

## Pathology of Avascular Necrosis in the Femoral Head of Spontaneously Hypertensive Rats

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**ABSTRACT :** Naturally occurring avascular necrosis in the femoral head of SHR, which resembles Perthes' disease in man, was observed histologically. Of 96 epiphyses from 48 SHR, 34 epiphyses from 27 SHR showed a hallmark of necrosis during the period of 10 to 40 weeks after birth. The complete necrosis over the whole epiphyseal nucleus without revascularization was seen at the age of 10 and 15 weeks. Then the necrotic epiphysis was gradually repaired by the invasion of vascularized granulation tissue, and finally the healing of the necrosis was complete before the age of 40 weeks.

An investigation into the pathogenesis of the healing infarction seemed that it would be of use in elucidating the cause of Perthes' disease.

### INTRODUCTION

Spontaneously Hypertensive Rats (SHR) have been used internationally as an excellent animal model of essential hypertension in man in part because of their spontaneous development and accompanying cardiovascular lesions<sup>3) 4) 10)</sup>. Recently, we found that avascular necrosis occurred frequently in the epiphysis of the femoral head of growing SHR from 9 weeks after birth, and suggested that SHR might also be an ideal model of Perthes' disease as well<sup>6)</sup>. However, the pathological changes of avascular necrosis in SHR are not known in detail.

The purpose of this study is to confirm the natural course after the onset of avascular necrosis in SHR histologically, and then to compare the findings with the published pathological changes of Perthes' disease.

### MATERIALS AND METHODS

Forty eight male SHR (Okamoto-Aoki strain), at the age of five weeks, were purchased from Charles River Japan Co., Ltd., Kanagawa. All animals were kept in ordinary rat cages with a standard stock chow diet in the Laboratory Animal Center for Biomedical Research, Nagasaki University School of Medicine.

The bilateral femurs of 10 SHR at the age of 10 weeks, 12 SHR at 15 weeks, 16 SHR at 20 weeks and 10 SHR at 40 weeks were extracted under ether anesthesia. For histologic examination, a total of 96 femurs from 48 SHR were fixed in 10 percent formalin and the proximal femurs were embedded in paraffin after decalcification. Thin coronal sections at the insertion of the teres ligament were stained by hematoxylin-eosin and Ráliš' tetrachrome method<sup>12)</sup> which dyes osteoid deep blue.

Of 96 epiphyses, 34 epiphyses from 27 SHR showed a hallmark of avascular necrosis, that



Fig. 1. A hallmark of avascular necrosis. Viable osteocytes are not seen in lacunae of bone trabeculae. Spontaneously Hypertensive Rat at the age of 15 weeks. H&E stain,  $\times 100$ .

is, a complete disappearance of viable osteocytes in lacunae of bone trabeculae of the epiphysis (Fig. 1). Those were used to investigate the natural course of avascular necrosis in the epiphysis of the femoral head of SHR.

## RESULTS

In all of the 96 hip joints, there was no intracapsular hemorrhage or effusion, macroscopically. Furthermore no abnormal findings, such as synovial thickening, inflammatory cell infiltration or fibrosis, were recognized in the joint

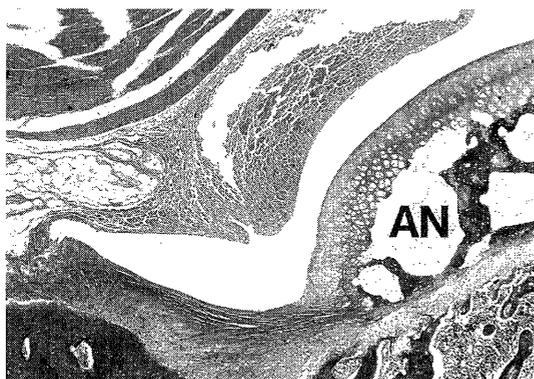


Fig. 2. There are no abnormalities in the joint capsule, though avascular necrosis of the epiphysis (AN) is seen. Spontaneously Hypertensive Rat at 10 weeks. H & E stain,  $\times 40$ .

capsule or the teres ligament by histologic examination (Fig. 2). The number of the epiphysis with a hallmark of avascular necrosis at 10, 15, 20 and 40 weeks was 3, 15, 10 and 6, respectively.

From the histologic findings in bone marrow between dead bone trabeculae in the epiphysis, various appearances of avascular necrosis were divided into four phases as follows.

Phase 1: Complete necrosis was seen over the whole epiphyseal nucleus which appeared in the lateral epiphysis and rarely near the insertion of the teres ligament (Fig. 3A and 3B). Viable marrow cells disappeared completely and there was no blood supply. Osteocytes within lacunae also fell into necrosis and became ghost cells. Those findings were observed in three epiphyses of SHR at the age of 10 weeks and four at 15 weeks. Phase 2: Compared with phase 1, a minute vascularized granulation tissue was recognized in the lateral margin of the wide-spread necrosis in five epiphyses at 15 weeks and two at 20 weeks (Fig. 4). Phase 3: Marrow space was almost replaced by reparative tissue composed mainly of fibroblastic cells and dilated blood vessels (Fig. 5). Osteoid was seen frequently in reparative tissue. Hematopoietic tissue appeared partially in several specimens. In all the specimens of this phase, appositional new bone was laid down around the dead bone trabeculae which lacunae had become empty. Five epiphyses at 15 weeks and three at 20 weeks were included in this phase. Phase 4: The remaining 12 epiphyses at 15, 20 and 40 weeks showed the deposition of normal hematopoietic tissue between the dead bone trabeculae which contained the apposition of mature viable bone (Fig. 6). The repair of the necrosis of marrow was suggested to be complete histologically, though the bone trabeculae was still thicker and irregular until the end of the observation period.

Correlation between the age of SHR and the number of the epiphysis in each phase is summarized in Table 1. It would indicate that avascular necrosis developed under the age of 20 weeks and thereafter the repair of the necrosis progressed gradually as SHR got older.

Of all 34 epiphyses, none indicated the findings of repeated infarctions which was observed in the epiphysis of Perthes' disease<sup>7) 9)</sup>.

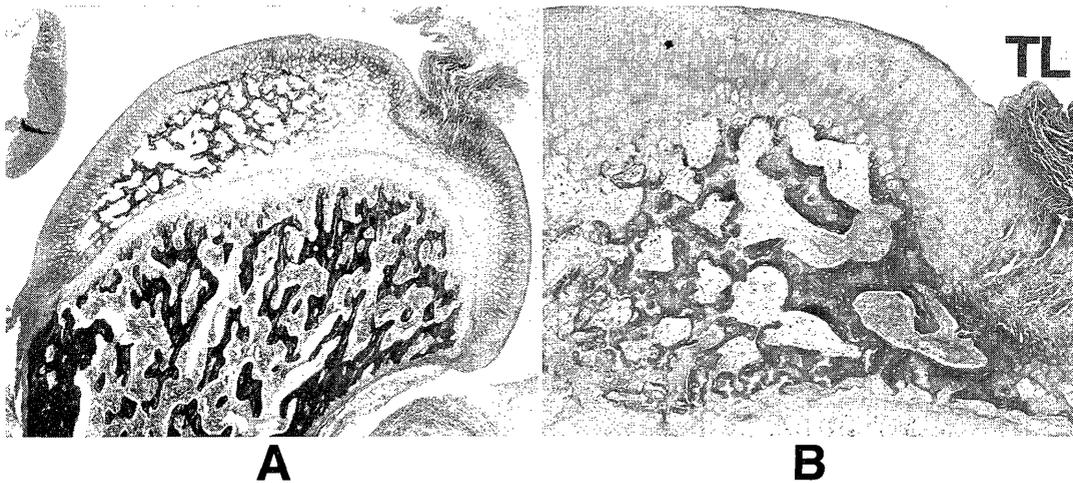


Fig. 3. The appearances of phase 1. A : Viable marrow cells over the whole epiphyseal nucleus in the lateral epiphysis disappear completely. Spontaneously Hypertensive Rat at 10 weeks. H & E stain,  $\times 13$ . B : Avascular necrosis in the epiphyseal nucleus near the insertion of the teres ligament (TL). Spontaneously Hypertensive Rat at 15 weeks. H & E stain,  $\times 40$ .



Fig. 4. Phase 2 in Spontaneously Hypertensive Rat at 15 weeks. Note the invasion of vascularized granulation tissue in the lateral side of the necrotic epiphysis. H & E stain,  $\times 60$ .

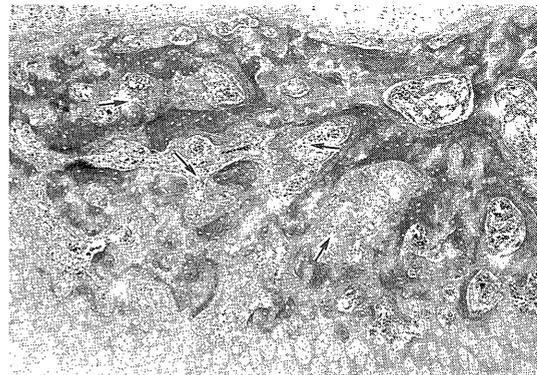


Fig. 5. Phase 3 in Spontaneously Hypertensive Rat at 15 weeks. New bone formation is abundant (arrows) and hematopoietic tissue already appears in small parts of marrow (left side). H & E stain,  $\times 60$ .

### DISCUSSION

Naturally occurring avascular necrosis, resembling Perthes' disease in man, appeared frequently in the femoral head of growing SHR<sup>6)</sup>. As to the site of the infarction, The complete necrosis over the whole epiphyseal nucleus would deny vascular occlusion in the inside of the epiphyseal nucleus, and vice versa, no abnormalities in the

joint capsule or the teres ligament suggested the inside of the femoral head. From this point of view, it seems that avascular necrosis in SHR results from the vascular occlusion in the cartilaginous tissue surrounding the epiphyseal nucleus, that is in the outside of the epiphyseal nucleus but the inside of the femoral head.

HARRISON and BURWELL<sup>5)</sup> reported that Perthes' disease was caused by the intraepiphyseal occlusion of blood flow. Furthermore, PONSETI *et al.*<sup>11)</sup>

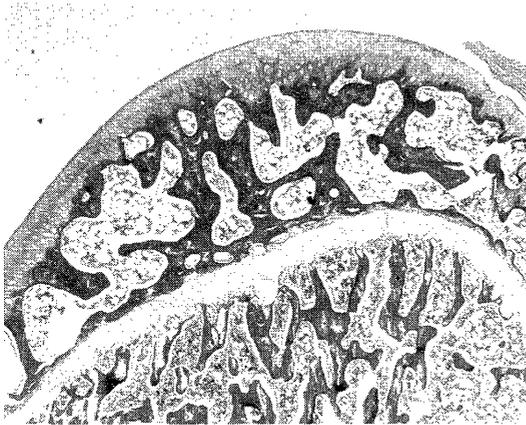


Fig. 6. Phase 4 in Spontaneously Hypertensive Rat at 40 weeks. Normal hematopoietic tissue is seen between thick bone trabeculae. H & E stain,  $\times 18$ .

suggested that the occlusion would be due to the breakdown and disorganization of the epiphyseal cartilage in Perthes' disease. The cause of avascular necrosis in SHR might be similar to that of Perthes' disease.

With regard to the process of the repair of the necrosis in the present study, the necrotic marrow was invaded by vascularized granulation tissue in the early phase. The osteoid formation became plentiful and bone marrow was replaced gradually with normal hematopoietic tissue. Simultaneously, necrotic bone trabeculae was remodelled with appositional new bone which was evident in the later phase. The frequency of the necrosis at 40 weeks was about a half of that at 15 weeks. This lesser frequency at 40 weeks is thought to be based upon the disappearance of a hallmark of the necrosis due to the completion of the repair. The repair in SHR was similar to that of avascular necrosis produced by transient vascular occlusion of the femoral head with experimental procedures in animals<sup>1) 8) 13)</sup>.

It is well known that the fundamental pathological process of Perthes' disease is also necrosis caused by infarction and subsequent revascularization of the epiphysis of the femoral head similar to avascular necrosis in SHR. However, the clinical outcome of Perthes' disease is not uniform<sup>2)</sup>. As to the chronicity and the slowness of the process of the repair, INOUE *et al.*<sup>7)</sup>

Table 1. The number of epiphysis with avascular necrosis in each phase.

Phase	Age (weeks)			
	10	15	20	40
1	3	4		
2		5	2	
3		5	3	
4		1	5	6

and McKibbin and Ráliš<sup>9)</sup> suggested that Perthes' disease was resulted from not one but more than one episode of major infarction.

Although repeated infarctions were not observed in SHR, the pathological changes of avascular necrosis in SHR were similar to those of Perthes' disease. To investigate the pathogenesis of the healing infarction in SHR seemed that it would be of use in elucidating the etiology of Perthes' disease.

#### ACKNOWLEDGEMENT

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