EXPERIMENTALLY PRODUCED OSTEONECROSIS AS A RESULT OF FAT EMBOLISM

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Circumstantial evidence is accumulating to indict continuous or intermittent fat embolism of bone as a possible initiating event in the evolution and pathogenesis of avascular necrosis. On the basis of Virchow's belief that investigation of a disease condition should be concentrated on the dynamic process rather than on the end result, experimental studies were undertaken with the hypothesis that fat embolism of bone may be an initiating event in producing osteonecrosis (Jones, 1971).

This hypothesis has been partially confirmed by Jaffe and co-workers (1972), who administered corticosteroids systemically to rabbits over a period of 9 weeks. They found significant intraosseous fat embolism after 3 weeks, which was predominantly confined to subchondral capillaries of the humeral and femoral heads. These findings agree with those of Moran (1962), who identified intravascular fat emboli in the kidney, brain, and lung in his corticosteroid-treated rabbits, beginning at 3 weeks. However, no histological or roentgenographic evidence of aseptic necrosis was found by Jaffee *et al.* (1972), although the relatively short follow-up period may have been a limiting factor. Despite osteoporosis, there was no evidence of vasculitis or local changes consistent with a hemorrhagic diathesis.

Mankin *et al.* (1972) concluded that systemic corticosteroids do not impair chondrocyte viability, although they significantly interfere with matrix protein and polysaccharide synthesis of rabbit articular cartilage.

Additional support for this hypothesis has recently been reported by Fisher *et al.* (1972), who also produced hypercortisonism in rabbits. Hyperlipemia developed, which was associated with increasing fatty metamorphosis of the liver and secondary pulmonary and systemic fat embolism. Fat embolism of subchondral arterioles and capillaries of the femoral and humeral heads was demonstrated in 40 of the 60 treated animals. Furthermore, a significant increase in osteocytic death was found on histological examination of these heads. Despite marked osteoporosis, which developed during the six-month study, there was no evidence of intramedullary hemorrhage or thrombosis, vasculitis, inflammation, or fractures.

Fat embolism in rabbit femora has been demonstrated for five weeks after a single infusion of fat into the distal aorta (Jones and Sakovich, 1966). Further studies have been performed in an attempt to demonstrate avascular necrosis resulting from intraosseous fat embolism, since several investigators, using various compression–decompression techniques, had previously been unable to produce dysbaric osteonecrosis in experimental animals.

MATERIAL AND METHODS

In addition to the 26 rabbits that, as previously reported, showed evidence of intraosseous fat embolism (Jones and Sakovich, 1966), four other animals were infused to obtain long-term results. An average dose of 1.2 ml of Lipiodol (iodized oil, U.S.P.) was infused into the distal aortas of the animals over a period averaging 22.5 minutes. The 30 rabbits either died or were killed at various intervals, ranging from immediately after infusion to 26 weeks later (Table I).

Similar studies were made of four control rabbits. Two were anesthetized but not subjected to the operative or infusion procedures. Another underwent the operative procedure with ligation of the left common femoral artery, but without insertion of a catheter. The fourth had an infusion catheter inserted, but no Lipiodol was infused. Each control animal was killed within two hours of the procedure.

Autogenous rabbit or human fat was not infused in these preliminary experiments. Fat

Table I. LIPIODOL DOSAGE AND INTERVAL BETWEEN INFUSION AND DEATH CORRELATED WITH HISTOLOGICAL EVIDENCE OF FAT EMBOLISM AND AVASCULAR OSTEONECROSIS IN RABBIT FEMORA

Animal No.	Killed or died	Interval between infusion and death	Infusion amount (ml)	Infusion duration (min)	Histological evidence of fat embolism*	Histological evidence of avascular osteonecrosis
1	D	0 min	1.0	30	Marked	None
2	D	10 min	1.0	25	Marked	None
3	D	90 min	2.2	18	Marked	None
4	D	12 hr	1.0	30	Marked	None
5	D	17 hr	1.0	17	Marked	None
6	K	17 hr	2.2	21	Marked	None
7	D	18 hr	1.0	25	Marked	None
8	D	24 hr	1.0	25	Marked	None
9	D	35 hr	2.0	20	Marked	None
10	K	44 hr	2.2	20	Marked	None
11	D	56 hr	1.5	20	Marked	None
12	D	68 hr	1.0	25	Marked	None
13	D	83 hr	2.0	20	Marked	None
14	D	84 hr	1.0	20	Marked	Trace
15	D	84 hr	1.5	20	Marked	None
16	D	4½ days	1.0	20	Moderate	Trace
17	K	6 days	1.0	25	Moderate	Trace
18	D	7½ days	1.0	20	Moderate	Trace
19	K	10 days	1.0	23	Moderate	Trace
20	K	2 weeks	1.0	20	Moderate	Slight
21	K	3 weeks	1.0	25	Moderate	Trace
22	K	4 weeks	1.0	20	Moderate	Slight
23	K	5 weeks	1.0	25	Moderate	Marked
24	K	6 weeks	1.0	25	Slight	Marked
25	K	8 weeks	1.0	25	Trace	Marked
26	K	10 weeks	1.8	20	None	Slight
27	K	17 weeks	1.0	20	None	Slight
28	K	21 weeks	1.0	22	None	Trace
29	K	24 weeks	1.0	20	None	Trace
30	K	26 weeks	1.0	30	None	Trace
		Average:	1.2	22.5		

*Jones and Sakovich, 1966

obtained from human long bones and subcutaneous tissues is almost entirely neutral, a high proportion of its fatty-acid constituents being unsaturated (Peltier *et al.*, 1956). Lipiodol is different from rabbit and human cellular lipids (Forestier, 1927; Strain and Berliner, 1964), since it is a vegetable neutral fat (poppy-seed oil) in which the unsaturated fatty acids have been iodinated to the extent of 40% by weight. Although both mammalian fat and Lipiodol assume embolic-sized proportions when introduced into the bloodstream, there are differences in molecular heterogeneity and in such physical characteristics as relative viscosity, specific gravity, solubility, and surface tension, which would alter some of the experimental findings. This possibility will be explored in later studies in which autogenous mammalian fats will be used.

Postmortem pelvic roentgenograms were taken of the undissected rabbits with the hind limbs

fixed in normal hip posture, *i.e.*, flexion, abduction, and external rotation (Wilkinson, 1962). Densitometric studies were performed with the Macbeth-Ansco Densitometer. Even though the vascularity of the L proximal femur had been partially compromised by the operative procedure, comparative measurements were taken from the A-P view of the pelvis, and the relative density of the R and L femoral heads was evaluated. In general, the lower the densitometric value (by transmitted light), the greater the osseous roentgenographic density. If a roentgenogram was technically acceptable, with respect to the variables of exposure and equivalent positioning of the femora and pelvis in relation to the beam, then a relative densitometric difference of 0.05 units or more between the R and L femoral heads was considered significant (Table II).

The normal vascular system supplying the proximal femur of the adult rabbit is shown in Fig. 1. The opportunity to obtain collateral circulation in the rabbit femoral head is limited, since the entire head is intracapsular. The only sources of blood supply are through the medial epiphyseal artery of the ligamentum teres, through the epiphyseal vessels from the periosteal attachment of the capsule to the margin of the head, and through the metaphyseal vessels. The R femur was the only bone studied for evidence of avascular necrosis. It was disarticulated and removed for examination; frozen histological sections, as well as fixed hematoxylin and eosin-stained sections, were prepared.

Radioautographs were also made of sections of the proximal R femur from all rabbits killed 5 weeks or longer after the Lipiodol infusion, except the animal killed at 10 weeks. Radioactive phosphorus (sodium phosphate ^{32}P) at a dose of 10 microcuries per kilogram of body weight was injected intravenously 1 hour prior to necropsy. Radioactive phosphorus is a preferential bone-seeking isotope. If the intraosseous circulation is adequate, maximum uptake in the femoral head probably occurs within 1 hour after administering ^{32}P intravenously (Boyd, 1964; Boyd and Calandruccio, 1963). The method of Salomon and Ray (1964) was modified because the relatively short half-life of ^{32}P (14.3 days) necessitated briefer exposures (7 to 14 days). The formalin-fixed bone blocks were embedded with methylmethacrylate and immersed in either Eastman-Kodak NTB3 or Ilford L-4 liquid emulsion (Kopriwa and LeBlond, 1962). After immersion, the sections were coated with emulsion and stained with the methylene blue-azure method of Bélanger (1961).

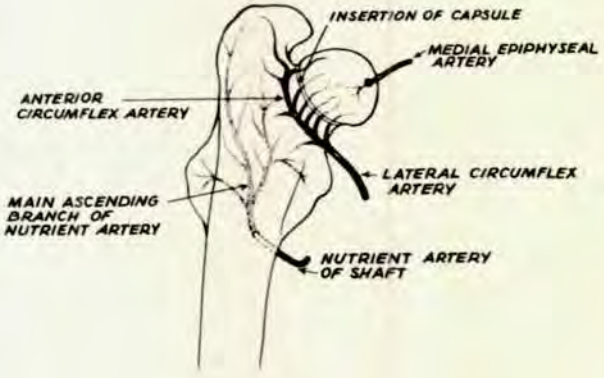

FIG. 1. Blood supply to femoral head in adult rabbit, which in many ways resembles arterial supply to human femoral head (Kistler, 1934; Lemoine, 1957; Rokkanen, 1962; Trueta and Harrison, 1953).

Table II. COMPARISON OF ROENTGENOGRAPHIC DENSITY OF FEMORAL HEADS OF CONTROL AND EXPERIMENTAL ANIMALS (DENSITOMETRIC UNITS)

	Weeks after infusion	Density of femoral head		Difference in density*
		Left	Right	
Control Rabbits	No infusion	1.12	1.12	0.00
Experimental Rabbits	Less than 5	1.38	1.37	0.01
	5	1.43	1.40	0.03
	6	1.32	1.21	0.11
	10	1.55	1.41	0.14

*A difference in roentgenographic density of 0.05 units or more is considered significant.

RESULTS

Mortality

Fifteen of the 30 rabbits died during the course of these experiments, 14 within the first 4½ days after the infusion (Table I). Their deaths could be attributed in some degree to pulmonary and systemic fat embolism. Rabbits surviving the first 4½ days usually lived, after which time few fat emboli were found in the brain and kidneys even though marked pulmonary embolism was still present (Fig. 2). Evidence that a fat globule

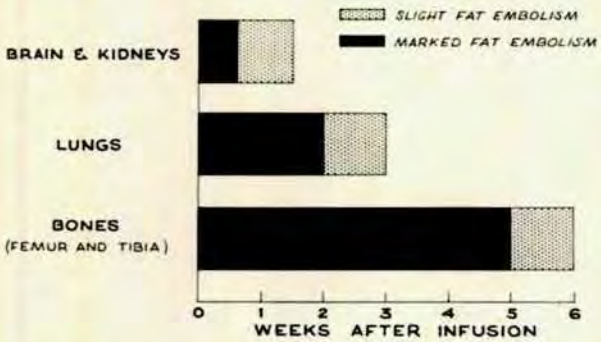

FIG. 2. Persistence of embolic fat in various rabbit organs after Lipiodol infusion.

was both intravascular and embolic was supported by its deformation into an oval or cylindrical configuration (Fig. 3), which indicated intravascular penetration and terminal impaction. The average Lipiodol dose administered to the animals that died (1.28 ml) did not differ significantly from the average dose infused into the 15 rabbits that lived (1.21 ml) (Table I).

Roentgenographic Findings

Minute intraosseous accumulations of radiopaque Lipiodol had previously been demonstrated in the undecalcified bone *in vivo* from 0 to 35 hours after infusion. Pelvic roentgenograms were not evaluated for evidence of osteosclerosis or early avascular necrosis during the first week after infusion because of potential difficulties in interpretation. There was no evidence of mottled or cystic radiolucencies, epiphyseal fragmentation, collapse, depression (flattening), subchondral fracture, angulation deformity, or secondary degenerative change in any of the control or experimental animals.

Naked-eye examination and magnification of the roentgenograms (up to × 40) with a Zeiss

FIG. 3. Deformed Lipiodol embolus 17 hr after infusion, impacted in capillary within epiphyseal region of femoral head, thereby blocking intraluminal blood flow (Animal 5, modified Felton stain, X 2000).

stereoscopic dissecting microscope revealed a questionable increase in roentgenographic density (osteosclerosis) in the R femoral heads of those animals killed at 6 and 10 weeks after the Lipiodol infusion (Fig. 4).

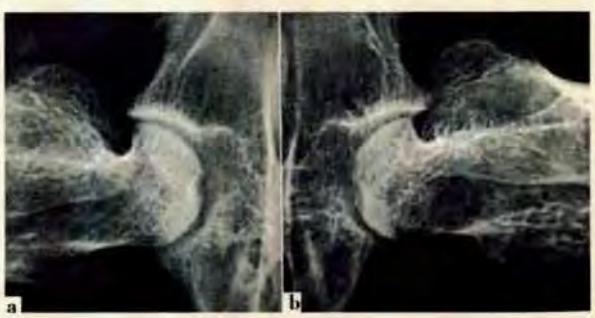

FIG. 4. Hips of Animal 26, killed 10 weeks after 20-min infusion of 1.8 ml of Lipiodol. (*a*) Slight osteosclerosis is present throughout R femoral head, compared with (*b*) less dense bone of L femoral head.

Densitometric determinations revealed a very slight increase in the relative radiodensity of the subchondral bone of the R femoral head, as compared with the L, at both 6 and 10 weeks after infusion. The greatest difference in relative density of the femoral heads was at 10 weeks after

infusion (0.14 unit) and at 6 weeks, although slightly less (0.11 unit) (Table II). There was no significant density change before 5 weeks or in the control rabbits.

Gross Appearance

No gross external alterations attributable to avascular necrosis were found in the R femur in any of the control or experimental rabbits.

Histological Findings

Control Animals. No ischemic or necrotic changes were seen either in the marrow cellular elements or in osteoblastic or osteocytic (osteogenetic) components of the femora from the four control rabbits. Scattered empty osteocyte lacunae were occasionally seen, primarily in interstitial lamellae.

Rabbit bone differs somewhat from human bone in that boundaries of individual osteons are not as prominent. Small Haversian canals can be seen, but concentric cylinders of lamellar bone and cementing lines are not as distinct as in human compact bone. The Haversian canals may encase one or two capillaries, the walls of which are composed of a single endothelial layer. In some of the smaller canals the capillary may occupy over 50% of the cross-sectional area. In the control animals, rarely was more than one layer of thin osteoblasts found lining the Haversian canals or covering trabecular and endosteal surfaces.

Small numbers of microscopic fissures, or microcracks (5 to 10 per section), were found between lamellae in otherwise intact trabeculae. These microcracks were present within the interior of the trabeculae; they did not appear to extend to bony surfaces adjoining the marrow spaces.

Lipiodol-infused Animals. *Immediately* after infusion, dilation and engorgement of several marrow sinusoids and capillaries were noted, indicating passive congestion. No hemorrhage in the marrow was apparent. Multiple fat globules were found in marrow capillaries and sinusoids shortly after the infusion (Fig. 5).

From 12 to 44 hours after infusion there was evidence of intraluminal erythrocyte disintegration and focal intracellular disorganization, plus disintegration involving the hematopoietic marrow. Islands of marrow cells showed loss of basophilic and nuclear staining. Fat cells and osteogenetic cells appeared normal.

From 56 to 83 hours after infusion, many hematopoietic cells had indistinct cytoplasmic

FIG. 5. Multiple Lipiodol fat globules within metaphyseal marrow, capillaries, and sinusoids of femoral head, 90 min after infusion (Animal 3, modified Felton stain, X 100).

borders (or they were missing altogether), homogenous coagulated cytoplasm, and condensed (pyknotic) or swollen (karyolytic) nuclei. Or they appeared as cloudlike masses of eosinophilic cell ghosts (Fig. 6). Minute areas of hematopoietic granular debris and acellular fibrinous necrosis were also noted (Fig. 7).

FIG. 6. Large cloudlike mass of eosinophilic cell ghosts of metaphyseal bone marrow, 68 hr after Lipiodol infusion, indicating focal infarction (Animal 12, hematoxylin and eosin stain, X 65).

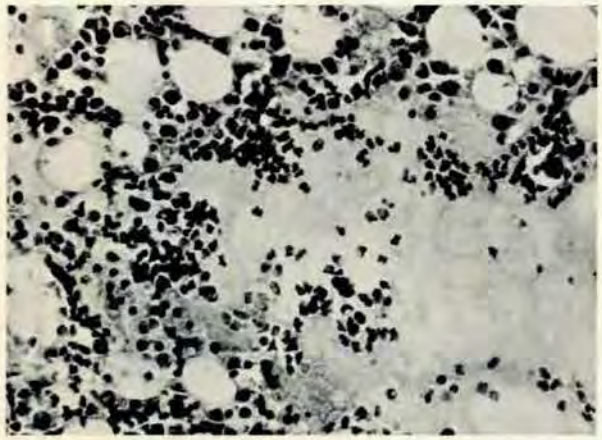

FIG. 7. Area of hematopoietic granular debris, 83 hr after Lipiodol infusion, with cellular ghosts and fibrinous necrosis (Animal 13, hematoxylin and eosin stain, X 380).

At 4½ days there was a slight decrease in marrow fat cellularity without focal necrosis. Scattered passive congestion, fibrin thrombi, and dissolution of vascular walls were evident. Osteocytes appeared normal, but few osteoblasts were found.

At 6 days even less marrow-fat cellularity was apparent and there were scattered areas of fatty liquefaction necrosis, with fat-cell dissolution and, in the metaphyseal marrow, cystic spaces or oil sacs of various sizes.

At 10 days necrosis of fat cells caused metaphyseal marrow fat to diminish.

By 2 weeks there was an increased number of microscopic cracks (20 to 30 per section) in the proximal metaphyseal marrow. The earliest microcracks were arranged linearly along the cementing lines in the interval between adjacent, concentric lamellae and, particularly, at the junction of two or more trabeculae. There was scattered focal loss of hematopoietic and adipose tissue pattern with fibrinous necrosis.

At 3 weeks diffuse intracellular disorganization and loss of cellular detail continued, especially with respect to marrow elements. No noteworthy osteocytic necrosis was found at this time.

At 4 weeks less osteoblastic proliferation was found. Along the periphery of the proximal femoral metaphysis there was minimal invasion by vascular granulation tissue, especially underlying some endosteal cortical surfaces. The osteocytic nuclei of several metaphyseal trabeculae were pyknotic or missing, especially in the surface lamellae.

At 5 to 8 weeks after infusion, there was histologic evidence of bone necrosis. Irregular foci of coagulation necrosis of marrow elements, representing focal infarctions, involved the epiphyseal region of the femoral heads. There was loss of tissue pattern in the intertrabecular spaces and autolysis with dissolution of cellular constituents in focal areas.

The borders of the infarcted areas had indistinct lines of demarcation. Bone and intertrabecular marrow spaces immediately beneath the articular cartilage of the femoral head were not extensively involved at 5 weeks, although separate scattered foci were apparent in the epiphysis (Fig. 8).

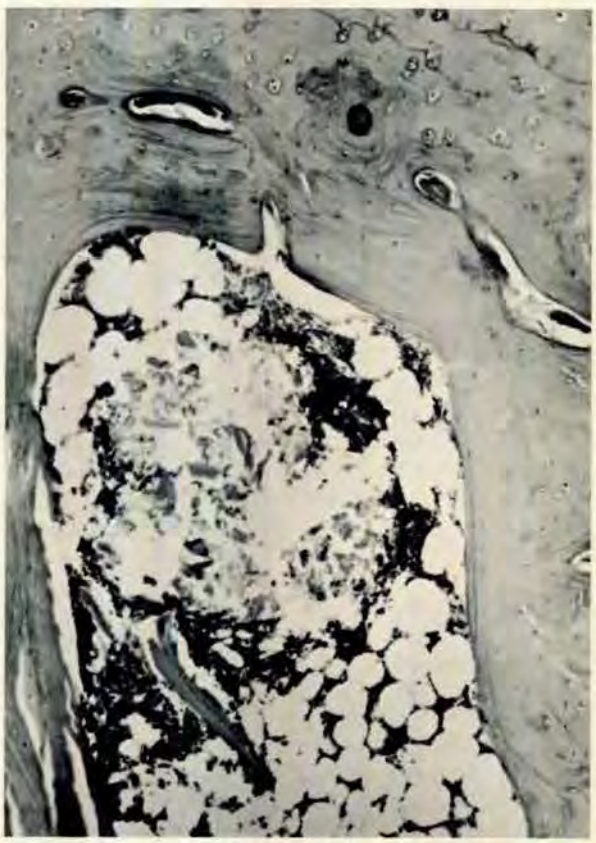

FIG. 8. Small anemic infarction lying within subchondral marrow of femoral head, 5 weeks after Lipiodol infusion. Trabecular microcracks and superficial cleavage planes are apparent, with sequestration of small fragments of bone into marrow space (Animal 23, hematoxylin and eosin stain, X 200).

At 6 and 8 weeks the punctate foci of necrotic debris had extended into the subchondral bone. During this period there were occasional oil cysts. The necrotic marrow spaces contained amorphous masses of granular debris, including necrotic bone fragments (Fig. 9). Although occasional macrophages were found, osteoclastic removal of bone was not extensive. There was no appositional new-bone formation and a minimum of peripheral fibroblastic proliferation. At least one-third of the lacunae in the subchondral bone of the femoral heads were devoid of osteocytic nuclei. Loss of nuclei was particularly evident within the interstitial and superficial trabecular lamellae.

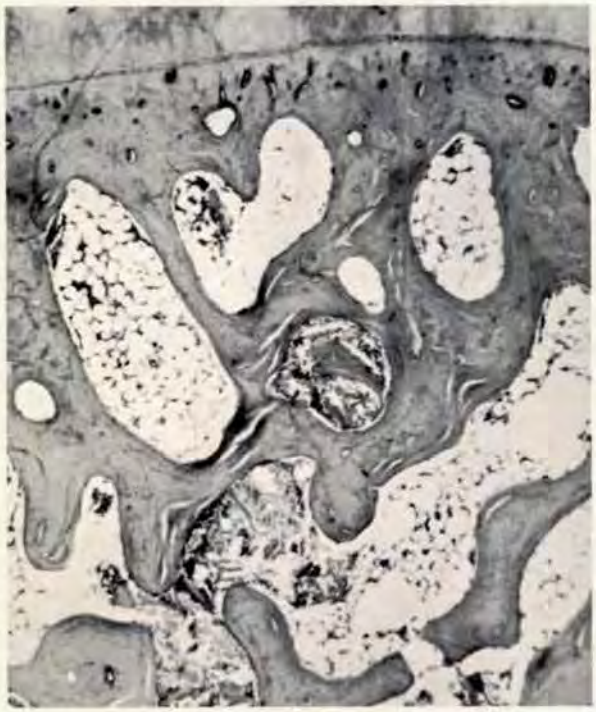

FIG. 9. Focal marrow infarctions and liquefaction necrosis, 6 weeks after Lipiodol infusion, with formation of small oil cysts in epiphyseal regions of femoral head (Animal 24, hematoxylin and eosin stain, X 100).

There was evidence of trabecular microdamage in the infarcted zones. Multiple microcracks (75 or more per section) were found at 5, 6, and 8 weeks. During this period it appeared that the microcracks had extended to trabecular surfaces, resulting in cleavage planes (Fig. 10) which separated the more superficial concentric lamellae.

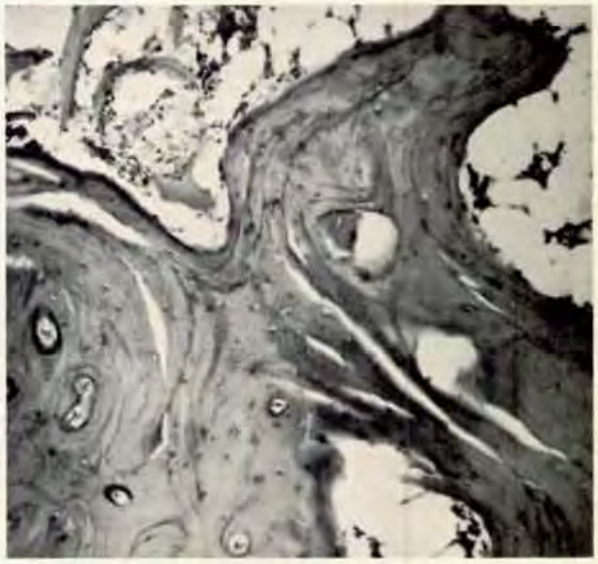

FIG. 10. Epiphyseal region of femoral head, 8 weeks after Lipiodol infusion, showing multiple microcracks separating along cleavage planes to trabecular surfaces (Animal 25, hematoxylin and eosin stain, X 200).

These superficial cleavage planes and the fragmentation of superficial lamellae (trabecular microfractures) were not found in the control animals.

Most of the fragmented bony spicules had lost their eosinophilic properties and were devoid of viable-appearing osteocytes. Thinning of bony trabeculae associated with superficial lamellar fragmentation was maximal at 8 weeks. Only ischemic changes were found in the distal metaphyseal and epiphyseal regions of the femur.

The articular cartilage appeared normal in staining characteristics, surface smoothness, layer thickness, and the number, size, distribution, and nucleation of the chondrocytes.

Beginning at 10 weeks after infusion there was evidence of regeneration of the ischemic and necrotic bone and marrow. Localized areas of osteoblastic activity were found with appositional new-bone formation.

Although an increased number of microcracks was noted at 10 and 17 weeks, there were few new fragmented and necrotic bone spicules. The size and number of fatty parenchymal elements in the proximal femoral metaphyseal regions continued to decrease. Although the hematopoietic cellular elements of the marrow had been recon-

stituted, there was still evidence of focal osteocytic death. Only about one-third of the subchondral lacunae were filled with viable-appearing osteocytes. There was no significant intertrabecular fibroblastic proliferation or connective-tissue scars. There were very few ghostlike cells or fatty cysts and little granular debris. Although liquefaction necrosis of fat cells had been previously observed, no calcified lipid was detected.

By 17 weeks few ischemic or necrotic changes were evident. The abnormal cells and the small amount of interstitial fibrous tissue had disappeared; the sinusoidal bed was restored and normal hematopoiesis apparently had been resumed. A marked proliferation of small adipose cells with enlarged nuclei was noted at this time.

At 21 to 26 weeks extensive reossification had occurred. The plump osteoblasts were two and three layers deep, covering the trabecular surfaces (Fig. 11) and walls of the Haversian canals.

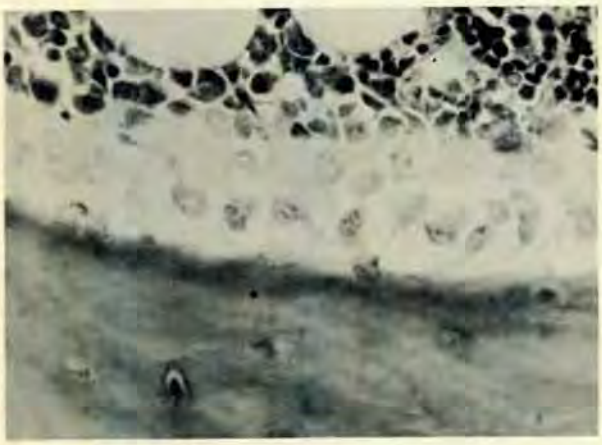

FIG. 11. Osteoblastic proliferation and appositional bone formation with normal marrow elements, 24 weeks after Lipiodol infusion (Animal 29, hematoxylin and eosin stain, X 900).

About a third of the lacunae were still vacant in the proximal subarticular cancellous bone at 21 weeks. Provisional new-bone formation was irregular and extensive; the deposits, of various sizes, were unevenly spaced.

Except for the persisting decreased numbers of osteocytes in the subchondral bone of the femoral head after 21 weeks, no evidence of focal infarcts remained (Fig. 12). Hematopoietic and fatty cellular elements appeared normal, although trabecular and cortical bone continued to show

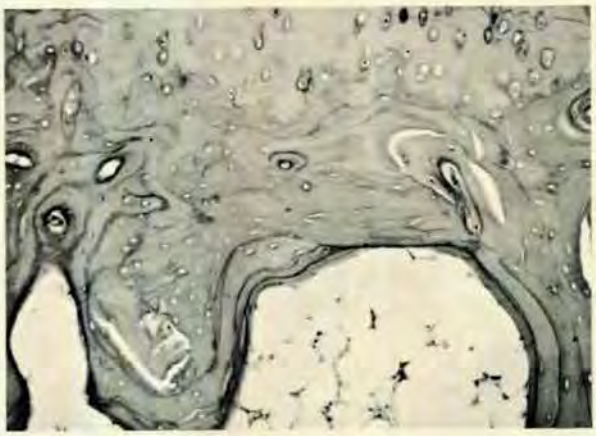

FIG. 12. Subchondral bone of femoral head of rabbit, 24 weeks after Lipiodol infusion. Marrow appears normal; there are increment lines with superficial lamellae containing viable-appearing osteocytes. Intratrabecular microcracks and lack of osteocytes will be noted in subchondral lacunae (Animal 29, hematoxylin and eosin stain, X 140).

histologic evidence of osteocytic necrosis. Regeneration of the hematopoietic and fatty marrow had occurred without organization of the infarcts or substitution by dense fibrous scar tissue (myelofibrosis).

Radioautographic Findings

The Eastman-Kodak NTB3 liquid emulsion yielded better results in qualitative radioactive autography than did the Ilford L-4 emulsion, presumably because of its greater sensitivity in delineating the emission of beta radiation from ^{32}P (Kopriwa and LeBlond, 1962).

Control Animals. Radioautograms of the control femora showed diffuse trabecular- and cortical-bone labeling with ^{32}P. Isotope accumulation was especially evident in areas immediately adjacent to capillaries.

Lipiodol-infused Animals. *At 5 to 8 weeks* there was spotty labeling of cortical bone and minimal labeling of trabecular bone in these animals, especially in the superficial lamellae within the necrotic foci. There was no labeling of the intertrabecular devitalized femoral-head fragments. Slight perivascular labeling was apparent in the bone adjacent to the necrotic focal infarctions.

From 17 to 26 weeks isotopic labeling was similar to that found in the control femora. Su-

perficial trabecular lamellae were labeled, but scattered areas lying deep within the interior of trabeculae remained unlabeled.

DISCUSSION

Avascular necrosis was not found in the femoral diaphyseal, distal metaphyseal, or epiphyseal regions of these rabbits. Although Lemoine (1957) produced osteochondritic changes in the upper femoral epiphysis of the rabbit after dividing the main capsular artery, similar changes could not be elicited in the distal femoral epiphysis, presumably because of the extent of collateral vasculature between the main artery and epiphyseal vessels.

Because of the great vascularity of bone, experimental division of an extraosseous vessel does not, except in isolated instances, simulate occlusion by emboli of that vessel's endings (Kistler, 1935). Because of the adequacy of extraosseous and intraosseous collateral vessels, ligation of the nutrient artery to the long bones of experimental animals only temporarily decreases intramedullary pressure and blood flow. End-arteries with few anastomoses favor embolic occlusion (Fernando and Movat, 1964). End-arteries are thought to be present beneath open epiphyseal plates and articular cartilages, which probably accounts for the frequent occurrence of metaphyseal and subchondral metastatic infarctions and necrosis. In these experiments, the emboli had especially obstructed subchondral arterioles and capillaries.

In addition to findings directly related to Lipiodol embolism and vascular tamponade, the femoral heads of the experimental animals revealed progressive evidence of focal tissue death and fragmentation. Passive congestion was evident throughout the first week after infusion. The earliest evidence of tissue death occurred within 48 hours and consisted of intraluminal erythrocyte shrinkage and degeneration and focal areas of hematopoietic marrow-cell disruption. Thereafter, until the tenth week after infusion, progressive focal necrosis and disorganization were evident, involving all marrow and bone elements.

At varying time intervals, the following sequence of tissue responses was observed:

1. Coagulation necrosis of hematopoietic marrow elements with focal disorganization.
2. Disappearance of adipose membranes, liquefaction necrosis of fatty-marrow elements with quantitative fat-cell loss, and appearance of cysts filled with liquid fat.
3. Disappearance of all osteoblasts from the trabeculae in large segmental or focal areas.
4. Degeneration of osteocyte nuclei with cellular shrinkage and, in some areas, complete disappearance of osteocytes.
5. Increasing numbers of fissures or microcracks in the bony metaphyseal trabeculae, with separation along the cementing lines and sequestration into the marrow spaces of many small fragments of dead bone.

Artifactitious tissue distortion, possibly resulting from technical processing, could also account for trabecular fragmentation. However, comparison of experimental and control animals revealed significant microdamage in the former at 5, 6, and 8 weeks. Several of the fragmented bony spicules had lost their eosinophilic properties and were devoid of viable-appearing osteocytes. Certainly additional studies, made with a variety of bone-processing techniques and tests for bone viability, are essential to verify the existence and significance of microdamage.

Bone and marrow infarction was localized in metaphyseal and epiphyseal regions of the femoral head. The majority of fat emboli had been found in arterioles and capillaries of these same regions from 2 to 6 weeks after the Lipiodol infusion.

After Lipiodol-fat embolism of intraosseous vessels, the following sequence of events is thought to result in bone necrosis. Tissues formerly supplied with blood for their nutrition become progressively hypoxic as the oxygen content of the stagnant blood is exhausted. It has been demonstrated experimentally that within 3 to 5 minutes after a sudden, complete interruption of blood supply to the femoral head, the viable cells have consumed all available oxygen (Woodhouse, 1964). Marrow capillaries and sinusoids become atonic and dilate. As they dilate they fill with blood and appear congested.

Lacking adequate collateral circulation, arterial and capillary occlusion produces focal anemic infarctions. As the process continues, infarcted tissues in the femoral-head region die because of anoxia and chemical injury from accumulated catabolic products, and they undergo coagulation and liquefaction necrosis.

Loss of structural integrity within a cell is irreversible and indicates unequivocal death (Johnson, 1964). However, osseous tissues do not all die simultaneously. Death is dependent upon the inherent susceptibility of the particular tissue to acute anoxia. The most sensitive parenchymal elements, the hematopoietic cells, therefore succumb first.

In our animals, slight focal evidence of marrow necrosis first appeared at 56 hours after

infusion. Marrow fat cells next showed focal changes, with liquefaction necrosis and formation of oil cysts, as well as cellular diminution 6 days after infusion; these changes were especially pronounced at 5 through 10 weeks. No osteoblasts were found lining trabecular surfaces in segmental infarcted areas at 4 through 8 weeks. Slight degeneration of the osteocyte nuclei, with cellular shrinkage and ultimate disappearance from lacunae, was first noted at 2 and 4 weeks. Osteocytes may be more resistant to hypoxia than are other osseous cellular elements. It is speculated that those osteocytes within concentric lamellae farthest from Haversian vessels are particularly susceptible to ischemia. Osteocytes within interstitial extra-Haversian bone, or in superficial lamellae of individual osteons, probably manifest the greatest degree of necrosis (Fig. 13).

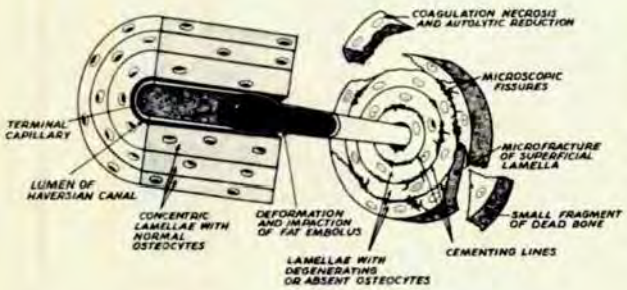

FIG. 13. Hypothetical mechanism whereby intraosseous fat embolism may result in osteonecrosis. (Jones, 1971. Illustration courtesy of publisher.)

However, the fact that osteocyte nuclei are stainable is not evidence of viability (Ray, 1964). Nor is the presence of scattered empty lacunae — as were occasionally noted in the interstitial lamellae of the control rabbits — evidence of bone death. It is therefore very difficult, if not impossible, to assess osteocyte viability on the basis of cellular morphology or staining characteristics. Kenzora (1972) performed radioautographs of cancellous and cortical bone from the femora and humeri of adult rabbits incubated with H^3-proline and H^3-cytidine. Only 65% of all osteocyte lacunae incorporated isotope immediately following total loss of all exogenous nutrition (19% of osteocytes did not incorporate isotope and 16% of lacunae were empty). Four days after loss of nutrition, no cells were labeled (60% of lacunae were empty and 40% were filled with cells). Biochemical determinations of DNA showed loss of osteocyte viability beginning about 4 hours after infarction.

Ascenzi and Bonucci (1971) determined fiber-bundle direction in successive lamellae and were the first to develop a micromechanical technique for determining shearing strength of fiber bundles in individual osteons. Swedlow and Katz (1972) also studied the ultrastructural properties of bone, using a combination of investigatory techniques. They found that strength and stiffness of bone are significantly related to collagen-fiber orientation. Regions with longitudinal or steeply spiraling collagen fibers are stronger and have higher elastic moduli than regions with more transverse fiber orientation. At the interlamellar interface, fiber alignment may change abruptly from circumferential to radial, and there may be a 90° change of fiber orientation between adjacent lamellae. It is likely that interlamellar microcracks are more likely to result from shearing stresses applied to adjacent lamellae having a marked change in fiber orientation.

Although microcracks were first observed in experimental animals by Rutishauser and Majno (1950), they have also been found routinely in bone from normal human adults (Frost, 1960). They are thought to develop when bones are cyclically loaded by muscle and body-weight forces during daily physical activity. These forces may cause interlamellar compression and shearing stresses that disrupt interlamellar bonds, resulting in microfatigue and trabecular microdamage.

Frost (1964) emphasized the importance of early detection and repair of microcracks in otherwise intact trabeculae. He considered osteocytes the logical key in microdamage detection, since microscopic cracks usually occur normally in extra-Haversian bone or inside trabeculae. Living osteocytes are thought to signal the activation of mesenchymal (osteoprogenitor) cells, which cause osteoclasts and osteoblasts to respond and repair the microdamage. Isolated instances of healing trabecular fractures have been found in human femoral heads by Todd and co-workers (1972).

In our experimentation, irregular accretions of woven bone were found surrounding the undisplaced trabecular fractures, evidence of repair with active new bone formation. There was complete reconstitution of the marrow; resolution occurred with little evidence of fibrous tissue substitution. With progressive revascularization of posttraumatic necrotic lesions, the fibroblastic network of granulation tissue (including capil-

laries and cells) ordinarily fills marrow spaces rapidly, tending to polarize toward bone surfaces. These cells then differentiate to become osteoblasts and produce new bone.

In subchondral bone, which is aptly called *cortex*, revascularization occurs through formation of extensive cutting cones in a process similar to that involved in primary bone healing. A dead bone trabecula is coated with a layer of woven bone, which may then be covered by a layer of living lamellar bone. However, classic revascularization of necrotic foci did not occur in the present study. Fibroblastic elements had vanished and the marrow appeared essentially normal at 17 weeks. The only indication of previous infarction was foci of osteocytic death.

It is interesting to correlate, chronologically, the histological evidence of fat embolism and focal osteonecrosis in the femoral heads of rabbits (Table I). Six weeks after a single shower of intraosseous fat emboli, evidence of the initiating event had vanished when there was focal necrosis. When the necrotic or healing heads were excised six weeks or later after the embolic shower, there was little or no evidence of the initiating event (Jones, 1971).

In these experiments, focal avascular necrosis was shown to result from fat embolism of bone. A single episode of fat emboli, even with temporary systemic recycling of the fat globules, did not produce gross or roentgenographic evidence of necrosis. Histologic evidence of necrosis in the rabbit is rather short-lived before regeneration of marrow occurs, although restitution of osteocytes is slower. But if the femoral head were to be bombarded with another shower of fat emboli 8 to 10 weeks after an initial episode, it is conceivable that additional minute bone infarctions would prevent healing. They might, as well, coalesce and result in gross architectural abnormalities.

Dysbarism, Fat Embolism, and Osteonecrosis

Nitrogen bubbles have been considered the primary factor in the etiology of dysbarism and related osteonecrosis. However, Kahlstrom and co-workers (1939) were unable to produce bone infarctions in the hind legs of dogs by arterial air embolism. Gersh *et al.* (1944) and Colonna and Jones (1948) failed to produce avascular necrosis in compression–decompression experiments, despite the presence of enlarging gas bubbles in bone marrow and within intramedullary vessels.

Reeves and co-workers (1972) reported on a series of 732 compressions involving 19 dogs; decompression sickness developed approximately 50% of the time. Roentgenograms of shoulder and hip joints were undertaken annually for five years, at which time necropsy was performed. Examination revealed no gross or microscopic evidence of osteonecrotic cortical or cancellous bone.

Bond and associates (1965) studied the hemodynamic alterations produced by intra-arterial gas emboli. They found that N_2 emboli initially cause vascular constriction and, probably, temporary obstruction. These events are followed by decreased resistance, interpreted as vascular relaxation, which persists until the emboli are removed either by traversing the capillary bed or by dissolution in the blood. Probably no more than 10% to 15% of the vessels remain blocked after gaseous embolism (Duff *et al.*, 1954). Nitrogen bubbles liberated from marrow fat are confined within the rigid medullary cavity and may build up sufficient intramedullary pressure to compress intraosseous vessels. Hills and Straley (1972) found experimentally that soon after compression there is a definite increase in bone blood flow and a decrease in marrow pressure, which seem to reverse soon after decompression. But the duration and extent of the ischemia they observed were only temporary and not considered sufficient to initiate osteonecrosis.

Shim *et al.* (1967) likewise were unable to produce dysbaric osteonecrosis experimentally in rabbits, despite a regimen of compression–decompression three times a week for four months in a hyperbaric chamber. Cystic lesions observed in the bone marrow of only one animal were similar to those reported by Colonna and Jones (1948). The evidence was insufficient to implicate these lesions as manifestations of osteonecrosis. Postmortem examinations revealed many gas bubbles within the blood vessels and tissue spaces of animals that died during the experiment, but none were found in the surviving animals. However, fat emboli were found in the lungs of most of the animals that died as well as in the lungs of the survivors.

Relative to the 1967 study (Shim *et al.*), fat stains were made of the proximal femur, lung, brain, liver, kidney, and heart of the animals involved (Jones and Shim, 1972). No evidence of intraosseous fat embolism of the femoral heads was found. Evidence of systemic fat embolism was insignificant, although pulmonary fat embolism was marked in the multiple compression–decompression group. There was also evidence

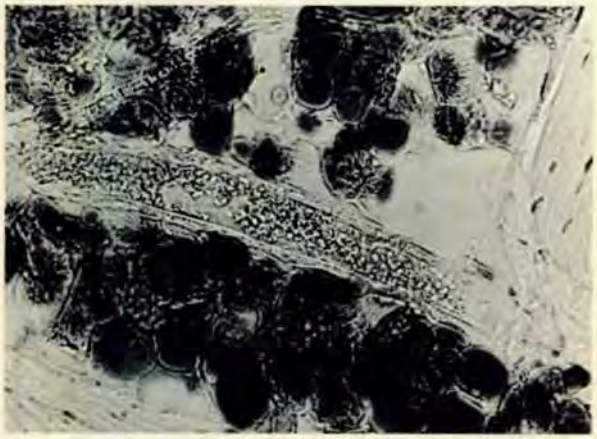

FIG. 14. Bone marrow of rabbit which died 1 hr after multiple compression-decompressions, revealing degenerating fat cells adjacent to capillary sinusoids (Oil Red O stain, X 250; tissue courtesy of Shim *et al.*, 1967).

of degeneration of marrow fat cells adjacent to sinusoids (Fig. 14). Occasional gas bubbles and probable intravascular fat globules were noted within the bone marrow of a rabbit that died within one hour following multiple compression–decompressions (Fig. 15).

Gersh *et al.* (1944) and Gersh (1945) demonstrated N_2 bubbles in adipose tissues following decompression sickness. Fat cells were subsequently disrupted by bubbles and depot fat was then mobilized in the blood. Cockett *et al.* (1972) documented fat embolism in the lungs, liver, and kidneys of dogs following overcompression to 165 ft for 1 hour and rapid decompression at the rate of 7 psig/min. There were also significant elevations of cholesterol, phospholipids, triglycerides, and total lipids 3 hours after "surfacing." LeQuire *et al.* (1959) decompressed rabbits and demonstrated increased cholesterol in the fat emboli that developed. Such levels of cholesterol, they believed, could not be derived from depot fat alone, and they suggested that the emboli were formed from unstable serum lipid.

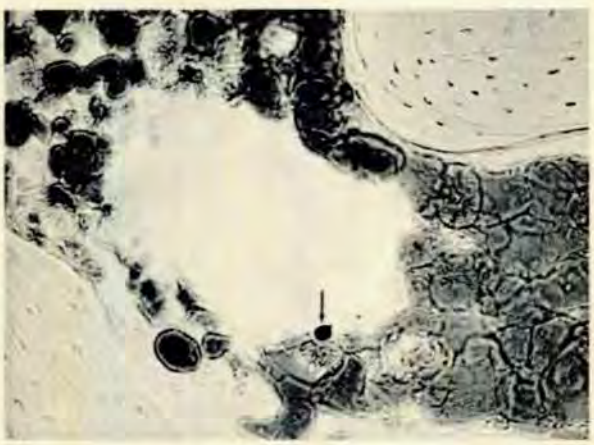

FIG. 15. Bone marrow of rabbit that died 1 hr after multiple compression-decompressions. Large gas bubble is seen adjacent to capillary-sinusoid, with a probable intravascular fat globule (arrow) (Oil Red O stain, X 200; tissue courtesy of Shim *et al.*, 1967).

Pauley and Cockett (1970) analyzed features common to fat embolism and dysbarism, including the virtually identical symptoms of the two syndromes and the latency period preceding their onset. Transcutaneous ultrasound (Doppler) measurements will demonstrate intravascular gas bubbles *in vivo* in the dysbarism syndrome (Evans *et al.*, 1972). Yet Kelly and coworkers (1972) (also using noninvasive ultrasound probes) have detected, in both dogs and humans, intravascular fat globules *in vivo* in the femoral venous effluent from a fracture. Blood tests revealed hyperlipemia and lung biopsies revealed fat embolism, which correlated well with ultrasonic recordings for fat globules.

Nitrogen bubbles probably trigger an elaborate chain of secondary events. Although the decompression-sickness syndrome initiated by N_2 is essentially reversible with recompression, certain secondary hemodynamic side effects are not thus reversible — including the fat-embolism syndrome. The coexistence of fat embolism and human dysbarism had been previously reported by several investigators (Haymaker and Davison, 1950; Haymaker and Johnston, 1955; Sillery, 1958; Rait, 1959; Odland, 1959; Robie *et al.*, 1960).

The strongest evidence supporting the concept that fat emboli are at least partially derived from disrupted adipose or other fatty-tissue depots (fatty liver or bone marrow) is the presence of marrow fragments within pulmonary vasculature following decompression. Pauley and Cockett (1970) studied the role of lipids in dysbarism. They concluded that changes in lipid stability probably occur because of injury to the fatty liver resulting from expanding N_2 bubbles. They suggested that unstable lipids, extruded from the liver, may form emboli and occlude the

pulmonary and systemic vasculature. Rheological changes — *i.e.*, sludging and aggregation of red-blood cells and platelets — are caused by unstable lipids and bubbles, resulting in impaired tissue perfusion, vascular damage, and fibrin thrombus propagation.

Philp and co-workers (1971) suggested that plasma lipids tend to disappear after rapid decompression. Philp *et al.* (1972) reviewed the interaction between blood and foreign surfaces, including gas bubbles. They suggested that intravascular proteins, platelet adhesion and aggregation, and coalescence and adhesion of plasma lipids to the blood–gas interface could contribute to fat embolism in dysbarism.

It is speculated that intraosseous fat embolism of terminal vessels then occurs, followed by focal intravascular coagulation and propagation of fibrin thrombi proximal to the occluding globule(s). The mechanism underlying the process may involve the release of some thromboplastin-like tissue material in response to cellular injury, with consequent activation of the coagulation process and incorporation of platelets in fibrin.

This response is thought to involve the interaction of vascular subendothelium and platelets to form the provisional hemostatic plug, followed by fibrin stabilization of the plug through coagulation (Harker and Slichter, 1972). In both the fat-embolism and dysbarism syndromes, destruction of both platelets and fibrinogen increases, resulting in thrombocytopenia and other hematological correlates. Fat embolism is known to precipitate disseminated intravascular coagulation (Lasch, 1969). The latter often goes unrecognized, because platelets and fibrinogen may be maintained at near-normal levels by compensatory increases in their production.

Pauley and Cockett (1970) noted that without recompression therapy, decompressed dogs survive when treated with Rheomacrodex (low-molecular-weight [40,000] dextran) or intravenous heparin (2 mg/kg). Hypothermia, which is effective in treating fat embolism, also appeared useful in treating dysbarism (Cockett *et al.*, 1965). The beneficial effects of dextran may be attributed to its capacity to clear plasma of coalescing lipid molecules, whereas the lipolytic action of heparin is attributed to activation of lipoprotein lipase. Gowdey and Philp (1965) also evaluated pharmacological adjuncts to recompression therapy. They suggested that heparin's capacity to reduce the incidence and severity of dysbarism is more likely associated with its lipemia-clearing activity than with its anticoagulant properties.

Pathophysiology

After these observations were synthesized, a hypothetical scheme was devised regarding the pathophysiology of dysbaric osteonecrosis. Nitrogen bubbles coalesce unstable lipids at the blood–gas interface and or cause the disruption and liberation of depot fat. These events result in intermittent systemic (intraosseous) fat embolism (Jones and Sakovich, 1966), which has been shown experimentally (Jaffe *et al.*, 1972; and Fisher *et al.*, 1972) to involve both the humeral and femoral heads, followed by intravascular coagulation, fibrin thrombus propagation, focal marrow necrosis, osteon anoxia, and osteocytic death.

Dead osteocytes cannot detect microdamage; therefore, no signal for repair is transmitted to osteoclasts and osteoblasts (Frost, 1964), which may have also been adversely affected by ischemia and necrosis. There is either insufficient repair, or no repair, of the microdamage. Microdamage is repeated, and the pathophysiology worsens, causing trabecular thinning, gross trabecular fragmentation, and fractures, with coagulation necrosis, autolytic reduction, and, inevitably, segmental subchondral collapse of functional significance.

SUMMARY

Focal and minute regions of avascular necrosis in bone and marrow have been produced in the metaphyseal and epiphyseal zones of the right femoral heads of rabbits by a single infusion of 1.2 ml of Lipiodol into the distal aorta. Fat emboli persisted up to 5 weeks after infusion and were particularly evident within subchondral vessels of the femoral head.

The presence of osteonecrosis was determined by roentgenographic, histologic, and radioautographic methods. Slightly increased bone density of questionable significance was observed at 6 and 10 weeks after infusion. The uptake of radioactive phosphorus was markedly reduced in the infarcted zones.

Histologically, necrosis involved all marrow and osseous elements in the infarcted regions. The earliest ischemic changes were detected at 4 days; by 10 weeks active repair was apparent. Hematopoietic marrow elements showed coagulation necrosis. Marrow fat cells were reduced in number and displayed liquefaction necrosis with the formation of oil cysts. Epiphyseal and

metaphyseal trabeculae lost osteoblastic lining cells. This loss was associated with death of osteocytes, formation of microfractures, and sequestration into the marrow space of many small fragments of dead bone. Although bone-marrow components had been completely regenerated by 17 weeks, foci of osteocytic death persisted.

The significance of these preliminary experimental findings remains unknown, since it is still not certain if tissue or intravascular gas bubbles are the primary cause of dysbaric osteonecrosis. It is known, however, that bubbles may have some secondary effect, such as the production of pulmonary and systemic (intraosseous) fat embolism, associated with fibrin thrombus propagation, and red-cell and platelet aggregation. It is speculated that the etiological agent in dysbaric osteonecrosis may not be reversible with recompression therapy alone, but may also require the use of various lipid-clearing agents.

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