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EFFECTS OF HYPERBARIC PRESSURE ON THE GROWTH PLATES OF RATS

Peter Walker, Edward Bates, Wui Chung, William Walsh and Andrew Leicester

Abstract

Children with open growth plates are exposed to raised atmospheric pressures when scuba diving and during treatment for medical conditions such as osteomyelitis and gas gangrene in a hyperbaric chamber. This study was to determine if raised pressures have any detrimental effect on growth plate potential.

Immature rats were exposed for different periods of time to raised atmospheric pressures in a hyperbaric chamber. The animals were then sacrificed and their tibias examined macroscopically, radiologically and histologically. No differences in growth were detected between those exposed and the control groups. It is our conclusion that there are no detrimental effects to the growth plate of rats as a result of the pressures used in this study.

Key Words

Dysbaric osteonecrosis, hyperbaric research.

Introduction

Longitudinal bone growth is confined predominantly to the growth plates located at each end of the long bones. Cartilage is added at the top of the plate and is replaced by bone at the bottom. The cartilaginous portion of the growth plate is divided, by its morphology and function, into reserve, proliferative and hypertrophic zones.

The relationship between oxygen tension and bone and cartilage formation is a complex one. It is possible that oxygen tension may be an important physiological control mechanism governing growth at the epiphysial plate.

Brighton[1] studied the effects on growth of the epiphysial plate in vitro under different oxygen tensions using the costochondral junctions of rats. He showed that the highest growth rate occurred in 21% oxygen and the lowest growth rate in 90% oxygen. This and other experiments indicate that there is an optimal oxygen concentration for growth to occur and that high oxygen concentrations are detrimental to growth.[2,3] The explanations for this oxygen toxicity are numerous, but are not fully understood.

Oxygen is carried in the blood in two ways, bound to haemoglobin and in solution. By increasing oxygen partial pressures, either by scuba diving or in a hyperbaric chamber, the amount of dissolved oxygen increases in a linear fashion.[4]

Effective treatment of disorders using increased pressure was introduced in the 19th century. It is used in the treatment of gas gangrene, decompression sickness, gas embolism, carbon monoxide poisoning, cyanide poisoning, acute peripheral arterial insufficiency, crush injury, refractory osteomyelitis and to improve the viability of skin grafts. The treatment of some disorders may be prolonged, involving several weeks of daily hyperbaric exposures.

Destructive bone lesions have long been recognised as a latent problem associated with exposure to compressed gas atmospheres in divers. Extensive surveys have shown the incidence of dysbaric osteonecrosis to range from 4% in Royal Navy Divers (almost all of whom had been

involved in experimental diving)[5] to 50% in Japanese shellfish divers.[6] The variation in incidence can be attributed to differences in frequency of exposure, degree of exposure to pressure and rate of decompression.

The high Japanese incidence has led to concern about repetitive diving in children and its effects on the growth plate. There is a widespread belief amongst diving instructors and medical personnel that diving may indeed be dangerous to the growth plates of children. There is no evidence in the literature to support or refute such a claim.

It is generally accepted that the formation of bubbles in tissue can occur during symptomless decompression carried out following conventional diving tables. Harvey stated that "the low tolerance of bone for inert gas supersaturation, may precipitate development of lesions when present day decompression tables are followed".[7]

The exact aetiology of osteonecrosis is unknown. Theories include the release of extravascular gas bubbles from fatty elements of bone, which exert sufficient pressure to compress blood vessels;[8] fat embolism produced experimentally;[9] release of vasoactive substances reducing blood flow to bones;[10] gas induced osmotic shift of fluids;[7] autoimmunity and dysproteinuria.[11] It is obvious from these proposed theories that the mechanism is not clear. What is known is that simple exposure to higher than normal pressures, without episodes of decompression illness, can cause areas of bone death. There seems no reason to believe why immature bone and more specifically growth plates cannot be similarly affected.

Numerous studies of mature bone have been performed in an attempt to simulate caisson disease. Colonna and Jones[12] were probably the first to examine the bones of animals exposed to repeated hyperbaric pressures. Smith and Stegall[13] produced radiological changes in miniature swine consistent with osteonecrosis. Successful attempts have been made to produce bone lesions in mice.[14]

An extensive search of the literature failed to find any long term studies on the effect of exposure to raised pressure on immature bone, especially the growth plate.

Using the rat as an experimental animal permits suitable numbers for statistical validation as well as allowing large numbers of animals to be exposed to identical environmental conditions. By using an animal model under standard physiological conditions we hoped to exclude any artificial results produced by in vitro models. We hoped to answer the following questions.

1 Are there any effects of hyperbaric pressure on the growth plates of rats?
2 Does increased frequency of exposures have an additive effect?
3 Is there a latent period between exposure and effect?
4 Can a single exposure have any effect?

From our results we hope to draw some conclusions regarding the possible effects of hyperbaric pressure on the growth plates of children during medical treatment as well as during underwater diving.

Methods

An pilot study using six rats was performed, under the supervision of the Ethics Committee and supervising veterinary surgeon, to determine if the protocol was safe. These rats were observed during the entire exposure through a glass window in the chamber. Throughout the pilot study and subsequent experiment there was no obvious change in behaviour to suggest any discomfort or symptoms of decompression sickness. The temperature in the chamber was recorded half-hourly.

After the rats were removed from the chamber they were observed for several hours and then several times a day for the next week. Again there were no signs of discomfort. It was decided that the protocol was entirely safe. During the subsequent exposures the rats continued to be monitored visually during and after the dives and on a daily basis thereafter. Beyond clinical observation no other tests of dysbaric stress were performed.

Thirty six male Sprague Dawley rats (4 weeks old) were placed at random into cages of six rats each. The rats were housed and kept under standard conditions under the guidance of the Animal Care and Ethics Committee (ACEC), in accordance with the NSW Government Animal Research Act (1985). Each rat was weighed initially and then at weekly intervals. Weight is a sensitive indicator of stress in rats.

The rats were exposed to a standard hyperbaric pressure protocol (Fig 1). The exposure was to the equivalent of 33 m of sea water for 100 minutes (including descent time of 15 minutes). Decompression stops were at 9 m for 10 minutes, 6 m for 40 minutes and 3 m for 75 minutes. This is a dive from the US Navy diving tables. Each group (6 rats per group) was exposed to increased pressure for 1 day, 5 days, 20 days or 40 days (Fig 2). At the beginning of the experiment all rats were 4 weeks old. Rats in groups 2, 3 and 4 were exposed five days a week at the same time each day. Twelve rats were not exposed to increased pressure and were used as controls.

At the end of the diving period half the rats from each cage were sacrificed using carbon dioxide inhalation. These rats (immediate sacrifice group) were all therefore twelve weeks old. The remaining rats (delayed sacrifice group) were sacrificed four weeks later (Fig 2).

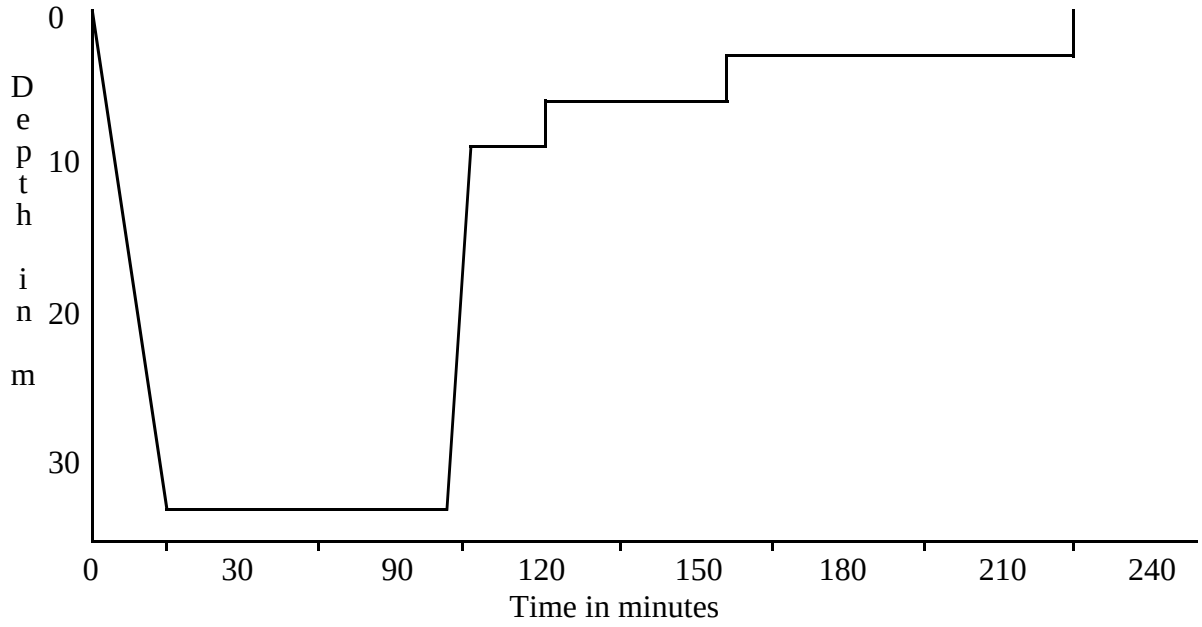

Figure 1. Hyperbaric pressure exposure protocol

Following sacrifice the femurs and tibias were dissected free of soft tissue and X-rayed using high resolution mammography film (Fig 3). Tibial lengths were measured from the tibial plateau to the tibial platform using a digital calliper.

The tibias were fixed in cold phosphate buffered formalin for 48 hours. Samples were demineralised in 0.5 M EDTA. Demineralised samples were sharply dissected and infiltrated with paraffin for sectioning. Five micron thick serial sections were taken through the middle of the upper tibial growth plate in the sagittal plane and stained with haematoxylin and eosin, Masson's trichome and alcian blue (Fig 4). Samples were examined under light microscopy using an Olympus BH-2 microscope. Morphological appearance was examined at 50x and 200x

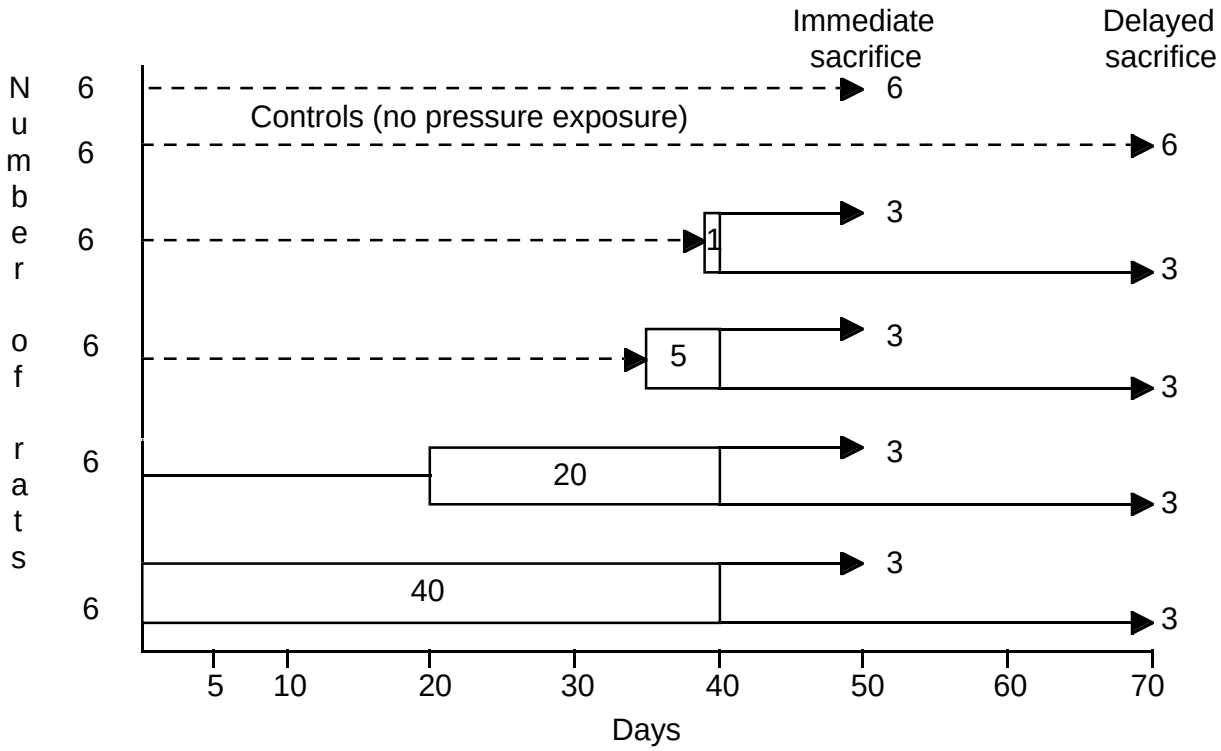

Figure 2. Periods of exposure and sacrifice times

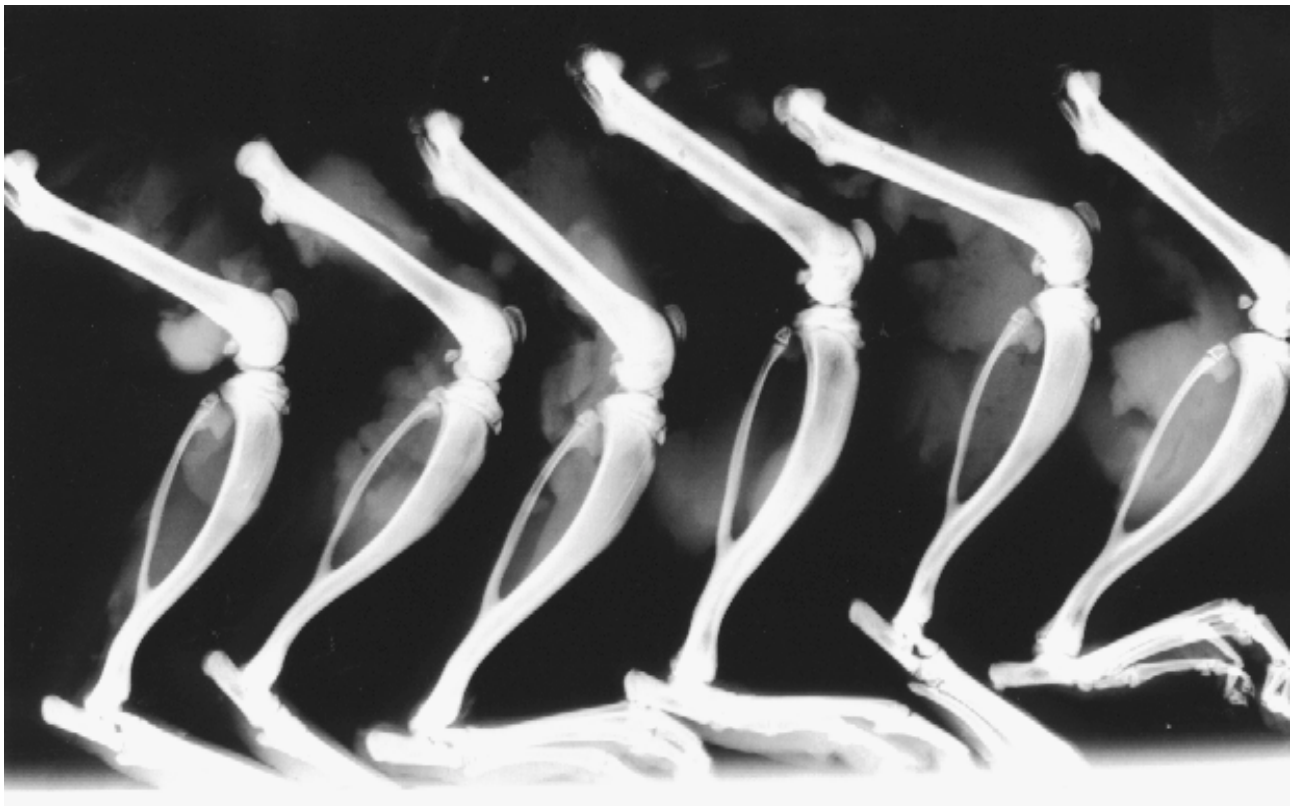

Figure 3. Radiographs of lower limbs of rats

magnification. Growth plate measurements were made using a high resolution Hitachi video camera connected to an Olympus BH-2 microscope and a MACIIVX equipped with a Scion Framegrabber. Morphometric measurements of the growth plate were made using NIH Image Software. The thickness of the growth plate for each section was measured in five different regions and averaged for each sample.

Data was statistically analysed using Statistical Analysis Software (SAS, Cary, NC, USA). A general linear model of an analysis of variance (ANOVA) was used to detect any difference between the groups.

Results

There were no signs of decompression illness. No rats developed a limp or any paralysis. The most sensitive indicator of stress in rats is their appetite and hence their weight. The rats in the pilot study and in the main experiment showed no differences in weight gain when compared with controls.

Tibial lengths and average thicknesses of the growth plate did not reveal any significant differences amongst the samples. The F ratio for group 1 and group 2 did not reveal any differences confirmed by post-hoc multiple comparisons.

Although no statistical differences were detected sample sizes were small. More sensitive methods such as electron microscopy or sophisticated histochemical techniques might have been able to detect subtle differences.

The slides were studied in detail by a pathologist with a special interest in growth plate pathology. The chondrocytes and surrounding matrix of the growth plate were normal. There was no evidence of haematopoietic cell necrosis, fat cell necrosis or osteonecrosis in the adjoining metaphyseal bone. No differences were found between any of the groups and the controls.

The X-rays were examined by a senior radiologist experienced in reading radiographs of rat bones. Several areas of questionable lysis, coarsening of trabeculations and some areas of sclerosis were seen, these were also present in the control groups, and assumed to be normal variants. There were no conclusive radiological findings.

Figure 4. Histological section of growth plate

Discussion

The proximal tibia of the Sprague Dawley rat is often used for quantitative histological study. Histomorphometric data from rats have usually proven an excellent predictor of human skeletal behaviour.[14] It has been shown by exposure of mature mice to hyperbaric pressures, that the proximal tibia is by far the most common region affected by osteonecrosis, the reasons for this being unknown.[8]

Despite adherence to strict diving protocols, osteonecrosis still occurs. In the Western world children are permitted to dive recreationally from before the age of 14 years, an age when rapid growth commonly occurs. This has led to concern regarding possible growth disorders amongst the recreational diving community. There has also been no questioning of the possible side effects of hyperbaric pressures for medical treatment. Only through well controlled studies will these questions be answered.

The rats used in this study were all exposed to well controlled realistic hyperbaric pressures outside the usual limits of recreational diving, which is limited to no-decompression diving. It was not our aim to produce decompression illness by exceeding known safe limits. All of the rats were the same age and sex making direct comparisons possible. It is our conclusion that exposure of immature rats to our hyperbaric pressure protocol failed to affect their interstitial or appositional growth. This finding answered our first quetion. As the answer was "No" the other three questions could not be answered for lack of dysbaric changes.

The physiological control mechanisms of growth at the epiphysial plate, especially the role of oxygen, are not yet fully understood. More studies are required to further our knowledge on this subject.

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THE WORLD AS IT IS

WAS IT DCS?

Russ Gately

Key Words
Biology, envenomation, decompression illness, marine animals, treatment

Our company was contracted to bury a submarine power cable running from Surabaya to Madura Island in Indonesia. The work started in late August 1996 and continued until early November. The diving crew was all commercial divers, with the least experienced member having 5 years in the business. Two members of the crew were diving medical technician (DMT) trained.

The work entailed setting up a jet sled on the cable, which was then pulled along the length of the cable and cut a trench, into which the cable settled. Visibility was zero throughout the job. There were strong currents which necessitated planning all diving operations to coincide with slack water periods.

On completion of a dive to check the progress of the operation, one diver complained of unusual sensations, described by him as "like electrical shocks".

The onset of these symptoms was within 20 minutes of surfacing and, over the next hour, the symptoms extended to involuntary muscle contractions, pins and needles in the hands and feet, general overall pain and nausea. These symptoms were treated as DCS related. The diver was put on oxygen and transported to the hyperbaric facility at the Surabaya Naval Hospital.

As the diving supervisor, I was somewhat at a loss to explain why this diver should have DCS symptoms as the dive to 26 m for 18 minutes was well inside the no-decompression limit. The dive was routine with normal ascent and descent rates. The only incident was a minor jellyfish sting to the face while he was undressing. The diver complained that the sting was painful and a small welt was evident on his top lip. This was treated with vinegar and the pain and welt disappeared within 15 minutes.

On arrival at the hyperbaric facility the diver was seen by an Indonesian Navy doctor who had studied diving medicine at Aberdeen in the UK. After a brief consultation he was put into the chamber and a Table 5 was initiated. On arrival at 18 m he reported that he felt better but the "electrical shock" feeling was still present. On ascent to 9 m all the symptoms returned, however the table was continued without extension. The diver was admitted to the hospital on completion and given daily Table 5 treatments.

While all this was going on the diving work continued. Two days after the incident, I carried out a dive to check the sled. The position of the sled was marked by a buoy-line which was used as the downline with the dive boat secured to it. The dive was to 26 metres with a bottom time of 12 minutes. While I was ascending the down line I was stung by jellyfish tentacles which were entangled around the line by the current. The initial sting was to the back of