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THREE VIEWS OF HIGH PRESSURE OXYGEN

Ed Salzman

Today I would like to tell three stories. They are on separate topics, all related to diving and relate to three areas of recent research activity of great interest to me. I will tell these stories simply by giving the theme then by describing how the theme came to be understood. Then I will comment on my understanding of what each of these topics means in diving medicine.

Topic number one has to do with aerobic performance in men subjected to greatly increased atmosphere pressures. A series of six dives has been performed to beyond five hundred metres in a hyperbaric chamber with the greatest pressure exposure at 68 atmospheres. The following observations relating to aerobic performance were made.

Firstly the individuals involved have been able to perform substantial exercise, albeit less than they could at the surface.

Secondly the maximal levels of ventilation that were possible were less than would be expected breathing denser gas at great depths, but they did out perform, by some 15-20%, the ventilatory levels that would have been expected from square root calculations on gas density.

Thirdly, and this is very important, the oxygenation of arterial blood was essentially appropriate, and correspond clearly to the inspired partial pressure of oxygen. They were breathing approximately 0.4% oxygen at 66 atm, giving a PO2 of inspired gas of approximately 354 mmHg and the PO2 measured in arterial blood was appropriate. Despite the normal oxygenation of arterial blood, the most interesting observation to me was the level of exercise under conditions that were otherwise typical for surface experiments.

Oxygen uptake was less, arterial pH was lower and the levels of lactic acid in the blood were higher. These observations can be interpreted most speculatively and most interestingly by the concept that these very greatly increased ambient pressures something happens to either the microcirculation or to intracellular metabolism, so that there is some impairment of aerobic metabolism or of oxygen transfer within the microcirculation. That is a speculation. One could also argue that blood vessels and cell membranes become more pervious to lactic acid and the higher concentrations in blood simply reflect a different distribution rather than a different level of production.

At any rate, this is an intriguing indirect observation. I leave it with you to think about. It may or may not relate to such problems as the high pressure nervous syndrome. But there clearly are some events that are occurring with aerobic metabolism at depth perhaps due to the pressure of the gas breathed that we are yet to sort out.

Topic number two again deals with oxygen. Very recently, two of my very bright young colleagues have performed an experiment which clearly proves to me that oxygen toxicity is very complicated in the mammalian systems. Oxygen toxicity is due to free radicals. They have in the same experiment also satisfied me that for the first time oxygen toxicity has been prevented in an animal model, in a specific rather than unspecific manner, with total prevention of biochemical or morphometric injuries to the lungs and to the brain. Now that has caught your attention, I will tell you about the experiment, and the problem.

The background comes from work begun about fifteen years ago that in the course of biologic oxidisation free radicals were formed, and that these free radicals, which were clearly known to be capable of destroying the architectural and metabolic integrity of cells, were blocked by both non-specific and specific mechanisms within the cells. They elucidated some of these specific mechanisms, with the emphasis upon superoxide dismutase, an enzyme occurring naturally in the cell that clearly prevented, under appropriate circumstances, the evolution of oxygen toxicity that would otherwise occur. They showed that in microorganisms that did not tolerate oxygen these defences were lacking, and that organisms that tolerated oxygen had these defences, and ultimately proved in a very unambiguous manner that the model for oxygen toxicity also applied in the species that do not have nucleii. Subsequently in another series of experiments, suggestive evidence accumulated gradually that free radical formations did in fact lead to oxygen toxicity in complicated mammalian systems, including man.

This work was morphometric and biochemical, to a great extent indirect. One of the very troubling features of the work was that if someone tried to prevent oxygen toxicity, be it in rats or guinea pigs, by infusing the specific defence enzymes such as superoxide dismutase and catalase into the blood, more often than not they were not able to prevent the evolution, in the shorter or longer interval, of oxygen toxicity. My colleague pointed out that these enzyme structures were very large and that cell membranes were relatively impervious and that it was not reasonable to expect to protect a complicated mammalian system from oxygen toxicity by delivering a defence mechanism outside the intracellular milieu where it was needed. And thereupon a very bright young biochemist conceived a marvellous experiment and has recently published preliminary data on it. He was quite familiar with the lysosomes. For those of you who do not know very much about them, as I did not a few months ago, lysosomes are artificial biomembranes which can be shaped like envelopes. One can put a specific enzyme or medication in these. They can be infused into the bloodstream, and when the artificial lysosome-enzyme envelopes abut against the endothelial lining of cells, they empty their contents into the cell. So a lysosome is essentially a postal service, which can supply anything one chooses to supply and deliver to cells that are within reach of the microcirculation. It is a system that overcomes the barrier to the delivery of large molecules from the extravascular compartment and the vascular compartment to the intracellular compartment.

Now, the experiment that he performed was a very simple one. We took a rat model that would die within sixty-five hours with exposure to one atmosphere of oxygen. And in which the parameters, by morphometric functional analysis and by chemical analysis had been very clearly demonstrated in both the lungs and the brain. He put into different models nothing, or superoxide dismutase or

catalase or he put in superoxide dismutase and catalase. What he found was that the animals that had essentially empty lysosomes died at sixty five hours. The animals that had superoxide dismutase lysosome delivered by this postal service lived longer and had a little less injury. The animals who had either catalase alone or catalase and superoxide dismutase by the lysosome system lived considerably longer, three times as long. Now that he has improved his system in terms of technical manufacture of the delivery system he has an animal model in which these rats live indefinitely, on 100% oxygen. There is no identifiable morphometric biochemical injury in the lungs or the brain in settings where otherwise one would expect this to occur.

I find this a tremendously exciting piece of work. I do not think that you can rush out and plan to administer these things so that Royal Australian Navy divers can work to five hundred feet breathing pure oxygen without injury, but this represents a very important breakthrough in an area of science that has always interested me because it is the experiment that really, for me, ties together the relationship with free radical formation to the induction of oxygen toxicity. It is, I believe, the first specific rather than non-specific implementation of an absolute defence against oxygen toxicity in the intact animal.

Topic number three is a very quick look through intracellular oxygen availability and utilization. In the past thirty years, work has gradually progressed in the development of methodology for studying oxygen transport to the cells and aerobic metabolism. We still do not know a great deal about it because the work is technically difficult and has evolved slowly. But we know a lot more now than we did three years ago.

We have progressed over the period of a generation and a half from a capacity to study mitochondrial enzymes, the users of oxygen in the body, in the test tubes by themselves, through the capacity to study these mitochondrial enzymes in situ in the intact animal in an experimental situation, to a methodology by which one can now study elements in intracellular mitochondrial metabolism non-invasively in an intact man. So this is a very exciting area in which to work.

The kinds of parameters that can be studied include the redox state Cytochrome AA3, the relative volume of haemoglobin in the surveyed field, as an analogue of the perfusion, and the oxygenation of haemoglobin within the surveyed field, and in the invasive preparation where fluorescence techniques can be applied to actual tissue tension of oxygen.

There are three observations that came out of this work that I think are of considerable interest to diving medicine. Observation number one is that in the intact animal full oxidation of the terminal mitochondrial enzyme cannot be accomplished at sea level. In order to fully oxidise cytochrome oxidase in a series of animal models, the requirements are approximately 3.7 atm of oxygen and 3% CO2.

Observation number two is that if one delivers oxygen to the bloodstream with an oxygen pressure of perhaps 2000 mmHg, one cannot at all assume that comparable deliveries of oxygen are occurring within the cells because in the intact animal there are tremendous adaptive mechanisms, notably including in the most studied system, the brain, the capacity for the microcirculation to constrict and for perfusion to fall. So that in a model of a cat's brain studied optically by the techniques that I am alluding to, at a PO2 of 2000 mmHg in arterial blood, if the circulatory system of the brain is clamped by hypocapnia, the PO_2 measured in the tissue may be as low as 130 mmHg. If, on the other hand, the microcirculation is unclamped by inducing hypercapnia and increasing blood flow tremendously then the PO_2 in the brain tissue can rise to 1500 mmHg, with a constant arterial PO_2 of approximately 2000 mmHg.

If one thinks about these things it is not terribly surprising, but what I am describing is the methodology that has been difficult to evolve, that in the past year is on firmer ground theoretically in terms of the lack of ambiguity of the measurements than ever before. A methodology which can indeed be adapted, not only to try to answer intriguing questions that people we work with in the laboratory like to ask, but methodologies that are capable of being miniaturised and employed non-invasively by a pilot with negative G-forces coming out of a dive, or in the diver to look at oxygenation and aerobic function of the central nervous system. Of course there is much more to say on these topics but time has run out.

Question
How does one make a lysosome and how often does one have to dose the animal with this?

Dr Salzman
I do not have the technical knowledge on how to make them, but they are not hard to make, and they are biologically safe. They are in fact approved by our American FDA, Federal Drug Administration, so you could use lysosomes as a method of delivering medication, benign and approved.

Question
How often were you dosing the rats?

Dr Salzman
I think he was doing it about every 48 hours. He has published one of his experiments.

This is an edited transcript of Dr Salzman's address. Any errors are the responsibility of SPUMS editorial staff and not Dr Salzman.

OCTOPUSES KILL TWO BREATHHOLD DIVERS

A report from Kiribati, formerly the Ellis Islands, tells of the death by drowning of two spearfishermen hunting octopuses. The local method is to let the octopus cling to the diver, who then surfaces and kills it by a bite between the eyes. Apparently while attempting this procedure in the lagoon at Tarawa, the largest island, octopuses larger than usual were encountered, with a 3 to 4 metre tip to tip size. These large octopuses were able to resist the victims' attempts to surface.

Such larger animals have been labelled "killers". Mr Kirata, the Kiribati Natural Resources Minister, is quoted as saying, "We are going to have to find another way of killing octopuses."