnover by cells of the skeletal system becomes important in that it allows one to evaluate the degree and manner of cellular participation in bone and cartilage matrix production. Adequate histo-cytochemical methods for studying glycine are not available, whereas biochemical methods by their very nature are unable to furnish in situ data. With the appearance of tritiated amino acids for "high resolution" autoradiography, a precise study of skeletal cells has become feasible.

* Research supported by the U. S. Atomic Energy Commission.

Several qualitative autoradiographic studies of bone employing H^3 -glycine have been reported (3, 24, 25, 26). The aim of the present study is to assess quantitatively the appearance, localization and distribution of H^3 in the femora of young mice following H^3 -glycine administration, in order to study the functional behavior and capacity of different skeletal cells in matrix production. The present study is a prelude to application of the same procedures in ageing animals.

MATERIALS AND METHODS

Thirty-six Brookhaven National Laboratory strain of inbred Swiss albino female mice, 5 weeks of age were used in this study. Each mouse was given a subcutaneous injection of 5 μ c of tritiated glycine per gram of body weight (specific activity = 50 mc/mM).¹ The mice were divided into groups of 3 each and killed at 5, 15, 30 minutes, 1, 4, 8, 16 hours, and 1, 2, 4, 7 and 14 days after administration of the labeled compound. Intact femora were removed, fixed for 24 hours in neutral buffered formalin then washed in running tap water for an additional 24 hours. The femora were decalcified in a 10% solution of disodium versenate. Tissues were dehydrated, and embedded in paraffin. Five micron sections were cut from paraffin blocks and mounted on albuminized slides. The tissue slides were dipped in NTB₃ Kodak liquid emulsion and stored for 17 days in a dry atmosphere at 4°C for exposure of the film. Following exposure, the slides were developed for 2 minutes in D19 developer, briefly rinsed in water, fixed for 2 minutes in Kodak sodium thio-sulfate solution and washed in running tap water for a minimum of 30 minutes. The slides were stained with hematoxylin and eosin.

Grain counts were made over whole, medial sections of labeled cells in order to be able to compare the labeling intensity of different cell types. If the cytoplasm of neighboring cells overlapped, they were excluded from the counts. Since the grain distribution overlying cells does not fit any of the distributions amenable to the usual statistical methods, a statistical analysis of the data has not been applied.

¹The labeled compound was purchased as glycine-2-H³ from New England Nuclear Corp., 575 Albany Street, Boston 18, Mass.

RESULTS

All types of skeletal cells were labeled with H^3 -glycine including newly formed osteocytes. Labeling was essentially cytoplasmic.

Five and Fifteen Minutes After Injection:

This period was characterized by increasing numbers of silver grains in autoradiographs over various cell types, especially osteoblasts at the metaphysis (Fig. 1). Cartilage cells were generally weakly labeled, except at the periphery of the epiphyseal plate. A few silver grains were also seen over osteoblasts. Bone cartilage and connective tissue matrices were not labeled.

Thirty Minutes After Injection:

Labeling of cells was highest at this time period. Metaphyseal-endosteal osteoblasts (Fig. 2) and cartilage cells at the proliferative and hypertrophic zones of the epiphyseal plate (Fig. 3) were covered by the largest number of grains. An accumulation of silver grains was also observed over the bone and cartilage matrix surrounding these cells. Osteogenic cells of the periosteum (pre-osteoblasts and osteoblasts) and the surrounding connective tissues were also heavily labeled but to a lesser degree than the cells of the metaphysis and epiphyseal plate. Cartilage cells and matrix of the articulating surfaces of the epiphysis were still less labeled. Cells and matrix of the fibrous layer of the periosteum, osteocytes (most of which are non-labeled), and osteoclasts represented a category having the least label.

One Hour After Injection:

Although labeling of autoradiographs appeared to be more intense than at the 30 minute time period, this increase in label was not seen over cells but over a greater proportion of matrix (Fig. 4). Any bone, cartilage or connective tissue matrix which was formed during the one hour period following H^3 -glycine administration was labeled. In these young animals, a considerable amount of matrix was produced during this short period of time, especially at the epiphyseal plate, metaphysis and diaphyseal (periosteal and endosteal) surfaces. The localization and distribution of label within bone matrix was seen as narrow bands of overlying silver grains, especially at various sites of the diaphysis. The location and movement of these diaphyseal bands with time will be discussed later to facilitate a better understanding of the events taking place during growth. Cartilage matrix was labeled diffusely throughout the study; never in sharp bands.

Four and Eight Hours After Injection:

These time periods were characterized by a loss of silver grains over the metaphyseal and periosteal osteoblasts and chondrocytes. A greater density of silver grains appeared over the cartilage matrix of the proliferative and hypertrophic zones of the epiphyseal plate.

The overlying lines of silver grains seen along metaphyseal trabeculae and diaphyseal bone surfaces have become denser. The number of silver grains over the periosteal matrix (osteogenic layer) varied in different regions. Labeling was higher at the posterior aspect of the femur and especially at the perichondrial zones.

Sixteen Hours and One Day After Injection:

Autoradiographs showed a continued loss of label in cells and the movement of silver lines away from the bony surfaces (Fig. 5). At the epiphyseal plate, labeling was seen at the degenerative zone, as well as, the proliferative and hypertrophic zones. Labeling of the proliferative and hypertrophic zones was, however, weaker than at the degenerative zone (Fig. 6). Labeled osteoclasts were still observed, however, changes in labeling intensity was not obvious and was generally weak.

Two and Four Days After Injection:

The autoradiographs were again weaker than the previous time periods, especially over skeletal cells. Metaphyseal and epiphyseal plate cells were essentially non-labeled. The abundant silver grains which were seen as lines over surfaces of newly formed metaphyseal trabeculae earlier, now appeared towards the center of the shaft and away from the epiphyseal plate. On the other hand, silver grains which were originally seen over the proliferative and hypertrophic zones, and later over the degenerative zone during growth by 1 day, now appeared over the cartilage matrix of new metaphyseal trabeculae (Fig. 7).

Although the labeling of periosteal matrix also became much weaker with increasing time, the perichondrial regions persistently contained more label than the rest of the osteogenic layer.

Seven Days After Injection:

Autoradiographs revealed very weak labeling of cells and matrices. Osteoblasts and chondrocytes were essentially non-labeled. The cartilaginous core of metaphyseal trabeculae appeared labeled much further away from the epiphyseal

plate than at earlier time periods. Labeling of trabecular surfaces which were seen shortly after H^3 -glycine administration have become on the most part, incorporated into cortical, diaphyseal bone.

Fourteen Days After Injection:

At this late time period following H^3 -glycine administration, the autoradiographs were essentially non-labeled except for the clearly demarcated diaphyseal labeling which appeared well within the cortical bone.

Table I and Figure 8 show the average number of grains counted over skeletal cells at different time periods following H^3 -glycine administration.

It is interesting to note that the uptake of H^3 -glycine by endosteal osteoblasts of the metaphysis was very high and appeared earlier than in other skeletal cells. Rapid increases in uptake also occurred at the epiphyseal plate, followed by the periosteum and articular cartilage. Osteocytes were generally less intensely labeled than other cell types. Osteoclasts were very weakly labeled throughout this study.

The peak of cellular labeling was observed at about 30 minutes after isotope administration. This was followed by a gradual decrease in labeling over all cell types as additional grains appeared over various skeletal matrices.

In Figure 9, the grain counts over periosteal cells along the anterior surface of the diaphysis were compared to cells at the posterior surface of the femur, and the combined data as a function of time. Since the cellular activity of the periosteal surfaces, as well as other surfaces vary along the shaft (see Fig. 10), a separate analysis was made to determine the differences in grain counting data. Figure 9 shows that significant differences can be seen at the early periods following H^3 -glycine administration. But, after peak labeling of cells the differences become less significant.

Localization and Migration of Growth Lines:

The appearance of silver grains over regions of newly formed bone has been mentioned earlier. These surfaces were found along the length of the diaphysis, both periosteally and endosteally (Fig. 10). Silver lines were also seen along proximal and distal metaphyseal trabeculae and the trabecular surfaces of the secondary centers of proximal and distal epiphyses. Figure 10 shows diagrammatically the femoral and diaphyseal localization of these lines with increasing time. More specifically, growth lines were found along the anterior periosteal surface extending from the proximal perichondrial region to the mid-diaphysis and from the distal fourth of the diaphysis to the distal perichondrial region. On the endosteal side of the medullary canal, growth lines were mainly seen on the posterior aspects of the proximal metaphysis and extending down the posterior, cortical endosteum, along the proximal third of the diaphysis.

A line was again seen at the posterior, distal third of the medullary canal. Both the anterior and posterior surfaces of the distal metaphyseal trabeculae were labeled, as well as the cortical endosteum along the anterior surface from the distal end of the medullary canal up to two-thirds of the shaft. On the posterior periosteal surface, a growth line was observed extending from the proximal perichondrial region, and along three-fourths of the diaphysis. The movement of these lines with time was extremely interesting to follow, since they reflected the growth events of the femur. From the time that silver lines made their appearance (30 minutes) to approximately 8 hours, additional labeled material was added causing a widening of these lines (Figs. 11, 12). This in itself represents some degree of movement and continued cellular deposition of label which does not end abruptly (see Figures 16 and 17).

Silver lines over the proximal two-thirds of the posterior periosteal surface and distal two-thirds of the anterior endosteal lining of the medullary canal were observed some distance away from the site of deposition at 16 hours. By 1 day, all silver lines were seen some distance away from the surfaces of origin. These silver lines were farther away from the free bone surfaces at each observation period (Figs. 13, 14, 15, 16). Both distal endosteal lines and lines at the proximal epiphysis became very irregular during growth as newly formed trabeculae became part of the diaphyseal cortical mass. The distal endosteal growth lines eventually reached the periosteal surfaces (Fig. 17). It was also interesting to note that the silver line at the anterior, distal periosteal surface with time became part of the articulating surface.

DISCUSSION

It is of interest to note that the four major skeletal cell compartments (i.e., periosteum, epiphyseal plate, metaphysis and articular cartilage), exhibited a maximum labeling over cells at 30 minutes following H^3 -glycine administration, although the grain counts were quite different (Table 1, Fig. 8). The data also showed that the rate of H^3 -glycine incorporation was more marked in some cell types. Maximum grain counts were seen over metaphyseal cells much earlier than over epiphyseal cells. Indicating that metaphyseal cells either incorporated the labeled material more rapidly or perhaps the circulation to metaphyseal cells was more adequate. At 30 minutes the grain counts were equal, however, a larger number of grains were observed over the matrix contiguous with metaphyseal osteoblasts. The highest turnover of glycine was exhibited by metaphyseal osteoblasts, followed by cartilage cells of the proliferative and hypertrophic zones of the epiphyseal plate, osteogenic cells of the periosteum and cartilage cells of the articulating surfaces. An analysis of the grain count data versus time shows that upon semilogarithmic plotting and analysis one obtains a reasonably good 2 exponential curve. The half-time of the curves is given in Table II. It seems possible that the 1st component represents turnover of soluble pre-collagen and the 2nd turnover of cytoplasmic proteins. Further studies are needed, however, to verify this statement. A similar sequence of activity was observed in previously reported studies involving H^3 -histidine (22, 23).

Another interesting point observed during this study was the fact that members of the same cell category located at different anatomical regions differed somewhat in their average grain counts. This difference was most obvious at 30 minutes.

H^3 -glycine labeling of osteoclasts was extremely weak throughout this study, as well as in rat cranial bone reported by Young (25, 26). In our H^3 -histidine studies, osteoclasts revealed much more labeling (22, 23). Yet, in collagen, histidine makes up less than 1% (4, 12), glycine 32.5% (1). The H^3 -glycine label of osteoclasts and at least a part of the H^3 -histidine label probably represent the amounts of these amino acids necessary for turnover of cytoplasmic proteins and not precursor collagen formation. A large contrast exists in the uptake of amino acids between osteoblasts and osteoclasts, however, one must not necessarily conclude on this basis, that these cells arise by alternate routes of specialization of the same precursor cell (25, 26), since (A) these cells do not represent the same functional state, and (B) the absence of a given cellular activity at any time does not indicate that the cellular potential for the particular activity is missing. An excellent example of this situation was seen in a study of the proliferative potential of periosteal osteoblasts in old mice. Old osteoblasts exhibited extremely low labeling indices with H^3 -thymidine. Following fracture, however, the labeling indices increased dramatically (20, 21). The present findings do not exclude the possibility that osteoblasts and osteoclasts represent different functional states of the same cell (17, 19).

The general findings of this study are consistent with those reported by Carneiro and Leblond (3) and Young (26). H^3 -glycine was first seen accumulating within skeletal cells and shortly within newly deposited bone and cartilage matrices. During growth the H^3 -glycine observed as a line of silver grains, appeared at an increasing distance away from the original site of incorporation.

The growth of bones has long been investigated by a variety of methods. Intravital staining of growing bone with madder was used by Cameron (2). Alizarin has also been used extensively (7, 8). More recently, isotopes such as P^{32} and C^{14} have been employed in the study of bone growth (9, 10). H^3 -glycine is useful in this respect, since it produces "high-resolution autoradiography".

The present study emphasizes several important features of femoral growth, namely; (A) that the distal anterior periosteal cells contributed matrix for epiphyseal growth. Cells are also contributed by the periosteum during epiphyseal growth (18). (B) That the proximal and distal femoral epiphyses differed in growth. The endosteum contributed to distal epiphyseal growth as previously reported with other isotopes (9, 10), while the periosteum contributed largely to the proximal epiphyseal growth and perhaps the most important feature, (C) that the activity of the subcortical endosteum differed widely from one region of the medullary canal to another. On the basis of this finding, it becomes evident that in studying the behavior of endosteal cells one must examine the entire length of the canal, so as not to produce an erroneous picture of the endosteal population.

SUMMARY

The localization, distribution and cellular turnover of tritium labeled-glycine was followed and quantitatively assessed autoradiographically in the femora of young mice. Initially the labeled amino acid was taken up by the cellular phase of the skeletal system, and was observed within cells 15 minutes after isotope administration. Thirty minutes after H^3 -glycine injection represented the peak of cellular uptake (assessed by autoradiographic grain density over cells). Shortly after cells incorporated H^3 -glycine, silver grains appeared over newly deposited bone and cartilage matrices. At the surfaces of bone, silver grains were arranged in bands which were followed up to 14 days after H^3 -glycine administration. These growth bands were found over deeper layers of bone with increasing time. The movement of these bands was illustrated and discussed. Labeling of cartilage matrix was diffuse.

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REFERENCES

1. BOWES, J. H., and KENTEN, R. H.: The amino-acid composition and titration curve of collagen. *Biochem., J.*, 43:358-365, 1948.
2. CAMERON, G. R.: The staining of calcium. *J. Path. Bact.*, 33:939-955, 1930.
3. CARNEIRO, J., and LEBLOND, C. P.: Role of osteoblasts and odontoblasts in secreting the collagen of bone and dentin, as shown by radioautography in mice given tritium-labeled glycine. *Exp. Cell Res.*, 18:291-300, 1959.
4. EASTOE, J. E.: The amino acid composition of mammalian collagen and gelatin. *Biochem. J.*, 61:589-600, 1955.
5. EASTOE, J. E.: The organic matrix of bone; In: *The Biochemistry and Physiology of Bone*, G. H. Bourne, editor, Academic Press, New York, 1956, pp. 81-105.
6. FRUTON, J. S., and SIMMONDS, S.: In: *General Biochemistry*, 2nd Ed. J. Wiley, New York, 1958, p. 53.
7. GOTTLIEB, B.: Die vitale färbung der kalkhaltigen gewebe. *Anat. Anz.* 46:179-194, 1914.
8. HOYTE, D. A. N.: Alizarin as an indicator of bone growth. *J. Anat.* 94: 432-442, 1960.
9. LEBLOND, C. P., WILKINSON, G. W., BÉLANGER, F., and ROBICHON, J.: Radioautographic visualization of bone formation in the rat. *Am. J. Anat.*, 86:289-341, 1950.

10. LEBLOND, C. P., BELANGER, L. F., and GREULICH, R. C.: Formation of bones and teeth as visualized by radioautography. *Ann. N. Y. Acad. Sci.*, 60:631-659, 1955.
11. McLEAN, F.C., and URIST, M. R.; In: *Bone - An Introduction to the Physiology of Skeletal Tissue*, 2nd ed., Univ. of Chicago Press, Chicago, 1961, p. 35.
12. NEUMAN, R. E.: The amino acid composition of gelatins, collagens and elastins from different sources. *Arch. Biochem.*, 24:280-298, 1949.
13. NEUBERGER, A. and SLACK, H. G. B.: The metabolism of collagen from liver, bone, skin and tendon in the normal rat. *Biochem. J.*, 53:47-52, 1953.
14. PRITCHARD, J. J.: The osteoblast; In: *The Biochemistry and Physiology of Bone*, G. H. Bourne, editor, Academic Press, New York, 1956, pp. 179-212.
15. ROUILLER, C.: Collagen fibers of connective tissue; In: *The Biochemistry and Physiology of Bone*, G. H. Bourne, editor, Academic Press, New York 1956, pp. 107-147.
16. SLACK, H. G. B.: Metabolism of collagen in the rat; In: *Nature and Structure of Collagen*, J. T. Randall, editor, Butterworths Scientific Pub., London, 1953, p. 51.
17. TONNA, E. A.: Osteoclasts and the aging skeleton; A cytochemical, cytological and autoradiographic study. *Anat. Rec.*, 137:251-270, 1960.
18. TONNA, E. A.: The cellular complement of the skeletal system studied autoradiographically with tritiated thymidine (H^3 TDR) during growth and aging. *J. Biophys. and Biochem. Cytol.*, 9:813-824, 1961.

19. TONNA, E. A. and CRONKITE, E. P.: Use of tritiated thymidine for the study of the origin of the osteoclast. *Nature*, 190:459-460, 1961.
20. TONNA, E. A. and CRONKITE, E. P.: Cellular response to fracture studied with tritiated thymidine. *J. Bone and Joint Surg.*, 43:352-362, 1961.
21. TONNA, E. A. and CRONKITE, E. P.: Changes in the skeletal cell proliferative responses to trauma concomitant with aging. *J. Bone and Joint Surgery* 44A:1557-1568, 1962.
22. TONNA, E. A.: An autoradiographic examination of the utilization of tritiated histidine by cells of the skeletal system. *Nature*, 19:1301-1302, 1962.
23. TONNA, E. A., CRONKITE, E. P. and PAVELEC, M.: An autoradiographic study of the localization and distribution of tritiated histidine in bone. *J. Histochem. and Cytochem.*, 10:601-610, 1962.
24. YOUNG, R. W.: Autoradiographic studies on bone and cartilage matrix formation in young rats injected with glycine- H^3 . *Anat. Rec.*, 142:335, 1962.
25. YOUNG, R. W.: Autoradiographic studies on nucleic acid metabolism and protein synthesis in the cells of bone. *J. Bone and Joint Surgery* 44A:1025, 1962.
26. YOUNG, R. W.: Autoradiographic studies on postnatal growth of the skull in young rats injected with tritiated glycine. *Anat. Rec.*, 143:1-13, 1962.

Fig. 1 An autoradiograph of the metaphyseal region, 15 minutes after H^3 -glycine administration. Metaphyseal osteoblasts reveal an early uptake of H^3 -glycine. Hemopoietic elements are not labeled at this time period. No grains appear over the matrix of bone. Hematoxylin and eosin stain. Oil immersion, x 540.

Fig. 2 An autoradiograph showing intense labeling of endosteal osteoblasts and silver grains appearing over newly deposited matrix, 30 minutes after H^3 -glycine administration. Labeling of megakaryocytes and hemopoietic elements is evident at this time period. Hematoxylin and eosin stain. Oil immersion, x 540.

FIGURE 1

FIGURE 2

Fig. 3 The autoradiograph of the epiphyseal plate of the distal femoral epiphysis, 30 minutes after H^3 -glycine administration shows intense labeling of cartilage cells. Some labeling of the cartilage matrix has already taken place. Hematoxylin and eosin stain. Oil immersion, x 540.

Fig. 4 The autoradiograph of metaphyseal trabeculae at the distal end of the femur, 1 hour after H^3 -glycine administration shows an abundance of silver grains mostly over recently deposited trabecular matrix. Endosteal osteoblasts show a paucity of label. Hematoxylin and eosin stain. Oil immersion, x 540.

FIGURE 3

FIGURE 4

Fig. 5 An autoradiograph of the metaphyseal trabeculae at the distal end of the femur, 16 hours after H^3 -glycine administration showing the loss of cellular label and the appearance of extracellular labeling bands. These bands appear away from the trabecular surfaces where deposition of new matrix occurred, (Compare with Figure 4). Note that the cartilaginous cores of the trabeculae are non-labeled. Hematoxylin and eosin stain. Oil immersion, x 540.

Fig. 6 An autoradiograph of the epiphyseal-metaphyseal junction, 16 hours after H^3 -glycine administration. Label which formally appeared at the proliferative and hypertrophic zones and in the matrix, now also appears at the degeneration zone of the plate. The metaphyseal cells are essentially non-labeled. Trabecular labeling within regions of newly deposited collagen matrix has moved farther away from the plate (Compare with Figures 3 and 4). Hematoxylin and eosin stain. Oil immersion, x 540.

FIGURE 5

FIGURE 6

Fig. 7 This autoradiograph of the trabeculae immediately next to the distal epiphyseal plate of the femur shows extensive labeling of the cartilage core, 2 days after H^3 -glycine administration. This label was formally observed at the proliferative and hypertrophic zones of the epiphyseal plate, and after 16 hours within the degenerative zone. Labeling of metaphyseal cells was essentially negative earlier. The initial intense deposition of label within trabecular surfaces appears much further away from the epiphyseal plate, because of growth (Compare with Figures 4, 5, and 6). Hematoxylin and eosin stain. Oil immersion, x 540.

FIGURE 7

Fig. 8 The bar diagram represents a plot of the average number of reduced silver grains over different skeletal cells observed in autoradiographs against time following administration of tritiated glycine. The data show the early uptake of H^3 -glycine especially by endosteal osteoblasts. The peak cell labeling is observed at 30 minutes. A diminished grain count after 30 minutes is representative of the reduced availability of the labeled compound and continued cellular output. Note that the grain count levels of the metaphysis and epiphyseal plate remain consistently higher than that of the periosteum and articular cartilage.

FIGURE 8

Fig. 9 The plot represents the average number of grains over cells of the osteogenic layer of the periosteum at different time periods following H^3 -glycine administration. The counts made at the proximal and distal halves of the femur are compared and the combined counts are also included. A small but consistent difference in grain counts of both regions is shown, especially at the earlier time periods. The difference is most significant at the period of highest uptake. Average grain counts were always higher for the proximal end.

FIGURE 9

Fig. 10 The accumulation of H^3 -glycine at newly deposited bone surfaces and its subsequent movement during growth are diagrammatically shown as dark lines representing autoradiographically, silver grains arranged in growth bands. Note that neither the periosteal nor endosteal surfaces along the shaft of the femur are entirely active in matrix production. Endosteal deposition of labeled material at the distal end of the femur becomes located with time at the subperiosteal surfaces, then actually removed. It is also interesting to note that labeled material at the anterior, distal periosteal surface becomes part of the articular cartilage.

THE INCORPORATION OF H^3 -GLYCINE
IN THE FEMORA OF YOUNG MICE

FIGURE 10

Fig. 11 An autoradiograph showing an accumulation of silver grains in the form of a band overlying the endosteal surface of the cortex 1 hour after H^3 -glycine administration. Hematoxylin and eosin stain. Oil immersion, x 540.

Fig. 12 An autoradiograph similar to Figure 11 showing a widening of the band of silver grains at 16 hours after H^3 -glycine administration. Hematoxylin and eosin stain. Oil immersion, x 540.

FIGURE 11

FIGURE 12

Fig. 13 An autoradiograph similar to Figure 12 showing the movement of the endosteal band of silver grains 4 days after H^3 -glycine administration. Note the lack of periosteal activity. Hematoxylin and eosin stain. Oil immersion, x 540.

Fig. 14 An autoradiograph 4 days after H^3 -glycine administration showing in a different region of the femoral shaft a band of silver grains which has moved away from the periosteal surface. Note in this photomicrograph the absence of endosteal activity (Compare with Figure 13). Hematoxylin and eosin stain. Oil immersion, x 540.

FIGURE 13

FIGURE 14

Fig. 15 This autoradiograph was prepared from a region of the femoral shaft where periosteal and endosteal matrix production was taking place at opposite surfaces, 14 days after H^3 -glycine administration. Hematoxylin and eosin stain. X 400.

Fig. 16 The autoradiograph shows endosteal label represented by a band of silver grains over the middle of the femoral shaft, 14 days after H^3 -glycine administration. Note the trail of silver grains on the endosteal side of the band as it moves towards the periosteal surface. This is evidence of continued cellular turnover of H^3 -glycine but in diminishing quantity. Hematoxylin and eosin stain. x 400.

FIGURE 15

FIGURE 16

Fig. 17 This photomicrograph was taken at the anterior distal end of the femoral diaphysis. The autoradiograph represents 14 days after H^3 -glycine administration. Note that the endosteal label represented by a band of silver grains has traversed the cortex of the shaft and part of it is seen at the subperiosteal surface. Hematoxylin and eosin stain. X 400.

FIGURE 17

TABLE I

AVERAGE NUMBER OF GRAINS COUNTED PER SKELETAL CELL AT DIFFERENT TIME PERIODS
FOLLOWING H³ - GLYCINE ADMINISTRATION TO YOUNG MICE*

TIME	PERIOSTEUM (Osteogenic layer)	EPIPHYSEAL PLATE (Proliferative and Hypertrophic zones)	METAPHYSIS (Endosteal Osteoblasts)	ARTICULATING SURFACES
5 min.	0.93	0.97	6.17	0.65
15 min.	2.17	3.59	13.54	1.26
30 min.	9.94	14.46	13.64	6.24
1 hr.	5.92	10.67	12.59	5.52
4 hrs.	4.70	7.78	6.51	4.50
8 hrs.	3.97	6.63	5.01	3.10
16 hrs.	1.52	4.48	2.59	2.27
1 day	1.65	5.87	2.12	2.20
2 days	1.00	3.05	1.93	2.32
4 days	0.72	2.07	1.17	1.47
7 days	0.29	0.64	0.40	0.71
14 days	0.12	0.25	0.11	0.51

*50-150 cells counted per average value.

TABLE II

Approximate Half-times of First and Second Components Obtained From Semilogarithmic Plots
of the Average Grain Counts of Mouse Skeletal Tissues Versus Time

Curve Components	Metaphysis	Epiphysis	Periosteum	Articular Cartilage
1st (hours)	2.5	4.0	4.0	3.5
2nd (days)	2.5	2.9	3.1	5.2