

原 著

## An Experimental Study on the Proliferation of Epiphyseal Cartilage Cells After Partial Resection of Epiphyseal Plate

by

KISABURO SAKAKIDA and BUNJI YAMASHITA

Department of Orthopedic Surgery, Kyoto Prefectural University of Medicine

Received for Publication March, 8, 1976.

Through numerous experimental studies have been performed on injuries of the epiphyseal plate, only a few have dealt with the chondrocyte dynamics caused by the injury and many aspects still remain uncertain.

We have studied local changes following partial resection of epiphyseal cartilage and the mode of proliferation and differentiation of remaining chondrocytes which escaped direct injury. This was accomplished by the use of  $^3\text{H}$ -thymidine autoradiography.

### Experimental Methods

Sixty Wistar rats of 50 gram body weight were used 3 weeks after birth. Two types of injuries were inflicted on one side, while the other side served as the control.

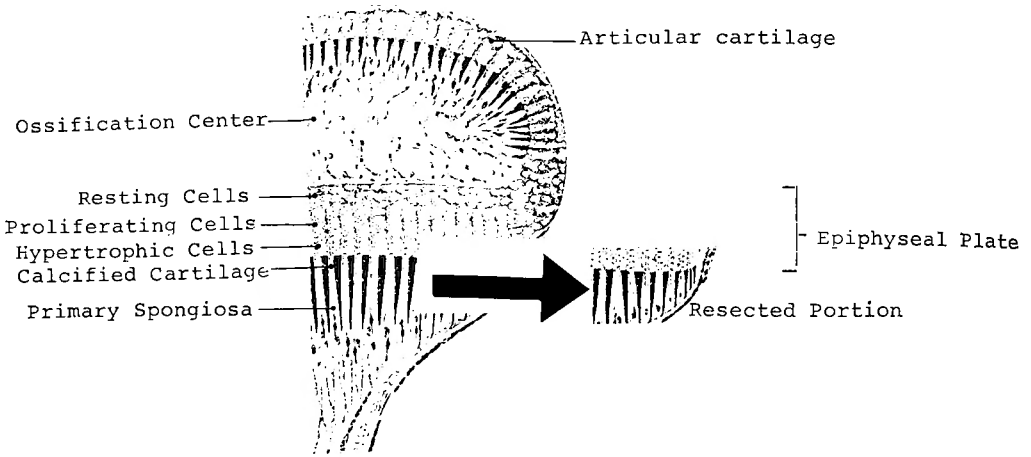
1) On the medial side of the proximal epiphyseal cartilage of the tibia, the medial 1/3 of the hypertrophic cell layer and primary spongiosa were resected with a scalpel under a microscope<sup>3)</sup>. This was followed by intraperitoneal injections of 2  $\mu\text{Ci}$ /gram body weight of  $^3\text{H}$ -thymidine 5 times every 6 hours over a period of 24 hours. The mode of repair was studied by cumulative labeling and pulse labeling methods.

2) In another group, again on the medial side of the proximal epiphyseal cartilage of the tibia, the medial 1/3 was resected from the resting cell layer to the primary spongiosa. (Fig.1) At 1, 2, 3 and 4 week intervals, 2  $\mu\text{Ci}$ /gram body weight  $^3\text{H}$ -thymidine was injected intraperitoneally 5 times every 6 hours over a period of 24 hours. The proximal end of the tibia was sampled every week, at 1 hour, 1 day, 2 days and 3 days after  $^3\text{H}$ -thymidine administration<sup>6)</sup>. The specimen was fixed in formalin, prepared into paraffin sections of 5  $\mu$  thickness, covered by a dipping method and subjected to microgranular development

Key words proliferating cells, experimental epiphyseal injuries, autoradiography, cell population kinetics, bone growth.

Present address : Department of Orthopedic Surgery, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto, Japan. 〒 602

Experiment (1)



Experiment (2)

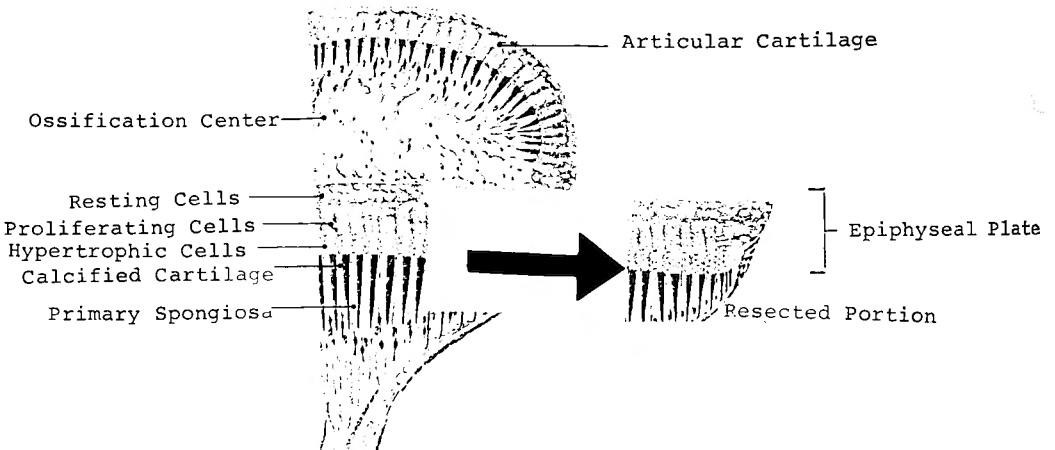


Fig. 1. Experimental methods

after 4 weeks of exposure to light in a cold dark place to obtain autoradiograph. The preparations were studied under a microscope after H-E and Safranin-O-fast green staining.

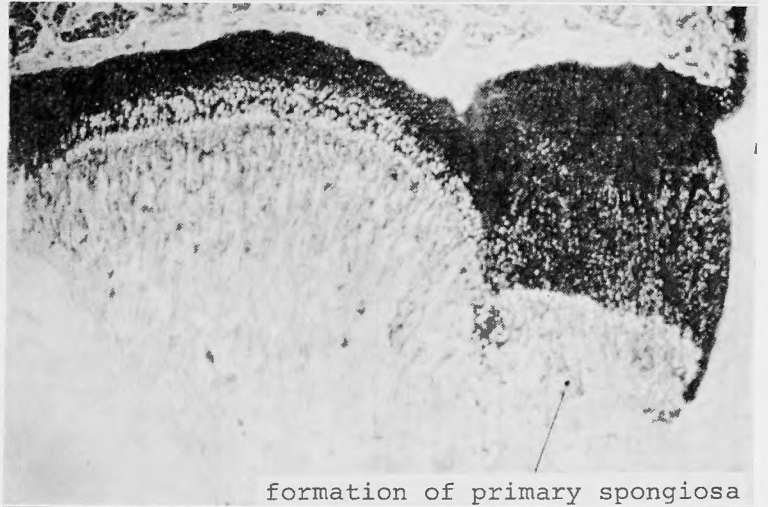
**Experimental Results**

1) After resection of the hypertrophic cell layer and primary spongiosa, the height of the epiphyseal cartilage at the site of injury increased 3 days after injury. Most of this consisted of hypertrophic cells. Hemorrhagic lesions were found on the metaphyseal side.

**Fig. 2.** Exp. 1. Post-op. 1 week. The height of the epiphyseal cartilage at the site of injury increased one week after injury.



**Fig. 3.** Exp. 1. Post-op. 2 weeks. The height of the epiphyseal cartilage increased on the lateral 2/3 of the proximal end of the tibia which escaped direct injury.

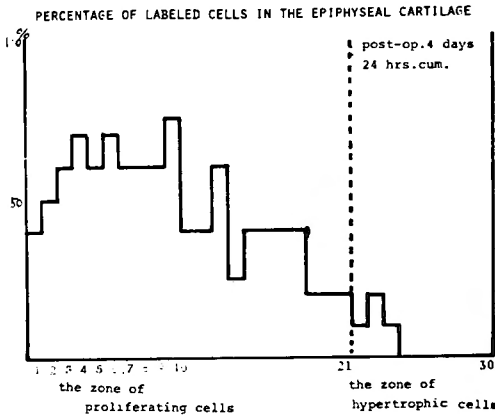


**Fig. 4.** Exp. 1. Post-op. 4 weeks. The proximal epiphyseal cartilage of the tibia was of almost uniform height medially and laterally.

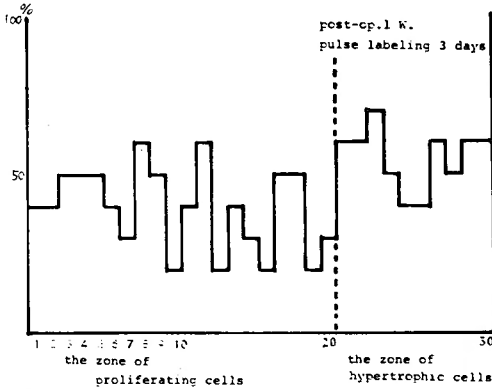


The height of the epiphyseal cartilage, moreover, increased on the lateral 2/3 of the proximal end of the tibia which escaped direct injury (Fig. 2). The number of cells between the margin of the proliferating cell layer to the tip of the hypertrophic cell layer averaged 30, being increased over the control value. In the cumulative labeling method over a period of 24 hours, the labeling index (abbreviated as L.I.) of cells up to the 24th cell of the proliferating cell layer was as high as 48%<sup>71</sup>.

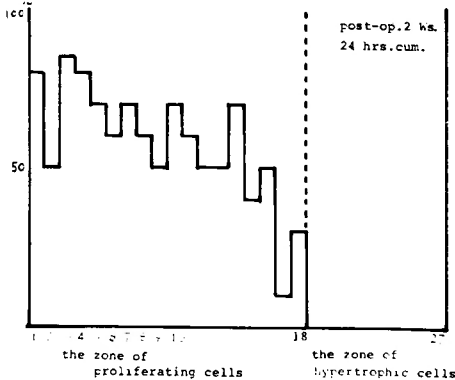
Two to 3 weeks after injury the increased height of the epiphyseal cartilage at the



(A) : Post op. 4 days, 24 hours cumulative labeling.



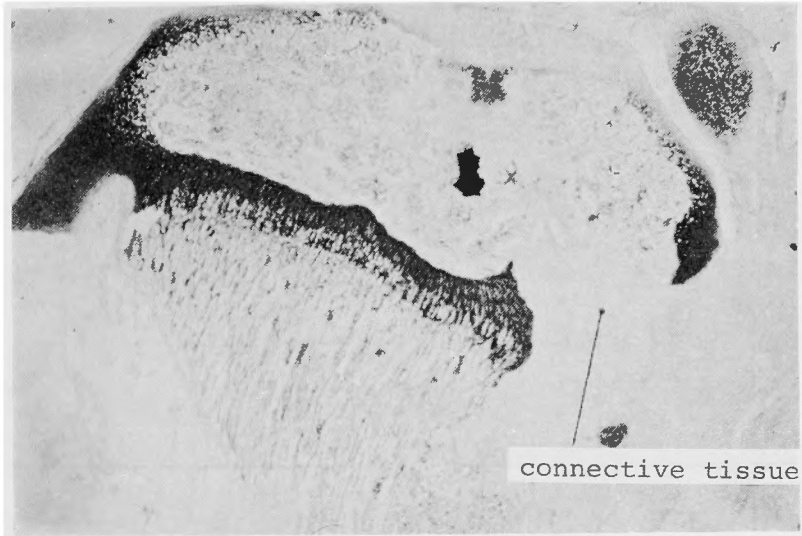
(B) : Post op. 1 week, pulse labeling, 3 days.



(C) : Post op. 2 weeks, 24 hours cumulative labeling.

Fig. 5. Percentage of labeled cells in the epiphyseal cartilage

site of injury gradually decreased in size. Approximating the time of reformation of the primary spongiosa on the metaphyseal side, the value was roughly equal to that of the



**Fig. 6-A.** Exp. 2. Post-op. 1 week. Invasion of the resected site by the connective tissue was apparent 1 week later.

epiphyseal cartilage on the lateral 2/3 which escaped direct injury (Fig. 3). At 4 weeks, the proximal epiphyseal cartilage of the tibia was of almost uniform height medially and laterally, making distinction of the damaged part difficult (Fig. 4 Fig. 5-A, B, C).

2) When resection of the epiphyseal cartilage was carried out from the resting cell layer to the primary spongiosa, invasion of the resected site by the connective tissue was apparent 1 week later (Fig. 6-A,B). The remaining epiphyseal cartilage, however, maintained its usual height and regular arrangement of chondrocytes.

At 1 hour after administration of <sup>3</sup>H-thymidine 1 week after injury, L. I. of the proliferating layer of the remaining epiphyseal cartilage was 54%, slightly higher than that of the controls, while the number of cell from the proliferating cell layer to the tip of the hypertrophic cell layer was similar to



**Fig. 6-B** Exp. 2. Post-op. 10 days. Invasion by bone tissue was noted at the site of the injury.

that of the control.

After 1 day, the chondrocytes labeled in the proliferating cell layer showed a stage of transition to the hypertrophic cell layer similar to that in the control (Fig. 7).<sup>7)</sup>

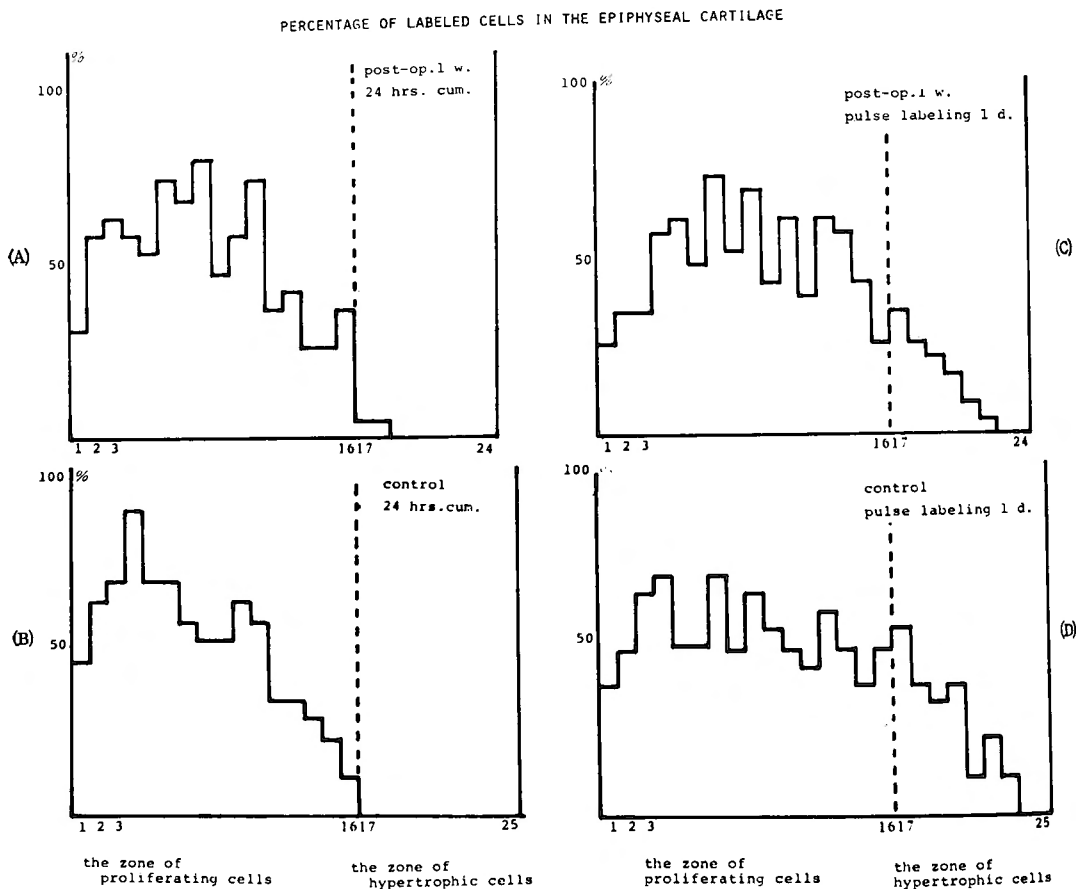


Fig. 7. Percentage of labeled cells in the epiphyseal cartilage

- (A) : Post-op. 1 week, 24 hours cumulative labeling.  
 (B) : Control  
 (C) : Post-op. 1 week, Pulse labeling, 1 day.  
 (D) : Control

Two weeks after injury, varus deformity became pronounced and invasion by bone tissue was noted at the site of the injury (Fig. 8)<sup>3)5)9)</sup>. At 1 hour after <sup>3</sup>H-thymidine administration<sup>2)9)</sup>, the height of the remaining epiphyseal cartilage was decreased to as low as 19 cells, with a concomitant decrease of L. I. of the proliferating cell layer to 37%.<sup>7)</sup> After 1 day, the chondrocytes labeled in the proliferating cell layer had shifted towards the direction of hypertrophic cell layer about 5 cell distance as in the controls. (Fig. 9)<sup>7)</sup>

At 3 weeks after injury, a bony bridge connecting the epiphysis and metaphysis was noted at the site of the resection, and early closure of the epiphyseal line was seen on the medial side.<sup>3),4)</sup> The columnar arrangement of the epiphyseal chondrocytes was irregular

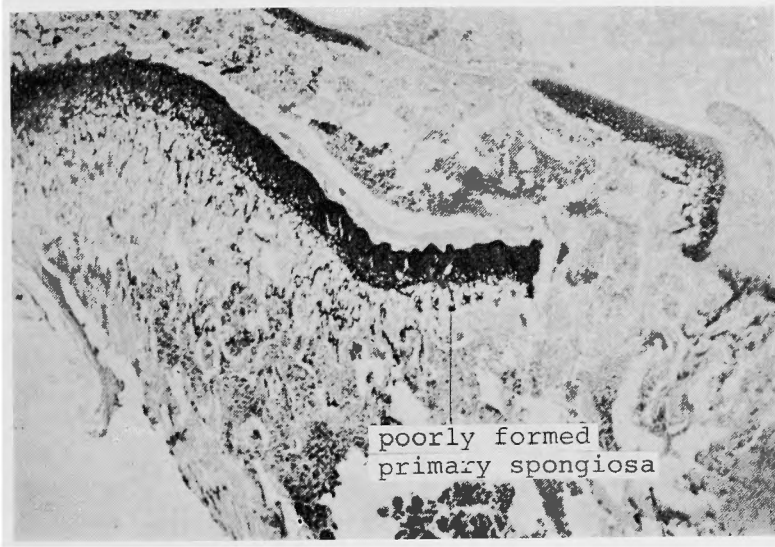


Fig. 8. Exp. 2. Post-op. 2 weeks. Varus deformity became pronounced.

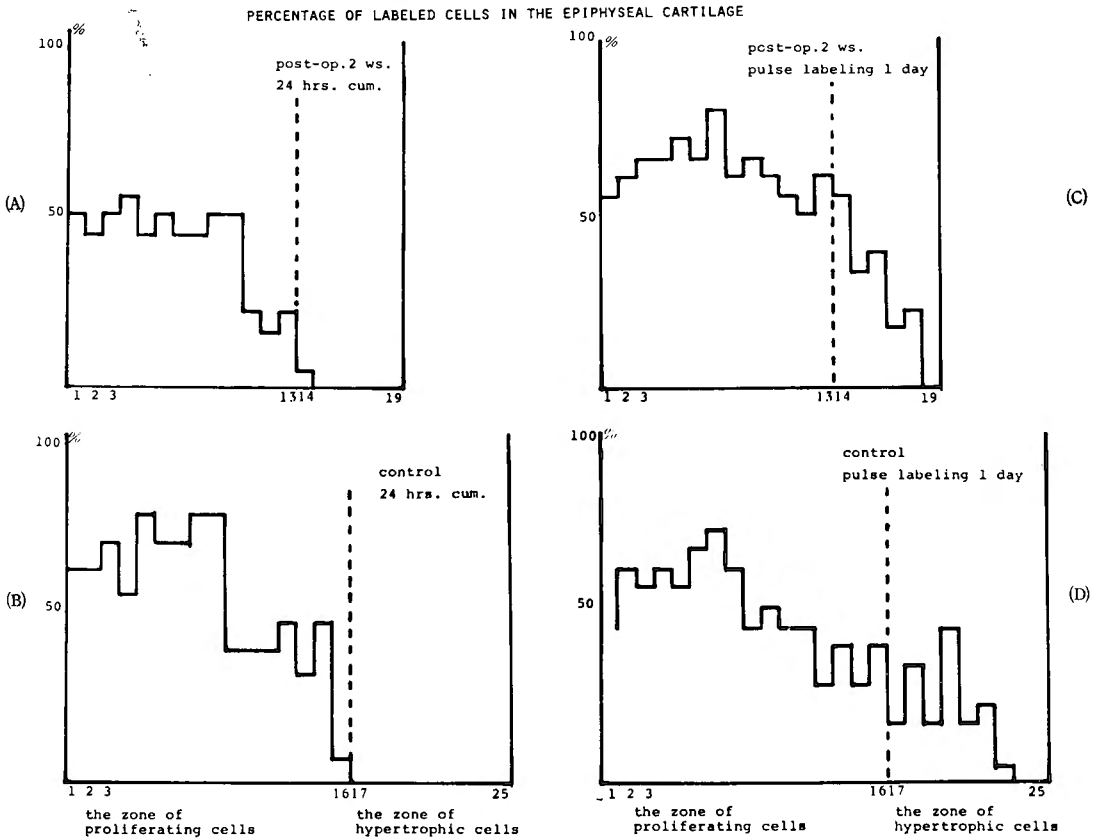
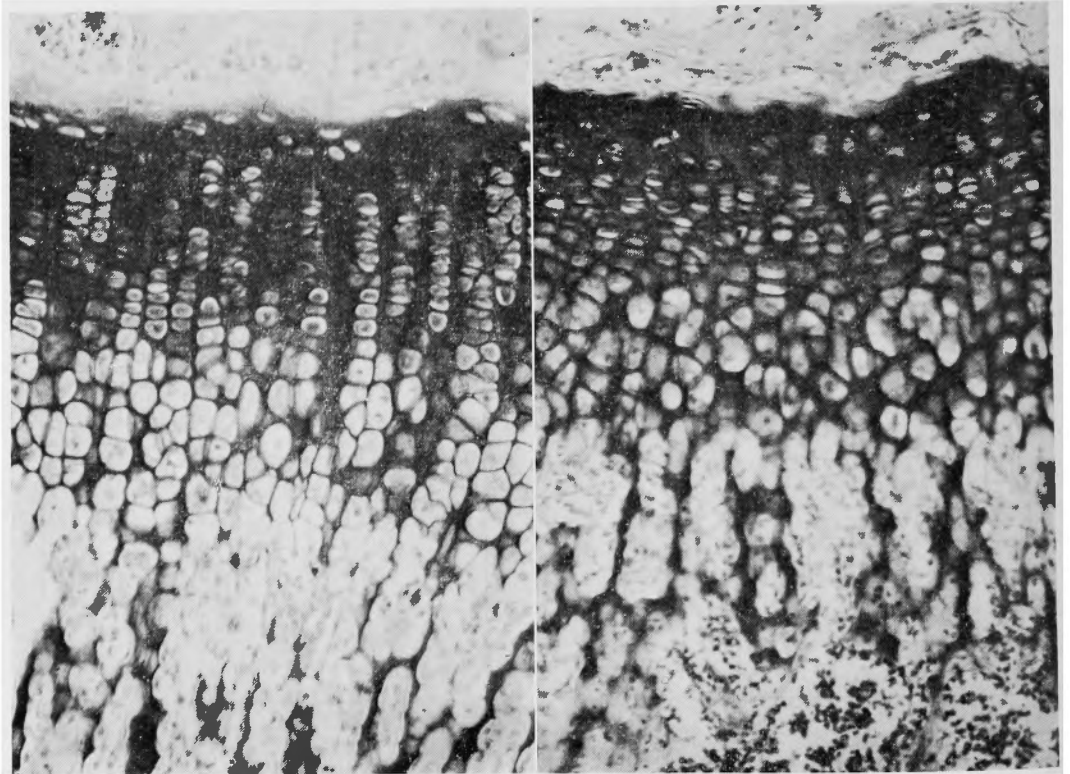


Fig. 9. Percentage of labeled cells in the epiphyseal cartilage  
 (A) : Post-op. 2 weeks, 24 hours cumulative labeling.  
 (B) : Control  
 (C) : Post-op. 2 weeks, Pulse labeling, 1 day.  
 (D) : Control



Fig. 10. Exp.2. Post-op. 3 weeks. A bony bridge connecting the epiphysis and metaphysis was noted at the site of the resection, and early closure of the epiphyseal line was seen on the medial side.



(A)

(B)

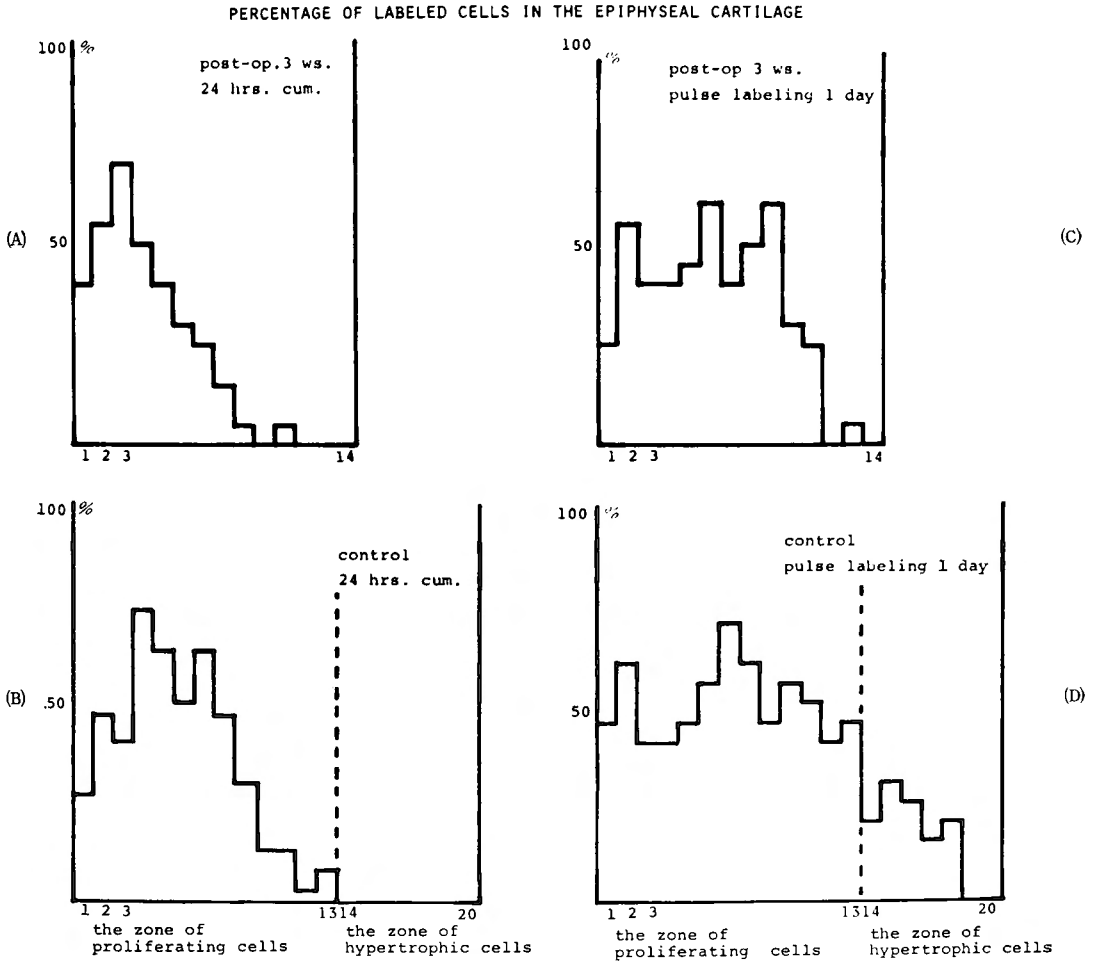
Fig. 11. (A) : Control, (B) : Post-op. 3 weeks,  $\times 50$



in the remaining lateral 2/3, with a decrease in height to an average thickness of 14 cells. The primary spongiosa was irregular on the metaphyseal side (Fig. 10, Fig. 11).

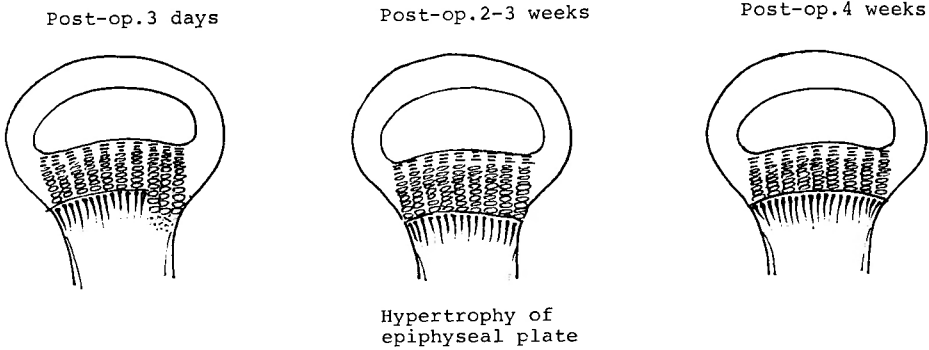
One hour after  $^3\text{H}$ -thymidine administration, the L. I. of the proliferating cell layer was 30%, indicating a further decrease from the value obtained at 2 weeks.

One day later, the shift of the labeled cells towards the hypertrophic cell layer was only a distance of 2 cell (Fig. 12, Fig. 13).



**Fig. 12.** Percentage of labeled cells in the epiphyseal cartilage  
 (A) : Post-op. 3 weeks, 24 hours cumulative labeling.  
 (B) : Control  
 (C) : Post-op. 3 weeks, Pulse labeling, 1 day.  
 (D) : Control

## Experiment (1)



## Experiment (2)

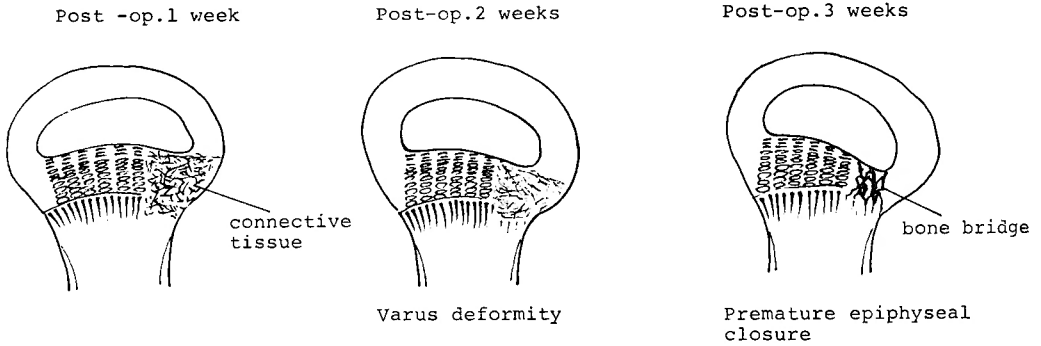


Fig. 13. Experimental results

## Discussion

In injuries of the epiphyseal plate, and results vary markedly depending upon the site of injury of the epiphyseal cartilage.<sup>4)</sup> If the injury occurs at the hypertrophic cell layer, the height of the epiphyseal cartilage is increased markedly. As the primary spongiosa is formed on the side of the metaphysis, however, this discrepancy is said to decrease, gradually approaching the control level. In our experiments with an injury of the hypertrophic cell layer of the epiphyseal cartilage, similar findings were obtained.

Interestingly the height of the lateral epiphyseal cartilage which escaped direct injury, is temporarily increased, with high L. I. values in the proliferating cell layer. In other words, an injury to the hypertrophic cell layer on the medial side of the epiphyseal cartilage appears to cause a transient and reactive increase of proliferating activity of the epiphyseal chondrocytes on the uninjured lateral side.

Resection of epiphyseal cartilage from the resting cell layer on the medial side of the proximal tibial epiphysis to the primary spongiosa led to the invasion of the injured sites by connective tissue followed by a gradual formation of a bony bridge and decrease in the height of the remaining epiphyseal cartilage again, approximately in agreement with the reports by various investigators.

<sup>3</sup>H-thymidine autoradiography used to the cell dynamics of chondrocytes during this period revealed the beginning of the fall of L. I. of the proliferating cell layer of the epiphyseal cartilage about 2 weeks after injury, with the shift of the labeled cells towards the hypertrophic cell layer as in the controls. After 3 weeks, the L. I. was further decreased, with a concomitant decrease in the speed of shift of the labeled cells.

Following resection of the epiphyseal cartilage from the proliferating cell layer to the primary spongiosa, the rate of cell-division of the remaining epiphyseal cartilage is decreased, with the consequent delay in the process of hypertrophy, maturation and necrosis of the proliferating chondrocytes, probably leading to the decrease in the height of the epiphyseal cartilage.

### Summary

Two kinds of injuries were inflicted on the medial 1/3 of the proximal epiphyseal cartilage of the tibia of Wistar rats of 50 gram body weight 3 weeks after birth. The mode of repair was studied by means of <sup>3</sup>H-thymidine autoradiography and the following results were obtained.

1. After resection of the hypertrophic cell layer and primary spongiosa of the epiphyseal cartilage, the height of the epiphyseal cartilage was increased markedly at the site of injury, followed by a return to the control level.

A reactive increase of proliferating activity was noted in the epiphyseal cartilage on the lateral 2/3 of the epiphyseal cartilage which escaped direct injury.

2. Resection of the epiphyseal cartilage from the resting cell layer of the epiphyseal cartilage to primary spongiosa resulted in formation of bony bridge at the site of injury and early closure of the epiphyseal line, with a decrease in height of the remaining epiphyseal cartilage.

The decrease of the height of epiphyseal cartilage is thought to be due to the decreased capacity of the proliferating layer.

### References

- 1) Bloom, W. and Fawcett, W.A. · A Textbook of Histology, Ed. 9, Philadelphia, Saunders, 1968.
- 2) Brashear, H.R. : Epiphyseal Fractures. J.B.J.S., 41-A:1055, 1959.
- 3) Friedenber, Z. B. : Reaction of the Epiphysis to Partial Surgical Resection. J.B.J.S., 39-A : 332, 1957.
- 4) Ford, L. T. and Key, J. A.: A Study of Experimental Trauma to the Distal Femoral Epiphysis in Rabbits. J.B.J.S., 38-A : 84, 1956.
- 5) Key, J. A. and Ford, L.T. : A Study of Experimental Trauma to the Distal Femoral Epiphysis in Rabbits. -II. J.B.J.S. 40-A:887, 1958.
- 6) Kember, N.F.: Cell Population Kinetics of Bone Growth; The First Ten Years of Autoradiographic Studies with Tritiated Thymidine. Clin. Orthop., 76 : 213, 1971.
- 7) Kember, N. F. : Cell Division in Enchondral Ossification ; A Study of Cell Proliferation in Rat Bones by the Method of Tritiated Thymidine Autoradiography. J.B.J.S., 42-B : 824, 1960.
- 8) Sakakida, K. : A Study of the Effect of Bone Peg Transplantation through Epiphyseal Plate on Longitudinal Growth of Bone. J. Jap. Orthop. Ass., 34 : 75, 1960.
- 9) Siffert, R. S. : The Effect of Staples and Longitudinal Wires on Epiphyseal Growth. J.B.J.S., 38-A : 1077, 1956.

## 和文抄録

骨端線部分切除における骨端軟骨細胞  
の増殖に関する実験的研究

京都府立医科大学整形外科学教室

榊田喜三郎, 山下 文治

骨端線損傷についての実験的研究は古来より多数みられるが、損傷により生ずる軟骨細胞の動態に関する報告は少く、また不明の点も多い。

そこで生後3週で体重50gのWistar系ラットの脛骨近位骨端軟骨の内側1/3部に1) 肥大軟骨細胞およびprimary spongiosaの切除、および2) 静止軟骨細胞から増殖細胞、肥大軟骨細胞をへてprimary spongiosaにいたるまでの切除の2種類の損傷を与え、これらの部分切除によって生ずる損傷部の変化および直接損傷を受けていない残存軟骨細胞の増殖と分化の様態を<sup>3</sup>H-thymidineのautoradiographyを用いて観察し次の結果を得た。

1. 骨端軟骨の肥大細胞層およびprimary spongiosaの切除の場合、損傷部の骨端軟骨は当初著明

にその高さを増加するが、やがて対照と同じ高さに戻り修復された。また直接損傷を受けていない外側1/3部の骨端軟骨には損傷初期に反応性の増殖活性の亢進が認められたが一過性であった。従って肥大軟骨層以下の部分損傷では可逆的变化を呈する。

2. 骨端軟骨の静止軟骨細胞層から増殖細胞層を含みprimary spongiosaにいたるまでの全層部分切除の場合、損傷部に結合織の浸入がみられ、やがて骨性架橋を生じて骨端線の早期閉鎖を示し、内反変形を来すとともに、残存する骨端軟骨にも高さの減少が認められた。すなわち静止軟骨細胞以下の骨端軟骨全層に亘る部分切除ではこれらの変化は非可逆的であるが、骨端軟骨の高さの減少は増殖細胞層の増殖活性の低下によるものと考えられる。