EXPERIMENTALLY INDUCED OSTEONECROSIS IN MINIATURE SWINE

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Ohta *et al.* (1965) have reported on the incidence of aseptic bone necrosis in professional divers at Ariake Bay in Japan. The Bay's seabed, from which shellfish are harvested from October until March, lies at a depth of from 10 to 30 meters.

There are approximately 400 professional divers living in Takesaki, a small village on Ariake Bay. At 16 to 17 years of age, the boys begin training as divers. By the age of 18 to 20, they are working as professional divers and continue as such until they are about 40 to 45 years old. Each diver spends two 4-hour shifts on the sea bottom, interrupted by a 30- to 60-minute rest period during the noon meal. During his underwater work shift, the diver never surfaces.

Of the 301 Ariake Bay divers examined, 50.5% showed some form of bone lesions radiographically. Men between the ages of 20 and 29 years had a 35% incidence of demonstrable bone lesions; those between 30 and 39 years, a 68% incidence; those between 40 and 49 years, a 78% incidence; and those 50 and over, a 71% incidence.

To study further the observed incidence of osteonecrosis in relation to compression–decompression stress, we exposed miniature swine to standard profiles of 60 FSW for 6 hours. The profile was repeated daily with a decompression rate of 30 feet per minute (fpm) for 35 to 50 "dives." Initially the animals tolerated the exposures with no external signs of decompression sickness. After 25 to 30 dives, however, the animals began to manifest signs of bends after each one, although they did not always require recompression. To protect the animals' lives, the decompression rate was reduced to 1.33 fpm at this point. Even with this prolonged decompression period, dysbarism did occur in some animals.

Hemodynamic and blood-chemistry studies included platelet count, complete blood count, hematocrit, hemoglobin, reticulocyte count, clotting times, prothrombin time, partial thromboplastin time, quantitative fibrinogen, and platelet adhesiveness. Serum studies included uric acid, alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT), lactic acid dehydrogenase (LDH), creatine phosphokinase (CPK), calcium, and phosphorus. Lipid chemistries included phospholipids, triglycerides, cholesterol, cholesterol esters, and total lipids.

Red and white cell counts, hematocrit, hemoglobin, and reticulocyte counts showed no significant changes following these exposure profiles. Control and postdive platelet counts for four animals are shown in Fig 1. All four showed a

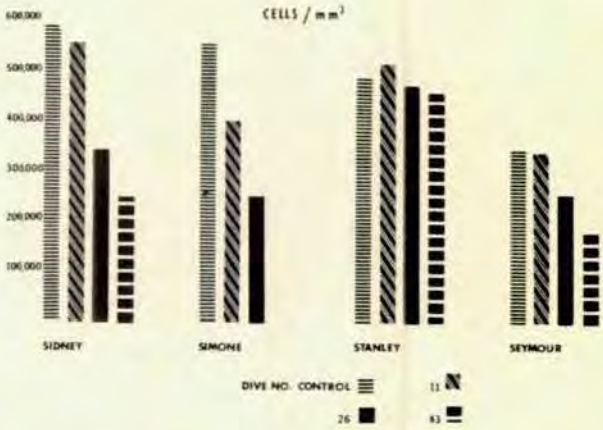

FIG. 1. Platelet changes in a group of 4 miniature pigs simultaneously exposed to 60-FSW/6-hr profiles, followed by 2-min decompression. Samples drawn after Dives 11, 26, and 43 are compared with baseline values.

decrease from their predive control sample following the chamber exposures. An additional animal — which was serially measured during a 60-FSW/6-hr exposure profile with a 30-fpm decompression rate — had a control platelet count of 430,000/mm³. One hour postdive the count had dropped to 320,000/mm³. The 24-hr count was 200,000/mm³, and the 48-hr count, 120,000/mm³. The control platelet count of another pig was 400,000/mm³ after he had "sur-

faced" from 60 FSW/6 hr, but the count had decreased to 74,000/mm³ one hour later.

Clotting, prothrombin, and partial thromboplastin times showed no significant changes. In the case of one typical pig, quantitative fibrinogen levels increased markedly after the 15th dive, following which the decompression rate was changed from 1.33 fpm to 30 fpm (Fig. 2). Platelet adhesiveness increased as much as 38% following these same exposures. Note that the exposure profiles underwent several changes throughout the period of this animal's diving history.

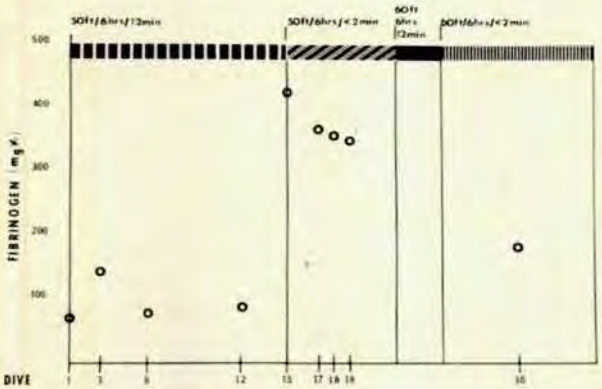

FIG. 2. Fibrinogen responses of TRF-strain miniature pig (Sarah) after her first fast decompression. From a control value of 73 mg %, fibrinogen level was elevated to 427 mg % in sample drawn 1 hr after surfacing in 2 min from 50 FSW/6 hr.

Uric acid, alkaline phosphatase, SGOT, LDH, calcium, and phosphorus values showed no significant change following these exposures. Creatine phosphokinase measurements, which were routinely elevated as the number of exposures increased, are shown in Fig. 3. Phospholipids, triglycerides, cholesterol, cholesterol esters, and total lipid values revealed no significant change.

As the purpose of these experiments was to produce aseptic bone necrosis, they were conducted on the tacit assumption that bends is a decompression phenomenon, probably caused by an inadequate decompression procedure. Although it was intended to induce dysbarism in the animals, it was not intended that the exposures be fatal. Nonetheless several animals were lost to acute decompression sickness after 35 to 40 asymptomatic exposures.

There now follows a brief radiographic history

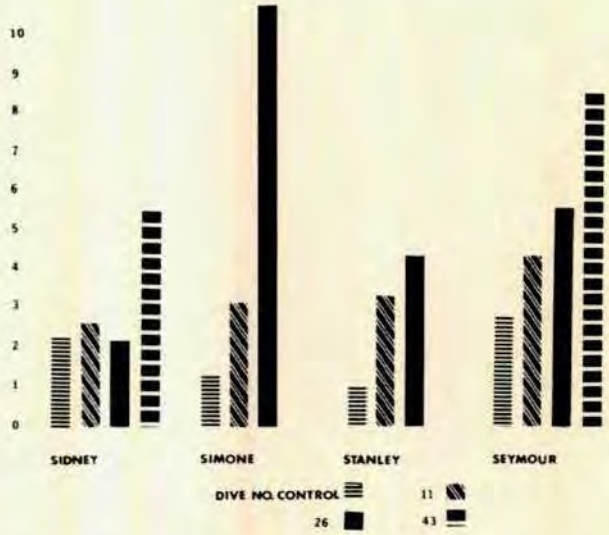

FIG. 3. Creatine phosphokinase levels in a group of 4 miniature pigs simultaneously exposed to 60 FSW/6-hr profiles with 2-min decompression. Changes after Dives 11, 26, and 43 are compared with baseline values.

and histopathologic study of several typical animals.

1. Sarah, a 3-year-old TRF-strain miniature pig, underwent a series of 35 exposures. The control radiographs of her femoral and humeral heads and shafts were reported unremarkable. By 22 April 1971 (Fig. 4*a*) — 2½ months after her initial hyperbaric exposure — a slight irregularly marginated radiolucency, measuring 3 cm, appeared in the subtrochanteric area of her left femur. Sclerotic areas of bony trabeculation developed in the metaphyseal portion of the femurs bilaterally by 1 July 1971. Further progression in the densities, which now had the definite appearance of bone infarcts, was reported on 14 September 1971 (Fig. 4*b*). Specimens removed from one femur at biopsy on 25 September 1971 contained nonviable cortical and cancellous bone (Fig. 5) and fat necrosis (Fig. 6). (The radiograph shown in Fig. 7 demonstrates the biopsy site.) A diagnosis of aseptic bone necrosis was made.

By November 1971 Sarah began to limp on her right front leg. A radiograph taken that month revealed an irregular sclerotic deformity in the proximal shafts of the humeri, predominantly on the right. Further progression in the femoral bony infarcts was also noted at that time. Fol-

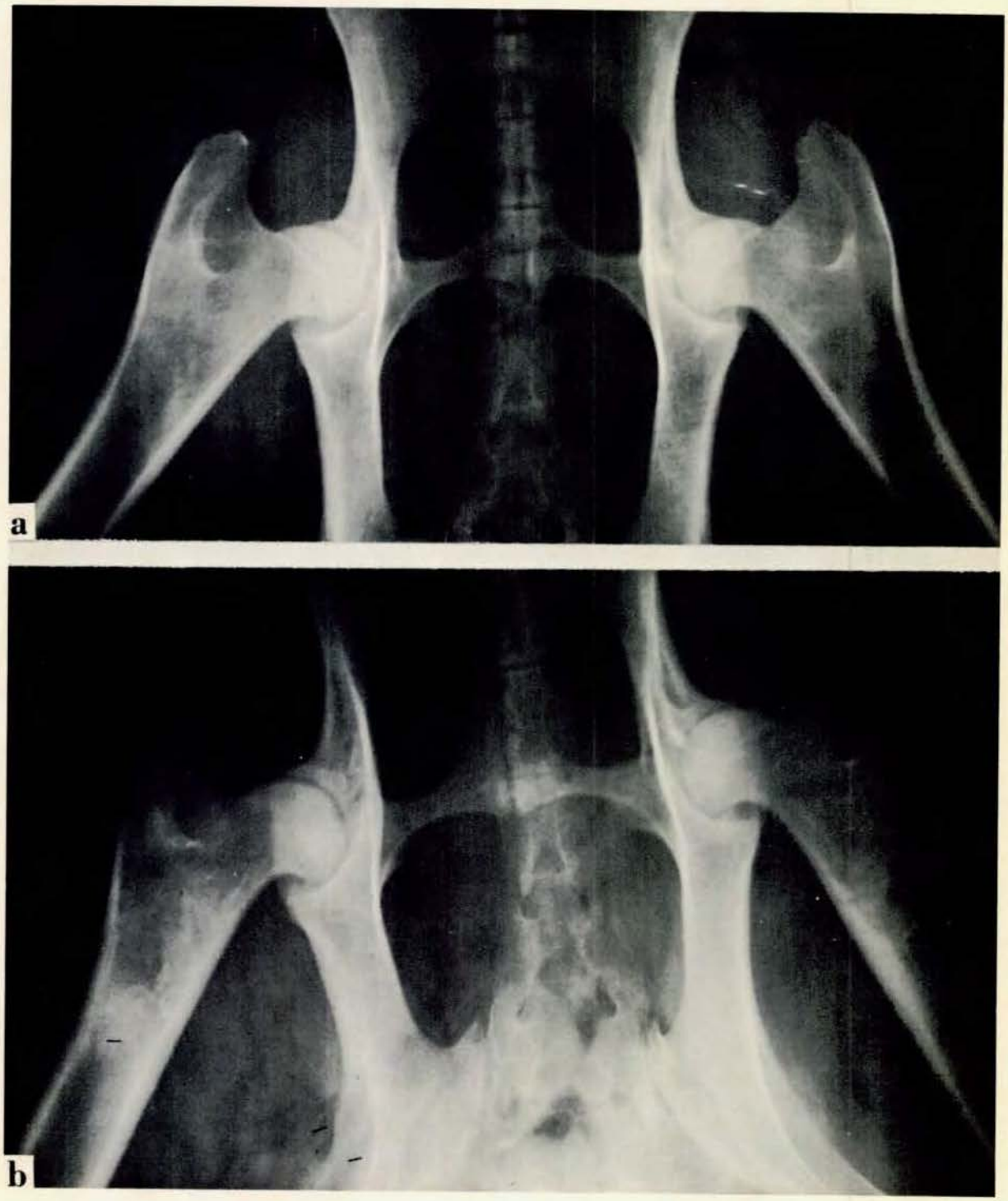

FIG. 4. Femurs of miniature pig Sarah. (*a*) Irregularly marginated lucency (arrow) appeared in subtrochanteric area of L femur 2½ months after animal was first exposed to compression-decompression stress; (*b*) 5 months later, lesion in L femur had progressed and a second lesion appeared in R femur.

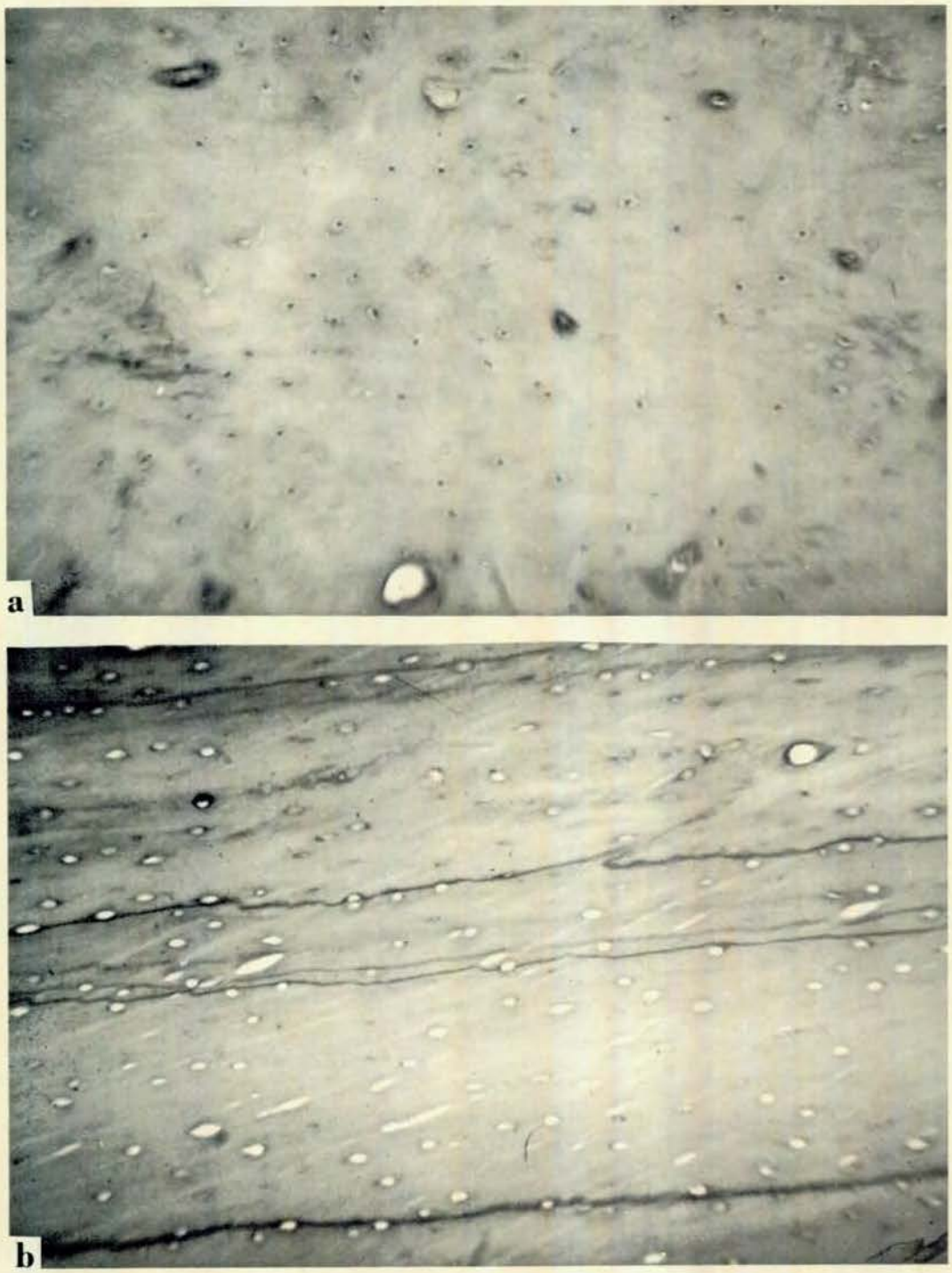

FIG. 5. Biopsied specimens from femur of miniature pig Sarah. (*a*) Small black dots are osteocytes within lacunae of bony matrix in normal specimen. (*b*) White spaces are lacunae devoid of osteocytes, a picture consistent with appearance of aseptic bone necrosis. Larger white spaces are Haversian canal systems whose vessels have degenerated.

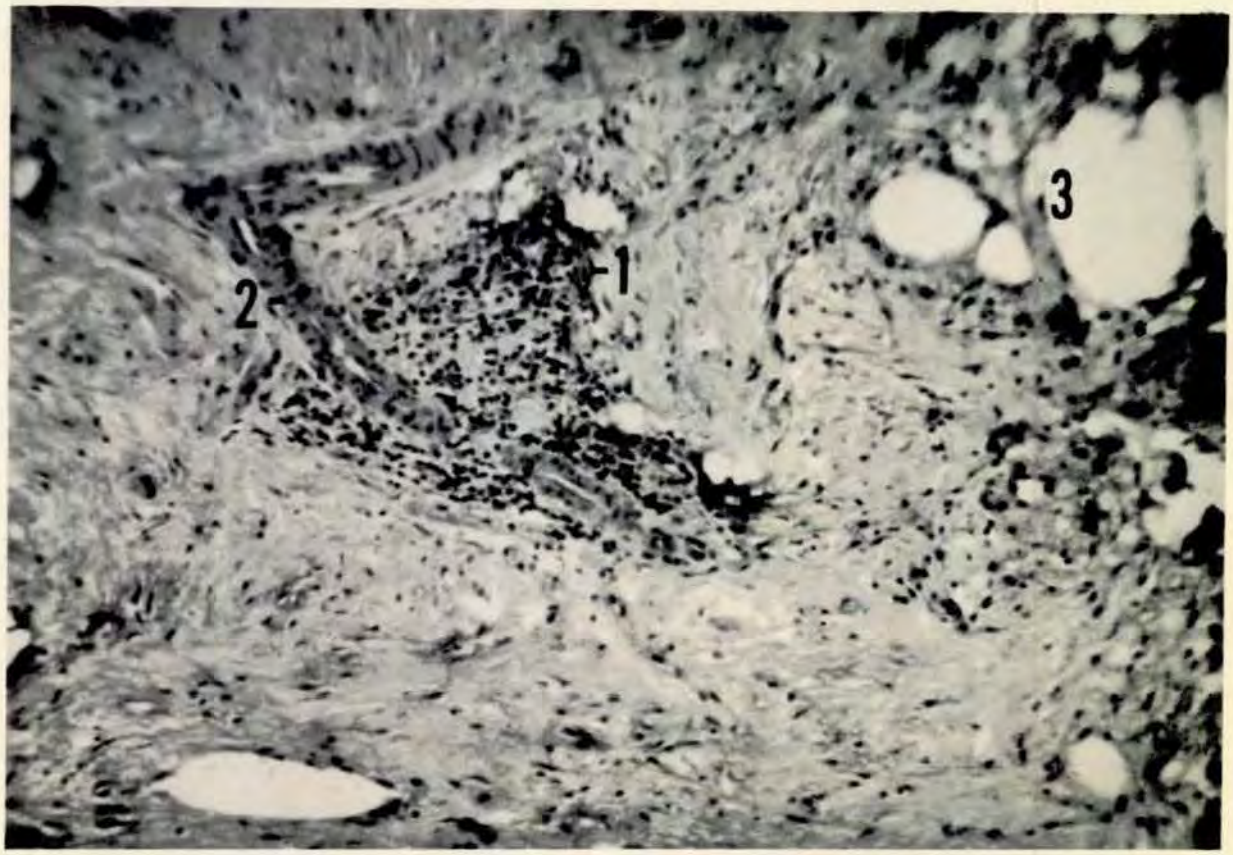

FIG. 6. Fat-marrow necrosis in biopsied specimen of pig femur (Sarah). Lymphocytic infiltration is seen (1) in early stage of fibrosis with compression of a small vessel and (2) in dense tissue. In area marked (3) are seen a few normal fatty marrow cells.

low-up films in January 1972 showed progression in trabecular distortion and periosteal reaction in the femoral midshafts with some remottling and healing occurring around the biopsy site. Humeral radiographs showed increased cortical thickening as well as endosteal and periosteal proliferation (Fig. 8).

2. Sidney, a 2-year-old Hormel-strain miniature pig, underwent 80 dives. After 33 exposures over a period of two months, a bilateral radiolucency was discovered in one femoral metaphysis. After 44 dives, radiographs showed definite new, mixed, sclerotic, and radiolucent patterns in the metaphyseal areas of both femurs. There were, as well, new findings of endosteal proliferation with calcification in one humerus (Fig. 9) — the probable result, in our opinion, of the animal's continuous exposure to an inadequate decompression profile.

3. Bentley, a 3-year-old Hanford-strain miniature pig, developed a severe case of decompression sickness on the 22nd dive. He remained paraplegic after four days of treatment and was then sacrificed. Histologic studies revealed bubbles, plasma pooling, red-cell aggregation, and platelet clumps indicative of possible disseminated intravascular coagulation.

4. Simone, a 2-year-old Hormel-strain miniature pig, died of acute decompression sickness within an hour after surfacing from her 48th dive. Areas of advanced bone necrosis, not seen radiographically, were found in a histologic study of her tissues; there was intravascular vacuolation throughout her whole system. Since these

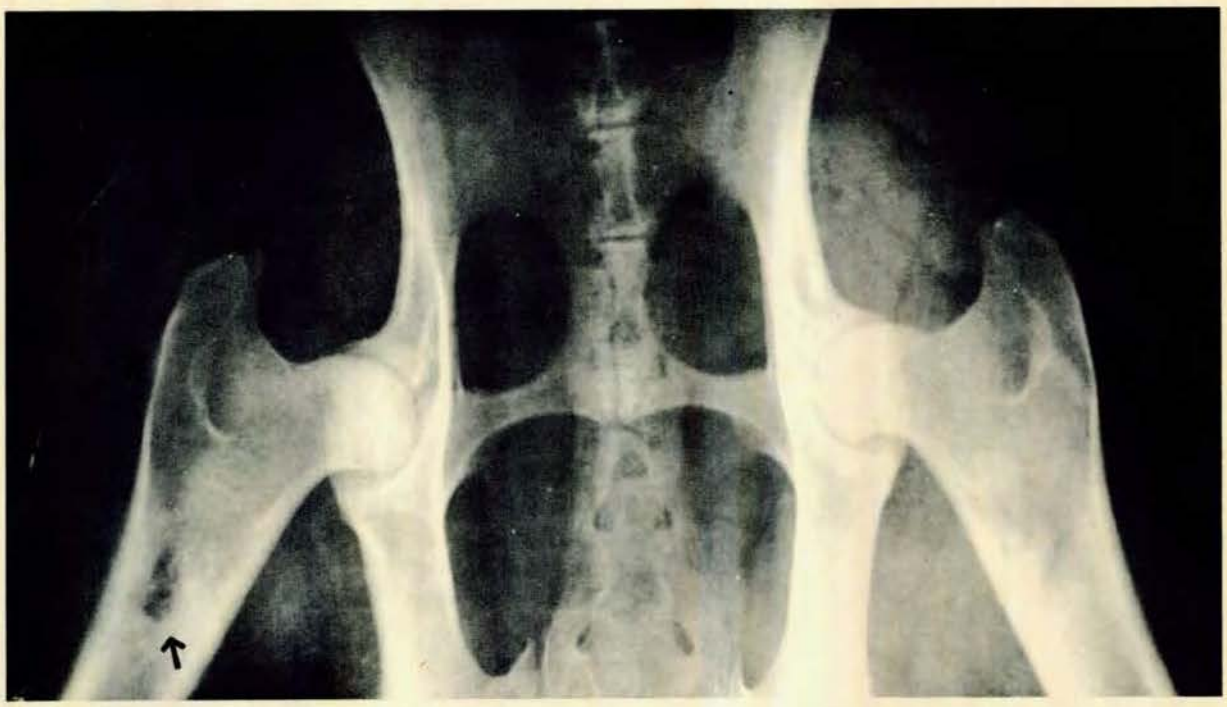

FIG. 7. Further progression in sclerotic bony infarcts in femurs of miniature pig Sarah; radiograph taken 7 months after that in Fig. 4*a*. Site of biopsy, performed 2 months earlier, is marked by arrow.

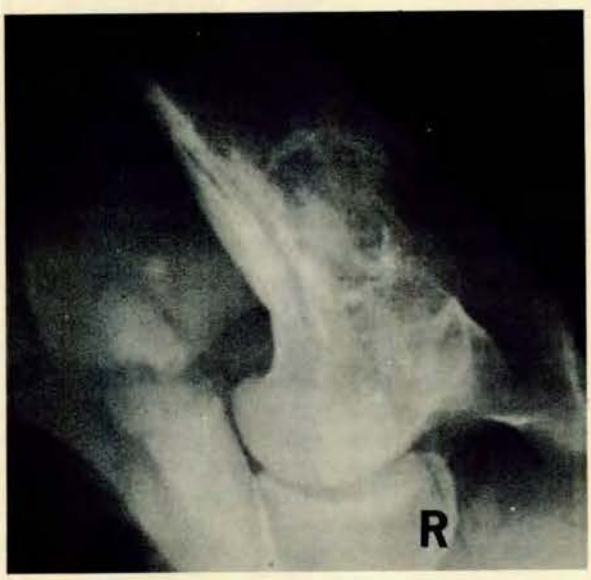

FIG. 8. Irregular sclerotic deformities in proximal humerus of miniature pig Sarah, consistent with aseptic bone necrosis developing bilaterally in femurs. X-ray was taken 9 months after that in Fig. 4*a* and almost 3 months after that in Fig. 7.

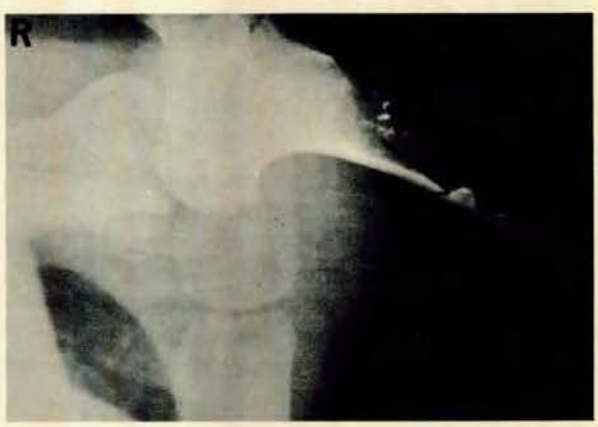

FIG. 9. Humerus of Hormel-strain miniature pig (Sidney) after 44 "dives," revealing endosteal proliferation and scattered calcifications.

spaces did not take up a fat stain, it is possible that the vacuoles were bubbles. New appositional bone containing osteocytes being laid down around a section of dead bone is seen in Fig. 10.

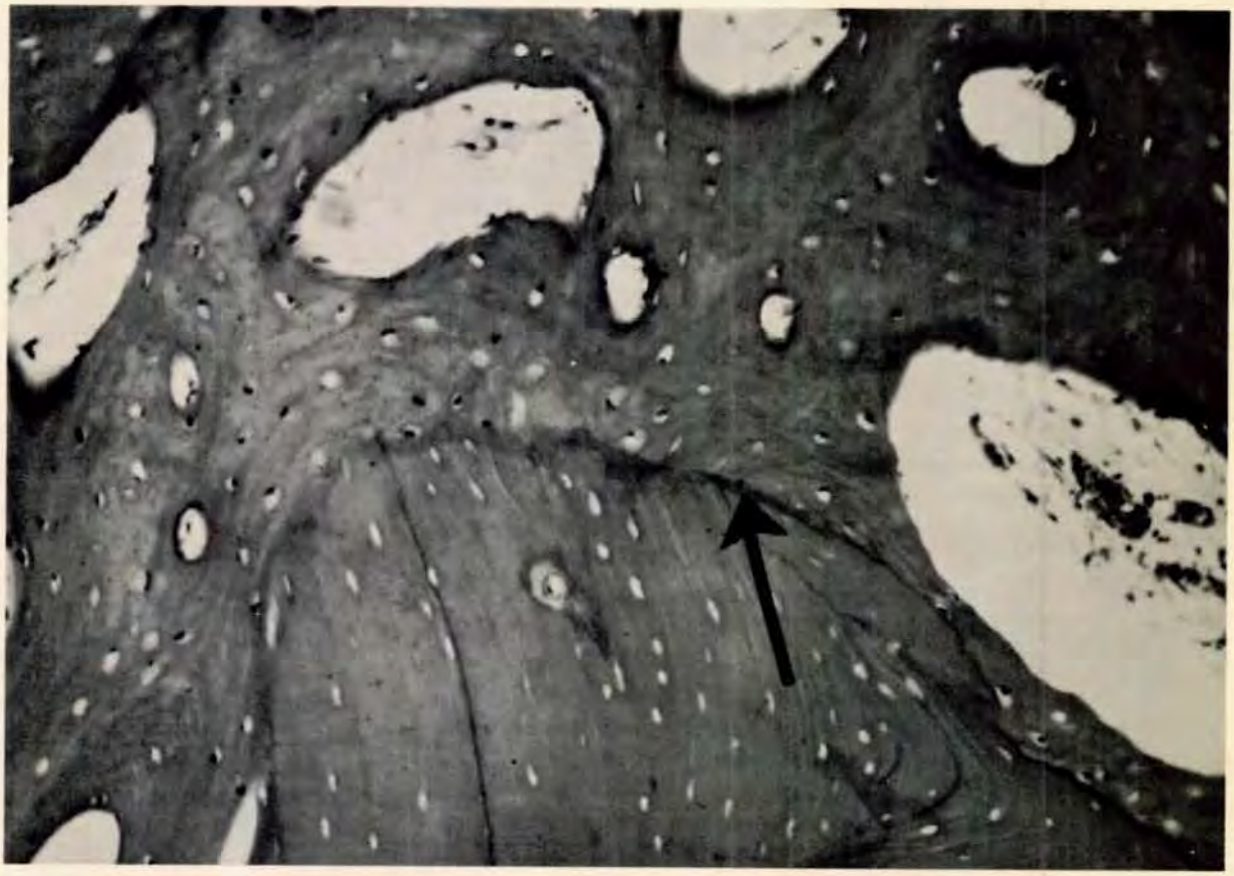

FIG. 10. New appositional bone formation in miniature pig (Simone) occurring on nonviable bone structure with definite line of demarcation (arrow). This animal had no radiographic evidence of necrosis.

As illustrated in this study, the most profound consequence of exposure to inadequate decompression profiles is aseptic bone necrosis. The study also produced initial evidence of severe hematologic changes, which occur simultaneously with bubble formation or as a result of it. All links in the chain stretching from initial hyperbaric insult to osteonecrosis have, of course, not been identified. Nevertheless, the connection between bubble formation — with alterations in the clotting mechanism — and the resulting infarcts in the small vasculature supplying nutrition to bone cells is generally recognized.

Ongoing studies are being designed to investigate and isolate more specifically the observed hemostatic and cellular alterations that occur when decompression rates are inadequate. Only then can the gap be filled between bends and bubbles on the one hand and bone necrosis on the other.

REFERENCE

Ohta, Y., Matsunga, H., and Shigeto, O. [1965]. Divers in Japan and their bone lesions. Unpublished data.