1,4,11,12) and interpretations made on data taken from whole bone mineral should take these differences into account.

Summary. Quantitative x-ray diffraction analyses of histological tissue zones representing progressive stages of endochondral and periosteal bone formation reveal that both amorphous and apatitic calcium phosphate salts appear throughout the entire mineralization process. The amorphous-crystalline mineral sequence exhibited by these tissue zones suggests that calcification in both cartilage and bone may entail an initial deposition of amorphous calcium phosphate followed by the conversion of this kinetically metastable, non-crystalline mineral phase to crystalline apatite.

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Hypoxic Decompression and Fat Embolism.* (32000)

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Although known to occur commonly following skeletal fractures, pulmonary fat emboli have also been observed in the absence of overt trauma(1,2,3). Such a situation exists in fatal subatmospheric decompression sickness, in which fat has frequently been found in the pulmonary and systemic vessels (1). The mechanism of fat embolization during decompression, and the source of the fat are not clear. Animal experiments attempting to relate subatmospheric decompression to fat embolization have been few and inconclusive(4,5).

This study was performed to determine if pulmonary fat emboli could be produced by hypoxic subatmospheric decompression. In an attempt to facilitate fat embolization, cholesterol hyperlipemia and ethionine fatty liver were induced prior to decompression. The results indicate that none of the factors tested predisposed to fat embolization in the presence of hypoxic decompression. Nor was decompression alone associated with fat emboli.

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TABLE I. Summary of Results.

Group description	No. of rabbits	Wt in g (avg)	Pulmonary fat emboli	Fatty liver	Serum cholesterol in mg % (avg)
Decompressed					
A. Normal	5	2,800	0	0	112
B. Cholesterol-fed	7	2,700	0	Marked	2,100
C. Ethionine-treated	6	2,800	Occasional	"	—
Non-decompressed controls					
D. Normal rabbits	5	2,850	0	0	—
E. Cholesterol-fed	5	2,700	0	Marked	2,153
F. Ethionine-treated	5	2,750	Occasional	"	—

Materials and methods. Young adult New Zealand white rabbits of both sexes were used in all experiments. They were maintained on Purina Rabbit Chow and water *ad libitum* except for those animals on the high cholesterol diet. The experimental plan is summarized in Table I. The animals were divided into 6 groups, A-F. Group A consisted of 5 normal rabbits, Group B of 7 rabbits maintained for 65 to 75 days on a 2% cholesterol diet (Nutritional Biochemicals Corp., Cleveland, Ohio) and water *ad libitum,* and Group C, 6 rabbits that received intraperitoneally 1.0 g of DL-Ethionine (Calbiochem, Los Angeles, Calif.) dissolved in 100 cc of distilled water 48 hours prior to decompression. The animals in Groups A, B, and C were decompressed to 226 mm/Hg (30,000 feet).

As controls, the following 3 groups were employed: Group D, 5 normal rabbits, Group E, 5 rabbits on the 2% cholesterol diet, and Group F, 5 rabbits administered ethionine 48 hours before sacrifice. These animals were killed by an intravenous overdose of phenobarbital without decompression.

The decompressions in Group A, B, and C were carried out in a 340 cubic liter chamber (Vacudyne Corp., Chicago, Ill.). Mechanical vacuum and air inlet were manually controlled. Animals were individually decompressed to a simulated altitude of 30,000 feet by reduction of the ambient pressure to 226 mm/Hg. Ambient air was constantly drawn into the chamber through the air inlet. Approximately one-half of the animals died spontaneously within 2 hours in the chamber. Those animals that survived over 2 hours were quickly killed by further decompression to 87 mm/Hg (50,000 feet).

In order to study the effects of variable rate of decompression, 10 rabbits were used. Five were administered the 2% cholesterol diet, and the remainder were maintained on regular diet. Both groups were decompressed individually at the following rates: 2,500, 5,000, 10,000, 15,000, and 20,000 feet per minute to a final pressure of 54 mm/Hg (60,000 feet).

All animals were autopsied within 10 minutes of death. Samples of brain, heart, lungs, liver, spleen, kidney, and adrenal were fixed in 10% neutral-buffered formalin. The lungs were fixed by intratracheal perfusion of the fixative, and sections were taken from each lobe. All tissues were embedded in paraffin and stained with hemotoxylin and eosin. Frozen sections, 10 and 20 μ thick, were cut from formalin-fixed lung and liver and stained with oil red 0 for the demonstration of neutral fat. As a positive control for the fat staining method, a small quantity of subcutaneous fat was injected intravenously into the femoral vein of a rabbit, and fat emboli were consistently demonstrated in the pulmonary vessels. Heart blood was used for the determination of serum cholesterol.

Results. Decompression of normal rabbits. The rabbits decompressed to 226 mm/Hg (30,000 feet) (Group A) showed no histologic features at autopsy that were significantly different from the non-decompressed control rabbits (Group D). Of the 5 animals in Group A, 2 lived for over 2 hours and were sacrificed, while the remaining 3 rabbits died spontaneously in less than 1 hour. No differences could be detected between those animals that died spontaneously and those that were sacrificed. Oil red 0 stains of lung sections did not reveal fat emboli in any of these animals.

Decompression of cholesterol-fed rabbits.

All the animals that had been on the high cholesterol diet (Groups B and E) showed histiocytic cell infiltrates in almost every organ examined. These cells contained lipid-laden cytoplasmic droplets that appeared bright red with the oil red 0 stain. The hepatic cells also contained numerous neutral lipid droplets.

No histologic differences could be found between the animals that had been decompressed (Group B) and the non-decompressed hypercholesterolemic controls (Group E). Of the 7 rabbits decompressed, 3 died in less than 1 hour, 3 within 1 hour and 15 minutes, and 1 survived for over 2 hours.

Decompression of ethionine-treated rabbits. Of the 6 animals decompressed (Group C), 4 survived over 2 hours and were sacrificed while the remaining 2 died spontaneously at 60 minutes and 70 minutes. Autopsies of the rabbits showed that the cytoplasm of the hepatic cells was replaced by numerous oil red 0-positive droplets. Occasional fat emboli were present in the pulmonary vessels of both the animals that had been decompressed (Group C) and the ones that were not (Group F). The decompression did not result in an increase of fat emboli in the lung.

Decompression of normal rabbits at variable rates. All rabbits decompressed to 54 mm/Hg (60,000 feet) died spontaneously during the decompression and showed some degree of pulmonary hemorrhage. Gas bubbles were found in the inferior vena cava of the animals decompressed at the rate of 20,000 feet per minute. Oil red O stains for pulmonary fat emboli were negative. These animals could not be differentiated histologically from the normal rabbits (Group A) decompressed to 226 mm/Hg (30,000 feet).

Decompression of cholesterol-fed rabbits at variable rates. This group of rabbits was also decompressed at variable rates. Histologic findings were only those associated with the cholesterol feeding. This group could not be differentiated from the other cholesterol-fed rabbits (Group B and E). No fat emboli were found in the lungs.

Discussion. The occurrence of pulmonary fat emboli in man following decompression sickness is well-documented(1,6). One of the earliest reports was that of Haymaker and Davison(6) in which they described 5 cases of fatal decompression sickness with some degree of fat embolism at autopsy. In a recent review, 14 of 17 autopsied cases of decompression sickness demonstrated intravascular fat emboli(1). Rait(4) stressed the frequent coexistence of fatty change of the liver and pulmonary fat emboli and noted similarities between the clinical syndrome seen with pulmonary fat emboli and that observed in decompression sickness. He suggested that embolization of liver fat to the lungs and brain produced the clinical pictures observed in fatal and near-fatal decompression sickness.

Experimental efforts to produce fat emboli by decompression have produced equivocal results. LeQuire *et al*(7) decompressed 19 rabbits to approximately 350 mm/Hg (20,000 feet) and found that 11 died within 1 to 12 hours in the chamber. Autopsy of these animals demonstrated sudanophilic material in pulmonary arterioles and capillaries. However, Henn and Wünsche(5) observed only minimal quantities of intravascular fat in the lungs of guinea pigs and rabbits following decompression to a simulated altitude 60,000 feet. Fat emboli were not found in the lungs or brains of obese rats exposed to 33,500 feet altitude(8).

In the present experiment, neither prolonged decompression at 226 mm/Hg (30,000 feet) nor rapid decompression to 54 mm/Hg (60,000 feet) was successful in producing lipid embolization in the lungs of normal rabbits. It is difficult to explain the discrepancy between these results and those of LeQuire *et al*(7) who reported pulmonary fat emboli in rabbits after decompression to a simulated altitude of only 20,000 feet. A possible explanation may lie in the rate of decompression or in the degree of hypoxia during the decompression procedure.

The experiment employing a variable rate of decompression was performed in an effort to test the hypothesis of Henn and Wünsche (5); namely, that 3 factors are necessary for the production of fat emboli by decompression: 1) lack of premature suffocation due to low oxygen tension, 2) maintenance of circulation, and 3) decompression of sufficient

rapidity to rupture fat cells, yet slow enough to prevent premature death of the animal from bubble formation. Their data for rats suggested that the optimal rate of decompression for the formation of fat emboli was approximately 8,500 to 19,500 feet/minute. In the present experiment, varying the rate of decompression within this range failed to produce fat emboli in the pulmonary vessels.

Hartroft and Ridout(9) demonstrated that large fatty cysts in the livers of rats may communicate with the hepatic sinusoids resulting in pulmonary and systemic fat emboli. Vascular perfusion of rat liver made fatty by a choline-free diet will show lipid in the perfusate, whereas the perfusate of a normal liver will be fat-free(10). Although it seems likely that fat may enter the vascular system from hepatic cells under some circumstances, this mechanism has not been established in decompression sickness. Following decompression of rats with dietary-induced fatty livers, Rait(4) was unable to demonstrate a conclusive increase in fat emboli.

In the present experiment, extensive accumulation of lipid in hepatic cells was produced by administration of ethionine 48 hours prior to the decompression. Decompression of these rabbits did not result in an increased number of pulmonary fat emboli compared to the control rabbits that were not decompressed. Likewise, the cholesterol-fed rabbits also demonstrated extensive fatty change of the liver, but no pulmonary fat emboli were found after decompression. The results indicate that it is difficult to produce fat emboli in the rabbit by atmospheric decompression, even in the presence of fatty liver.

Davis and Musselman(3) felt that fat "embolism" may be the result of destabilization of lipid transport mechanisms in the blood with intravascular agglutination of lipid. Conditions of stress would induce release of fatty acids with a decrease in the stability of serum proteins. This in turn would lead to agglutination of lipids, appearing as fat emboli in the vessels. The administration of cholesterol in the present experiment was intended to induce excessive amounts of circulating lipid, so that the stress of hypoxia and reduced pressure might result in lipid agglutination appearing as fat emboli. Although the rabbit serum was markedly hypercholesterolemic, there was no evidence of significant fat emboli in the animals that had been decompressed to 226 mm/Hg (30,000 feet), or in those that had been decompressed to 54 mm/Hg (60,000 feet). If *in situ* agglutination of lipid does occur, evidently the stresses of hypoxia and decompression are insufficient to induce such a sequence, even in the presence of severely hypercholesterolemic serum.

Summary. To investigate a possible relationship between fat emboli and hypoxic decompression, rabbits were exposed to simulated altitudes of 30,000 feet and 60,000 feet in a decompression chamber. Normal rabbits, cholesterol-fed rabbits, and ethionine-treated rabbits were decompressed and autopsied, and a search was made for fat emboli in the lungs. None of the animals developed a significant number of emboli compared to control animals that were similarly treated but not decompressed. These data suggest that hypoxic decompression in the rabbit will not result in the formation of fat emboli, even in the presence of fatty liver or hyperlipemia. The factors responsible for fat emboli in fatal human decompression sickness remain unclear.

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