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# DYSBARIC OSTEONECROSIS (CAISSON DISEASE OF BONE): ARE ACTIVE OXYGEN SPECIES AND THE ENDOCRINE SYSTEM RESPONSIBLE, AND CAN CONTROL OF THE PRODUCTION OF FREE RADICALS AND THEIR REACTION PRODUCTS CONFER PROTECTION?

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The development of osteonecrosis after exposure to altered air pressures is consistent with cellular injury brought about by active oxygen species. The syndrome is considered to arise as a result of an unusual combination of circumstances in which hyperoxia itself, together with the additive responses of the endocrine system to hyperoxia, hypothermia and exertion, each appear to play a part; the net result is thought to increase the mitochondrial generation of superoxide. It is suggested that effective prophylaxis may be possible primarily by establishing a nutritional status that is adequate to ensure that the functional activities of radical-scavenging systems are not hampered by deficiencies either of essential trace elements or of vitamin E. Pharmacological pretreatments designed both to decrease excessive levels of superoxide through increased catalysis of anionic dismutation and to attenuate enzyme-dependent peroxidation may provide an additional line of defence.

KEY WORDS: dysbaria, endocrine system, free radicals, hyperbaric oxygen, hypothermia, mitochondrial injury, osteonecrosis, trace element deficiency.

## INTRODUCTION

The earliest observation that necrosis of the long bones may develop following exposure of workmen to compressed air probably dates back for almost a century[1] (see[2–4] for reviews). Osteonecrosis is also encountered in divers,[3–7] more particularly after exposure to greater depths.[5,6] From 1975–1979 the incidence of the lesion in divers appeared to be increasing,[6] a situation to which improved diagnostic technique may have contributed, but the condition is much more commonly encountered in men who have worked in compressed air.[8,9] The development of bone necrosis depends both on the pressure to which individuals are subjected and on the time of exposure.[6,10] Although the association of Type I decompression sickness, commonly known as the bends, with the subsequent development of osteonecrosis is strong, the correlation is not absolute;[5–7,11] not only has necrosis been subsequently encountered in only a small

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proportion of men who claimed to have experienced decompression sickness,[5,6] but the lesion has also been recorded in individuals with no history of the bends.[6,7] In consequence, controversy continues to surround attempts to explain the origin of the tissue injury. More recently, however, a key role for oxygen in the development of the condition has been advanced in terms of injury to fat cells of the bone marrow resulting in cellular swelling;[12] a detailed analysis of the underlying events which are thought to occur is provided below.

## OXYGEN RADICALS AND CELL DEATH

The unique central role played by oxygen in the initiation of cell death has received increasing recognition over the past 10–15 years.[13–22] The key steps in the production and control of active oxygen species have been reviewed.[15–17,22] The events leading up to cellular injury and death are considered to involve oxygen activation, lipid peroxidation, and interference with energy production.[14] The sequence of morphological changes accompanying these events includes slight clumping of chromatin, distortion and blebbing of the plasma membrane, swelling of mitochondria, condensation of the inner mitochondrial compartment, and an increase in cellular volume associated with sodium entry.[13,14] The suggestion has been made that the moment of cell death coincides with the onset of irreversibility of high-amplitude mitochondrial swelling.[14]

Major parts in the protection of biological systems against injury caused by free radicals derived from oxygen are played by vitamin E and by certain trace elements essential for the activities of enzymic scavengers. In eukaryotic cells the mitochondrial form of superoxide dismutase is mostly manganese-dependent, while the cytosolic form mostly contains copper and zinc.[17,23] Peroxidised fatty acids, which disrupt mitochondrial energy production by uncoupling oxidative phosphorylation,[24,25] and hydrogen peroxide are decomposed by glutathione peroxidase,[26] which contains selenium.[27]

## FREE RADICAL FORMATION IN HYPEROXIA

In the rat, studies of mitochondria isolated from liver,[28,29] heart[30] and lung[31] all indicate that the yields both of superoxide[30] and of hydrogen peroxide[28,30,31] increase as the oxygen tension is raised. Some degree of protection for the lung against the toxic effects of increased amounts of superoxide and hydrogen peroxide resulting from exposure to 1 ATA oxygen is provided by the intravenous injection of liposomes encapsulating superoxide dismutase and catalase.[32] Superoxide dismutase activity was 44% lower in the lungs of copper-deficient rats than in those of animals receiving adequate amounts of the element; the copper-deficient group exhibited increased mortality and enhanced pulmonary toxicity at elevated partial pressures (0.85 and 4 ATA) of oxygen.[33] Lung injury in terms of morphological damage[34] and depressed ATP concentrations[34,35] has been reported after exposure of animals to 1–2 ATA oxygen for periods in excess of 18 h, yet evidence of lipid peroxidation in the form of an increased tissue content of material reacting positively with thiobarbituric acid (TBAM) was not seen.[34,35] In contrast, exposure to higher pressures (3.7 ATA) of oxygen for 4–6 hr served to increase the TBAM concentration in the brain.[36]

Decreases in the ATP contents of brain, liver and kidney were described after 90 min exposure to oxygen, but a pressure of 5 ATA was necessary before all three organs were affected.[37] In the heart reports of increased levels of conjugated dienes and depletion of unsaturated fatty acids after exposing rats to hyperbaric oxygen for 26–29 hr have appeared.[30] Over periods of time of up to 15 min shifts in the redox state of pyridine nucleotides towards oxidation occur *in vivo* in liver, kidney[38,39] and brain,[39] but in these experiments the oxygen pressure was 9 ATA; no comparable changes were seen at 1 ATA.[39] However, hydrogen peroxide levels in liver appear unchanged *in vivo* over the range 0.2–6 ATA oxygen.[29]

Extrapolation of these findings to man is very difficult. Certainly the toxic effects of elevated oxygen tensions can extend beyond organs coming into immediate contact with the atmosphere, such as lung, blood and eye, and include damage to liver, kidney, heart and brain (see 15, 16 for detailed reviews). A net rise in free radical production as a straightforward response to hyperoxia seems likely, provided that in sensitive tissues the oxygen tension does indeed rise as the external partial pressure of the gas is raised, and in addition that corresponding increases in oxygen activation occur. Careful distinction should be made between primary oxygen toxicity and secondary effects arising from impairment of lung function.

## THE ENDOCRINE SYSTEM IN FREE RADICAL GENERATION AT ELEVATED PRESSURES

Up to now involvement of the endocrine system in the development of dysbaric osteonecrosis has not been suspected, but in recent years evidence to support this view has been accumulating. Studies both in animals and in man suggest that superoxide production as well as lipid peroxidation may increase as a consequence of the conditions to which divers especially[40,41] and compressed air workers are necessarily exposed. In man, plasma levels of noradrenaline are elevated in response to exposure to cold air[42,43] and to air at 4 ATA pressure during submersion in water at 25°C.[44] In addition, sharp rises in the plasma concentrations of both catecholamines, especially of noradrenaline, occur during exercise.[45] Physical training, however, attenuates these increases,[45] which may therefore be less pronounced in men accustomed to performing prolonged manual work.

In rats, the production of superoxide by rat liver mitochondria increases in response to noradrenaline,[46,47] rising to a peak 4 hr after intraperitoneal injection;[47] thyroxine given 1 and 2 days previously also enhances superoxide generation.[46] Exposure to cold produced a similar but less marked effect in the same system.[46] Levels of TABM, taken to indicate lipid peroxidation, were found to be elevated in livers from rats previously chilled to near-freezing in a physically-restricting environment.[48] In addition, bilateral adrenalectomy decreased the TBAM content of rat brains both in untreated animals and following whole-body exposure to 3.7 ATA oxygen for 4–6 hr.[37] The inferences are drawn that both elevated circulating levels of noradrenaline resulting from hypothermia and hyperoxia, as well as normal background concentrations of catecholamines, appear to furnish significant contributions to free radical generation and lipid peroxidation in certain body tissues, and that similar changes in the fat cells of bone marrow may be sufficient to initiate local cellular injury.

The role of thyroxine in the generation of active oxygen species is less easy to assess. The information available refers almost exclusively to the rat. Oxygen toxicity is

enhanced by thyroxine[49,50] and by thyroid preparations,[50,51] and is diminished as a result of depressing thyroid activity,[51] of thyroidectomy,[49] or of hypophysectomy.[50] Sustained exposure to hyperbaric oxygen for several days eventually lowers circulating thyroxine levels by about a quarter,[52] but the conditions employed would be unacceptably severe in man. Although hypothermia increases circulating levels of thyroxine in the blood,[53] both hypothermia and noradrenaline stimulate the rate of deiodination of thyroxine,[54] and the net effect can be to depress plasma concentrations of thyroxine.[55] In quantitative terms the overall contribution of thyroxine to increased free radical generation under conditions of hyperoxia and hypothermia is certainly unpredictable, and may prove to be relatively minor.

## FREE RADICALS IN HYPOBARIA

Although aseptic bone necrosis may arise as a consequence of exposure to hyperbaric oxygen,[2–10] it has also been described as occurring in individuals exposed to air pressures of 0.09 ATA or less[56] and in aviators flying at high altitudes without the benefit of pressurised cabins.[57] Such incidences are extremely rare, and may be due to causes[58] other than hypoxia. If genuine, such cases seem hard to reconcile with an hypothesis based on the concept of cell injury and death brought on by augmented oxygen activation resulting partly from hormonal responses to an increase in oxygen tension and partly to the increase itself. However, one common factor which may be encountered at high altitudes as well as in diving is hypothermia, the effects of which on plasma noradrenaline in man[42–44] and on lipid peroxidation in the rat[48] are discussed above.

Whereas circulating levels of certain hormones may alter in response to hypoxia, the available data call for cautious interpretation. In three studies[59–61] hypoxia (0.11–0.14 ATA oxygen) in the short term had no significant effect on plasma concentrations of noradrenaline, but rises were recorded under the same conditions during exercise.[59,61] In contrast, urinary excretion of noradrenaline rose at altitudes corresponding to 0.12–0.13 ATA oxygen,[62–64] rises being noted on the second[62] and third[64] day. Small increases in plasma thyroxine have also been described under similar conditions;[64–66] measurements were made at intervals of a day or more. The basal consumption of oxygen increased by 15–20% within 24 h of transferring subjects from 1,600 m (0.16 ATA oxygen) to 4,300 m (0.115 ATA oxygen); the basal rate gradually fell back towards normal after a few days.[64]

The difficulty in relating the actuality of hypoxia to these findings[59–66] is that periods of exposure for aviators are of the order of h rather than min[59–61] or days.[62–66] The modest extents of the physiological responses may not be unconnected with the rarity of the lesion following hypobaric exposure.[56,57] Aseptic bone necrosis can also arise at normobaric pressures of oxygen in response to a variety of stimuli, not all of which have been identified,[58] but some of which are associated with increased free radical activity (for example, iron intoxication, irradiation, thermal injury or trauma) or endocrine disturbance (for example, phaeochromocytoma). It is therefore conceivable that the reported lesions[56,57] developed as a result of the aggravation by hypobaric exposure of an existing predisposition.

Nonetheless, both in the rat and in man free radical generation can be stimulated by hypoxia. Evidence of augmented lipid peroxidation has been found in the serum, aortic wall and brain of rats kept in 0.15 ATA oxygen for 2 weeks; peroxidation

increased still further in animals deficient in vitamin E.[67] Small increases in serum TBAM were found in men exposed to −15°C for 30 min at altitudes corresponding to oxygen pressures of 0.08 and 0.16 ATA, but no changes were seen in individuals who had been previously adapted to these conditions.[68]

## DISCUSSION

The importance of preventing cellular injury in the form of an uptake of fluid by those cells encased in the matrix of the long bones appears to be particularly relevant to the development of osteonecrosis.[12,69] Bone is unable to compensate for cellular swelling by structural alteration; the net effect of an increase in the volume of cells situated in the marrow cavity will be to decrease the diameter of the blood vessels supplying the inside of the bone, and blood flow will consequently diminish.[12,69] For the majority of cells situated elsewhere in the body, the problem does not arise.

Various aspects of the development of the lesion have been considered in detail by Walder and his associates.[11,12,69,70] Blood flow through the marrow of the femurs of rabbits[69,70] and miniature swine[11] decreased during exposure to hyperbaric air and to subsequent decompression; no comparable effect was seen in rabbit gastrocnemius muscle under the same conditions.[69]

The identity of the cell types undergoing injury in the bone may have a critical bearing on the problem for two reasons. The long bones of the adult human contain a high proportion of fatty marrow;[2,69,70] early measurements put the fat content as high as 96%.[71] Fat cells isolated from the epididymal fat pad of the rat have been exposed to various gas mixtures, including air and pure oxygen, at 6 ATA pressure. Increases in cellular volume were only recorded with those mixtures in which the partial pressure of oxygen exceeded 0.2 ATA.[12] Although oxygen tension within the circulatory system falls continuously as removal from arterial blood and utilisation proceed, the amount of dissolved oxygen in blood leaving the lungs may rise in response to hyperbaria. Uptake of oxygen by fatty tissues in the immediate vicinity of capillaries may be enhanced in consequence, especially as the solubility of oxygen in oils and in fat at 37°C is over four times greater than the solubility in water.[72,73]

Second, the $pK_a$ of the protonated form of superoxide ($HO_2\cdot$, the hydroperoxy radical) is 4.7;[74] at a physiological pH (7.4) only 0.2% of superoxide is present as $HO_2\cdot$. However, superoxide decay, with or without lipid peroxidation, occurs more rapidly when the physical state of fatty acids alters from a dispersed to a micellar form;[75] the likelihood that hydroperoxy radical formation is favoured by the kind of environment provided by fat cells cannot be excluded.

Detailed consideration of the sequence of events that links dysbaric exposure with the development of bone lesions, including assessment of the relative importance of cellular injury arising primarily from the cytotoxic effect of active oxygen species or from secondary hypoxia as a consequence of impairment of blood flow, lies outside the area of available information. While each of the various influences considered here might not lead separately to the development of dysbaric osteonecrosis, the critical factor in the causation of the syndrome may be the unusual combination of circumstances present during diving and working in compressed air. Moreover, the length of the period elapsing between the causative episode and the development of definite bone lesions, which can vary from 4–12 months[3,4] or longer,[4] is substantial in terms of the usual progress of necrosis, and appears to indicate a progression of damage in which two phases, the one acute and the other secondary, can be distinguished.

## PREVENTIVE MEASURES

Osteonecrosis is an irreversible degenerative condition for which the optimal measures would be effective forms of prophylaxis. Ideally, prophylaxis should extend not only to the control of sites of formation of active oxygen, but also to the detoxication of cytotoxic lipid peroxides. The value of adequate nutrition should not be underestimated as a critical first line of defence. Only a proportion of workers at risk actually develops bone necrosis;[3,6–8,10] in some of these individuals dietary intakes of trace elements essential for the provision of adequate functional levels of superoxide dismutases (copper, zinc, manganese) and glutathione peroxidase (selenium), as well as of vitamin E, may not have been adequate. Support for this idea comes from various studies in which the activity of the copper–zinc form of superoxide dismutase fell as a consequence of copper deprivation (cf. Reference 33). In man, for example, superoxide dismutase activity in erythrocytes was below the normal range in copper-deficient subjects.[76–78] Lower levels of enzymic activity were also recorded in certain organs of mice which had been deprived of copper; of all the tissues studied, by far the greatest decrease (83%) was recorded in the bone marrow.[79] Less information is available with regard to other trace elements and to vitamin E. Reports of untoward effects in bone marrow as a consequence of zinc or manganese deficiency have not been traced, although manganese deprivation from birth in young rats resulted in a lower activity of the manganese-dependent form of the enzyme and increased lipid peroxidation in liver mitochondria.[80] In Keshan disease, a syndrome caused by selenium deficiency,[81] examinations of the long bones and of bone marrow were not described in postmortem reports.[82] However, changes suggestive of delayed erythrocyte maturation have been described in the bone marrow of pigs simultaneously deficient in selenium and vitamin E.[83]

Theoretical considerations may prompt further modes of preventive treatment, but the newness of the concepts described in the present communication emphasizes the tentative nature of the suggestions. One area for preventive treatment might include the scavenging of superoxide by conversion to hydrogen peroxide. Beneficial effects in terms of increasing survival against radiation damage,[84,85] burn shock[86] and endotoxin[87,88] have been claimed by treating rodents with the copper–zinc form of bovine superoxide dismutase, but both its rapid clearance from the bloodstream following intravenous injection, as shown by the rat,[32] and its potential antigenicity point to the unsuitability of the procedure in man. However, superoxide dismutating activity has been reported in a number of water-soluble complexes of copper with organic acids, bases and drugs[89–91] including diisopropylsalicylic acid,[91] but the usefulness of these agents in man awaits conclusive demonstration. In addition, if it can be demonstrated that peroxidative attack on bone marrow lipid is enzymically mediated, pretreatment with an appropriate non-steroidal anti-inflammatory agent might attenuate the resulting injury.

Last, attempts to diminish the increase in circulating noradrenaline levels seen in response to forms of stress[42–45,48] by prior adrenergic blockade may appear attractive as a further way of protecting bone marrow from hyperoxic injury. This view is strengthened by the finding that pretreatment of rats with certain adrenergic blockers effectively stops the increase in superoxide generation brought about by noradrenaline.[47] On the other hand, adrenergic blockade can interfere with the maintenance of body temperature by men exposed to cold,[42] and the possibility that medication along these lines might magnify the risk to divers from hypothermia to a dangerous extent[41,42] ought carefully to be considered.

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