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Experimentally Induced Dysbaric Osteonecrosis in Sheep: a Histopathological Analysis

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ABSTRACT

Six mature female sheep were subjected to a series of 0.5, 1, 2 and 4 hour-hyperbaric exposures followed by acute decompressions over a 2.5 year period to simulate recreational and commercial diving. Sheep were euthanized 16 months after the last hyperbaric exposure. Roentgenographic examination of the sheep's long bones revealed dysbaric osteonecrosis (DON) lesions in all the six sheep. Femurs from two of the six sheep with DON were examined for pathological changes. A histopathological evaluation was performed on undecalcified sections of the femurs which showed widespread fatty marrow necrosis of the shafts. Proliferation of granulation tissue, calcification, and ossification were coexistent around the necrotic foci. Endosteal new bone formation also occurred. The significance of fatty marrow necrosis is discussed in comparison with human cases concerning the development and progression of DON.

Key words: Dysbaric osteonecrosis (DON), Sheep, Undecalcified sections, Bone compartment syndrome, Vascular embolism.

INTRODUCTION

Dysbaric osteonecrosis (DON) is a form of nontraumatic aseptic necrosis of bone prevalent in caisson workers (Davidson, 1976) and divers (Kawashima, 1976). DON can often disable them, and the disease represents an important public health problem, especially among Japanese professional divers (Kawashima, 1976). DON has now been experimentally induced in sheep (Lehner et al., 1990). With an experimental model of DON, we can study the development of this condition and recommend diving procedures designed to avoid DON.

We previously induced DON in the long bones of sheep with repetitive 24-hour hyperbaric exposures (Lehner et al., 1990). For this series of experiments, we assessed whether DON

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could be induced with hyperbaric exposures of shorter durations.

HYPERBARIC EXPOSURE AND BONE PREPARATION

Experimental procedures that induced DON in sheep were conducted at the University of Wisconsin-Madison. Six, 2-year-old, crossbred female sheep (85–110 Kg) underwent a series of 29–30 hyperbaric exposures of 0.5 to 4 hours durations over a 2.5 year period. Decompression to surface pressure was at a rate equivalent to 11 meters of sea water pressure (MSW)/min. Persistent limb lifting, a sign of limb bends, was often observed the sheep after their hyperbaric exposures. Signs of limb lifting and non-weight bearing in sometimes lasted up to 8 days. Signs of mild chokes in the sheep were also commonly observed. The maximum pressures of the hyperbaric exposures reached 2.7 atmospheres absolute (ATA), equivalent to 17 MSW, in the 4 hour exposures and 4.3 ATA, 33 MSW, in the brief 0.5 hour exposures (Table 1).

Table 1. Experimental procedures

Animals: Sheep (#227: 92 kg, #249: 108 kg) 6 Years Old at Autopsy
Female, Crossbred

Hyperbaric Exposures:

Duration, h	Maximum Pressures, ATA
0.5	4.31
1	3.80
2	2.38
4	2.68

Compression Rate at 0.7 ATA/min
Decompression Rate at 1.1 ATA/min
Sheeep #227 was subjected to 29 hyperbaric exposures in 2.5 years and euthanized 16 months after the last exposure.
Sheep #249 was subjected to 30 hyperbaric exposures in 2.5 years and euthanized 16 months after the last exposure.

The sheep were euthanized 16 months after the last hyperbaric exposure and were autopsied. At the time of autopsy, perfusion fixation was performed with 10% neutral buffered formalin and contrast media (Barium sulphate: Novapaque and Micropaque) through the heart for systemic fixation and later roentgenographic examination.

The femurs were disarticulated and removed from the sheep cadavers. The heads and shafts of the femurs of two (#227 and #249) of the six sheep were sampled as indicated in Fig. 1. Two transverse sections were cut from the proximal and distal shafts, each approximately 6–7 cm from the proximal and distal ends. The femoral ends were cut into longitudinal sections. Femoral head sections included articular cartilage, subchondral cortical and cancellous bone and marrow. Femoral shaft sections included cortical bone and marrow.

For the contact microradiographic and histopathological examinations, undecalcified sections of bone were prepared at Kagoshima University. Bone blocks (1.5–2.0 mm thick)

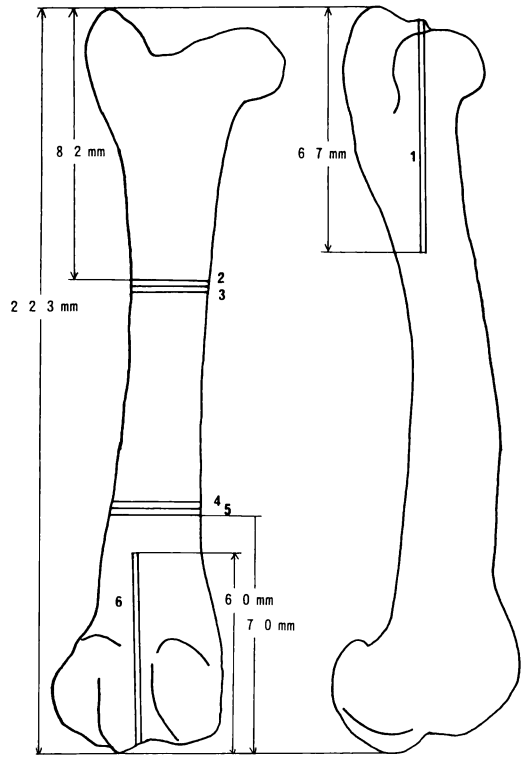


Fig. 1. Schematic illustration of bone sampling from a femur of sheep.

were embedded in polyethylene resin and then each was sectioned using an Exact's cutting machine (BS 3,000) and ground to a 50 micrometer thickness using an Exact's precision grinder (MG 4,000). Bone sections were analyzed by contact microradiography using a Softex' soft X-ray photographic instrument (CSM-2), and the sections were then stained with methylene-blue and basic fuchsin for histopathological analysis.

FINDINGS OF DON IN SHEEP

Radiological Changes in the Femurs: Prominent medullary opacities were evident in the distal femurs of all the six experimental sheep. In particular the right femur of Sheep #227 and both femurs of Sheep #249 showed prominent changes. Medullary opacities were considered to be evidence of DON development in the long bones of the sheep.

Macroscopic Findings: Sectioned bone specimens from the sheep femurs contained fatty marrow necrosis which is a common finding in DON. Fatty marrow necrosis appeared as opaque, shiny, yellow-white inclusions in the marrow cavity. The distributions of necrotic foci of sheep #227 and #249 are schematically illustrated in Figs. 2 and 3. Red marrow was present in some areas of the proximal femoral head. Apparent necrotic foci occurred in the right femur of Sheep #227 (Figs. 4 and 5) and in both femurs of Sheep #249.

Histological Findings in Sectioned Bone Specimens: In spite of variations in the size of the

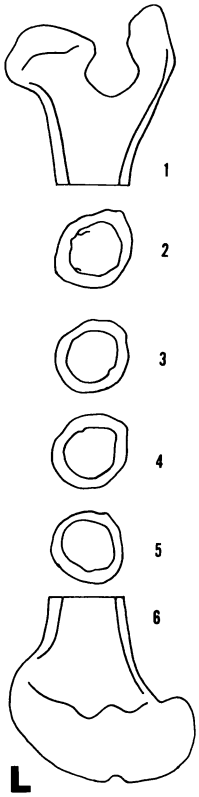
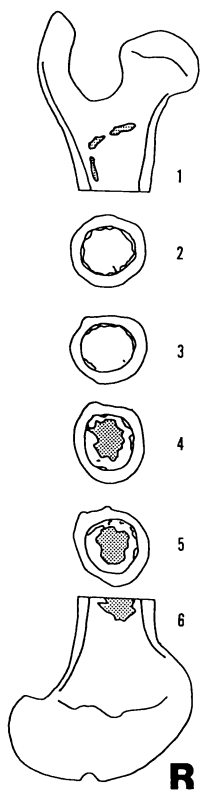
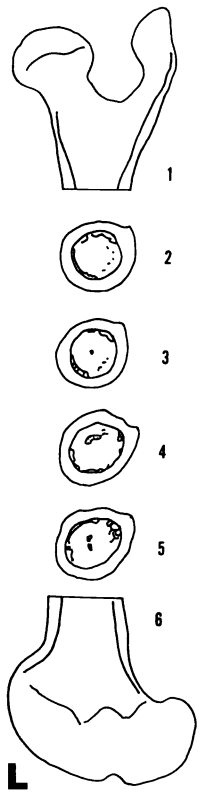


Fig. 2. Schematic drawing of the distribution of necrotic foci in both the right (R) and left (L) femurs of Seep #227. Dotted areas indicate the necrotic foci. Note the almost normal left femur except for a slight, irregular endosteal thickening of the cortical bone.





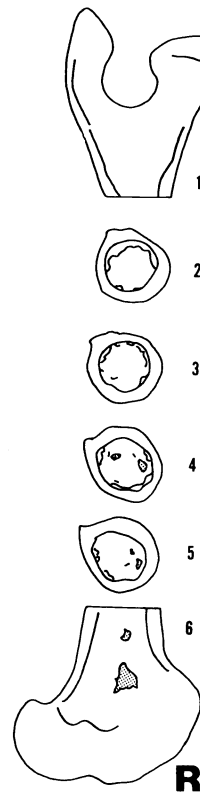


Fig. 3. Distribution of necrotic foci (dotted areas) in both the right (R) and left (L) femurs of Sheep #249.

necrotic foci, the histopathological findings of marrow necrosis were similar.

Necrotic fatty tissue showed partial liquefaction (Fig. 4b). Individual necrotic fat cells were interspersed with thin layers of basophilic material, suggesting pericellular calcification. The foci of the necrotic fatty tissue were globally enveloped by fibro-osseous tissue. Foamy mononuclear or multinucleated histiocytic cells, which seemed to contain fat droplets in their cytoplasms, were aggregated in the fibrous layer in which collagenosis had considerably advanced. Ossification took place intermittently around the necrotic foci. The most predominant calcification took place around necrotic fat cells in peripheral areas of the necrotic foci, and these fat cells appeared as "cores" of ossification which showed definitive lamellar structure (Figs. 5, and 6).

Endosteal new bone formation was widespread in the shafts of the four femurs. Endosteal thickening by the new bone formation was more extensive in the femur which sustained more extensive necrotic changes in the bone marrow tissue (Fig. 4b). Vascular channels in the marrow were obliterated due to collapse or thrombosis in the necrotic foci, but in viable areas they appeared patent and normal.

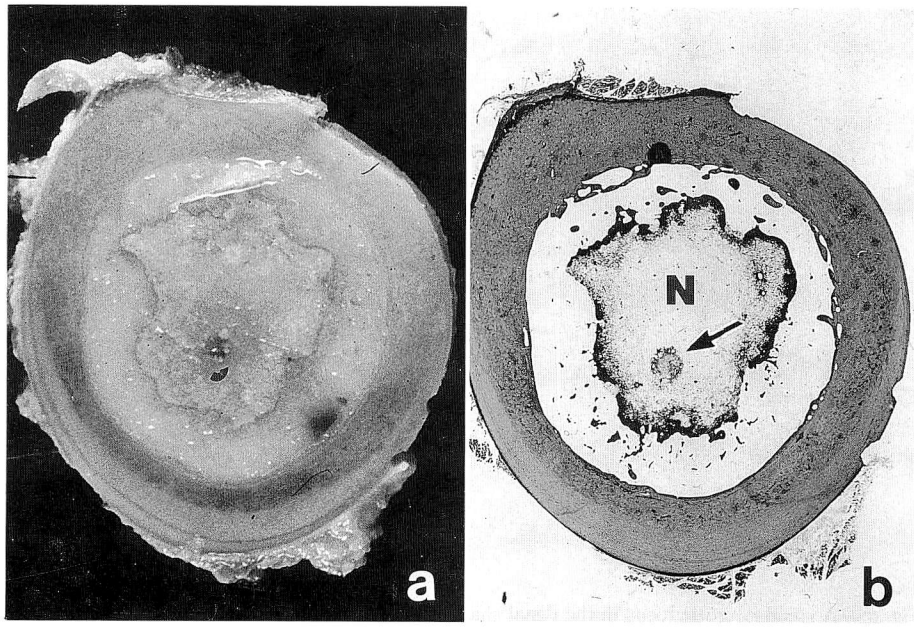


Fig. 4. (a: left): Transverse section of the distal shaft from the right femur of Sheep #227.
(b: right): Undecalcified histological preparation stained with methylene-blue and basic fuchsin (MBF) of the same material as in Fig. 4a. Focal liquefaction of fat (arrow) occurred in the necrotic focus (N). Endosteal new bone formation was present in the cortical bone. ×2.4.

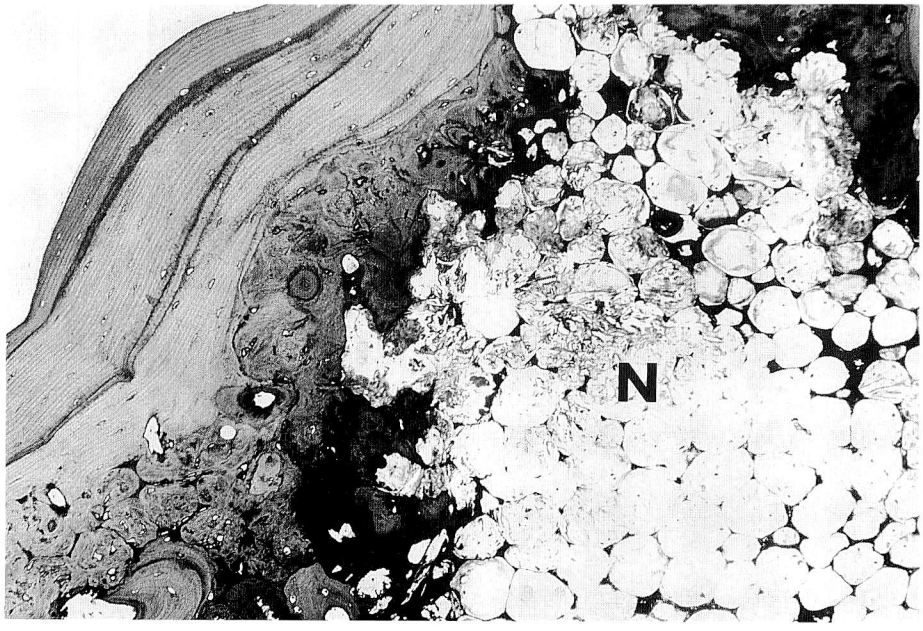


Fig. 5. Higher magnification of the necrotic focus (N) which appears in Fig. 4b. Necrotic fatty marrow is surrounded by a fibro-osseous layer. Individual necrotic fat cells are enveloped by basophilic material (calcified tissue). Methylene blue and basic fuchsin (MBF), ×750.

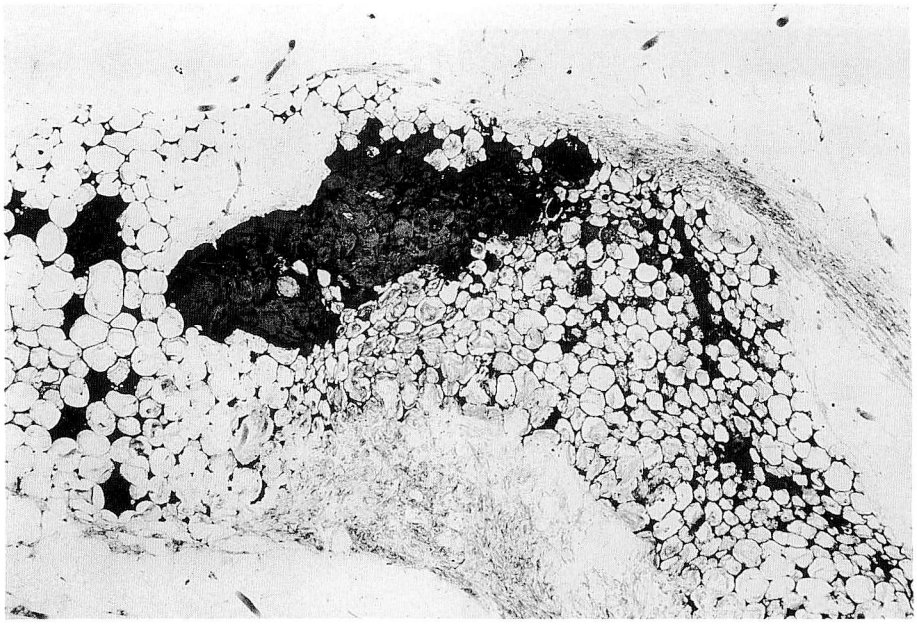


Fig. 6. A small necrotic focus in the distal shaft of the left femur of Sheep #249. MBF, ×375.

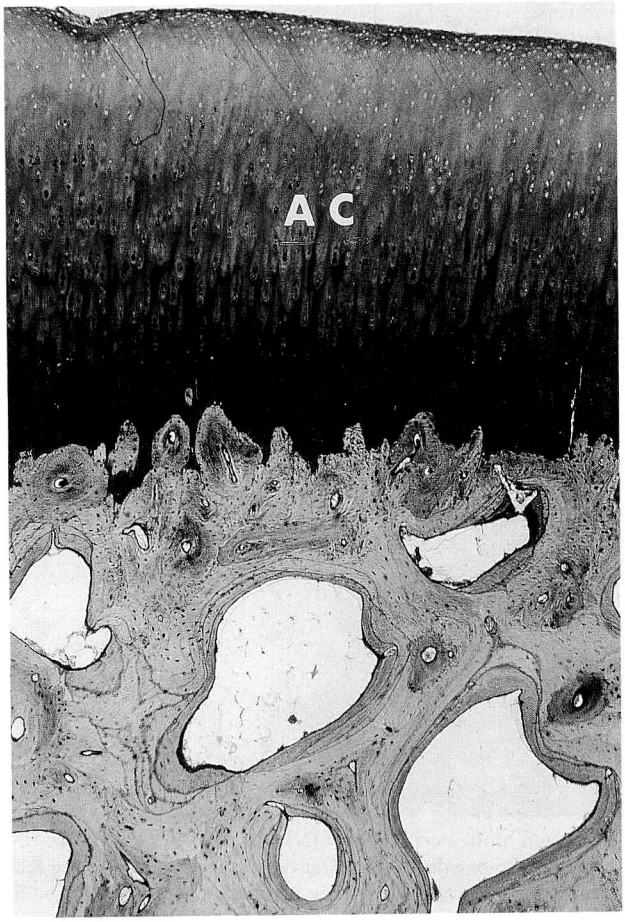


Fig. 7. Normal appearance of articular cartilage (AC) and juxtaarticular bone from the proximal head of the right femur of Sheep #227. MBF, ×300.

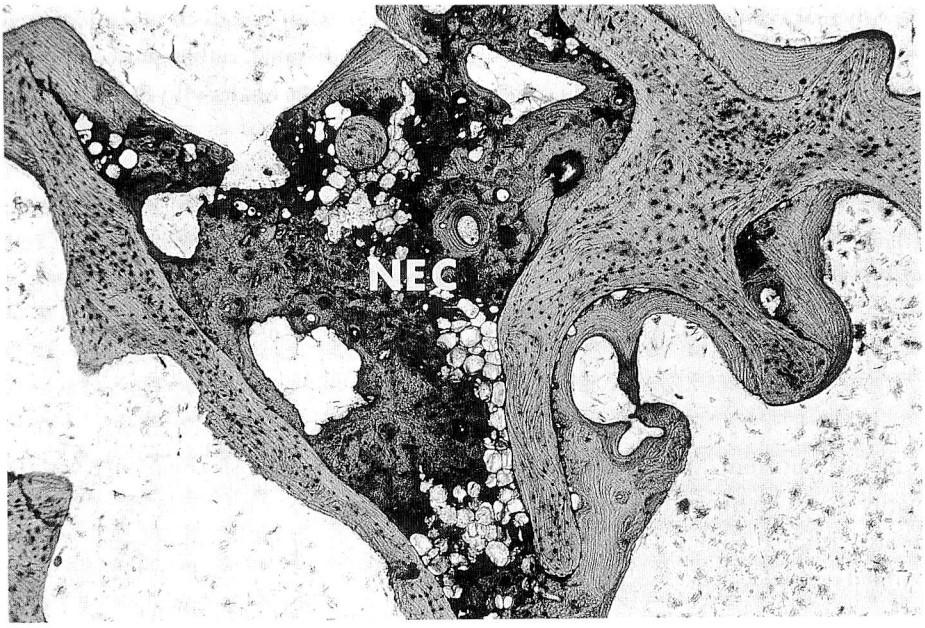


Fig. 8. A small necrotic focus (NEC) in the intertrabecular marrow space of the proximal metaphysis in the right femur of Sheep #227. The bone trabeculae appear viable, and appositional new bone formation is conspicuous. MBF, ×300.

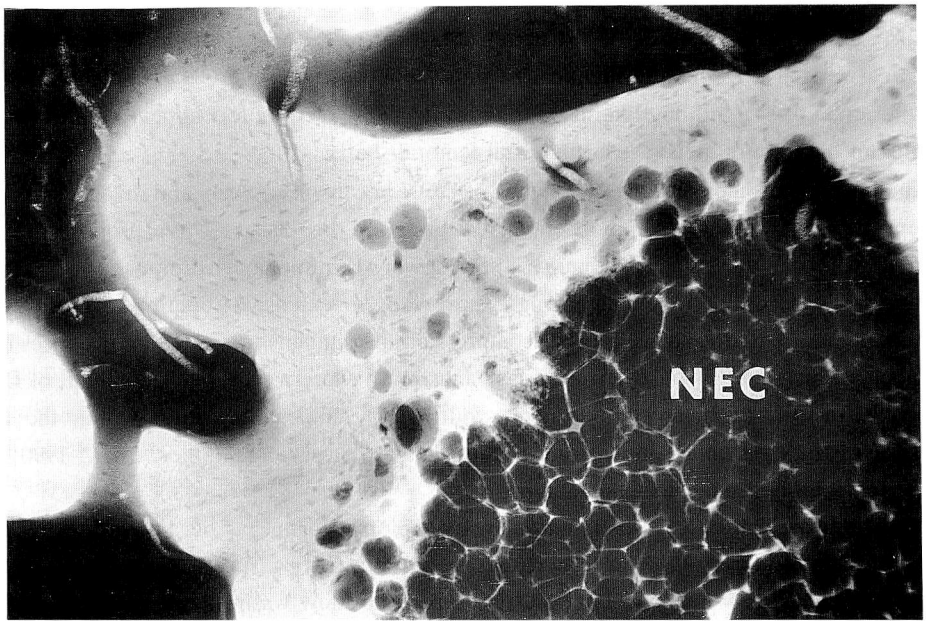


Fig. 9. A contact microradiograph of the same necrotic focus in Fig. 5. The necrotic focus (NEC) is surrounded by a radiopaque area (calcified or ossified), and individual necrotic fat cells are also enveloped by radiopaque material. The small blood vessels containing contrast media are radiopaque. ×750.

The articular cartilage appeared intact (Fig. 7). There was no marked necrotic change in the subchondral bone, in which bone trabeculae appeared viable, although necrotic foci in the right femurs of Sheep #227 and #249 extended from the shafts into the proximal and distal metaphyses (Fig. 8).

A contact microradiographic survey of the undecalcified thin sections revealed that the necrotic foci were enveloped by an irregularly-shaped membranous and radiopaque layer. This layer was composed of calcified or ossified material. Moreover, individual fat cells in the necrotic foci were also surrounded by radiopaque, calcified membranes (Fig. 9).

DISCUSSION

In the present study, we found evidence of a fatty marrow necrosis compatible with DON primarily in the shafts of femurs from both sheep subjected to relatively short hyperbaric exposures from 0.5 to 4 hours durations. As a result of histopathological evaluation performed on undecalcified sections of the femurs, fatty marrow necrosis was associated with fibrosis, histiocytic aggregation, calcification, and ossification around the necrotic foci. Endosteal new bone formation also occurred. In DON, both tissue calcification and ossification appear to be closely linked to fat cell necrosis. We previously reported this change as the ossification of fat cell ghosts in long bone lesions of DON (Lehner et al., 1990).

The histopathological features of DON in sheep used in the present study appeared to be essentially the same as those in sheep with repetitive 24-hour hyperbaric exposures (Lehner et al., 1990). The changes of the latter, however, were much more aggressive. The features were also essentially identical to those found in diver's DON (Kawashima, 1976, Kitano et al., 1984), although in divers the prevalence of necrotic foci appeared to be almost as high in the femoral heads as in the distal femoral shafts.

The findings in sheep and the prevalence of DON in caisson workers and divers exposed to compressed air suggest a common etiological basis for this disease. We propose that the development of DON typically requires the following conditions: 1) prolonged exposure to compressed air, either in a single long exposure or in extensive repetitive exposures, 2) abundant fatty marrow, and 3) rapid decompression, presumably at a rate that produces large numbers of nitrogen gas bubbles in the fatty marrow. Moreover, the development of DON probably involves a bone compartment syndrome. Bubbles may form within the fatty marrow of the long bones and elevate intramedullary pressure (Lanphier, et al., 1990) and cause ischemia that leads to tissue necrosis.

Fatty marrow can become a significant reservoir of nitrogen gas because nitrogen gas is about five times more soluble in fatty tissues than in non-fatty tissues. Since the blood flow rates in fatty marrow are low, fatty marrow represents slow wash-in and wash-out rates of nitrogen gas. The marrow cavity surrounded by rigid bone is anatomically a semi-closed compartment. In another study, we observed elevated marrow pressures in sheep long bones of symptomatic limbs, with limb lifting after 24-hour hyperbaric exposures (Lanphier et al., 1990). When bubble formation from the dissolved nitrogen gas into fat cells occurs

rapidly within a compartment, as in the marrow cavity of long bones, a bone compartment syndrome may develop as tissue pressure increases. In that case, blood flow and tissue nitrogen gas wash-out would virtually cease.

Another pathogenic mechanism which may play an important role in development is intravascular obstruction. Intravascular nitrogen gas bubbles with bubble embolization can promote blood coagulation (Kawashima et al., 1977, Kitano et al., 1978). Nitrogen gas bubbles produced by decompression may cause fat cells to rupture and release fat globules into the marrow sinusoids and embolize the marrow vessels (Kitano & Hayashi, 1981, Jones, 1985). The fat embolization process also activates blood coagulation. Circulatory disruption due to intravascular blood coagulation, in association with nitrogen gas bubble emboli and fat emboli, would contribute to the development and progression of DON. However, we found no definitive evidence to support this acute pathogenic mechanism in the sheep femurs examined. This is likely due to the fact that autopsies were performed late, 16 months after the last hyperbaric exposures of the sheep.

In summary, we believe that DON results from a form of bone compartment syndrome initiated by bubble formation in the fatty marrow which causes circulatory disruption due to increased tissue pressure. Additionally, blood coagulation associated with fat and bubble embolization may also play an important role in DON pathogenesis. Extensive repetitive hyperbaric exposures and air dives with rapid decompression carry the risk of DON, even under hyperbaric exposures of shorter durations.

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