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Intravenous Administration of Perfluorocarbon Emulsions as a Non-Recompression Therapy for Decompression Sickness

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INTRAVENOUS ADMINISTRATION OF PERFLUOROCARBON EMULSIONS AS A NON-RECOMPRESSION THERAPY FOR DECOMPRESSION SICKNESS

A Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

by

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List of Abbreviations

a ..... arterial

A ..... alveolar

AAALAC ..... Association for Assessment and Accreditation of Laboratory Animal Care

AGE ..... arterial gas embolism/emboli

ANOVA. ..... analysis of variance

AP ..... arterial pressure

ATA ..... atmospheres absolute

ATG ..... atmospheres gauge

C_aO_2 ..... arterial oxygen content

CCO ..... continuous cardiac output

CI ..... cardiac index (indexed to body weight)

CO ..... cardiac output

CO_2 ..... carbon dioxide

CSF ..... cerebrospinal fluid

$C_{\bar{v}}O_2$ ..... mixed venous oxygen content

CVP ..... central venous pressure

DCS ..... decompression sickness

$\dot{D}O_2$ ..... oxygen delivery per minute

DoD ..... Department of Defense

ER ..... oxygen extraction ratio

F_IHe ..... fraction of inspired helium

F_IN_2 ..... fraction of inspired nitrogen

F_IO_2 ..... fraction of inspired oxygen

fsw ..... feet of seawater

Hb ..... hemoglobin concentration

He ..... helium

I.D. ..... internal diameter

I.M. ..... intramuscular

I.V. ..... intravenous

IACUC ..... Institutional Animal Care and Use Committee

msw ..... metres of seawater

N_2 ..... nitrogen

NIH ..... National Institutes of Health

O_2 ..... oxygen

P_a ..... ambient pressure

PAP ..... pulmonary arterial pressure

P_b ..... bubble internal pressure

pCO_2 ..... partial pressure of carbon dioxide

PFC ..... perfluorocarbon

pH ..... negative logarithm of hydrogen ion concentration

pO_2 ..... partial pressure of oxygen

P_t ..... tissue pressure

P_y ..... pressure exerted by surface tension

r ..... radius

RGBM ..... Reduced Gradient Bubble Model

S_aO_2 ..... arterial hemoglobin oxygen saturation

$S_{\bar{v}}O_2$ ..... mixed venous hemoglobin oxygen saturation

$\bar{v}$ ..... mixed venous

VGE ..... venous gas embolism/emboli

$\dot{V}O_2$ ..... oxygen consumption per minute

VPM ..... Varying Permeability Model

List of Units of Measure

dL ..... decilitre

Fr ..... French

fsw ..... feet of seawater

G ..... Gauge

kg ..... kilogram

kPa ..... kilopascal

L ..... litre

m ..... metre

mg ..... milligram

mL ..... millilitre

mm ..... millimetre

mmHg ..... millimetre of mercury

psi ..... pounds per square inch

µm ..... micrometre

Abstract

INTRAVENOUS ADMINISTRATION OF PERFLUOROCARBON EMULSIONS AS A NON-RECOMPRESSION THERAPY FOR DECOMPRESSION SICKNESS

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A Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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Decompression sickness (DCS) results from a sudden decrease in ambient pressure leading to super-saturation of tissues with inert gas and subsequent bubble formation within both tissues and blood. Perfluorocarbons (PFC) are able to dissolve vast amounts of non-polar gases. The administration of intravenous (I.V.) PFC emulsions reduce both

morbidity and mortality of DCS, but the mechanism of this protective effect has not yet been demonstrated.

Juvenile Dorper cross sheep between 16 and 24 kg (n=31) were anaesthetized and instrumented for physiological monitoring, the administration of I.V. fluids and sampling of arterial and mixed venous blood. Animals were placed in a hyperbaric chamber and compressed to 6.0 atmospheres absolute for 30 minutes, then rapidly decompressed. Upon chamber exit animals were randomly assigned to receive 6cc/kg of either PFC or saline control over 5 minutes beginning immediately after chamber exit. They were also randomized to receive one of 4 breathing gases post-chamber: 100% O_2, 80/20 N_2/O_2, 50/50 HeO_2, or 80/20 HeO_2. Blood samples were drawn at 5, 10, 15, 30, 60, and 90 minutes to examine whole-body oxygenation. Respiratory gases were monitored and recorded in real-time using mass spectroscopy to examine nitrogen washout.

PFC administration increased arterial oxygen content (16.30±0.27 vs. 14.75±0.25 mL/dL, $p<0.0001$), oxygen delivery (14.83±0.28 vs. 13.44±0.25 mL/minute/kg, $p=0.0004$), and tissue oxygen consumption (3.37±0.14 vs. 2.76±0.13 mL/minute/kg, $p=0.0018$) over saline control, but did not increase mixed venous oxygen content (12.45±0.26 vs. 11.74±0.24 mL/dL, $p=0.0558$) or extraction ratio (0.23±0.012 vs. 0.21±0.011, $p=0.1869$).

PFC administration lowered the plateau of the curve, increasing the amount of nitrogen washout vs. saline control (22.22±1.566 vs. 15.98±1.380 mmHg, $p= 0.0074$). Breathing 80/20 HeO_2 increased the decay constant of the curve, increasing the rate of washout vs. breathing 100% O_2 (0.03176±0.001044 vs. 0.03096±0.0009402, $p=0.5777$).

PFC improves whole-body oxygenation after severe DCS and increases the amount of nitrogen washout. Although the effects of both PFC and 80/20 HeO_2 breathing were statistically significant the magnitude of the nitrogen washout effect is quite small, and unlikely to be clinically significant. Thus it is likely that the improved oxygenation is responsible for the previously-observed therapeutic effects of PFC in treating DCS.

CHAPTER 1

GENERAL INTRODUCTION

A Short History of Diving

Breath-Hold Diving

The first human entry into the world beneath the sea was performed using breath-hold diving. Exactly when and where this first took place has been lost to the ages, but there are archaeological claims that Neanderthals may have dived for food. We cannot be sure if they were diving, or rather were harvesting shellfish by wading at low tide(33, 125). We do know that by 4500 BC, diving had developed from limited and fear-filled excursions into a thriving industry that supplied the communities with shells, food, and pearls(33, 125).

From those early times until now fishermen have used breath-hold diving to harvest products from the sea as diverse as fish, shellfish, pearls, shells, and sponges. Breath-hold diving was the only method available to access anything deeper than a few feet beneath the surface up until the nineteenth century when helmet diving equipment was developed(33, 125). Breath-hold diving is still used today. Not only is it a recreational and competitive sport, but also used as a traditional fishing method by groups such as the Ama diving women of Japan and Korea, and pearl divers of the Tuamoto Archipelago(88, 125, 134). Originally the male divers were fishermen while the female divers collected shells and seaweeds that are important to Korean and Japanese cuisine. More recently diving duties have been restricted to the women while the men serve as tenders. This change has been

attributed by some to greater endurance of the women in cold water, while others pay homage to the traditional folklore that diving reduces the virility of males(134).

Diving also has a long history of being used for strategic purposes. Divers are known to have been involved in military operations as far back as the Trojan Wars (1194-1184 BC). Divers were used to sabotage enemy ships by boring holes in the hull below the waterline or cutting anchor ropes(33, 125). Divers were also used in the construction of underwater defenses to protect ports from attacking fleets(33, 125). Similarly, attackers would employ divers to remove obstructions.

In North America in the sixteenth, seventeenth and eighteenth centuries, skilled native divers were able to descend to depths of 30 metres and remain submerged for up to 15 minutes. The Spaniards exploited these native divers' abilities to harvest pearls, salvage sunken ships, and even to smuggle goods past customs(33, 125). Demand for such divers was very high, indicated by their value on the slave market where prices of up to 150 gold pieces were paid(33, 125)

Diving Equipment

The earliest diving equipment to emerge was the snorkel. These simple breathing tubes made from reeds or bamboo were developed in many different parts of the world. This simple device allows a diver to breathe air from the surface with his or her head underwater. Writings from Aristotle imply that the Greeks used snorkels, though exactly what for is not clear(33, 106, 107, 125). Columbus also reported that the North American natives used snorkels for hunting waterfowl. They would submerge themselves and breathe through a reed while approaching birds floating on the water, and were then able to capture

the birds with nets, spears, and even with their bare hands(33, 106, 107, 125). Similar methods were also used by the Australian aborigine to hunt wild duck. Snorkels, however, do have serious limitations. Respiratory dead space increases with snorkel length. In addition, deeper dives place a greater load on the respiratory muscles. Thus, snorkels are not useful deeper than 1-2 feet(33, 106, 107).

Once these limitations were noted, the snorkel spawned the idea of a diver carrying a gas supply with them. An Assyrian drawing dated 900 BC is believed by some to picture an early diving set(33, 107, 125). The drawing depicts a man with a tube in his mouth and the tube connected to a bladder or bag. That such diving sets were not in use centuries later suggests that either this arrangement did not work well, or that the above interpretation could be incorrect, and that what is depicted is, in fact, something more akin to a float or life preserver(33, 107, 125). Leonardo da Vinci also developed ideas for diving sets. His sketches depict not only breathing equipment, but fins as well(33, 106, 107, 125). In one sketch he illustrates a long snorkel, which would not have worked well. In another idea he wrote of, and sketched ideas for providing a diver with a 'wine skin to contain the breath'. This is most likely the first design of a self-contained breathing apparatus(33, 106, 107, 125). These sketches by da Vinci appear tentative, so it is most likely safe to assume that there was no practical diving equipment being used in Europe in da Vinci's time(33, 106, 107, 125).

Diving bells were the first successful method of allowing divers to remain at depths greater than could be reached with a snorkel and for durations longer than they could hold their breath. A diving bell consists of a chamber, weighted so it could sink and open at the

bottom, in which one or more people could be lowered under water. The early use of diving bells was limited to short durations in shallow water. The air contained within the bell would not support life for extended periods and its compression when submerged would limit the depth to which the bell could be lowered(33, 106, 107, 125). Use of this type of diving bell dates back to the mid-to-late 15th century(33, 106, 107, 125). A giant step forward was developed in 1691 by Sir Edmund Halley, after whom the comet is named, when he patented a diving bell which could be re-supplied with air in barrels(33, 106, 107, 125). Weighted barrels of air were hauled from the surface and the air could be released into the bell. This development led to the more widespread use of diving bells for tasks such as salvage, treasure recovery, and construction. Dives to depths of 20 metres for durations as long as 90 minutes were recorded using this apparatus(33, 106, 107, 125). Similar diving bells, developed earlier and which probably served as the basis for Halley's idea and patent, were used in Sweden for salvage. Between 1659 and 1665 divers were able to salvage 50 bronze canons weighing over 1000 kg each from the warship *Vasa* which sank in 30 metres of water in Stockholm harbour. The guns were recovered by divers in diving bells assisted by ropes from the surface(33, 106, 107, 125). That this was accomplished is truly amazing. It would be a difficult task now, even with the best of modern equipment.

During the second half of the 18th century air pumps were developed that were both reliable and powerful enough to pump air against the pressures experienced by divers(33, 106, 107, 125). These pumps led to the development of what are now called 'open helmets' for diving. In 1819 Augustus Siebe, a German coppersmith living in London,

developed a diving rig consisting of a copper helmet riveted to a leather jacket. Air would be pumped into the helmet, but there was no way to control the amount of air entering the suit. Excess air would simply bubble out of the jacket at the waist(33, 88, 107). Siebe's original suit was quite successful, and was used in the salvage of the British warship *H.M.S. Royal George*(33, 88, 107). Its greatest shortcoming was that if the diver should fall, or if the pump or hose should leak it would quickly fill with water, be difficult to get out of, and the diver would most likely drown(33, 88, 107). Siebe continued his innovative design of diving apparati, and by 1837 he had developed a waterproof, full-body suit which could be bolted to a breastplate and helmet(33, 88, 125). With the emergence of this suit the diver could safely work in any position because the entire body was enveloped in the suit. With the suit now being a closed environment, inlet and exhaust valves were incorporated to allow the divers to regulate their buoyancy. This design was so successful that it remains essentially unchanged today. The United States Navy Mark V deep sea diving suit, which was in use up until 1986, is almost an exact copy of Siebe's original diving suit, with some refinement in materials and improvement in the valves(88).

Following the development of the diving suit it became possible for divers to dive to ever greater depths, and to remain at such depths for longer durations than had been possible with previous equipment. These deeper, longer dives were not accompanied by what we would think of today as appropriate decompression, and decompression sickness (DCS) was being noted in more and more divers(88, 125).

Decompression Sickness

The first recorded instance of what would now be referred to as DCS was recorded in 1670 by Sir Robert Boyle. In what is now thought of as a landmark experiment in alternobaric physiology, Boyle placed a viper in a vacuum chamber and was able to generate symptoms of DCS. In his journal he noted "*I once observed a Viper furiously tortured in our Exhausted Receiver … that had manifested a conspicuous Bubble moving to and fro in the waterish humour of one of its eyes*"(12, 88). In this experiment Boyle recorded that a rapid decrease in ambient pressure could result in the production of gas bubbles in body tissues.

The earliest report of symptoms which are now believed to have been DCS in humans dates back to the 1840's. The British warship *H.M.S. Royal George* sank at the Spithead anchorage near the Isle of Wight, and the wreck was a danger to navigation. Divers were employed to clear the wreck, and Colonel Pasley, the commander of the operation, noted symptoms of rheumatism and over-fatigue in many of his divers. These symptoms were attributed to dampness and cold, so divers were provided with fresh, dry undergarments. Other divers suffered paralysis which was attributed to zeal and over-exertion(88, 125, 169). It is most likely that these men were suffering from DCS, especially given that their dives exceeded the now-accepted safe diving limits by a factor of 3(125, 169).

The first records of DCS as such in humans come from a French mining engineer named Triger. Coal mines below the level of the water table were pressurized with air in order to keep the water out. In 1841 Triger noticed that some of the miners suffered cramps

and muscle pains after leaving the compressed air environment of the mine(125, 169). By 1854 DCS was beginning to be understood. Pol and Watelle, two physicians in the employ of the Douchy Coal Company of Douchy, France began a systematic investigation of the symptoms experienced by caisson workers. They noticed that this disease was universally associated with leaving the compressed air environment, and noted that "*one pays only on leaving*"(88, 132). Pol and Watelle were also the first to notice that a return to the compressed air environment was effective in alleviating symptoms, and that younger men of 18 who had "*not reached their greatest mature physical strength*" suffered less from the symptoms of DCS than did men in their mid-30's "*who were in their prime*"(88, 132).

Over the next 30 years several small steps were made in the understanding of DCS. Boyle's original experiments were repeated in 1857 by Hoppe-Seyler, at which time he described the obstruction of pulmonary blood vessels by bubbles, and noted that the heart was unable to adequately pump blood under these conditions(77, 169). He also suggested that the intravascular release of gas bubbles he had observed might be the mechanism underlying some of the cases of sudden death observed in caisson workers and divers. Hoppe-Seyler was also the first to suggest recompression as a therapy for DCS(77, 169). In 1869, Le Roy de Mericourt, a French naval surgeon, reported on an occupational illness he observed amongst sponge divers in the Mediterranean which was attributed to the breathing of compressed air. He equated this with the illness observed in caisson workers and other divers(92, 125). The first review of compressed-air sickness research was conducted by Freidburg in 1872. He collected descriptions of symptoms of workers who received insufficient decompression after exposure to high pressure. He also compared the

clinical course of severe and fatal cases of DCS to that of the venous air emboli occasionally seen in obstetrics and surgery(125). He believed that rapid decompression resulted in the release of gas which had been taken up into tissue during the high-pressure exposure, and that the blood was filled with gas bubbles which interfered with circulation in the heart and lungs(125). Soon thereafter in 1873 Andrew Smith described what was becoming known as 'caisson disease' as a disease depending on increased atmospheric pressure, but always developing after reduction of the pressure(125, 144). This illness, he noted, was characterized by moderate-to-severe pain in one or more of the extremities and sometimes in the trunk. In some cases, there may be elements of paralysis which, when they appear, are most frequently confined to the lower extremities. Cerebral symptoms such as headache, vertigo, convulsions, and loss of consciousness may also be present(125, 144).

The first rigorous scientific study of DCS was carried out by the French physiologist Paul Bert and was reported in his seminal book of 1878 entitled *La Pression Barometrique*(7, 14, 88, 125). Bert was able to demonstrate that the symptoms of DCS were associated with bubbles in tissue, that these bubbles were formed during rapid decompression, and that these same bubbles consisted primarily of nitrogen(7, 88, 125). Bert also used various concentrations of oxygen to speed decompression, demonstrating the utility of oxygen breathing for the treatment of DCS, and was the first to propose the idea of oxygen recompression as a therapy for DCS(7, 125).

The now common slang term 'the bends' evolved during the construction of the Brooklyn Bridge. This term was first noted by the above-mentioned Andrew Smith in 1894

while engaged as the medical advisor for the Brooklyn Bridge caisson work. The fashionable ladies of the period assumed a stooped posture while walking termed the 'Grecian bend'. When the workers emerged from the caissons afflicted by DCS, limping and hunched over from the pain in their legs, hips, and back, their colleagues would chide them for 'doing the Grecian bend'(88, 125).

The next major leap forward in the understanding and treatment of DCS came from an engineer rather than a physician or researcher. In 1889, efforts were underway to drive railway tunnels under the Hudson River. When the British engineer E. W. Moir took over as project superintendent the death rate among all workers from DCS was 25%. He erected a recompression chamber at the work site. Any worker showing signs of DCS was quickly recompressed and later decompressed at a slower rate. Up until this time, even though it was understood that recompression was able to alleviate the symptoms of DCS, since exposure to compressed air had caused the disease in the first place, physicians were loathe to recommend recompression as a cure(88, 125). Although he admitted in descriptions of his own work that he believed that his treatment was largely homeopathic, mortality from DCS amongst his workers decreased. By 1896 the recompression treatment Moir insisted on had reduced mortality to a mere 1.6% from 25% observed prior to his arrival(88, 111, 169).

By the dawn of the 20th century it was understood that DCS was a product of nitrogen gas bubbles evolving within the body as a result of decreasing pressure after exposure to a high-pressure environment. It was also accepted that a return to increased pressure could relieve the symptoms, but there were no decompression schedules or

procedures that could be followed to reduce the risk of generating DCS in the first place(88).

Decompression Planning

Haldanian Theory

In the early part of the 20th century there was a great deal of controversy regarding exactly how divers and caisson workers should be decompressed(165, 169). It was generally believed that the best way to avoid DCS was to ascend to the surface at a slow, uniform rate. There was no consensus regarding what constituted an appropriately slow rate(1, 165). The *Royal Navy Diving Manual* specified an ascent rate of 5 feet/minute, while the French physiologist Paul Bert was advocating for an ascent rate of 3 feet/minute. The German physiologists Heller, Mager and von Schrotter recommended an ascent rate of 1.5 feet/minute(165). In spite of all these recommendations, DCS was still alarmingly common amongst divers and caisson workers(165).

The first step towards safe diving was a product of the work of the Scottish physiologist John Scott Haldane. His critical supersaturation ratio hypothesis formed the basis of the first decompression tables. Exactly how Haldane persuaded the Royal Navy to investigate his hypothesis is not known. Navy submarines were sinking, the divers salvaging them were at risk, and the little-known fact that his older brother was the Minister of War may have influenced the decision(4, 165).

By Haldane's time Bert had established that the signs and symptoms of DCS were often associated with nitrogen bubbles in blood and tissue(7, 165). He was also struck by the powerful impact that long exposures at depth had on the appearance of DCS. For

example a salvage diver working for the Siebbe-Gorman company salvaged £70,000 in gold bullion from a wreck sitting in 162 feet of seawater (fsw) over the course of 33 safe dives with 25-minute bottom times. For a single dive he extended his bottom time to 45 minutes. After this dive he developed paralysis from which he never fully recovered(165). Likewise another diver working for Siebbe-Gorman salvaged silver from several wrecks at depths ranging from 174 to 180 fsw with bottom times ranging from 8 to 13 minutes. After approximately 70 safe dives using this profile he extended his bottom time to 40 minutes. Upon surfacing he experienced great pain and ruptured blood vessels with blood accumulating under the skin. His condition was so serious that a priest was called and administered last rites, but he survived and was back working a week later(165).

These cases, and others like them, suggested to Haldane that a diver's tissues absorbed nitrogen in a time-dependent manner while under pressure as arterial blood carried dissolved nitrogen from the lungs to other body tissues. When the diver ascended and the pressure decreased some excess nitrogen in tissue could be safely tolerated, but too much would cause bubbles to form and result in DCS(165).

To examine what sort of time-depth combinations could be tolerated Haldane performed a series of experiments in goats. He exposed them to 2.36 atmospheres absolute (ATA) for 2 hours and then rapidly decompressed them to surface pressure (1 ATA). This pressure drop of 1.36 ATA produced DCS in some animals(55, 169). He also found that the same absolute decrease in pressure of 1.36 ATA from 6 ATA did not produce DCS, but rather a decrease of 3 ATA was required to cause DCS(55, 169). From this he deduced that DCS was not a product of an absolute decrease in pressure. Instead, he theorized, DCS was

caused by a relative decrease in pressure. More specifically he hypothesized that pressure could be decreased in a 2:1 ratio without causing DCS. Thus, if a dive was conducted to 6 ATA, the diver could safely ascend to 3 ATA where a stop needed to be made or if a dive were conducted to 4 ATA, the diver could safely ascend to 2 ATA where a stop needed to be made(55, 169). This is known as the 'critical ratio' supersaturation hypothesis and led to the idea of 'stage decompression'. From here, Haldane made some assumptions: First, that the uptake and elimination of nitrogen followed an exponential pattern. Second, that the body could be represented mathematically using 5 different 'tissue compartments' with nitrogen loading and unloading half-times of 5, 10, 20, 40 and 75 minutes. These 'tisue compartments' are purely mathematical constructs. They do not directly equate to any tissue in the body. From these data and assumptions Haldane devised the first dive tables. Haldane's 'Table I' was used for dives to depths of up to 204 fsw with decompression times up to 30 minutes – see Table 1. This table proved to be very successful and virtually eliminated DCS, but over time was found to call for decompression stops that were both deeper and longer than were necessary in practice(165, 169). Haldane's 'Table II' was used for dives with bottom times exceeding 1 hour requiring over 30 minutes of decompression. It was found to generate decompression schedules that were insufficient to prevent DCS(165, 169) – see Table 2.

Table 1: Table I of appendix IV from J. S. Haldane's 'The prevention of compressed air illness'(55)

STOPPAGES DURING THE ASCENT OF A DIVER AFTER ORDINARY LIMITS OF TIME FROM SURFACE.

Depth Feet	Depth Fathoms	Pressure Pounds per square inch	Time from surface to beginning of ascent	Approximate time to first stop	Stoppages in minutes at different depths* 60 ft.	50 ft.	40 ft.	30 ft.	20 ft.	10 ft.	Total time for ascent in mins.
0-36	0-6	0-16	No limit	-	-	-	-	-	-	-	0-1
36-42	6-7	16-18.5	Over 3 hours	1	-	-	-	-	-	5	6
42-48	7-8	18.5-21	Up to 1 hour	-	-	-	-	-	-	-	1.5
			1-3 hours	1.5	-	-	-	-	-	5	6.5
			Over 3 hours	1.5	-	-	-	-	-	10	11.5
48-54	8-9	21-24	Up to 0.5 hour	-	-	-	-	-	-	-	2
			0.5-1.5 hours	2	-	-	-	-	-	5	7
			1.5-3 hours	2	-	-	-	-	-	10	12
			Over 3 hours	2	-	-	-	-	-	20	22
54-60	9-10	24-26.5	Up to 20 mins.	-	-	-	-	-	-	-	2
			20-45 mins.	2	-	-	-	-	-	5	7
			0.75-1.5 hours	2	-	-	-	-	-	10	12
			1.5-3 hours	2	-	-	-	-	5	15	22
			Over 3 hours	2	-	-	-	-	10	20	32
60-66	10-11	26.5-29.5	Up to 0.25 hour	2	-	-	-	-	-	-	2
			0.25-0.5 hour	2	-	-	-	-	-	5	7
			0.5-1 hour	2	-	-	-	-	3	10	15
			1-2 hours	2	-	-	-	-	5	15	22
			2-3 hours	2	-	-	-	-	10	20	32
66-72	11-12	29.5-32	Up to 0.25 hour	2	-	-	-	-	-	2	4
			0.25-0.5 hour	2	-	-	-	-	3	5	10
			0.5-1 hour	2	-	-	-	-	5	12	19
			1-2 hours	2	-	-	-	-	10	20	32
72-78	12-13	32-34.5	Up to 20 mins.	2	-	-	-	-	-	5	7
			20-45 mins.	2	-	-	-	-	5	10	17
			0.75-1.5 hours	2	-	-	-	-	10	20	32
78-84	13-14	34.5-37	Up to 20 mins.	2	-	-	-	-	-	5	7
			20-45 mins.	2	-	-	-	-	5	15	22
			0.75-1.25 hours	2	-	-	-	-	10	20	32
84-90	14-15	37-40	Up to 10 mins.	2	-	-	-	-	-	3	5
			10-20 mins.	2	-	-	-	-	3	5	10
			20-40 mins.	2	-	-	-	-	5	15	22
			40-60 mins.	2	-	-	-	3	10	15	30
90-96	15-16	40-42.5	Up to 10 mins.	3	-	-	-	-	-	3	6
			10-20 mins.	2	-	-	-	-	3	5	10
			20-35 mins.	2	-	-	-	-	5	15	22
			35-55 mins.	2	-	-	-	3	10	15	30
96-108	16-18	42.5-48	Up to 15 mins.	3	-	-	-	-	3	5	11
			15-30 mins.	3	-	-	-	3	7	10	23
			30-40 mins.	3	-	-	-	5	10	15	33
.08-120	18-20	48-53.5	Up to 15 mins.	3	-	-	-	2	3	7	15
			15-25 mins.	3	-	-	-	5	5	10	23
			25-35 mins.	3	-	-	-	5	10	15	33
.20-132	20-22	53.5-59	Up to 15 mins.	3	-	-	-	2	5	7	17
			15-30 mins.	3	-	-	-	5	10	15	33
.32-144	22-24	59-64.5	Up to 12 mins.	3	-	-	-	3	5	5	16
			12-25 mins.	3			2	5	10	12	32
.44-156	24-26	64.5-70	Up to 10 mins.	3	-	-	-	3	5	5	16
			10-20 mins.	3	-	-	2	5	10	12	32
.56-168	26-28	70-75	Up to 10 mins.	3	-	-	2	3	5	5	18
			10-16 mins.	3	-	2	3	5	7	10	30
.68-180	28-30	75-80.5	Up to 9 mins.	3	-	-	2	3	5	5	18
			9-14 mins.	3	-	2	3	5	7	10	30
.80-192	30-32	80.5-86	Up to 13 mins.	3	-	2	3	5	7	10	30
.92-204	32-34	86-91.5	Up to 12 mins.	3	2	2	3	5	7	10	32

Table 2: Table II of appendix IV from J. S. Haldane's 'The prevention of compressed air illness'(55)

STOPPAGES DURING THE ASCENT OF A DIVER AFTER DELAY BEYOND THE ORDINARY LIMITS OF TIME FROM SURFACE

Depth Feet	Depth Fathoms	Pressure Pounds per square inch	Time fom surface to beginning of ascent	Approximate time to first stop	Stoppages in minutes at different depths 80ft	70ft	60ft	50ft	40ft	30ft	20ft	Total time for ascent 10ft	mins.
60-66	10-11	26.5-29.5	Over 3 hours	2	-	-	-	-	-	-	10	30	42
66-72	11-12	29.5-32	2-3 hours	2	-	-	-	-	-	-	10	30	42
			Over 3 hours	2	-	-	-	-	-	-	20	30	52
72-78	12-13	32-34.5	1.5-2.5 hours	2	-	-	-	-	-	-	20	25	47
			Over 2.5 hours	2	-	-	-	-	-	-	30	30	62
78-84	13-14	34.5-37	1.25-2 hours	2	-	-	-	-	-	-	15	30	47
			2-3 hours	2	-	-	-	-	-	5	30	30	67
			Over 3 hours	2	-	-	-	-	-	10	30	35	77
84-90	14-15	37-40	1-1.5 hours	2	-	-	-	-	-	5	15	25	47
			1.5-2.5 hours	2	-	-	-	-	-	5	30	35	72
			Over 2.5 hours	2	-	-	-	-	-	20	35	35	92
90-96	15-16	40-42.5	1-1.5 hours	2	-	-	-	-	-	5	15	30	52
			1.5-2.5 hours	2	-	-	-	-	-	10	30	35	77
			Over 2.5 hours	2	-	-	-	-	-	30	35	35	102
96-108	16-18	42.5-48	40-60 minutes	2	-	-	-	-	-	10	15	20	47
			1-2 hours	2	-	-	-	-	5	15	25	35	82
			Over 2 hours	2	-	-	-	-	15	30	35	40	122
108-120	18-20	48-53.5	35-60 minutes	2	-	-	-	-	5	10	15	25	57
			1-2 hours	2	-	-	-	-	10	20	30	35	97
			Over 2 hours	2	-	-	-	-	30	35	35	40	142
120-132	20-22	53.5-59	0.5-0.75 hours	3	-	-	-	-	5	10	15	20	53
			0.75-1.5 hours	3	-	-	-	5	10	20	30	30	98
			Over 1.5 hours	3	-	-	-	15	30	35	40	40	163
132-144	22-24	59-64.5	25-45 minutes	3	-	-	-	3	5	10	15	25	61
			0.75-1.5 hours	3	-	-	-	10	10	20	30	35	108
			Over 1.5 hours	3	-	-	-	30	30	35	40	40	178
144-156	24-26	64.5-70	20-35 minutes	3	-	-	-	3	5	10	15	20	56
			35-60 minutes	3	-	-	-	7	10	15	30	30	95
			Over 1 hour	3	-	-	20	25	30	35	40	40	193
156-168	26-28	70-75	16-30 minutes	3	-		-	3	5	10	15	20	56
			0.5-1 hour	3	-	-	3	10	10	15	30	30	101
			Over 1 hour	3	-	5	25	25	30	35	40	40	203
168-182* (168-180)	28-30	75-80.5	14-20 minutes	3	-	-	-	3	3	7	10	15	41
			20-30 minutes	3	-	-	2	2	3	10	15	25	60
			0.5-1 hour	3	-	3	3	7	10	20	30	35	111
			Over 1 hour	3	-	15	25	30	30	35	40	40	218
182-194* (180-192)	30-32	80.5-86	13-20 minutes	3	-	-	-	3	3	7	15	15	46
			20-30 minutes	3	-	-	3	3	5	10	15	25	64
			0.5-1 hour	3	-	3	5	10	12	20	30	35	118
			Over 1 hour	3	5	20	25	30	30	35	40	40	228
194-206* (192-204)	32-34	86-91.5	12-20 minutes	3	-	-	3	3	5	7	10	20	51
			20-30 minutes	3	-	3	3	3	5	10	20	20	67
			0.5-1 hour	3	3	3	5	10	15	20	30	35	124
			Over 1 hour	3	15	20	25	30	30	35	40	40	238

Neo-Haldanian Developments

As Haldene's ideas and findings were carried to other countries, including France, Russia, and the United States, Haldane's original tables were modified in search of safe and efficient decompression schedules. The United States Navy found that Haldane's original 2:1 supersaturation ratio was too conservative for short dives, limited by nitrogen accumulation in tissues with short nitrogen loading half-times, producing decompression schedules that were longer than necessary. At the same time this 2:1 ratio was found not to be conservative enough for long duration dives where decompression is limited by tissues with long nitrogen loading half-times, resulting in an unacceptably high number of cases of DCS(169). These experiences indicated that Haldane's critical supersaturation ratio was not fixed at 2:1 under all conditions. Work for the United States Navy by Yarbrough in the 1930's found that the critical supersaturation ratio was different for each of the hypothetical tissue compartments in the mathematical model, but also varied with the duration of exposure, or dive time(169, 187). Subsequent work by Des Granges, Dwyer, and Workman showed that the critical supersaturation ratio also varied with depth(27, 36, 169, 185). This ultimately led to the replacement of the critical supersaturation ratio with a series of tables of 'M values' by Workman. (169, 185) – see Table 3. These showed the maximum allowable supersaturation for each of the hypothetical tissue compartments in the model at each depth. This work represented an enormous leap forward in the understanding of the physiology of DCS and, with the addition of a 120-minute nitrogen loading half-time tissue compartment to the model, formed the basis of the 1956 United States Navy dive tables, which, with a few minor modifications, are still in use today(165,

169). In fact, the vast majority of dive tables, or decompression tables in use today are direct outgrowths of Haldanian and neo-Haldanian approaches and understanding.

Table 3: Workman M-values(185). The maximum allowed (absolute) partial pressure of nitrogen M in a hypothetical tissue compartment characterized by the half-time τ is defined as follows:

$$M = M_0 + \Delta MD$$

Where M = partial pressure limit for each tissue compartment (τ) in metres of seawater (msw)
M_0 = partial pressure limit at sea-level (zero depth) (msw)
ΔM = increase of M per metre of depth (msw/m)
D = depth (m)

τ (minutes)	M_0 (msw)	ΔM
5	31.7	1.80
10	26.8	1.60
20	21.9	1.50
40	17.0	1.40
80	16.4	1.30
120	15.8	1.20
160	15.5	1.15
200	15.5	1.10
240	15.2	1.10

In more recent years as divers have been venturing ever deeper for longer durations even these neo-Haldanian tables proved to be inadequate. More and more tissue compartments were needed in the model to produce decompression schedules with adequate safety. These models grew from the original 5 compartments in Haldane's model to as many as 20 with nitrogen loading half-times as long as 1000 minutes for extreme exposures(169).

Other Hypotheses

As often happens in any area of research, shortly after Haldane's pioneering work dissenting opinions began to appear. In 1912, Sir Leonard Hill produced both experimental and theoretical evidence questioning the value of stage decompression over continuous, uniform decompression(67). Hill believed that Haldane's stage decompression allowed gas bubbles to form in tissue and then attempted to limit their growth by applying decompression stops whereas using a slow, linear decompression could prevent bubble formation altogether. Although Hill's techniques are now used extensively in decompression from long duration saturation exposures such as are used in very deep water by the oil and gas industry, his inability to explain why Haldane's technique was able to avoid DCS, combined with the practical value of the ease-of-use of Haldane's tables meant that Haldane won out with the British Royal Navy in the early 1900's(67, 169).

Decades later, in the early 1960's, Brian Hills & Hugh Le Messurier observed that Okinawan pearl divers using air as a breathing gas were able to dive to depths of 90 metres for durations of up to one hour, twice a day, six days per week. These people, as a group, had accumulated enormous experience. They were working without any preconceived scientific framework and were eventually able to produce safe decompression procedures. These procedures were arrived at through trial and error and were very expensive in terms of lives lost during development. Their procedures required approximately 33% less decompression time than the United States Navy tables(93). Hills therefore suggested that DCS develops in the early stages of ascent. He believed that adding deeper decompression

stops rather than extended shallow stops would decrease the incidence of DCS. A study he conducted in the United Kingdom demonstrated that this theory was correct. By moving the conventional 3 metre (m) decompression stop to 6 m, the incidence of decompression sickness was decreased by 40%(70, 169).

Observations such as these led to Hills postulating a 'thermodynamic' model of DCS and introduced the concept of 'unsaturation' to the understanding of bubble prevention and resolution(69, 71, 72, 75). The concept of unsaturation is best illustrated by examining representative values for the partial pressures of respiratory gases for a person under normal atmospheric pressure - see Table 4.

Table 4: Partial pressures of respiratory gases at 1 ATA(174)

Sample	Gas Partial Pressure				
	O_2	CO_2	N_2	H_2O	Total
	(mmHg)	(mmHg)	(mmHg)	(mmHg)	(mmHg)
Inspired Air	158	0.3	596	5.7	760
Expired Air	116	32	565	47	760
Alveolar Air	100	40	573	47	760
Arterial Blood	100	40	573	47	760
Venous Blood	40	46	573	47	706
Tissues	≤30	≥50	573	47	700

It is clear from Table 4 that the total gas tension in tissues is less than the ambient barometric pressure, thus the tissue is ordinarily unsaturated, and others have suggested that the degree of unsaturation may be higher than the 60 mmHg indicated in Table 4 above(75, 169). The importance of this tissue unsaturation in diving is that an instantaneous reduction in pressure equal to at least 60 mmHg (an ascent of 2.6 fsw) is

required before tissues become saturated with gas(70, 75, 169). After this ascent the alveolar partial pressure of nitrogen is less than the tissue partial pressure of nitrogen so a pressure gradient is established, and to balance this gradient nitrogen is eliminated from tissue, re-establishing tissue unsaturation(70, 75, 169). This suggests that the speed at which gas is taken into tissue or eliminated from tissue is critically important to the development of DCS. For example, when astronauts are operating outside the space vehicle in space suits, the suit is pressurized to 0.2-0.3 ATA. In order for astronauts to operate in this low-pressure environment they must first pre-breathe 100% oxygen for 3 hours to remove nitrogen from their bodies. If the astronaut breathes room air for as little as 5 minutes after this denitrogenation they will develop DCS at suit pressure(169). Only the 'fast' tissues, those with very short nitrogen loading half-times could be involved in this sequence. This is likely a result of the fact that several nitrogen gradients are developed at the same time by the depressurization. Not only is there a gradient from these 'fast' tissues to alveolar gas, but there is also a gradient from these 'fast' tissues to 'slower' tissues, and they will continue to absorb gas from the faster tissues, even after the pressure has stabilized, until the whole system reaches equilibrium. In this way the 'fast' tissues serve not only as the depot to which nitrogen is added to and removed from the body, but also as a source of nitrogen to slower tissue compartments, as illustrated in Figure 1.

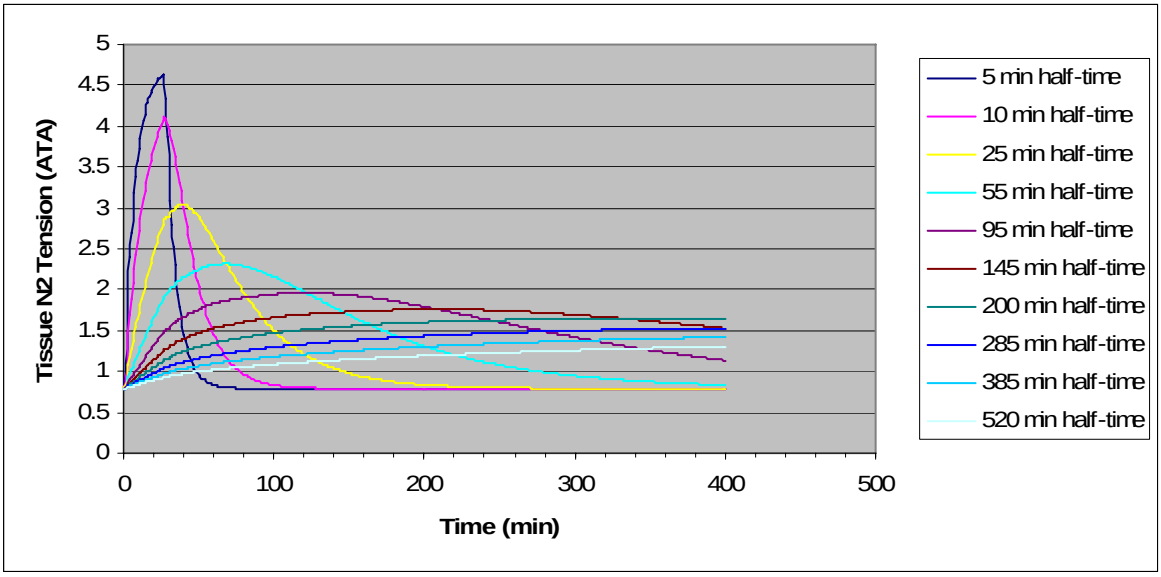


Figure 1: Graphical representation of tissue on- and off-gassing.

Nitrogen is loaded into tissues based on the partial pressure to which it is exposed. This figure represents how N_2 would move into and out of theoretical tissue compartments with half-times ranging from 5 to 520 minutes during and after a dive to 6.0 ATA (165 fsw) for 30 minutes. Notice that gas continues to load into compartments with slower half-times even after the dive is over. This is because the nitrogen in the faster half-time compartments moves not only out of the body via the lungs, but into slower half-time compartments which are less filled.

The next leap in understanding DCS began in 1951 when Harvey postulated the importance of bubble nuclei in the formation of bubbles in DCS(63). This went largely unnoticed until 1977 when Yount *et al.* integrated Harvey's ideas about bubble nuclei with the concept of unsaturation(194). This led to new theories about bubble formation and growth and began a revolution in thinking about decompression planning. Up until this time all dive tables were based to some degree on the work of Haldane. The one critical failing of Haldanian and neo-Haldanian approaches is the fact that there is the inherent

assumption that all gases remain dissolved. If gas should come out of solution and form bubbles, it is effectively lost to the calculations, despite the fact that it was well-understood that bubbles were the root of DCS.

Yount proposed that there are stable gas micronuclei that can act as seeds for bubble growth(194). Greatly simplified, bubble growth is a function of internal pressure vs. external pressures acting on the bubble. The internal pressure (P_b) is due to the gas molecules contained in the bubble, and is a product of the combined partial pressures of all dissolved gases in the immediate environment of the bubble. The external pressures include the ambient pressure (P_a), tension due to tissue displacement (P_t), and the surface tension of the bubble (P_y). If $P_b > P_a + P_t + P_y$ then the bubble will grow, if $P_b < P_a + P_t + P_y$ then the bubble will shrink(169, 194). Bubble growth can follow decompression when P_b reflects the pressure of gases (especially nitrogen) at a greater depth and P_a is decreasing. Similarly, bubbles can grow in areas of turbulent blood flow where P_t is reduced. The effect of P_y will vary with the size of the bubble according to the Law of LaPlace: $P_y = 2y/r$ where P_y = pressure due to surface tension, y = surface tension and r = radius of the bubble. For large bubbles P_y is reduced, and may become negligible. For very small bubbles P_y may become so great as to force the bubble back into solution(169, 194). Given the above, it is difficult to understand how extremely small bubbles are able to persist except in protected environments. Further work by Yount demonstrated that even though bubbles of diameters greater than 1 μm should float to the surface of standing liquids or gels and bubbles smaller than this should dissolve within a

few seconds, that there are stable microbubbles present in most liquids and gels which can act as seeds for the development of DCS(190, 191, 194, 195).

These findings gave birth to a new way of thinking about DCS. Ultimately they resulted in a radical change in decompression planning – what are now called dual phase models. These models account for dissolved gas much like neo-Haldanian models do, but also track free gas in the form of bubbles. They seek to shape a decompression profile in order to minimize the number of bubbles formed, as well as their total volume(178-181, 188, 191-193). The two best developed models of this type are the Varying Permeability Model (VMP) and the Reduced Gradient Bubble Model (RGBM)(176-179, 181, 188, 190, 191, 193). These represent the most advanced decompression models currently in use.

These dual-phase models operate fundamentally differently than the dissolved-phase (Haldanian and neo-Haldanian) models. Dissolved-phase models seek to prevent DCS by removing excess nitrogen as quickly as possible. This is done by ascending to depths as shallow as is safe, followed by long, shallow decompression stops. Dual-phase models, on the other hand, seek to minimize the number and, more importantly, the total volume of bubbles free in circulation. This is achieved by requiring short decompression stops beginning at depths much greater than dissolved-phase models would recommend. This keeps the ambient pressure relatively high, promoting gas dissolution out of the bubbles and elimination via the lungs, resulting in bubble shrinkage. These dual phase models result in decompression schedules which are both shorter and statistically produce fewer incidents of DCS(176-179, 181, 188, 190, 191, 193).

RGBM in particular has been used to plan and execute extraordinary dives which would not have been possible using dissolved-phase models. For example, on 17 December, 2007 two divers from the Woodville Karst Plains Project, Jarrod Jablonski and Casey MacKinlay, entered the Turner Kink near Tallahassee, FL. They descended to a depth of 300 fsw where they entered an underwater cave system. They penetrated an amazing 11.25 km into the cave system before turning around and exiting the way they came in. The cave penetration itself required 6 hours, and an additional 14 hours of decompression time was required before the two divers could exit the water. Neither of these divers developed any signs or symptoms of DCS(61, 182). This dive would not have been possible prior to the advent of dual-phase models for decompression planning.

Physiology and Pathophysiology of Decompression Sickness

What is Decompression Sickness?

DCS is a much less well understood phenomenon than researchers in the field would like people generally to believe. Understanding the physiology and pathophysiology of DCS is complicated by several factors. Chief among them is the simple fact that a diagnosis of DCS is entirely based on history and symptoms. There exist no medical tests to either rule in or to rule out DCS. Furthermore, DCS is a stochastic phenomenon. If ten people were all subjected to the same dive profile designed to produce some symptoms of DCS, some would suffer from DCS symptoms while others would not. Among those that developed symptoms, there would be a wide range of presentations from mild joint pain to serious neurological symptoms such as parasthesia and paralysis - see Table 5. Similarly, if one person were subjected to the same dive profile in the example above on ten different

occasions, they would most likely develop symptoms of DCS on some occasions, and not on others, and just as above, the symptoms they develop are likely to vary from one exposure to the next. At this time there doesn't exist a clear understanding of all the factors that contribute to DCS, let alone how they affect the onset and development of DCS. For these reasons DCS remains very difficult to predict in any terms other than statistical probabilities.

Table 5: Frequency of signs and symptoms in 935 cases of decompression sickness(138)

Sign or Symptom	Number of Instances Within 935 Cases	Percentage of Instances Within 935 Cases	Number of Instances Manifested Initially	Percentage of Initial Manifestations
Localized pain	858	91.8	744	76.6
Numbness or paresthesia	199	21.2	41	4.3
Muscular weakness	193	20.6	8	0.8
Skin rash	140	14.9	42	4.4
Dizziness or vertigo	80	8.5	24	2.5
Nausea or vomiting	74	7.9	8	0.8
Visual disturbances	64	6.8	14	1.4
Paralysis	57	6.1	2	0.2
Headache	37	3.9	5	0.5
Unconsciousness	26	2.7	6	0.6
Urinary disturbances	24	2.5	0	-
Dyspnea (“chokes”)	19	2.0	4	0.4
Personality change	15	1.6	0	-
Agitation or Restlessness	13	1.3	0	-
Fatigue	12	1.2	2	0.2
Muscular twitching	12	1.2	0	-
Convulsions	11	1.1	0	-
Incoordination	9	0.9	0	-
Equilibrium disturbances	7	0.7	0	-
Localized edema	5	0.5	0	-
Intestinal disturbance	4	0.4	0	-
Auditory disturbance	3	0.3	0	-
Cranial nerve involvement	2	0.2	0	-
Aphasia	2	0.2	0	-
Hemoptysis	2	0.2	0	-
Subcutaneous emphysema	1	0.1	0	-

The study of the physiology and pathophysiology of DCS is also complicated by the fact that DCS is a symptomatic diagnosis. Firstly, it is unethical to conduct randomized trials in people, deliberately subjecting them to a condition which could cause permanent injury or death. Likewise the invasive measurements needed to best understand what is happening in the midst of DCS are also not ethical for human use. Secondly, studies in animals are complicated by the simple fact that animals are not able to report on their symptoms. This makes it very difficult to ascertain to what degree the symptoms generated in animal experiments mimic what is observed in humans. None the less, great strides have been made in understanding the physiology and pathophysiology of DCS over the last 340 years, as was touched upon above. These advances will now be elaborated upon and examined from the perspective of physiology, rather than that of history.

The consensus in the hyperbaric community is that DCS is the result of the formation of gas bubbles in tissue and anatomic spaces unsuited to the presence of gas upon decompression, however, the presence of such gas bubbles does not inevitably lead to DCS(34, 45, 170). Gas bubbles in tissue may produce no symptoms at all or may produce symptoms ranging from pruritis, fatigue, or joint and muscle soreness to permanent paralysis, convulsions, and death(34). The term ‘decompression sickness’ is generally used to refer to the pathologic response to the development of bubbles in tissue and blood, while the term ‘decompression illness’ is used as a general term to denote any injury or illness which is a consequence of a reduction in ambient pressure, including, but not limited to DCS, barotraumas, as well as both arterial and venous gas emboli.

DCS, as defined above, is a product of a rapid decrease in ambient pressure(34). At equilibrium the amount of gas dissolved in any given tissue is proportional to the partial pressure of that gas to which the tissue is exposed according to the physical principal known as Henry's law. The gas partial pressure to which tissues are exposed is primarily a product of ambient pressure and the gas mix being breathed. When ambient pressure decreases, bubbles will form in the tissue unless the gas can either be taken up by tissue, as would be the case with O_2 or CO_2, or carried down a concentration gradient and ultimately equilibrate with the gas partial pressures in the alveoli, from which they can be exhaled through the lungs, as would be the case for inert gases(34). When this reduction of ambient pressure is sufficiently gradual, the removal of excess gas proceeds safely(34, 55, 67). When ambient pressure is decreased both too much and too rapidly it is believed that gas bubbles form and result in the symptoms of DCS, but exactly how and where in the body these bubbles form remains poorly understood(32, 170). What is understood is that part of the mechanism of injury in DCS is the mechanical effects of these bubbles, be that the compression and distortion of tissue surrounding the growing bubble, or obstruction of blood flow by bubbles lodged in arterioles, capillaries or venules(34, 169, 170).

How and Why Bubbles Form

Water, and thus water-based fluids, can tolerate very high levels of supersaturation before bubbles begin to form *de novo* within the water itself. In fact, in pure water, supersaturation itself will not produce bubbles until this supersaturation is approximately 1000 ATA. That is to say, if water exposed to room air is normally saturated with 0.79 ATA of nitrogen, then the nitrogen partial pressure would need to exceed 790 ATA in

order to generate *de novo* bubble formation as a result of supersaturation alone(66, 169). Since DCS can develop with supersaturations considerably less than this, there must be other mechanisms involved in addition to supersaturation by itself. There are two mechanisms for the formation of bubbles, with much lower degrees of supersaturation necessary, which are believed to be at play in the body. The first of these is the process by which bubbles can grow from pre-existing, stable micronuclei already present in tissue(115, 169, 189). With any sort of imperfection in a surface, very small pockets of gas can develop and remain stable in environments which, without the stabilizing effects of the crevice in which they reside, would collapse and dissolve. The presence of such stable 'micronuclei' has been previously demonstrated by Yount *et al*(190, 191, 194, 195). When supersaturation of the liquid develops, gas molecules can diffuse into these micronuclei and they will expand, bulging out of the crevice into the surrounding liquid. Surface tension at the gas-liquid interface tends to oppose this growth, but it will continue as long as the liquid is sufficiently supersaturated for gas to diffuse into the forming bubble. When the forming bubble reaches a critical size, surface tensions will pinch off the budding bubble and release it into the liquid leaving the micronucleus behind to repeat the process so long as the state of supersaturation persists(115, 169, 189). This is the process by which bubbles are released from carbonated beverages and why the bubbles appear to stream from certain points on the wall of the glass. This is illustrated in figure 2 below.

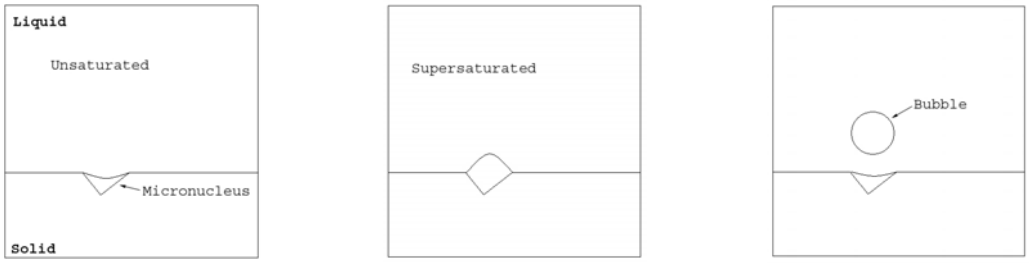


Figure 2: Nucleation of bubbles from pre-existing micronuclei with decompression.

Stable gas micronuclei exist within microscopic imperfections in surfaces. When the surrounding liquids are supersaturated with gas, gas will dissolve into the micronucleus causing it to grow and bud out into the liquid. Once the bubble reaches a critical size surface tension effects will cause the bubble to be pinched off and released into the liquid leaving the micronucleus behind to repeat the process.

The second mechanism likely at play is called tribonucleation, or cavitation nucleation. Local areas of very low pressure are created within the body by several factors including vertical motion in areas of turbulent blood circulation, and the shearing forces created within joints as solid structures move away from each other. The extremely low pressures generated in these environments can exceed 1000 ATA below ambient pressure, and is sufficient to allow for the spontaneous formation of bubble nuclei, which can then grow as gas from the supersaturated blood and tissue diffuses into the bubble(109, 166, 169). This same process can take place even when tissue is not supersaturated with gas. The formation and collapse of bubbles formed by tribonucleation is responsible for the sound of knuckles cracking(34, 165, 169).

Where Bubbles Form and Where They Are Found

Where such bubbles form is even less well understood than how they form. In theory, bubbles can form in any liquid environment, including within cells, in extracellular fluid spaces, and within the blood and lymphatic circulations, as well as in cerebrospinal fluid (CSF). The intracellular formation and growth of bubbles would result in membrane rupture and functional loss similar to what is observed with the formation of intracellular ice crystals in frostbite. Bubbles have been observed to form and grow in decompressed hen's eggs, but unicellular organisms and erythrocytes in suspension have proven to be very resistant to intracellular bubble nucleation(62, 65, 121). None the less, it has been inferred that intracellular gas bubbles do form in mammals from observed mitochondrial membrane abnormalities and intracellular bubbles have been observed in some adipocytes of rapidly decompressed rodents(6, 46). Some of the space-occupying lesions observed in myelin sheaths may have begun as intracellular bubbles(6, 40, 46, 158). Although intracellular bubbles probably do form, their clinical importance is likely overshadowed by other events(34).

Bubbles may also form in the extracellular, extravascular space. In fact, the earliest report of DCS commented on the presence of a bubble in the aqueous humour of a viper's eye(12). Similar observations have also been recorded in dogs(22). Likewise, bubbles have been reported to be present in the urine, CSF, and tear film of acutely decompressed animals(55, 156). It is unlikely that bubbles formed in these extracellular fluids produce any clinical symptoms(34). During the development of the first deep water diving tables, numerous holes were found to be present in fixed tissue of the spinal cords of goats that

had developed DCS(55, 60). Since that time many other investigators have reported the presence of empty spaces in the white matter of fixed spinal cords that compress and distort surrounding structures after decompression(40, 108, 122, 158). These spaces were found to be more common in decompressed animals than in control animals, thus it is unlikely that they were purely fixation artifacts(40, 108). They appeared to compress surrounding structures suggesting that the empty spaces were growing bubbles(40, 108). It is difficult to determine if the origin of these bubbles was intracellular, extracellular, or intravascular due to the distortion of surrounding tissues. Gas bubbles that appear to be extracellular have been observed in the peripheral nerve myelin of decompressed guinea pigs, suggesting that the spinal cord lesions may, indeed, have been extracellular in origin(34, 47).

The most common location where gas bubbles either form, or end up is in the intravascular space. Intravascular bubbles produce symptoms by interfering with blood circulation. Arterial gas bubbles are more likely to result in serious pathologic consequences than are venous bubbles(34, 170). Intravascular gas bubbles have been directly observed *in vivo* after decompression in the arteries and veins of the hamster cheek pouch, in the spinal epidural veins, posterior spinal arteries, and cranial arteries and veins of dogs(13, 15, 47, 56, 101). There have also been numerous reports of intravascular gas bubbles in humans after fatal DCS(24, 64, 123). The *in vivo* occurrence of intravascular bubbles in humans is inferred from the observation of abnormal ultrasound signals using Doppler flow meters and ultrasound scans(5, 38, 82). These techniques are only able to detect moving bubbles and small bubbles are difficult to distinguish from solid

emboli(105). The Doppler signal is auditory and is usually recorded and later graded by experienced observers. This type of analysis lends itself to bias if the observers are aware of the experimental conditions. Any movement of the transducer is also very likely to generate signal artifacts. Despite these limitations, Doppler ultrasound is an accepted tool in DCS research(34).

Intravascular bubbles associated with DCS are most often confined to the venous side of the circulation. Arterial bubbles are rarely observed, but when present they usually accompany serious symptoms of DCS(34). It is unlikely that bubbles form directly in the arteries since the relatively high arterial blood pressure opposes their formation and the arterial blood, having just passed through the lungs, has inert gas tensions close to ambient pressure(34). Arterial bubbles are usually a result of one of two causes: pulmonary barotrauma or right-to-left shunts, either in the heart or in the lungs. Pulmonary barotrauma is most often a product of air trapped within the broncho-alveolar tree expanding on decompression. This results in tearing of the lung parenchyma and vasculature and air being forced into the pulmonary veins with overpressurizations as small as 29.5 kPa or 4.2 psi(136, 186). Right-to-left shunts in the heart usually take the form of atrial septal defects, such as a patent foramen ovale. Approximately 20-30% of the human population have defects such as this(157). Several studies have indicated that patent foramen ovale is much more common among divers that develop severe DCS than in the general population(113, 183). This suggests that atrial septal defects are commonly associated with severe DCS, but whether or not they increase the risk of developing DCS, and to what degree, is unknown(34). The lungs are ordinarily a very efficient filter of the bubbles present in the

venous circulation after decompression(16, 73). However massive numbers of bubbles in the venous circulation can overwhelm the ability of pulmonary capillaries to filter out such microemboli(16, 73). Large numbers of bubbles also lead to the opening of pulmonary arteriovenous shunts, as well as increased pulmonary arterial pressure, both of which contribute to passage of bubbles through the lungs into the arterial circulation(18, 114). These mechanisms are likely involved in cerebral DCS as it has been observed that after severe DCS in dogs known to be without atrial septal defects, the first bubbles to appear in the cerebral circulation appear in the arteries(34)

Non-Mechanical Effects of Bubbles

Bubbles causing distortion of tissues and obstructing blood flow would be injurious in and of itself, even if the body did not react in any way to the presence of the bubbles. However, blood is a highly reactive, living tissue which is primed to respond to any deviation from its normal environment(34). The presence of bubbles in circulation is sufficient to trigger many of these responses(34). Endothelial cells are key players in the regulation of blood coagulation and the regulation of microcirculatory blood flow. Endothelial cells are often damaged by the passage of bubbles, even bubbles small enough to pass through the vessel without lodging and obstructing blood flow(74, 164). The blood gas interface is capable of denaturing proteins because the hydrophobic portions of the proteins will have a lower free energy when surrounded by air than by blood plasma(95). This protein denaturation can lead to the accumulation of free fat globules and bubble formation can provoke the release of free fatty acids from cell membranes(34, 64, 96). The accumulation of such products can result in the formation of fat emboli, which have been

observed in several pathologic studies of DCS, and may contribute to the central nervous system damage associated with DCS(34, 64, 96).

The presence of bubbles is also able to activate coagulation and complement systems. Bubbling of air through cell-free blood plasma has been shown to decrease clotting time, presumably through the activation of the contact system activator known as Hageman factor(57, 58). Hageman factor activates the intrinsic pathway for thrombin formation and activates the system for kinin formation(41, 119). This results in pain, vasodilation, edema formation, leukocyte chemotaxis, and activation of the plasma fibrinolysis system(41, 119). The net effect of the activation of these systems is progressive clotting and subsequent ischemia at and around the site of the bubble(41, 119). These activated factors are also distributed systemically and likely contribute to leukocyte trapping in the lungs and systemic reactions to decompression such as fatigue(34). Complement activation by bubbles has been noted in rabbit serum and rabbits depleted of complement have been found to be resistant to DCS(20, 171, 173). While complement activation is certainly seen with DCS, exactly how it contributes to the pathology of DCS is poorly understood due to conflicting reports in the literature regarding the correlation of activated complement with severity of signs and symptoms of DCS(76, 153, 172).

Human studies have shown increased packed cell volume and decreased platelet counts after decompression(127). These findings are consistent with the development of edema and activation of thrombin, which would further impair blood flow in the microcirculation by increasing blood viscosity(34). Electron microscopy studies have shown that leukocytes and platelets adhere to both circulating bubbles and damaged

endothelium(128). These cells have also been shown to accumulate in areas of low blood flow after arterial air embolism in the brain(35, 59, 118). Leukocyte depletion has been shown to reduce the severity of neurophysiologic damage induced by cerebral air embolism(35, 59, 118).

Bubbles, it would appear, are the root cause of the signs and symptoms of DCS. The injury induced by the presence of bubbles is not limited to direct mechanical interaction of bubbles with tissue. The presence of bubbles, particularly in the blood, will also activate multiple biochemical pathways that ultimately lead to thrombosis and edema, as discussed above. This can have detrimental effects which outlast the presence of the bubbles that triggered the activation in the first place.

Perfluorocarbons

Perfluorocarbons and Oxygen

The emergence of perfluorocarbon (PFC) emulsions as medical therapeutics is largely a product of the dangers of viral transmission associated with blood transfusion in the early-to-mid 1980's(147). With the advent and refinement of viral marker testing in the 1990's, the risk of viral transmission decreased dramatically, but there remain risks associated with blood transfusion that can never be eliminated such as ABO and Rh incompatability, graft-vs.-host disease, volume overload, and immunosuppression(147). In part as an effort to deal with these risks, the development of safe and efficacious intravenous (I.V.) oxygen therapeutics, or 'blood substitutes', continues to be investigated. Perfluorocarbon emulsions are one of the lines of products currently being investigated.

Perfluorocarbons are a synthetic class of small molecules composed of linear or cyclic carbon backbones in which the carbon atoms have been fully substituted with fluorine or other halogens(154, 155). These compounds were originally developed during the Manhattan Project for use as insulators in nuclear piles(141, 154). Perfluorocarbons also serve as the building blocks of products such as Teflon (polytetrafluoroethylene; DuPont, Wilmington, DE) and Scotchgard (fluorochemical products; 3M, St. Paul, MN)(141, 154). During the 1950's and 1960's there was a great deal of research involving PFCs which ultimately culminated in the above-mentioned Teflon. During this research it was surreptitiously noted that PFCs were able to dissolve vast amounts of non-polar gases such as oxygen, carbon dioxide, and nitrogen(117). Unlike hemoglobin, which carries oxygen by binding it to the ferrous center in the heme moiety, PFCs carry oxygen passively(147, 154). Perfluorocarbons dissolve oxygen in proportion to the pO_2 to which they are exposed(147, 154). Therefore the more the pO_2 can be increased, the more oxygen can be carried(147, 154). Many PFCs in their pure liquid form can dissolve 50-60 mL of oxygen per 100 mL of PFC at a pO_2 of 760 mmHg (See table 6)(147).

Table 6: Oxygen solubility of various perfluorochemicals(49).

Compound	O_2 Solubility (mL O_2/100 mL of compound at PO_2 760 mmHg)
Perfluorodihexyl ether	55.42
Perfluorodibutyl sulfur tetrafluoride	48.02
Perfluorotriisobutylamine	44.37
Perfluoro-(*N*-ethylmorpholine)	50.10
Perfluoro-*N*,*N*-dipropylmethylamine	52.60
Perfluorotriethylamine	53.86
Perfluoro-*N*-methylpiperidine	41.33
Perfluoro-*N*-methylmorpholine	37.57
Perfluoro-*N*,*N*-dimethyl-*N*-hexylamine	51.51
Perfluoro-*N*-butylmorpholine	50.59
Perfluoro-4-(*N*,*N*-dimethyl-2-aminoethyl)-morpholine	45.07
Perfluoropentyl ether	49.67

The medical world quickly picked up on this observation and in 1966 it was first demonstrated that a PFC liquid could be used to supply oxygen in organ perfusion models(51). In the mid-to-late 1970's experimental work began on liquid breathing systems(21). Goldfish, mice, rats, and even cats were submerged in different liquid environments(21). Mice and goldfish were placed in low-viscosity silicone oils with oxygen solubilities approximately 1.5 times the oxygen content of whole blood(21). Mice were able to survive in this environment, but only if the temperature was kept below 18 °C, and they all died within 10 minutes of being removed from the oil(21). Goldfish were able to survive for weeks in silicone oils bubbled with oxygen(21). Perfluorocarbons proved to be a much more satisfactory medium for liquid breathing. An oxygen electrode placed in the brain of a mouse prior to immersion showed the same level of tissue oxygenation while breathing oxygenated perfluorobutyltetrahydrofurans as when breathing room air(21). After removal from the PFC the mice could survive for weeks(21).

Perfluorocarbon Emulsions

Around the same time, pure PFCs were being emulsified for possible use as 'blood substitutes'. Several emulsions were developed using the PFCs perfluorotributylamine and perfluorodecalin(48). It was found that these emulsions, when properly prepared, could be used to perform a total blood volume exchange in rats, reducing their hematocrit below 1%, and that these rats could survive for weeks after such transfusions(48). These so-called 'bloodless' rats were found to be able to carry out their normal functions, regenerate normal blood cells and plasma proteins, and grow and develop normally(48). It was believed at the time that such rats could prove to be powerful research tools(116, 129-131,

140, 159). At least initially, they were lacking all plasma proteins, and thus any desired combination of plasma proteins could be generated simply by adding them to the rat(116, 129-131, 140, 159). Such models have been used sporadically to study various conditions such as cyanide toxicity, carbon monoxide toxicity, transcapillary transport, as well as in imaging studies where the refractive effects of haemoglobin need to be eliminated(116, 129-131, 140, 159).

Thus far only a single PFC emulsion has been approved by the FDA for use in humans, the first-generation PFC emulsion Fluosol-DA(20%) (Green Cross Corp, Osaka, Japan), and then only for the prevention or treatment of distal myocardial ischemia during coronary angioplasty(147). Although it was fairly successful in this application, its use was cumbersome. The development of angioplasty systems which allow coronary flow to continue, combined with a side effect profile which included complement release and activation, hypotension, white cell depression, and uptake by the reticuloendothelial system resulting in hepatomegaly and splenomegaly resulted in Fluosol-DA(20%) never gaining enough popularity among clinicians for widespread use(29, 90, 91, 147). Ultimately it was withdrawn from the market. Newer second- and third-generation PFC emulsions are currently under development. These products contain higher concentrations of PFCs, smaller particle sizes, and different emulsifiers which do not provoke such aggressive immune responses(147). The PFC emulsions currently under development provoke mild flu-like symptoms such as malaise, muscle aches, and a mild fever, as well as transient thrombocytopenia 3-4 days after administration of the PFC emulsion(19, 52, 141, 145, 146, 155). However these mild side-effects are quite tolerable, especially in the

light of the much-improved oxygen carrying capacity of these newer products(19, 52, 141, 145, 146, 155).

Perfluorocarbons and Decompression Sickness

PFC emulsions have the ability to enhance the transport of gases other than oxygen. All non-polar gases are soluble in PFCs and of particular interest is nitrogen. In pure PFCs nitrogen may be up to 10,000 times more soluble than in plasma(147). Nitrogen is the primary component of room air and as such can become a problem in many surgical procedures. Microscopic air emboli are a common problem in cardiopulmonary bypass, and larger air emboli are serious complications of neurosurgical, orthopaedic, and some gynaecological procedures(17, 53, 85, 120, 126). Animal studies have shown that PFC emulsions administered preoperatively can greatly reduce, or prevent entirely, the harmful effects of venous air embolism, intracoronary air embolism, and cerebral arterial gas embolism(110, 149, 150, 162). Data obtained using early formulations of PFC emulsions suggests strongly that the capacity of the PFC emulsion to dissolve and carry nitrogen is one of the likely mechanisms by which this protection is afforded(110, 149, 150, 162).

It is the ability of PFC emulsions to dissolve and remove larger amounts of nitrogen that first attracted attention in the realm of DCS research. Since excess nitrogen trapped in tissue is believed to be the root cause of DCS, a therapy which could either act as a sink for that nitrogen, increasing the effective volume of distribution sufficiently that bubbling is limited, or that can speed the elimination of excess nitrogen from the body, again, limiting bubble formation and growth, is an obvious target for DCS research. The use of PFCs for the prevention or treatment of DCS has taken many different forms. Likely the most

esoteric of these was the enteric administration of pure, degassed PFCs(142). Although this idea was patented over 12 years ago, there has been no active research on this topic since. Far more common is the idea that either I.V. PFC emulsions or pure, oxygenated PFC liquids as breathing media can be used to treat or prevent DCS. The ideal situation is to prevent DCS altogether. Several studies have demonstrated that breathing liquid, oxygenated PFCs during hyperbaric exposures followed by rapid or even explosive decompression is able to prevent the development of DCS, while air-breathing control animals exposed to the same compression/decompression produced large numbers of animals with severe DCS(50, 89, 103, 175). Diving performed using liquid breathing would most likely make DCS irrelevant, but there are enormous hurdles which would need to be overcome. The respiratory muscles are not designed to move a high-viscosity liquid in and out of the lungs and would quickly be exhausted by doing so. This would necessitate a closed-circuit ventilator capable of pumping the PFC in and out of the lungs, as well as adding oxygen to, and removing carbon dioxide from the breathing liquid. There are also tremendous psychological factors which would need to be overcome. Inhaling a liquid causes a sensation of drowning and produces panic(2, 11). These factors combine to make what may be an ideal solution to the problem very impractical.

Research has instead focused primarily on the I.V. use of PFC emulsions to treat or prevent DCS. Lundgren *et al*. demonstrated that I.V. PFC emulsions could greatly enhance nitrogen washout at surface pressure while breathing oxygen(99). Although no compression/decompression was associated with this experiment, it demonstrated that I.V. PFC emulsions might be able to prevent DCS when used prophylactically to remove the

nitrogen normally present in the body prior to a dive. Unfortunately this sort of treatment is unlikely to be useful in the real world. Mahon *et al.* found that although I.V. PFCs administered at depth enhanced nitrogen elimination, they also enhanced acute oxygen toxicity manifested by seizure activity(104). Since acute oxygen toxicity is of much greater immediate danger to a diver in the water, I.V. PFC emulsions are unlikely to be administered before a diver has left the water.

Intravenous PFC emulsions used after exit from the water in order to treat DCS, rather than to prevent it, appear much more likely to be clinically useful. Several studies have demonstrated that the administration of I.V. PFC emulsions after the onset of severe DCS is able to dramatically reduce morbidity and mortality in a variety of species(23, 31, 100, 151). That the administration of I.V. PFC emulsions can reduce both the severity of DCS symptoms and reduce the lethality of severe DCS is now generally accepted within the hyperbaric community, but the mechanism of this protective effect has not yet been demonstrated.

The studies described in this dissertation were purposed to examine two possible mechanisms for the protective effects afforded by I.V. PFC administration in severe cardiopulmonary DCS: First, that the intravenous administration of PFC emulsions acutely following decompression is able to increase oxygen delivery at the whole-body level, and presumably to prevent or lessen hypoxic death or injury of tissue to which there is little or no blood flow as a result of bubble obstruction. Second, that the intravenous administration of PFC emulsions acutely following decompression is able to increase the removal of

excess nitrogen from the body, thus relieving the obstructions caused by intravascular gas bubbles.

CHAPTER 2

INTRAVENOUS PERFLUOROCARBON EMULSIONS INCREASE WHOLE-BODY OXYGENATION AFTER SEVERE DECOMPRESSION SICKNESS

Introduction

Breathing compressed air increases the amount of nitrogen dissolved in body fluids(54, 112, 135). Factors such as depth, thus the ambient pressure, and the duration of the dive are the primary determinants of the amount of excess nitrogen absorbed(26, 54, 112, 135). As a diver ascends and ambient pressure decreases it is possible for the dissolved gas tensions in tissue to exceed ambient pressure. This supersaturated state leads to the formation and growth of free gas bubbles, resulting in venous gas emboli (VGE) and possible arterial gas emboli (AGE)(26, 32). It is believed that these bubbles within the vasculature are the root cause of decompression sickness (DCS)(26, 32). There are likely multiple pathophysiological mechanisms at play in DCS including impairment of microcirculation by inert gas bubbles, increased blood viscosity, damage to endothelium, and activation of complement. The physiochemical discontinuity of the gas-blood interface can also denature proteins promoting the release of fatty acids from cell membranes leading to the formation of fat emboli(26, 32). When intravascular bubbles obstruct capillaries or venules, it often leads to ischemia followed by reperfusion induced oxidative tissue damage(37).

Perfluorocarbon emulsions (PFCs) are intravenous emulsions of fluoridated hydrocarbons within phospholipid micro-particle micelles(155). PFCs have been

developed in medicine as intravenous oxygen therapeutics(155). PFCs accomplish this in a way which is fundamentally different from how whole blood transports the majority of its oxygen content. Oxygen, or any other gas, carried by PFCs is not bound, as oxygen is to hemoglobin, rather it is dissolved in the PFC itself. PFC, because of its non-polarity, can dissolve up to 60 volume percent oxygen(117). Plasma can only dissolve 0.034 volume percent but whole blood at 14 gm/dl hemoglobin can chemically bind up to 21 volume percent oxygen. The oxygen dissolved in PFC is all available for tissue usage whereas that chemically bound by hemoglobin is restricted (usually only 23% can be released for metabolism). Microcirculatory changes such as edema, vasospasm, white cell activation and vessel plugging result in decreased erythrocyte delivery of oxygen to watershed neurons, yet plasma flow may continue without red cells(160). PFC, due to its extremely small particle size (~0.1-0.4 μm) can be delivered in this trickle-flow of plasma(9, 137, 155). Plasma flow oxygen delivery by PFCs is enough to keep tissue alive, as seen with Fluosol DA-20%, a first generation PFC which reduced myocardial infarction and garnered FDA approval(86, 87).

PFC is also effective in treating DCS, AGE and VGE(23, 31, 147-149, 151, 196). Using a saturation dive model with direct ascent to the surface, Dromsky *et al.* found that the administration of I.V. PFC while breathing 95% oxygen was able to decrease the lethality of this decompression insult, decrease the incidence of DCS, and decrease the number of neurological events(31). Similarly it was found that I.V. PFC can improve outcomes after massive VGE, cerebral AGE, and coronary AGE(148-150). The administration of I.V. PFC has also been shown to increase nitrogen washout through the

lungs after VGE(196). Clearly PFC administration is of benefit in the treatment of decompression illnesses, but the exact mechanism of this benefit has not yet been elucidated. Is this benefit a result of the PFCs ability to increase nitrogen washout, and thus, presumably, to remove the bubbles obstructing circulation faster? Is it a product of improving the oxygen supply and thus metabolic state of tissue to which blood flow and oxygen supply has been disturbed? Is it some combination of both of these events? The research described here was designed to investigate the effect of I.V. PFC emulsions administered acutely after surfacing on whole-body oxygenation in an ovine model of severe DCS.

Materials and Methods

All animal experiments performed for this project were done in strict accordance with the National Institutes of Health (NIH) "Guide for the Care and Use of Laboratory Animals" and were approved by both the Department of Defense (DoD) and the Virginia Commonwealth University Institutional Animal Care and Use Committee (IACUC). Juvenile Dorper cross sheep of either sex (Robinson Services, Inc., Mocksville, NC) weighing 14-24 kg were housed in United States Department of Agriculture (USDA)- and Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-approved facilities in social flocks with free access to food and water on a 12-hour light/dark cycle. Sheep were allowed a minimum of 3 days for acclimatization and veterinary inspection prior to their use in any experiment.

Sheep Instrumentation and Preparation

Prior to the experiment sheep were muzzled for a period of 48 hours in order to prevent access to food and providing free access to water while remaining with the remainder of their flock in order to limit animal stress. Sheep were initially sedated with ketamine/xylazine (20.0/2.0 mg/kg I.M.) and the animal was placed supine on the surgical table. All animals were promptly intubated with a 9.0 mm internal diameter (I.D.) cuffed endotracheal tube (Hudson RCI, Temecula, CA) and ventilated with 50/50 nitrogen/oxygen using a Siemens 900C servo ventilator (Siemens Corp., New York, NY) set to a tidal volume of approximately 10 mL/kg adjusted to maintain arterial pCO_2 at 40 mmHg ± 5 mmHg. An orogastric tube fashioned from TYGON® R-3603 tubing (Satin-Gobain Performance Plastics Corp., Akron, OH) was advanced into the rumen to allow for fluid drainage and to allow gas accumulated in the gut during the air dive to vent upon decompression. A MAC® 2-port introducer sheath (Arrow International Inc., Reading, PA) was placed in the right external jugular vein to allow for the administration of fluids and the anesthetic cocktail. Once I.V. access was secured, administration of the triple drip anesthetic cocktail (ketamine/xylazine/guaifenesin 2.0/0.1/50.0 mg/mL in 5% dextrose) was begun immediately at 1.0-2.0 mL/kg/hr titrated to maintain a surgical plane of anesthesia using a Harvard Apparatus PHD 2000 programmable syringe pump (Harvard Apparatus, Holliston, MA). The right femoral artery was canulated with an 18 gauge (G) femoral arterial catheter (Arrow International Inc., Reading, PA) for monitoring of arterial pressure (AP) and arterial blood sampling. The right femoral vein was canulated with a 4 French (Fr) double lumen catheter (Arrow International Inc., Reading, PA) for the

administration of the anesthetic cocktail while inside the hyperbaric chamber and for the administration of the treatment drug after exiting the hyperbaric chamber. The left femoral vein was canulated for the placement of a 7.5 Fr CCOmbo® continuous cardiac output (CCO) pulmonary artery catheter to allow for CCO monitoring, central venous (CVP) and pulmonary arterial pressure (PAP) monitoring, and central venous blood sampling. Respiratory gases were continuously monitored using an MGA 1100 respiratory mass spectrometer (Perkin-Elmer, Norwalk, CT). Following surgical manipulations all animals were allowed to stabilize for a minimum of 30 minutes. After stabilization animals were weaned off the ventilator until capable of spontaneously breathing prior to being placed inside the hyperbaric chamber.

Inclusion/Exclusion Criteria

At the end of the stabilization period all animals were required to meet certain inclusion criteria prior to being weaned off the ventilator and placed in the hyperbaric chamber. The inclusion criteria were as follows: mean arterial pressure above 60 mmHg, arterial pO_2 above 200 mmHg, arterial pH 7.45 ± 5, arterial pCO_2 40 ± 5 mmHg. All animals not meeting the above inclusion criteria were excluded from the study and euthanized.

Sheep Dry Dive Procedures

All sheep that met the study inclusion criteria (n=31) were weaned off the ventilator until capable of breathing spontaneously, then disconnected from all monitoring equipment and placed into a Reimers Systems model #17-48-100 Research Hyperbaric Chamber (Reimers Systems, Inc., Springfield, VA). During the dry dive procedures all animals

breathed room air and general anesthesia was maintained using a continuous infusion of triple drip as described above. All sheep were subjected to the following dive profile: Beginning at 1.0 ATA the chamber was compressed at a rate of 1.0 ATA/minute to a pressure of 2.0 ATA. From 2.0 ATA the chamber was compressed at a rate of 2.0 ATA/minute to a pressure of 6.0 ATA (165 feet of seawater (fsw), or 608 kilopascals (kPa)). The pressure of 6.0 ATA was maintained for 27 minutes, after which sheep were immediately decompressed to 1.0 ATA at a rate of 2.0 ATA/minute.

Post-Decompression Monitoring

Upon complete decompression (considered time=0) all animals were quickly removed from the hyperbaric chamber and all monitoring equipment was reconnected. At this point animals were randomized to receive I.V. infusion of either 6.0 mL/kg Oxycyte® PFC (n=15) (Synthetic Blood International, Costa Mesa, CA) or saline control (n=16) and one of four breathing gases; 100% oxygen (n=8), 80/20% nitrogen/oxygen (n=8), 50/50% helium/oxygen (n=7), or 80/20% helium oxygen (n=8). Animals were randomized using a block randomization scheme such that for every 8 experiments performed one animal fell into each of the 8 treatment groups, but the order within each block was random. Neuromuscular blockade with I.V. pancuronium (0.1 mg/kg) given acutely post-chamber was necessary in order to accurately measure respiratory gases with the mass spectrometer. All animals were monitored for 90 minutes after decompression, during which time both arterial and mixed venous blood samples were drawn and analyzed using a Radiometer OSM 3 Hemoximeter and a radiometer ABL 700 blood gas analyzer (Radiometer America, Westlake, OH) at 5, 10, 15, 30, 60 and 90 minutes after decompression. Data from all

instruments was recorded directly to hard drive storage using the BioPac system and Acqknowledge v. 3.90 software (BioPac Systems, Inc. Goleta, CA) After 90 minutes all animals were euthanized.

Later offline analyses were performed in order to determine arterial and mixed venous blood oxygen content (C_aO_2, $C_{\bar{v}}O_2$), oxygen delivery ($\dot{D}O_2$), tissue oxygen consumption ($\dot{V}O_2$), and oxygen extraction ratio (ER). The following formulae were used for the calculations:

Arterial oxygen content:

$$C_aO_2 = (1.34 \times Hb \times S_aO_2) + [(0.0031 \times P_aO_2 \times \alpha) + (0.01997 \times P_aO_2 \times \beta)] \quad (1)$$

Mixed venous oxygen content:

$$C_{\bar{v}}O_2 = (1.34 \times Hb \times S_{\bar{v}}O_2) + [(0.0031 \times P_{\bar{v}}O_2 \times \alpha) + (0.01997 \times P_{\bar{v}}O_2 \times \beta)] \quad (2)$$

Oxygen delivery:

$$\dot{D}O_2 = \frac{[CO \times (C_aO_2 \times 10)]}{weight} \quad (3)$$

Oxygen consumption:

$$\dot{V}O_2 = \frac{CO \times [(C_aO_2 - C_{\bar{v}}O_2) \times 10]}{weight} \quad (4)$$

Extraction ratio:

$$ER = \frac{\dot{V}O_2}{\dot{D}O_2} \quad (5)$$

Where C_aO_2 = arterial oxygen content in mL/dL
$C_{\bar{v}}O_2$ = mixed venous oxygen content in mL/dL
Hb = hemoglobin concentration in mg/dL
S_aO_2 = arterial oxygen saturation fraction
P_aO_2 = arterial oxygen tension in mmHg
$\dot{D}O_2$ = oxygen delivery in L/minute/kg body weight
$\dot{V}O_2$ = oxygen consumption in L/minute/kg body weight
ER = extraction ratio
CO = cardiac output in L/minute

0.0031 = oxygen solubility coefficient in plasma in mL/dL
0.01997 = oxygen solubility coefficient in PFC in mL/dL
α = blood fraction of circulation volume
β = PFC fraction of circulating volume

Statistical Methods

Unless otherwise stated all data were analyzed using repeated-measures analysis of variance (ANOVA) controlling for cardiac index, PFC administration, and breathing gas administered, followed by Least Squares Means Student's *t*-test, as appropriate, to determine if treatment and control groups were significantly different. Data are presented as Least Squares Means ± Standard Error. Differences considered statistically significant with p-values of less than 0.05. All statistical calculations were performed using the statistical software JMP 7 from SAS Institute (Cary, NC).

Results

One-way ANOVA performed on baseline data obtained during the stabilization period post surgery and pre-dive, controlling for breathing gas, indicated that there were no statistically significant differences between the PFC-treated group and the saline controls on any of the variables of interest (PFC vs. saline - cardiac index (indexed to body weight)(CI): 91.88 ± 0.65 vs. 100.84 ± 0.63 mL/minute/kg, p=0.3305; C_aO_2: 15.72 ± 0.53 vs. 14.67 ± 0.51 mL/dL, p=0.1680; $C_{\bar{v}}O_2$: 12.28 ± 0.48 vs. 10.95 ± 0.47 mL/dL, p=0.0610; $\dot{D}O_2$: 14.35 ± 0.90 vs. 14.65 ± 0.87 mL/minute/kg, p=0.8097; $\dot{V}O_2$: 3.09 ± 0.34 vs. 3.71 ± 0.33 mL/minute/kg, p=0.1931; ER: 0.22 ±0.019 vs. 0.25 ± 0.019, p=0.2895).

After compression/decompression it was found that, with regards to CI, the repeated measures ANOVA was significant as a whole model (F=14.91, df=5, p<0.0001) as well as with regards to perfluorocarbon administration (F=17.99, df=1, p<0.0001) and with regards to time (F=7.06, df=1, p=0.0087). Using Student's *t*-test cardiac index was found to decrease over the course of the experiment and it was found to be significantly lower in the PFC-treated group vs. the saline control group (82.66 ± 3.46 vs. 102.61 ± 3.18 mL/minute/kg, p<0.0001 – see figure 3). Due to the significant effect PFC administration had on CI, CI was controlled for in all further analyses.

The analysis of arterial oxygen content, as a whole model, was found to be significant (F=10.89, df=6, p<0.0001). This model was also found to be significant with respect to PFC administration (F=17.31, df=1, p<0.0001) and with respect to time (F=8.61, df=1, p=0.0038). Using Student's *t*-test It was found that C_aO_2 was significantly higher in the PFC-treated group vs. the saline group (16.30 ± 0.27 vs. 14.75 ± 0.25 mL/dL, p<0.0001) and increased over the course of the experiment – see figure 4.

With regard to mixed venous oxygen content, it was found that the whole model was significant (F=7.68, df=6, p<0.0001), but neither PFC administration (F=3.71, df=1, p=0.0558) nor time (F=1.16, df=1, p=0.2822) had a significant effect. Student's *t*-test indicated that $C_{\bar{v}}O_2$ was not significantly different in the PFC-treated group vs. the saline control group (12.45 ± 0.26 vs. 11.74 ± 0.24 mL/dL, p=0.0558) – see figure 5.

With regards to $\dot{D}O_2$, it was found that the whole model was significant (F=104.27, df=6, p<0.0001) and that PFC administration had a significant effect (F=12.97, df=1,

p=0.0004), but time did not (F=3.46, df=1, p=0.0648). Student's *t*-test showed that the effect of the PFC was to increase $\dot{D}O_2$ over the saline control group (14.83 ± 0.28 vs. 13.44 ± 0.25 mL/minute/kg, p=0.0004) – see figure 6.

Similar effects were observed with regard to $\dot{V}O_2$. The whole model was significant (F=26.22, df=6, p<0.0001), and both PFC (F=10.11, df=1, p=0.0018) and time (F=9.13, df=1, p=0.0029) were observed to have significant effects. Student's t-test showed that PFC increased $\dot{V}O_2$ vs. saline control (3.37 ± 0.14 vs. 2.76 ± 0.13 mL/minute/kg, p=0.0018) and $\dot{V}O_2$ was found to increase over the course of the experiment – see figure 7.

When extraction ratio, defined as $\dot{V}O_2 / \dot{D}O_2$, was analyzed it was found that the whole model was significant (F=4.94, df=6, p<0.0001). The effect of PFC was found not to be statistically significant (F=1.76, df=1, p=0.1869) while the effect of time was significant (F=5.85, df=1, p=0.0167). PFC administration did not significantly increase the ER over saline control (0.23 ± 0.012 vs. 0.21 ± 0.011, p=0.1869), but ER did increase over the course of the experiment – see figure 8.

Discussion and Conclusions

As has been seen before in other split-hoofed animal models, I.V. administration of PFCs resulted in decreased CI in this model(152). In the previous work conducted in pigs, pulmonary hypertension was reported to be the cause of the observed decrease in CI and similar observations were made here(152). When analysed using the same repeated measures ANOVA model described above pulmonary arterial pressure was found to be nearly doubled in the PFC-treated animals vs. the saline control (27.10 ± 1.12 vs. 15.50 ±

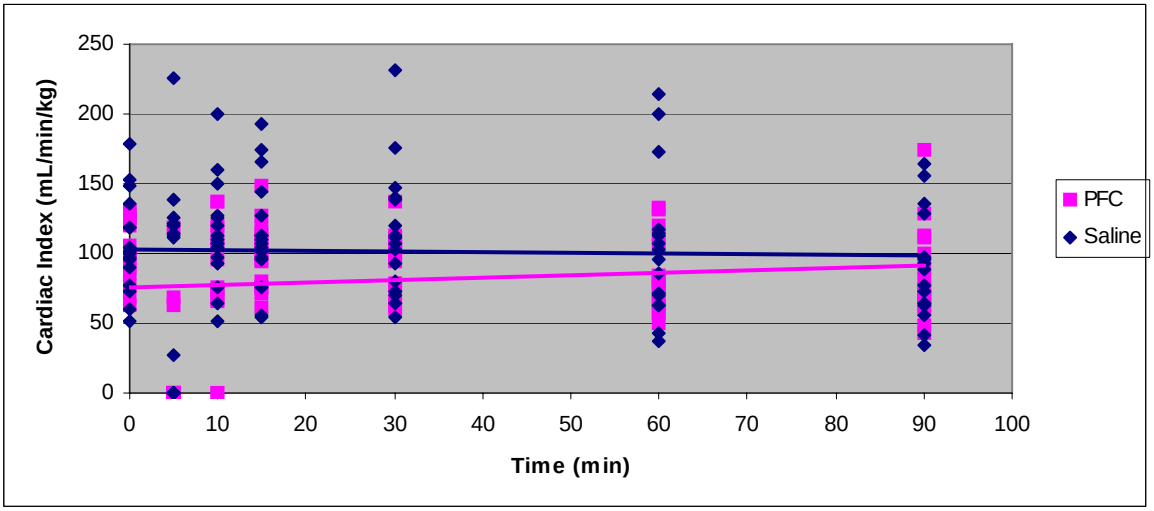
1.12, $p<0.0001$). This suggests that the problem of pulmonary hypertension leading to decreased CI will likely be present in all split-hoofed species.

It is clear from this study that I.V. PFC administration results in an increase in CaO_2. In the PFC-treated group arterial oxygen content is elevated nearly 11% over control. Even if the oxygen carried directly by the PFC is removed from the calculations, arterial oxygen content is still significantly higher in the PFC-treated vs. the saline-treated group (15.87 ± 0.26 vs. 14.73 ± 0.24 mL/dL, $p=0.0019$), so it would appear that PFC does more than simply carry more oxygen. Exactly what PFC is doing, in addition to its own nascent oxygen carrying ability, is unclear. Further analysis using the repeated measures ANOVA model showed both PaO_2 and SaO_2 were significantly lower in the PFC-treated vs. saline-treated group (200.03 ± 10.77 vs. 238.72 ± 9.90 mmHg, $p=0.0109$ and 93.52 ± 0.89 vs. 97.39 ± 0.81 %, $p=0.0021$, respectively). Hemoglobin (Hb) was elevated in the PFC-treated group vs. the saline control after compression/decompression (12.22 ± 0.17 vs. 10.72 ± 0.15 mg/dL, $p<0.0001$) but not when baseline results were analysed using one-way ANOVA (11.19 ± 0.38 vs. 10.61 ± 0.37 mg/dL, $p=0.2931$). It is possible that the presence of the PFC is inducing the release of erythrocytes from the spleen or other storage, accounting for the higher Hb, and thus the higher CaO_2 in the PFC-treated group.

Likewise, the observation that PFC administration results in increases in both $\dot{D}O_2$ and $\dot{V}O_2$ of 10% and 22%, respectively, demonstrates that the PFC is able to not only increase the amount of oxygen present in the blood, but is able to improve tissue access to that oxygen. This suggests that the mechanism of I.V. PFC improving tissue oxygenation is not simply its ability to dissolve and carry greater quantities of oxygen, but that it

facilitates oxygen delivery to cells. This may take the form of the PFC extravasating in capillary beds, taking dissolved oxygen with it. Alternatively the PFC emulsion particles, being approximately $1/100^{th}$ - $1/1000^{th}$ the size of an erythrocyte, may be able to pass through blood vessels where red cell flow has been blocked by bubbles, but a trickle flow of plasma remains(31, 151) – see figures 9 and 10. In this case the small amount of oxygen carried in the PFC may be sufficient to keep tissue viable which otherwise might succumb to hypoxic injury. More interestingly, PFC particles may act as a bridge, of sorts, facilitating the movement of oxygen from erythrocytes out into cells where it is needed. This possibility has very intriguing implications. As shown above, the amount of oxygen actually dissolved in PFC is relatively small. Hemoglobin binding oxygen remains the dominant mechanism for oxygen transport. Once in capillary beds the greatest impediment to oxygen offloading from hemoglobin into tissue is the plasma(133). Oxygen is very insoluble in plasma, and much more soluble in PFC. In this case the PFC would act almost as a transport vessel for oxygen, ferrying it from erythrocytes to tissue, a mechanism somewhat akin to facilitated diffusion across cell membranes. These possible mechanisms should be explored further in future studies.

It is clear from these results that improved tissue oxygenation at a whole-body level is likely responsible for at least a portion of the beneficial effects offered by the I.V. administration of PFC emulsions after severe decompression sickness.



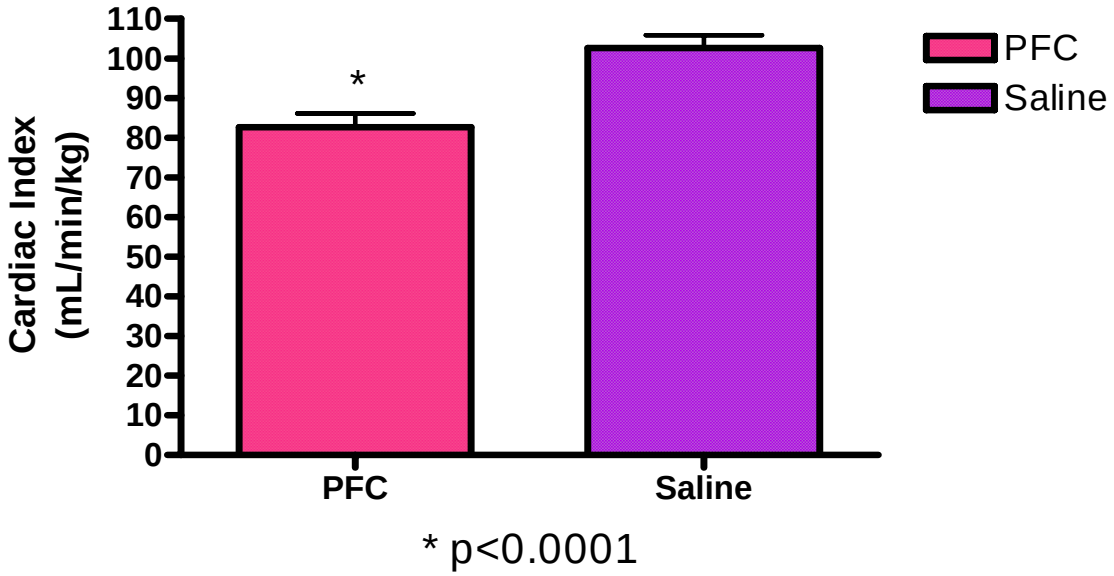


Figure 3: The effect of perfluorocarbon administration on cardiac index.

The effect of the administration of PFC emulsions, when the effects of time and breathing gas are controlled for, is to significantly decrease cardiac index vs. the saline control group (82.66 ± 3.46 vs. 102.61 ± 3.18 mL/minute/kg, $p<0.0001$).

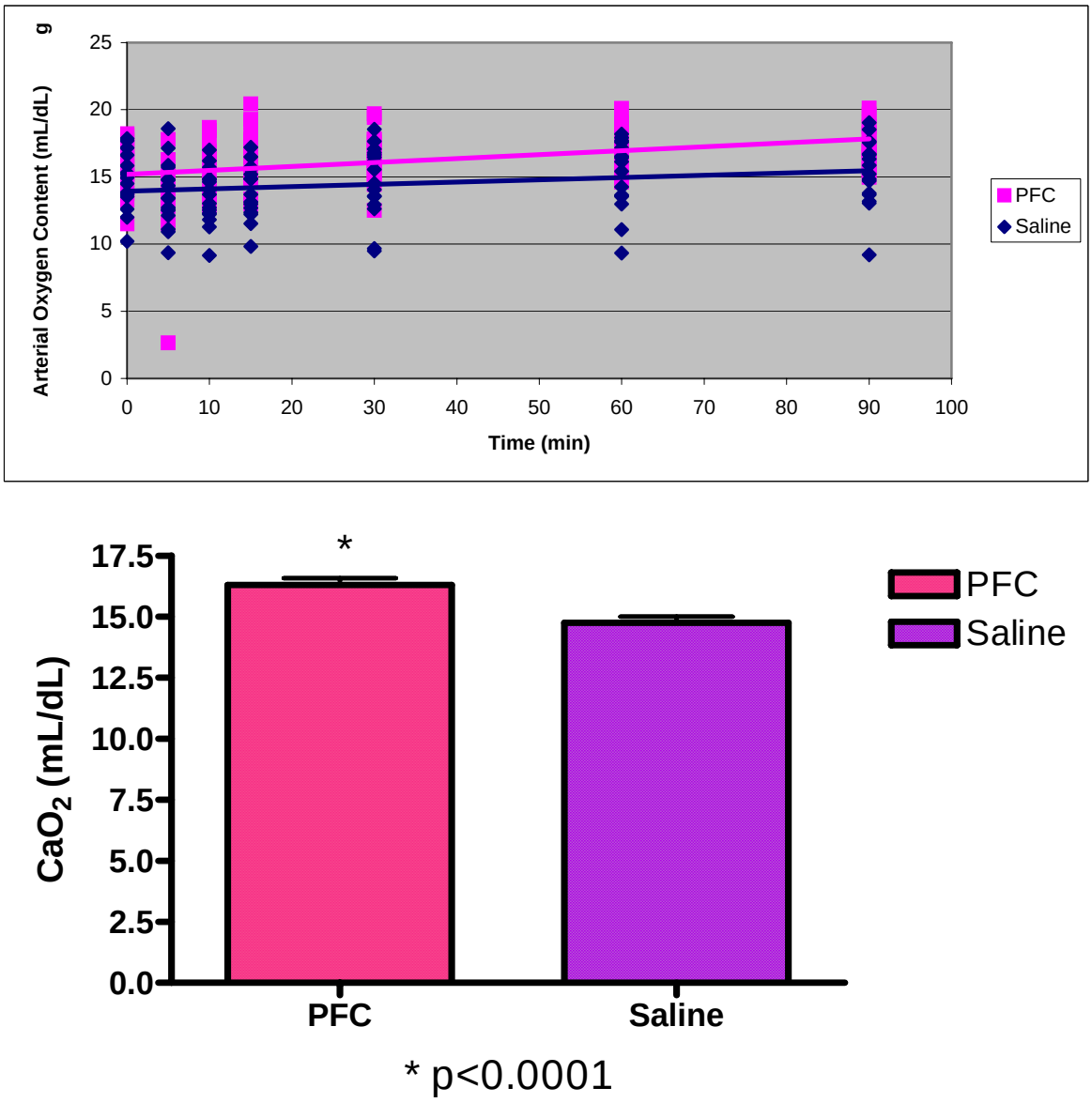
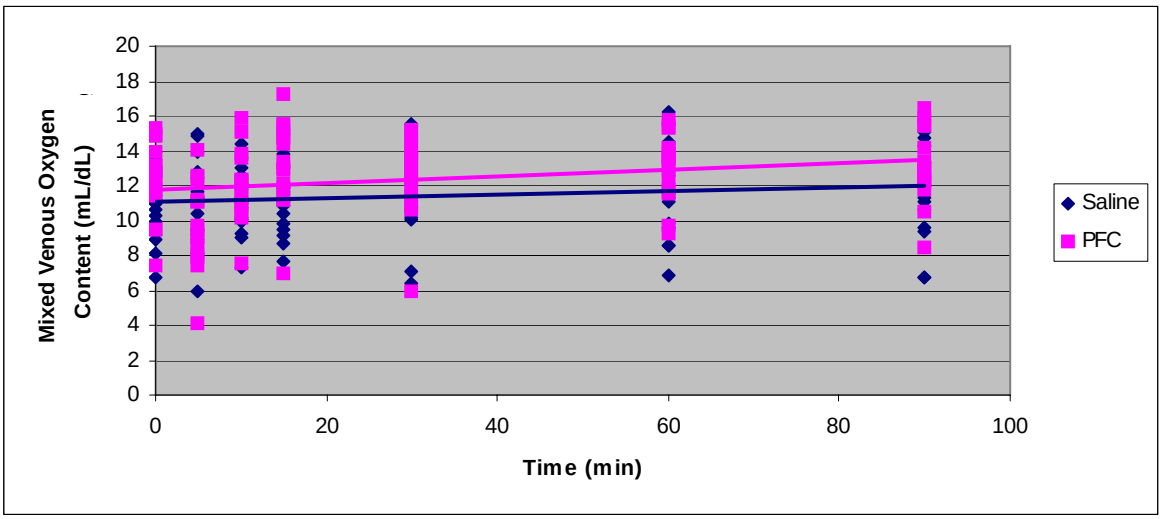


Figure 4: The effect of perfluorocarbon administration on arterial oxygen content.

The effect of the administration of PFC emulsions, when the effects of time, breathing gas, and CI are controlled for, is to significantly increase C_aO_2 vs. the saline control group (16.30 ± 0.27 vs. 14.75 ± 0.25 mL/dL, p<0.0001).



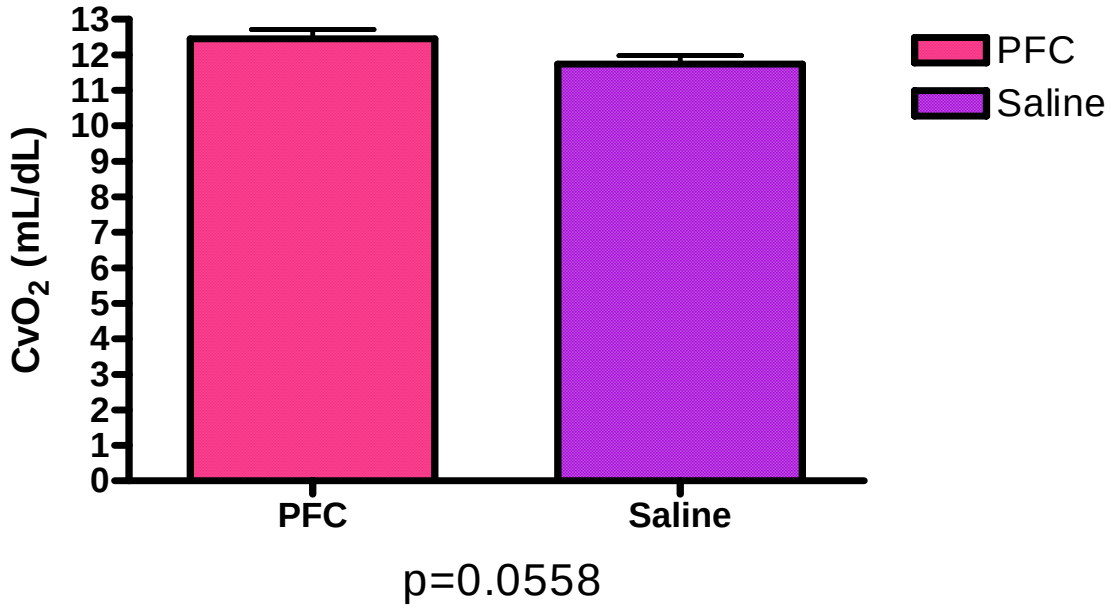
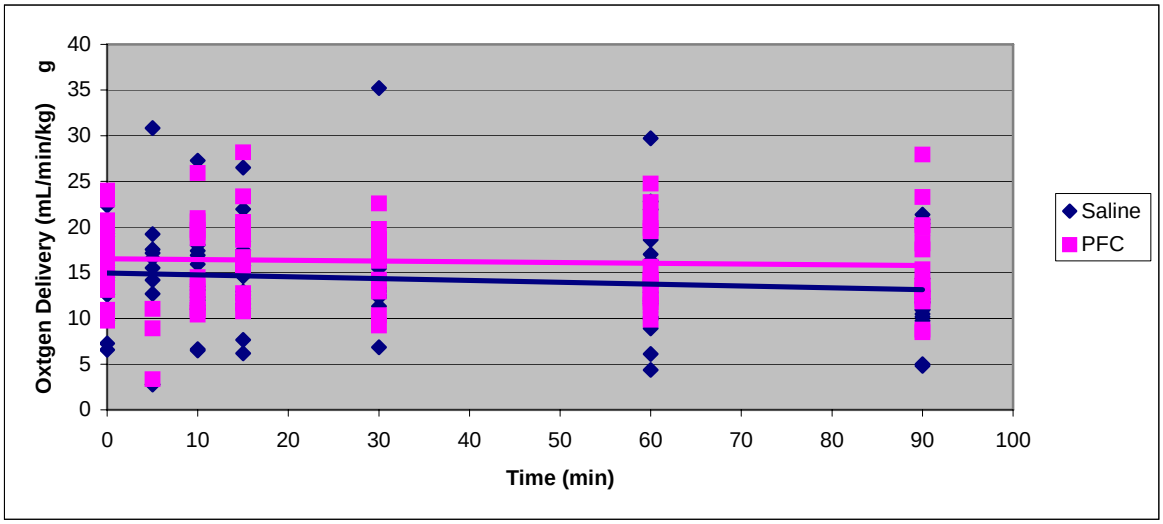


Figure 5: The effect of perfluorocarbon administration on mixed venous oxygen content.

When the effects of time, breathing gas, and CI are controlled for, PFC has no significant effect on $C_{\bar{v}}O_2$ vs. the saline control group (12.45 ± 0.26 vs. 11.74 ± 0.24 mL/dL, p=0.0558).



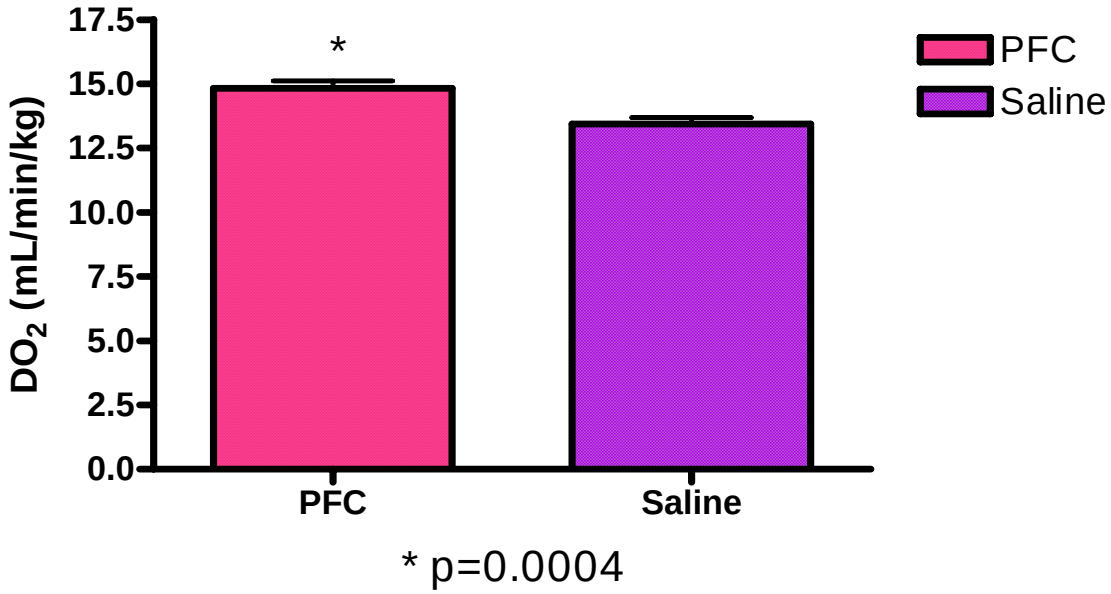
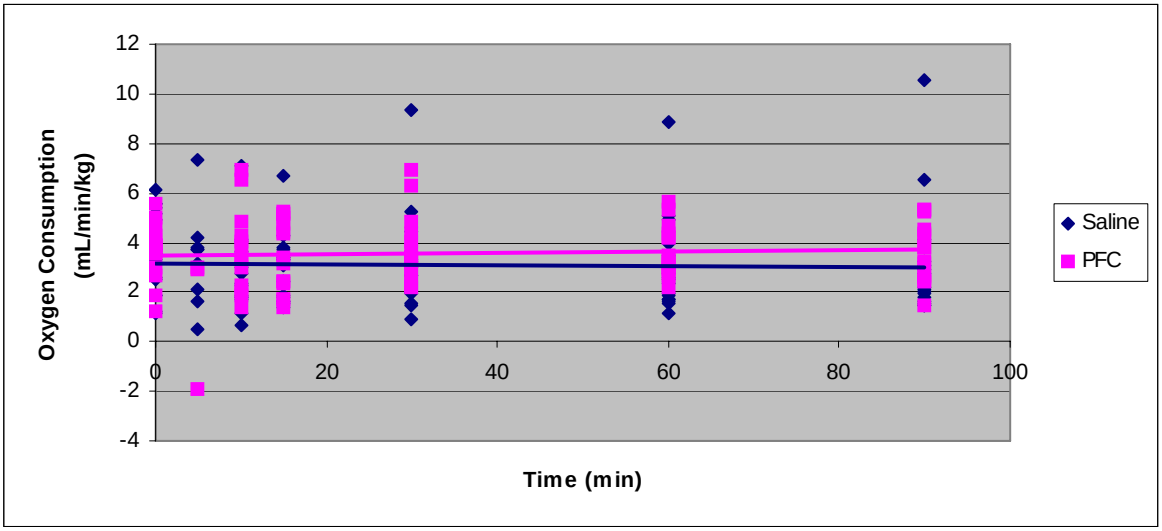


Figure 6: The effect of perfluorocarbon administration on oxygen delivery.

The effect of the administration of PFC emulsions, when the effects of time, breathing gas, and CI are controlled for, is to significantly increase $\dot{D}O_2$ vs. the saline control group (14.83 ± 0.28 vs. 13.44 ± 0.25 mL/minute/kg, p=0.0004).



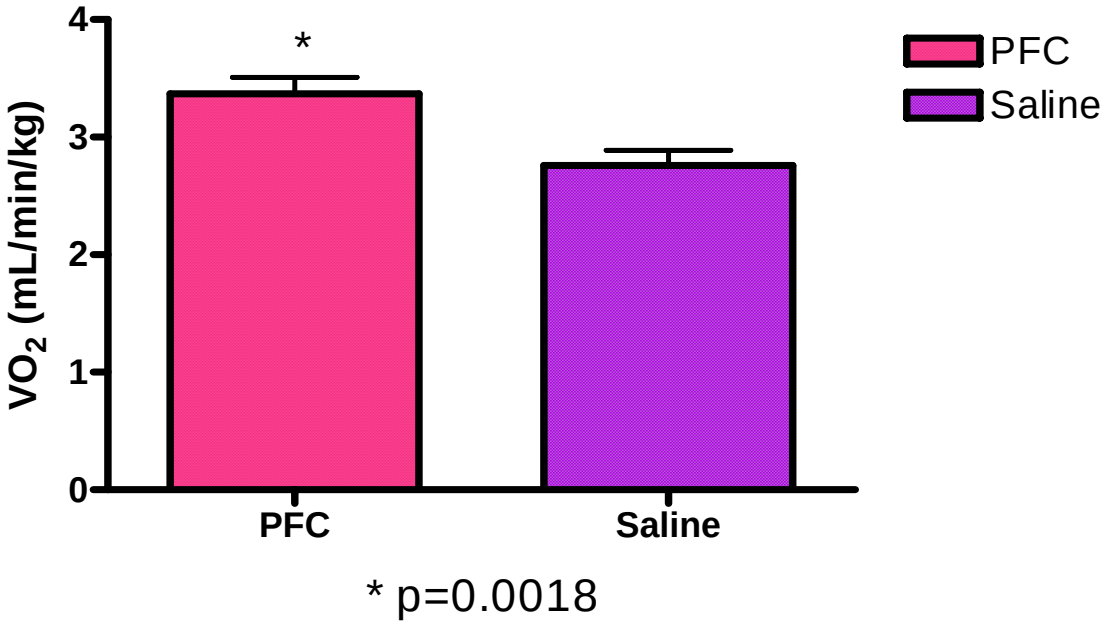
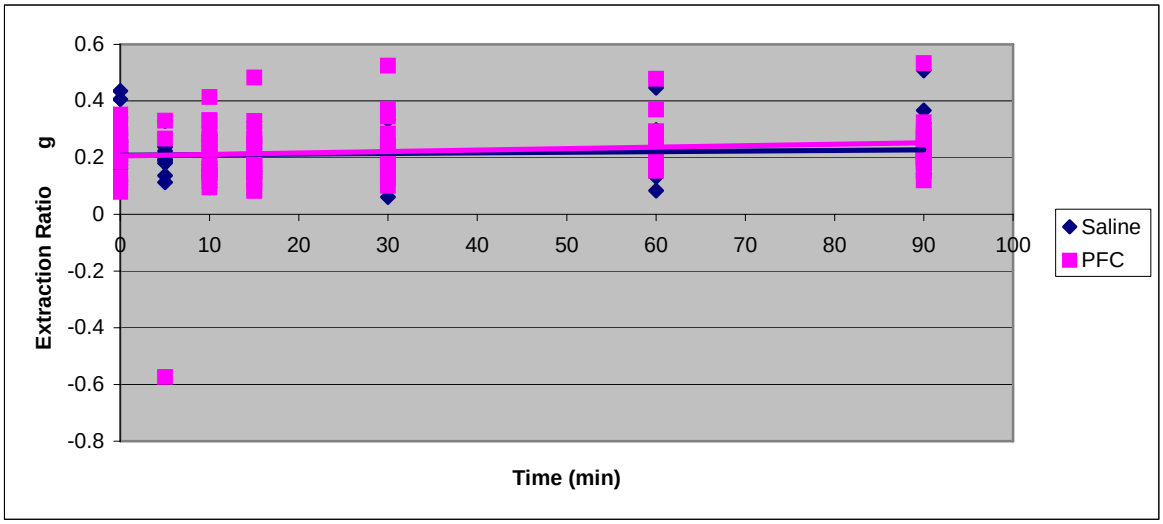


Figure 7: The effect of perfluorocarbon administration on oxygen consumption.

The effect of the administration of PFC emulsions, when the effects of time, breathing gas, and CI are controlled for, is to significantly increase $\dot{V}O_2$ vs. the saline control group (3.37 ± 0.14 vs. 2.76 ± 0.13 mL/kg/minute, p=0.0018).



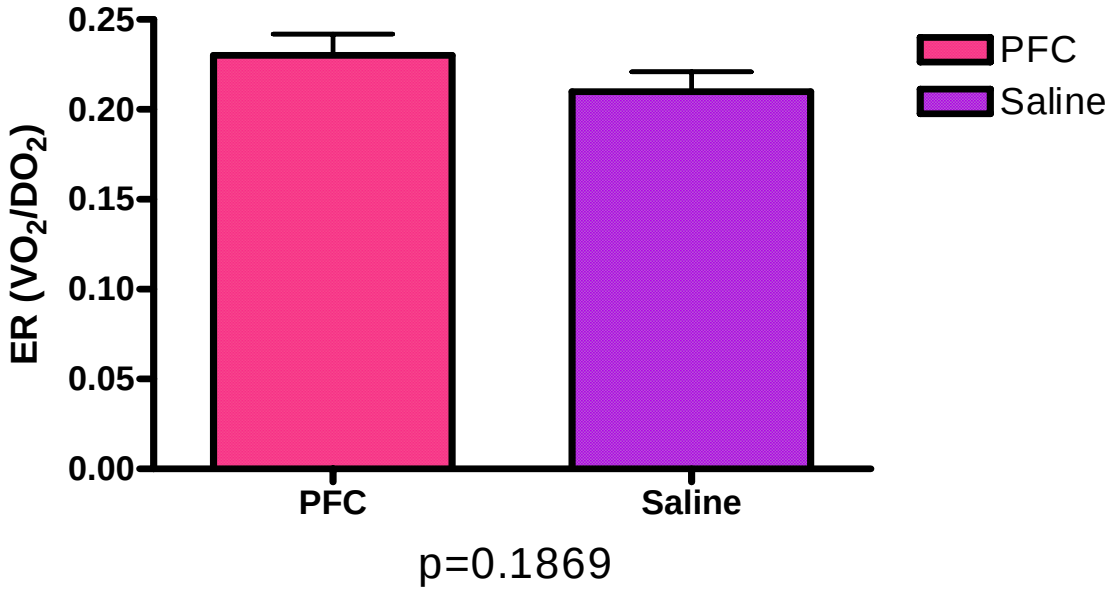


Figure 8: The effect of perfluorocarbon administration on extraction ratio.

When the effects of time, breathing gas, and CI are controlled for, PFC has no significant effect on ER vs. the saline control group (0.23 ± 0.012 vs. 0.21 ± 0.011, p=0.1869).

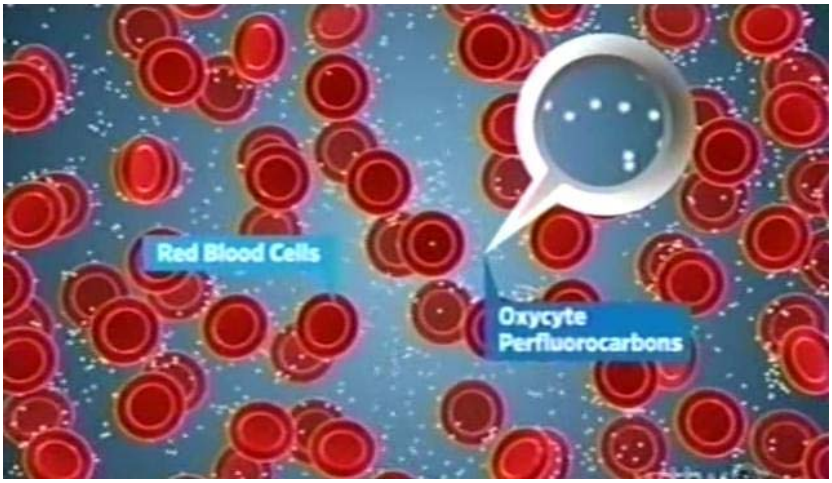


Figure 9: Erythrocyte size compared with PFC emulsion particle size.

A cartoon illustrating the relative size of red blood cells (diameter approx 6-8 μm) compared to Oxycyte® PFC emulsion particle size (approx 0.1-04 μm).

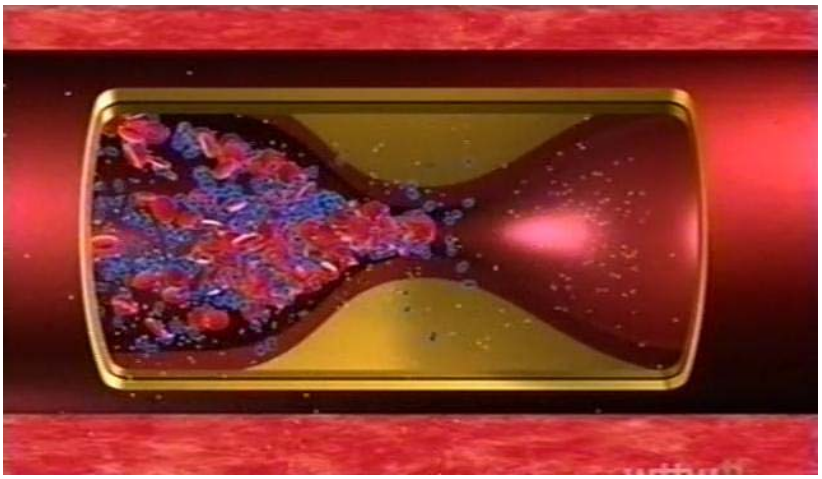


Figure 10: Perfluorocarbon emulsion particles can pass thrombi which block blood flow.

A cartoon illustrating that PFC emulsion particles, due to their small size (approx 0.1-0.4 μm), are able to flow past emboli such as bubbles, fat, blood clots, and constrictions which obstruct the flow of erythrocytes. By moving past such obstructions PFCs may be able to continue to deliver enough oxygen to tissues with no red cell flow to stave off hypoxic injury or death.

CHAPTER 3

INTRAVENOUS PERFLUOROCARBON EMULSIONS INCREASE THE EXTENT, BUT NOT THE RATE WHILE HELIUM-OXYGEN BREATHING INCREASES THE RATE, BUT NOT THE EXTENT OF NITROGEN WASHOUT AFTER SEVERE DECOMPRESSION SICKNESS

Introduction

Breathing compressed air increases the amount of nitrogen dissolved in body fluids(54, 112, 135). Factors such as depth, and thus the ambient pressure, and the duration of the dive are the primary determinants of the amount of excess nitrogen absorbed(26, 54, 112, 135). As a diver ascends and ambient pressure decreases, it is possible for the dissolved gas tensions in tissue to exceed ambient pressure. This supersaturated state leads to the formation and growth of free gas bubbles resulting in venous gas emboli (VGE) and possible arterial gas emboli (AGE)(26, 32). It is believed that these bubbles within the vasculature are the root cause of decompression sickness (DCS)(26, 32). There are likely multiple pathophysiologic mechanisms at play in DCS including impairment of microcirculation by inert gas bubbles, increased blood viscosity, damage to endothelium, and activation of complement. The physiochemical discontinuity of the gas-blood interface can also denature proteins promoting the release of fatty acids from cell membranes leading to the formation of fat emboli(26, 32). When intravascular bubbles obstruct capillaries or venules, it often leads to ischemia followed by reperfusion oxidative tissue damage(37).

Perfluorocarbon emulsions (PFCs) are intravenous (I.V.) emulsions of fluoridated hydrocarbons within phospholipid micro-particle micelles(155). PFCs have been developed in medicine as intravenous oxygen therapeutics(155). PFCs accomplish this in a way which is fundamentally different from how whole blood transports the majority of its oxygen content. Oxygen, or any other gas, carried by PFCs is not bound as oxygen is to hemoglobin, rather it is dissolved in the PFC itself. PFC, because of its non-polarity, can dissolve up to 60 volume percent oxygen(117). Plasma can only dissolve 0.034 volume percent oxygen, but whole blood at 14 gm/dl hemoglobin can chemically bind up to 21 volume percent oxygen. The oxygen dissolved in PFC is all available for tissue usage whereas oxygen chemically bound by hemoglobin is restricted (usually only 23% can be released for metabolism). Microcirculatory changes such as edema, vasospasm, white cell activation and vessel plugging result in decreased erythrocyte delivery of oxygen to watershed neurons, yet plasma flow continues without red cells(160). PFC, due to its extremely small particle size (~0.1-0.4 μm) can be delivered in this trickle-flow of plasma(9, 137, 155). Plasma flow oxygen delivery by PFCs is enough to keep tissue alive, as seen with Fluosol DA-20%, a first generation PFC which reduced myocardial infarction and garnered FDA approval(86, 87).

PFC is also effective in treating DCS, AGE and VGE(23, 31, 147-149, 151, 196). Using a saturation dive model with direct ascent to the surface, Dromsky *et al.* found that the administration of I.V. PFC while breathing 95% oxygen was able to decrease the lethality of this decompression insult, decrease the incidence of DCS, and decrease the number of neurological events(31). Similarly it has been found that I.V. PFC can improve

outcomes after massive VGE, cerebral AGE, and coronary AGE(148-150). The administration of I.V. PFC has also been shown to increase nitrogen washout through the lungs after venous air embolism(196). Clearly PFC administration is of benefit in the treatment of decompression illnesses, but the exact mechanism of this benefit has not yet been elucidated. Is this benefit a result of the PFCs ability to increase nitrogen washout, and thus, presumably, to remove the bubbles obstructing circulation faster? Is it a product of improving the oxygen supply and thus metabolic state of tissue to which blood flow and oxygen supply has been disturbed? Is it some combination of these two processes?

Helium-based breathing mixes also offer an interesting and relatively unexplored avenue of investigation. It is well understood that helium-based breathing gases improve ventilation and clinical condition in asthma and chronic obstructive pulmonary disease (COPD) patients(44, 139, 143). This improvement is a product of the physical properties of helium(43). Helium is approximately an order of magnitude less dense than either oxygen or nitrogen and helium-oxygen (HeO_2) mixtures are always less dense than nitrogen-oxygen mixtures(43). Inhaling HeO_2 mixtures alters ventilatory mechanics by modifying gas convection in the airways thus increasing alveolar ventilation(28, 30, 43). The dramatically decreased density of HeO_2 mixtures compared to nitrogen-oxygen mixtures results in airflow through conducting airways being much less turbulent(28, 30, 43, 124, 184). This laminar flow, in turn, results in less airway resistance and a much smaller pressure gradient being required to move gas through the tracheo-bronchial tree(28, 30, 43, 124, 184). Clinically this reduces the work of breathing and improves oxygen delivery to the alveoli(28, 30, 43, 124, 184). These improvements have been

observed not only in spontaneously breathing patients, but also in patients receiving both non-invasive positive pressure ventilation and mechanical ventilation(42, 83, 84, 94).

The research described here was designed to investigate the effect of I.V. PFC emulsions and helium-based breathing mixtures, both administered acutely after surfacing, on nitrogen washout in an ovine model of severe DCS.

Materials and Methods

This study was performed in conjunction with the whole-body oxygenation study described above in chapter 2.

All animal experiments performed for this project were done in strict accordance with the National Institutes of Health (NIH) "Guide for the Care and Use of Laboratory Animals" and were approved by both the Department of Defense (DoD) and the Virginia Commonwealth University Institutional Animal Care and Use Committee (IACUC). Juvenile Dorper cross sheep of either sex (Robinson Services, Inc., Mocksville, NC) weighing 15-20 kg were housed in United States Department of Agriculture (USDA)- and Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-approved facilities in social flocks with free access to food and water on a 12-hour light/dark cycle. Sheep were allowed a minimum of 3 days for acclimatization and veterinary inspection prior to their use in any experiment.

Sheep Instrumentation and Preparation

48 hours prior to the experiment, sheep were muzzled to prevent access to food and provide free access to water while remaining with the remainder of their flock in order to

limit animal stress. Sheep were initially sedated with ketamine/xylazine (20.0/2.0 mg/kg I.M.) and the animal was placed supine on the surgical table. All animals were promptly intubated with a 9.0 mm internal diameter (I.D.) cuffed endotracheal tube (Hudson RCI, Temecula, CA) and ventilated with 50/50 nitrogen/oxygen using a Siemens 900C servo ventilator (Siemens Corp., New York, NY) set to a tidal volume of approximately 10 mL/kg adjusted to maintain arterial pCO_2 at 40 mmHg ± 5 mmHg. An orogastric tube fashioned from TYGON® R-3603 tubing (Satin-Gobain Performance Plastics Corp., Akron, OH) was advanced into the rumen to allow for fluid drainage and to allow gas accumulated in the gut during the air dive to vent upon decompression. A MAC® 2-port introducer sheath (Arrow International Inc., Reading, PA) was placed in the right external jugular vein to allow for the administration of fluids and the anesthetic cocktail. Once I.V. access was secured, administration of the triple drip anesthetic cocktail (ketamine/xylazine/guaifenesin 2.0/0.1/50.0 mg/mL in 5% dextrose) was begun immediately at 1.0-2.0 mL/kg/hr, titrated to maintain a surgical plane of anesthesia using a Harvard Apparatus PHD 2000 programmable syringe pump (Harvard Apparatus, Holliston, MA). The right femoral artery was canulated with an 18 gauge (G) femoral arterial catheter (Arrow International Inc., Reading, PA) for monitoring of arterial pressure (AP) and arterial blood sampling. The right femoral vein was canulated with a 4 French (Fr) double lumen catheter (Arrow International Inc., Reading, PA) for the administration of the anesthetic cocktail while inside the hyperbaric chamber and for the administration of the treatment drug after exiting the hyperbaric chamber. The left femoral vein was canulated for the placement of a 7.5 Fr CCOmbo® continuous cardiac output (CCO)

pulmonary artery catheter to allow for CCO monitoring, central venous (CVP) and pulmonary arterial pressure (PAP) monitoring, and central venous blood sampling. Respiratory gases were continuously monitored using an MGA 1100 respiratory mass spectrometer (Perkin-Elmer, Norwalk, CT). Following surgical manipulations all animals were allowed to stabilize for a minimum of 30 minutes. After stabilization animals were weaned off the ventilator until capable of spontaneously breathing prior to being placed inside the hyperbaric chamber.

Inclusion/Exclusion Criteria

At the end of the stabilization period all animals were required to meet certain inclusion criteria prior to being weaned off the ventilator and placed in the hyperbaric chamber. The inclusion criteria were as follows: mean arterial pressure above 60 mmHg, arterial pO_2 above 200 mmHg, arterial pH 7.45 ± 5, arterial pCO_2 40 ± 5 mmHg. All animals not meeting the above inclusion criteria were excluded from the study and euthanized.

Sheep Dry Dive Procedures

All sheep that met the study inclusion criteria (n=31) were weaned off the ventilator until capable of breathing spontaneously, disconnected form all monitoring equipment and placed into a Reimers Systems model #17-48-100 Research Hyperbaric Chamber (Reimers Systems, Inc., Springfield, VA). During the dry dive procedures all animals breathed room air and general anesthesia was maintained using a continuous infusion of triple drip as described above. All sheep were subjected to the following dive profile: Beginning at 1.0 ATA the chamber was compressed at a rate of 1.0 ATA/minute to a pressure of 2.0 ATA.

From 2.0 ATA the chamber was compressed at a rate of 2.0 ATA/min to a pressure of 6.0 ATA (165 feet of seawater (fsw), or 608 kilopascals (kPa)). The pressure of 6.0 ATA was maintained for 27 minutes after which sheep were immediately decompressed to 1.0 ATA at a rate of 2.0 ATA/minute.

Post-Decompression Monitoring

Upon complete decompression (considered time=0) all animals were quickly removed from the hyperbaric chamber and all monitoring equipment was reconnected. At this point animals were randomized to receive I.V. infusion of either 6.0 mL/kg Oxycyte® PFC emulsion (n=15) (Synthetic Blood International, Costa Mesa, CA) or saline control (n=16) and one of four breathing gases; 100% oxygen (n=8), 80/20% nitrogen/oxygen (n=8), 50/50% helium/oxygen (n=7), or 80/20% helium oxygen (n=8). Animals were randomized using a block randomization scheme such that for every 8 experiments performed, one animal fell into each of the 8 treatment groups, but the order within each block was random. Data from all instruments was recorded directly to hard drive storage using the BioPac system and Acqknowledge v. 3.90 software (BioPac Systems, Inc. Goleta, CA). Neuromuscular blockade with I.V. pancuronium (0.1 mg/kg) administered acutely post-chamber was necessary in order to accurately measure respiratory gases with the mass spectrometer.

Statistical Methods

Nitrogen washout curves for the 5 minute period beginning at time=0. Breath-by-breath measures of end-tidal nitrogen were plotted against time and analyzed using non-

linear regression to fit a single exponential decay curve to the data using the following equation:

$$y = \alpha^{(-Kx)} + \beta$$

Where α = peak

K = decay constant

β = plateau

This curve-fitting generates 3 variables which can be examined: First, a value for the y-intercept, hereafter referred to as the 'peak'. Second, a value for the exponential decay constant, which represents the rapidity with which nitrogen is eliminated. Higher numbers indicate more rapid elimination. Third, a value for the level which the right-most portion of the curve asymptotically approaches, hereafter referred to as the 'plateau'. This number represents the extent to which nitrogen is eliminated with lower numbers indicating greater elimination. Since this study was examining only nitrogen washout and not initial body nitrogen loading, the peak was not analyzed. One-way ANOVA followed by Dunnett's test using the 100% oxygen-breathing group as the control group was employed in order to examine differences between groups in the decay constant and plateau. Student's *t*-test was used in cases where only 2 groups were being compared. Data are presented as Means ± Standard Error. Curve-fitting and statistical analyses were performed using GraphPad Prism 4 curve-fitting and statistical software (GraphPad Software, Inc. San Diego, CA).

Results

Before plotting nitrogen washout curves as described above all 8 groups were separated into 2 broad categories – those with nitrogen in their inhaled gas (Saline + Room Air (n=4) and PFC + Room Air (n=4) groups) and those without nitrogen in their inhaled gas (Saline + 100% O_2 (n=4), Saline + 50/50 HeO_2 (n=4), Saline + 80/20 HeO_2 (n=4), PFC + 100% O_2 (n=4), PFC + 50/50 HeO_2 (n=3), and PFC + 80/20 HeO_2 (n=4)). The 2 groups with nitrogen in their inhaled gas were analyzed separately from the other 6 groups because it is not possible for them to eliminate nitrogen to the extent the other groups can, given that their inhaled gas contains 80% nitrogen.

Fitting of the end-tidal nitrogen data from the groups with nitrogen in their inhaled gas (Saline + Room Air and PFC + Room Air) to the above single exponential decay equation resulted in the curves seen in Figure 11. Details of the nonlinear fit are shown in Table 7.

Table 7: Best-fit values resulting from the fit of a single exponential curve to the end-tidal nitrogen data obtained from the Saline + Room Air and the PFC + Room Air groups.

	Saline + Room Air	**PFC + Room Air**
Best-fit Values		
Decay Constant	0.02453	0.02871
Plateau	566.3	563.0
Standard Error		
Decay Constant	0.02017	0.008135
Plateau	1.136	0.6879
95% Confidence Interval		
Decay Constant	-0.01501 to 0.06408	0.01277 to 0.04466
Plateau	564.1 to 568.5	561.7 to 564.4

When the decay constants obtained from the Saline + Room Air and PFC + Room Air curve fittings were compared using Student's t-test it was found that there was no statistically significant difference (0.02453 ± 0.02017 vs. 0.02871 ± 0.008135 Saline vs. PFC, t=0.1922, p=0.8539). See figure 12.

When the plateaus obtained from the Saline + Room Air and PFC + Room Air curve fittings were compared using Student's t-test it was found that the plateau for the Saline + Room Air group was significantly higher than that for the PFC + Room Air group (566.3 ± 1.136 vs. 563.0 ± 0.6879 mmHg, t=2.485, p=0.0475). See figure 13.

Fitting of the end-tidal nitrogen data from the groups without nitrogen in their inhaled gas (Saline + 100% O_2, Saline + 50/50 HeO_2, Saline + 80/20 HeO_2, PFC + 100% O_2, PFC + 50/50 HeO_2, and PFC + 80/20 HeO_2) to the above single exponential decay equation resulted in the curves seen in figure 14. Details of the nonlinear fit are shown in Table 8.

One-way ANOVA was used to compare the decay constants obtained from the Saline + 100% O_2, Saline + 50/50 HeO_2, Saline + 80/20 HeO_2, PFC + 100% O_2, PFC + 50/50 HeO_2, and PFC + 80/20 HeO_2 groups. The ANOVA was found to be significant ($p<0.0001$). Dunnett's post-hoc test using the Saline + 100% O_2 group as the control group revealed that there were no statistically significant differences between the Saline + 100% O_2,group and the Saline + 80/20 HeO_2, PFC + 100% O_2, and PFC + 50/50 HeO_2 groups (0.03363 ± 0.001518 vs. 0.03067 ± 0.002362, 0.02758 ± 0.001444, and 0.02976 ± 0.002260, respectively). Dunnett's post-hoc test also found that the decay constants for the Saline + 50/50 HeO_2 and the PFC + 80/20 HeO_2 groups were significantly higher than that

of the Saline + 100% O_2 group (0.03363 ± 0.001518 vs. 0.04186 ± 0.001003, $p<0.05$; and 0.04481 ± 0.001343, $p<0.01$, respectively). See figure 15.

Table 8: Best-fit values resulting from the fit of a single exponential curve to the end-tidal nitrogen data obtained from the Saline + 100% O_2, Saline + 50/50 HeO_2, Saline + 80/20 HeO_2, PFC + 100% O_2, PFC + 50/50 HeO_2, and PFC + 80/20 HeO_2 groups.

	Saline + 100% O_2	**Saline + 50/50 HeO_2**	**Saline + 80/20 HeO_2**	**PFC + 100% O_2**	**PFC + 50/50 HeO_2**	**PFC + 80/20 HeO_2**
Best-fit Values						
Decay Constant	0.03363	0.04186	0.03067	0.02758	0.02976	0.04481
Plateau	26.17	23.40	21.22	16.82	15.88	17.00
Standard Error						
Decay Constant	0.001518	0.001003	0.002362	0.001444	0.002260	0.001343
Plateau	2.425	1.189	2.903	2.621	2.996	1.177
95% Confidence Interval						
Decay Constant	0.03066 to 0.03661	0.03989 to 0.04382	0.02604 to 0.03530	0.02475 to 0.03041	0.02533 to 0.03419	0.04218 to 0.04744
Plateau	21.41 to 30.92	21.07 to 25.73	15.53 to 26.91	11.68 to 21.95	10.01 to 21.76	14.69 to 19.30

When the plateaus obtained from the Saline + 100% O_2, Saline + 50/50 HeO_2, Saline + 80/20 HeO_2, PFC + 100% O_2, PFC + 50/50 HeO_2, and PFC + 80/20 HeO_2 curve fittings were compared to the Saline + 100% O_2 group using one-way ANOVA it was found that the whole model was significant ($p=0.0294$). Dunnett's post-hoc test revealed that there was no statistically significant difference between the Saline + 100% O_2 group

and the Saline + 50/50 HeO_2 and Saline + 80/20 HeO_2 groups (26.17 ± 2.425 vs. 23.40 ± 1.189 and 21.22 ± 2.903, respectively). Dunnett's post-hoc test also revealed that the plateaus for the PFC + 100% O_2, PFC + 50/50 HeO_2, and PFC + 80/20 HeO_2 groups were significantly lower than that of the Saline + 100% O_2 group (26.17 ± 2.425 vs. 16.82 ± 2.621, $p<0.05$; 15.88 ± 2.996, $p<0.05$; and 17.00 ± 1.177, $p<0.05$, respectively). See figure 16.

In order to better examine the specific effects of the PFC and the various breathing gas mixes independently, the data were pooled based on PFC (n=11) vs. saline (n=12) administration as well as by breathing gas administered (100% O_2 (n=8), 50/50 HeO_2 (n=7), or 80/20 HeO_2 (n=8)).

Fitting of the end-tidal nitrogen data from the pooled saline- and PFC-treated groups to the above single exponential decay equation resulted in the curves seen in figure 17. Details of the nonlinear fit are shown in Table 9.

Table 9: Best-fit values resulting from the fit of a single exponential curve to the end-tidal nitrogen data obtained from the saline- and the PFC-treated groups.

	Saline	**PFC**
Best-fit Values		
Decay Constant	0.03176	0.03096
Plateau	22.22	15.98
Standard Error		
Decay Constant	0.001044	0.0009402
Plateau	1.566	1.380
95% Confidence Interval		
Decay Constant	0.02971 to 0.03380	0.02912 to 0.03280
Plateau	19.15 to 25.29	13.28 to 18.69

When the decay constants obtained from the saline- and PFC -treated curve fittings were compared using Student's *t*-test it was found that there was no statistically significant difference (0.03176 ± 0.001044 vs. 0.03096 ± 0.0009402 saline vs. PFC, t=0.5656, p=0.5777). See figure 18.

When the plateaus obtained from the saline- and PFC-treated curve fittings were compared using Student's *t*-test it was found that the plateau for the saline-treated group was significantly higher than that for the PFC-treated group (22.22 ± 1.566 vs. 15.98 ± 1.380 mmHg, t=2.967, p= 0.0074). See figure 19.

Fitting of the end-tidal nitrogen data from the pooled breathing gas groups to the above single exponential decay equation resulted in the curves seen in figure 20. Details of the nonlinear fit are shown in Table 10.

Table 10: Best-fit values resulting from the fit of a single exponential curve to the end-tidal nitrogen data obtained from the 100% O_2-, 50/50 HeO_2-, and 80/20 HeO_2-breathing groups.

	100 % O_2	**50/50 HeO_2**	**80/20 HeO_2**
Best-fit Values			
Decay Constant	0.02649	0.02826	0.03786
Plateau	19.14	16.29	19.80
Standard Error			
Decay Constant	0.001056	0.001326	0.001518
Plateau	2.154	2.175	1.548
95% Confidence Interval			
Decay Constant	0.02442 to 0.02856	0.02566 to 0.03086	0.03488 to 0.04084
Plateau	14.91 to 23.36	12.03 to 20.55	16.76 to 22.83

When the decay constants obtained from the 50/50 HeO_2 and 80/20 HeO_2 curve fittings were compared to the 100% O_2 group using one-way ANOVA followed by

Dunnett's post-hoc test it was found that the ANOVA was significant ($p<0.0001$). Dunnett's post-hoc test showed that there was no statistically significant difference between the 100% O_2 group and the 50/50 HeO_2 group (0.02649 ± 0.001056 vs. 0.02826 ± 0.001326, $p>0.05$). It was also found that the decay constant for the 80/20 HeO_2 group was significantly higher than that of the 100% O_2 group (0.03786 ± 0.001518 vs. 0.03363 ± 0.001518, $p<0.01$). See figure 21.

When the plateaus obtained from the 50/50 HeO_2 and 80/20 HeO_2 curve fittings were compared to the 100% O_2 group using one-way ANOVA it was found that the ANOVA was not statistically significant ($p=0.4381$). See figure 22.

Discussion and Conclusions

As is intuitively obvious, the data gathered from the air-breathing animals demonstrates clearly that a low fraction of inspired nitrogen (F_IN_2) is required for effective nitrogen washout to take place. This should come as no surprise. Nitrogen washout, like any other process of diffusion, relies on a concentration gradient. So long as there is a high level of nitrogen in the alveolar gas there is little driving force to remove nitrogen from the blood. At the same time, the degree to which nitrogen can be removed is severely limited. Diffusion of nitrogen out of the blood into the alveolar gas will only proceed until the concentration of nitrogen is balanced across the alveolar wall. Because of this limit imposed on off-gassing, it makes no sense to compare the groups breathing room air with the other groups breathing 100% oxygen or heliox mixtures, the latter of which all have F_IO_2 of zero.

There were two major findings that came out of this project. Firstly, the administration of HeO_2 results in higher decay constant values without affecting plateau values. Secondly, the administration of PFC results in lower plateau values without affecting decay constant values. The decay constant is a measure of how quickly the end-tidal nitrogen is changing, or practically speaking, how quickly the nitrogen is being removed. Higher values indicate that the end-tidal nitrogen is decreasing faster, i.e. nitrogen washout is proceeding more quickly.

When the 6 treatment groups are analyzed separately it is interesting to see that the effects of helium were not dose dependant and would appear to be dependant on the intravenous therapy administered. When I.V. saline was administered, 50/50 HeO_2 resulted in a significant increase in the decay constant, while 80/20 HeO_2 showed no difference vs. 100% O_2. When I.V. PFC was administered the opposite was true - 80/20 HeO_2 resulted in a significant increase in the decay constant, while 50/50 HeO_2 showed no difference vs. 100% O_2. What this represents is unclear. It may represent a difference in the number and size of bubbles lodged in the pulmonary microcirculation which is unrelated to either PFC administration or breathing gas, but rather represents a manifestation of the stochastic nature of DCS and the relatively small group sizes. In this case examining the data pooled based on breathing gas is likely to be much more informative.

This pooled data indicated that the decay constant in the 80/20 HeO_2 group was significantly higher than the 100% O_2 group, but that the 50/50 HeO_2 group was not different. This suggests that a high F_IHe is required to have maximum impact on increasing the speed of nitrogen washout. This is not surprising. Since the benefits of helium lie

purely in its physical properties, primarily its density, these benefits will be lost as the F_IHe decreases. HeO_2 has been administered previously for the treatment of experimental DCS(3, 78-81). These studies have shown that breathing HeO_2 is of benefit both when used during recompression and at 1 atmosphere absolute (ATA) after decompression(3, 78-81). However, there is no record in the scientific literature of previous investigations examining the effect of HeO_2 on nitrogen washout. Previous studies have examined either death rates or used microscopic observation of the growth and shrinkage of bubbles deliberately placed in surgically exposed tissue (primarily adipose tissue)(3, 78-81). This study, therefore, represents the first examination of the mechanism of the previously-described benefits of HeO_2 breathing after DCS.

The effect of PFC administration was very different from that of helium. PFC resulted in a decrease in the plateau without affecting the decay constant. The plateau represents the level to which end-tidal nitrogen is decreased over the period of observation, and thus the extent to which nitrogen is removed. Lower values for the plateau indicate that more nitrogen has been removed. It was found that the administration of PFC resulted in lower plateau values across the board. This was observed amongst all the different breathing gases, including the room air groups – all the PFC-treated groups showed significantly lower plateau levels than the saline-treated groups. This indicates that PFC administration, while not affecting the speed of nitrogen washout, results in nitrogen washout proceeding to a greater extent. Only two previous studies have examined the effect of PFC administration on nitrogen washout and the findings of this study agree well with both of them(99, 196). Both of these previous studies were conducted at 1 ATA,

Lundgren *et al.* using a model where nothing was done to increase the amount of nitrogen dissolved in body tissues and Zhu *et al.* using a venous gas embolism model(99, 196). The present study represents the first demonstration that the ability of PFC to enhance nitrogen washout is retained in the DCS situation where blood flow through the pulmonary microcirculation is disrupted by the presence of large numbers of bubbles.

The findings of the current study offer insight into the mechanism for the previously observed protective effects of both HeO_2 breathing and I.V. PFC administration in treating DCS. This study demonstrates for the first time that the effect of HeO_2 is to increase the speed of nitrogen washout. This study also demonstrates for the first time that the effect of the PFC is to increase the amount of nitrogen washed out. Although we were able to demonstrate statistically significant effects of I.V. PFC, these results must be taken with a grain of salt since the magnitude of this effect was so small that it is likely it is of little or no clinical relevance. Up until this point it has been widely believed that the therapeutic benefit derived from the administration of PFC in cases of severe DCS were largely a product of their ability to dissolve inert gases such as N_2, and aid in the removal of excess N_2 from the body, thus alleviating the bubble obstructions and restoring normal tissue blood flow(23, 31, 98, 100, 102, 151, 175). This work has demonstrated that, although PFC administration does, indeed, increase nitrogen washout, that this effect is so small, a mere 6.24 mmHg difference, that it is not very likely that this explaines the dramatic protective effects previously observed with the administration of I.V. PFC after decompression(23, 31, 100, 102, 151, 175). This novel finding combined with the results from study 1 described above shed new light on the likely mechanism of the protection

afforded by PFC administration after DCS. These findings suggest strongly that improved tissue oxygenation is far more important than minimal increases in nitrogen washout.

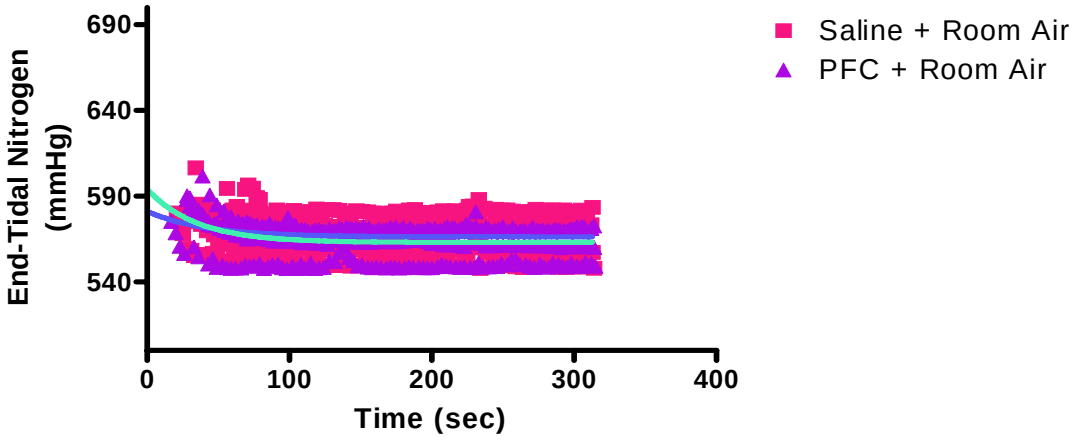


Figure 11: Nitrogen washout curves from the groups with nitrogen in their inspired gas.

Best-fit curves resulting from the fit of a single exponential curve to the end-tidal nitrogen data obtained from the Saline + Room Air and the PFC + Room Air groups. For Saline + Room Air R^2=0.02240, for PFC + Room Air R^2=0.1210.

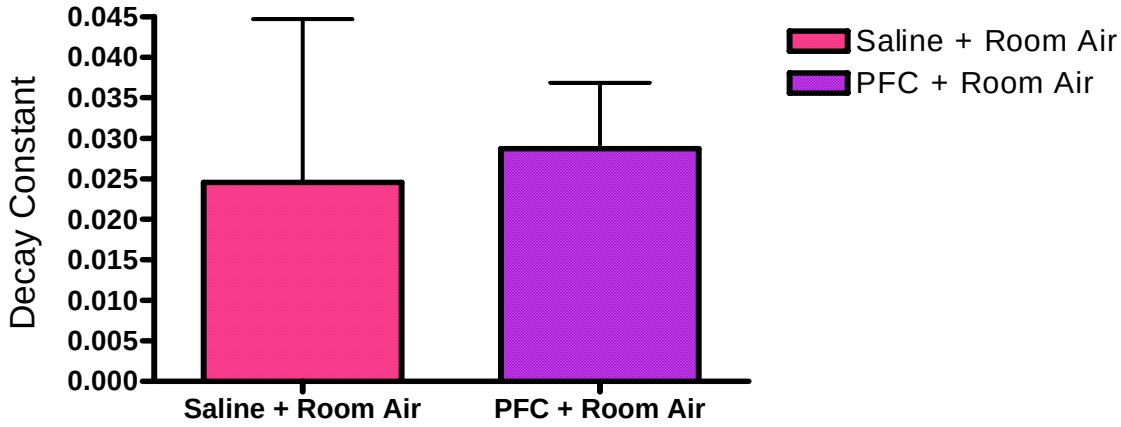


Figure 12: Comparison of the decay constants from the groups with nitrogen in their inspired gas.

The decay constants from the groups with nitrogen in their inhaled gas were compared using Student's t-test. It was found that there was no significant difference between the Saline + Room Air group and the PFC + Room Air groups.

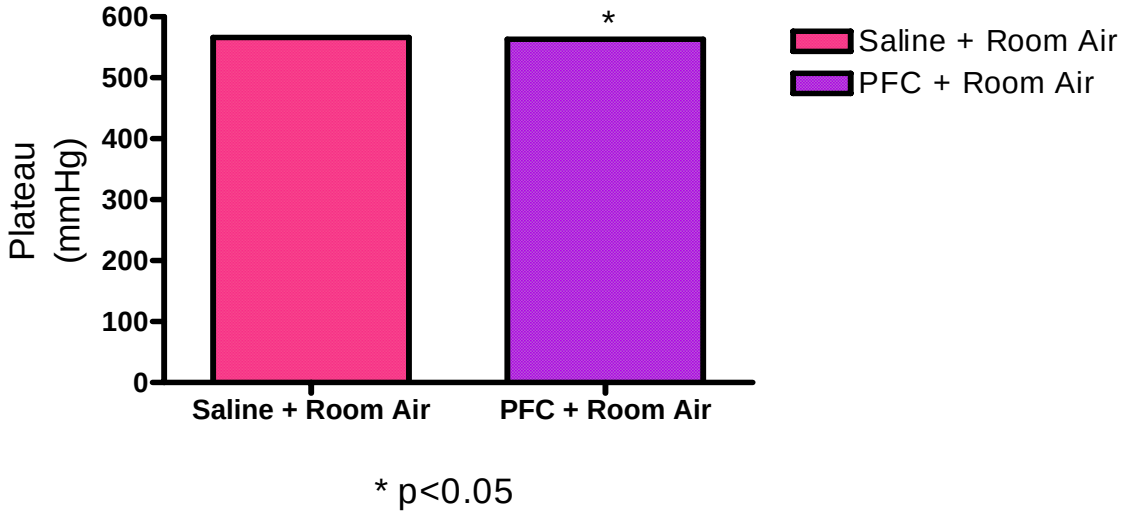


Figure 13: Comparison of the plateaus from the groups with nitrogen in their inspired gas.

The plateaus from the groups with nitrogen in their inhaled gas were compared using Student's t-test. It was found that the plateau for the PFC + Room Air group was significantly lower than that of the Saline + Room Air group.

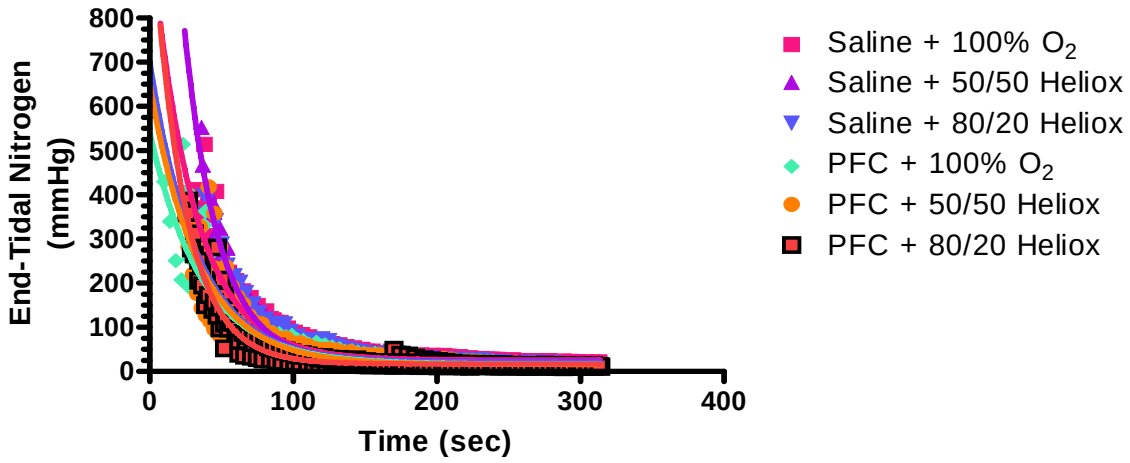


Figure 14: Nitrogen washout curves from the groups without nitrogen in their inspired gas.

Best-fit curves resulting from the fit of a single exponential curve to the end-tidal nitrogen data obtained from the Saline + 100% O_2 (R^2= 0.8691), Saline + 50/50 HeO_2 (R^2= 0.9614), Saline + 80/20 HeO_2 (R^2= 0.7483), PFC + 100% O_2 (R^2= 0.8300), PFC + 50/50 HeO_2 (R^2= 0.7516), and PFC + 80/20 HeO_2 (R^2= 0.9387) groups.

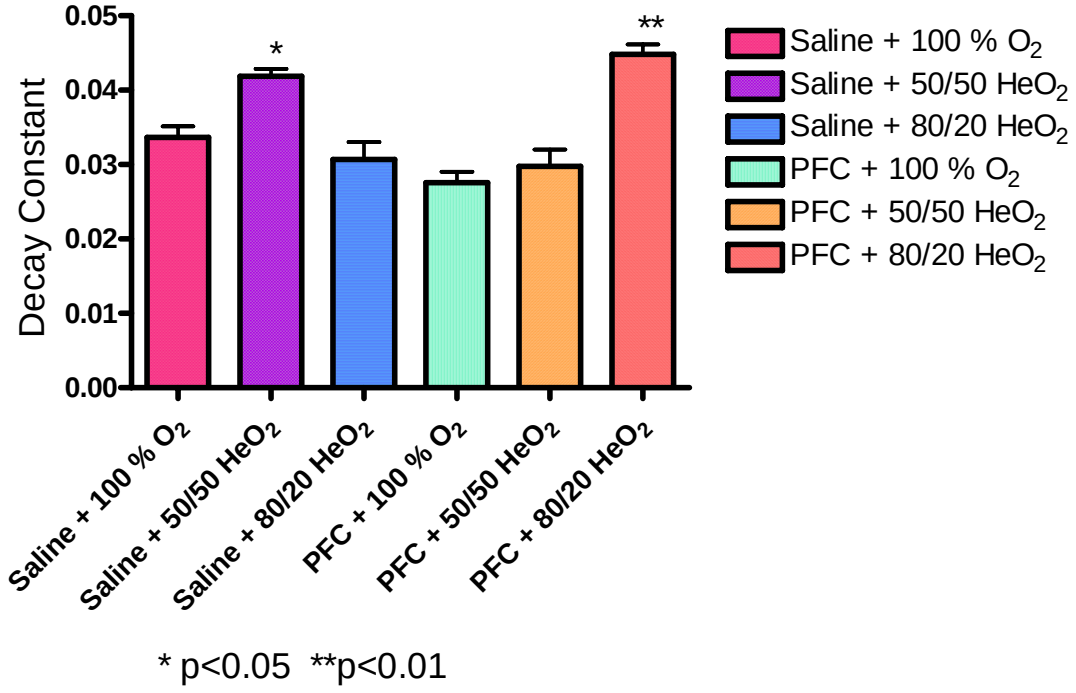


Figure 15: Comparison of the decay constants from the groups without nitrogen in their inspired gas.

The decay constants from the groups without nitrogen in their inhaled gas were compared to the decay constant of the Saline + 100% O_2 group using one-way ANOVA followed by Dunnett's test. It was found that decay constants for both the Saline + 50/50 HeO_2 and the PFC + 80/20 HeO_2 groups were significantly higher than that of the Saline + 100% O_2 group.

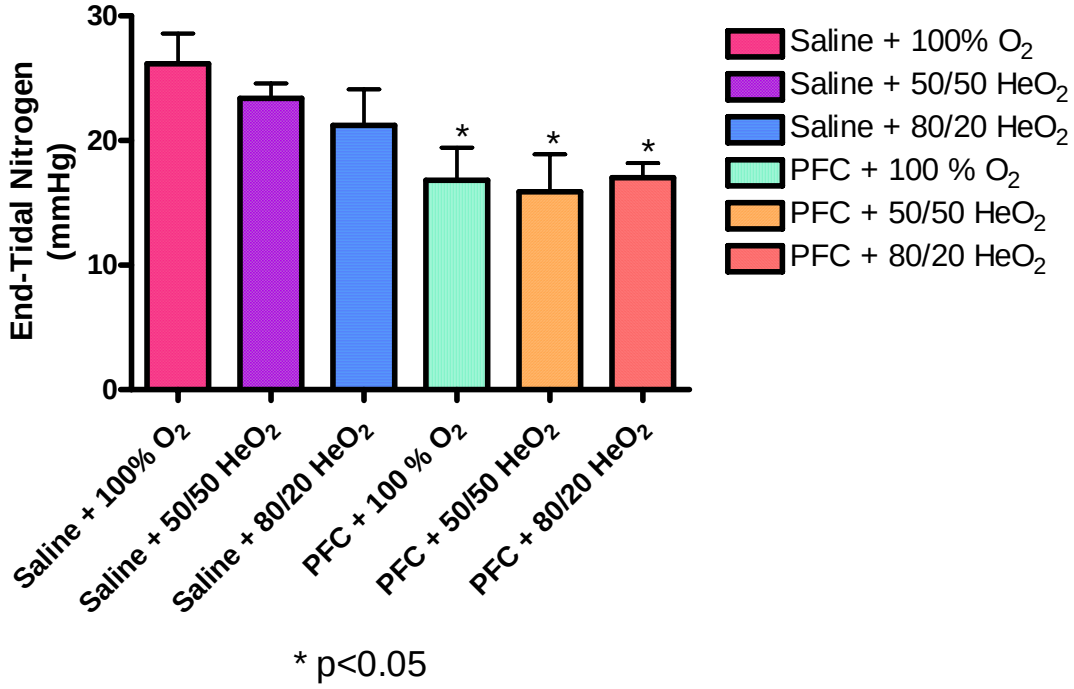


Figure 16: Comparison of the plateaus from the groups without nitrogen in their inspired gas.

The plateaus from the groups without nitrogen in their inhaled gas were compared to the plateau of the Saline + 100% O_2 group using one-way ANOVA followed by Dunnett's test. It was found that the plateaus for the PFC + 100% O_2, PFC + 50/50 HeO_2 and PFC + 80/20 HeO_2 groups were all significantly lower than that of the Saline + 100% O_2 group.

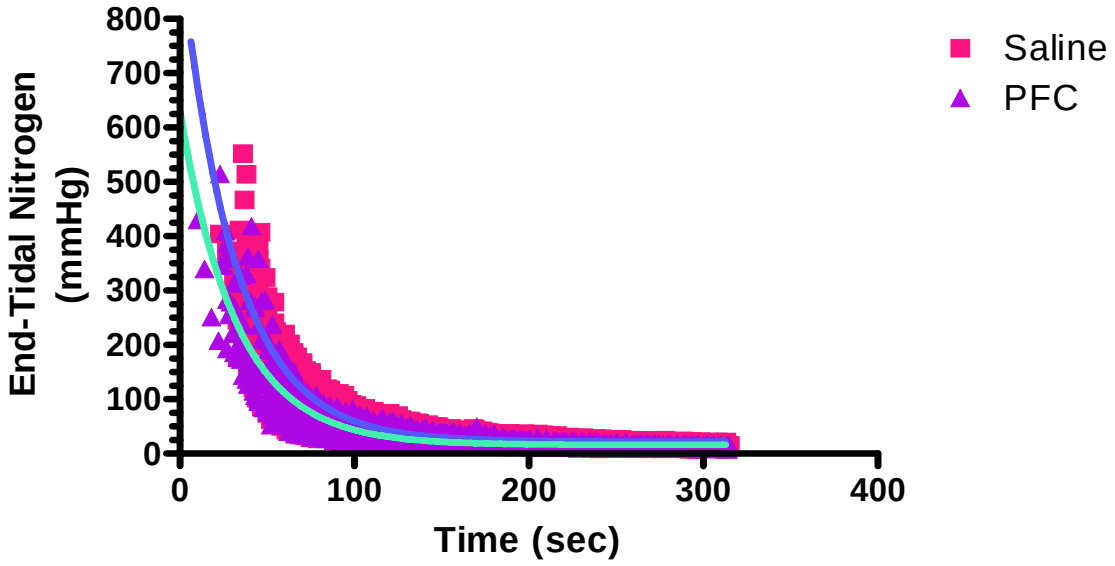


Figure 17: Nitrogen washout curves from groups pooled based on PFC administration.

Best-fit curves resulting from the fit of a single exponential curve to the end-tidal nitrogen data obtained from the pooled saline- and the PFC-treated groups. For saline R^2=0.8201, for PFC R^2= 0.8249.

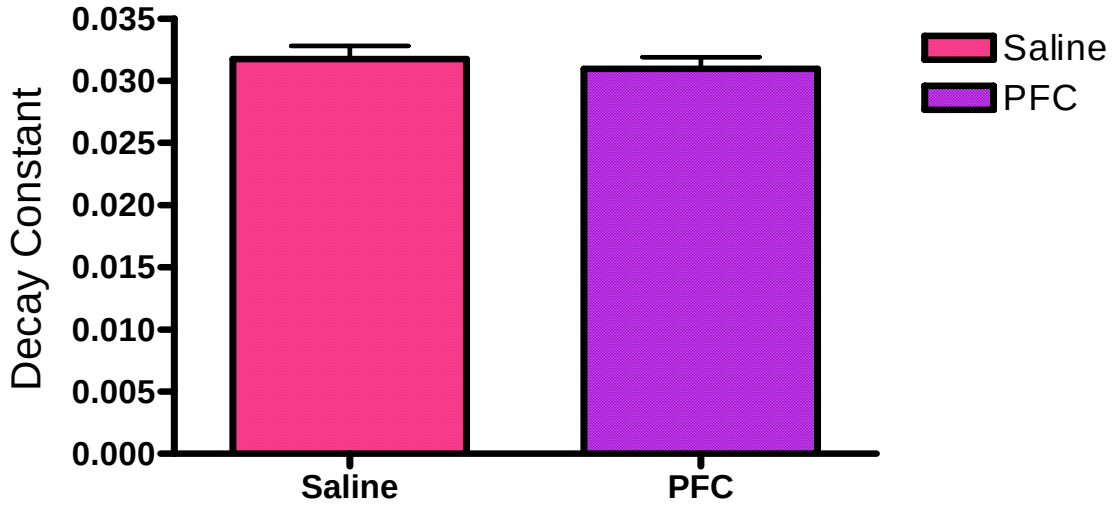


Figure 18: Comparison of the decay constants from the pooled saline- and PFC-treated groups.

The decay constants from the saline- and PFC-treated groups were compared using Student's t-test. It was found that there was no significant difference between the saline-group and the PFC-treated groups.

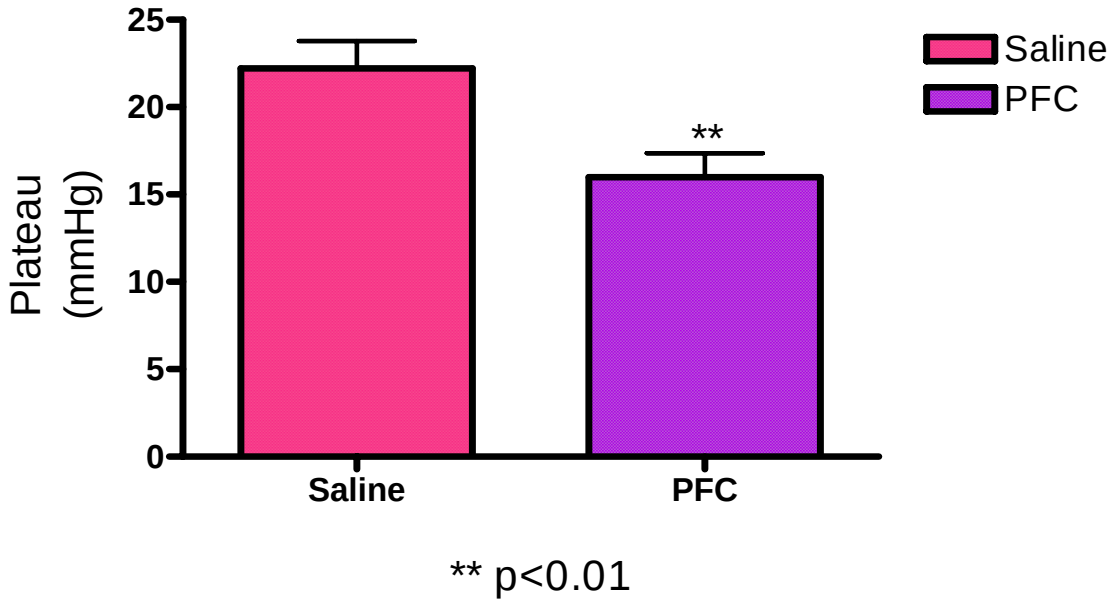


Figure 19: Comparison of the plateaus from the pooled saline- and PFC-treated groups

The plateaus from the saline- and PFC-treated groups were compared using Student's t-test. It was found that the plateau for the PFC-treated group was significantly lower than that of the saline-treated group.

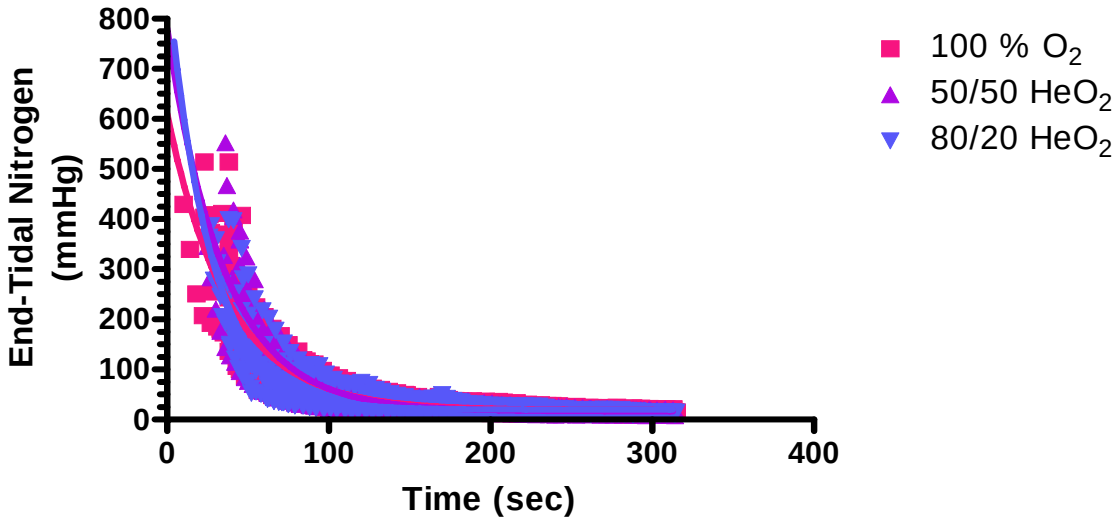


Figure 20: Nitrogen washout curves from groups pooled based on breathing gas administration.

Best-fit curves resulting from the fit of a single exponential curve to the end-tidal nitrogen data obtained from the pooled 100% O_2 (R^2= 0.8046), 50/50 HeO_2 (R^2= 0.7805), and 80/20 HeO_2 (R^2= 0.8198) breathing gas groups.

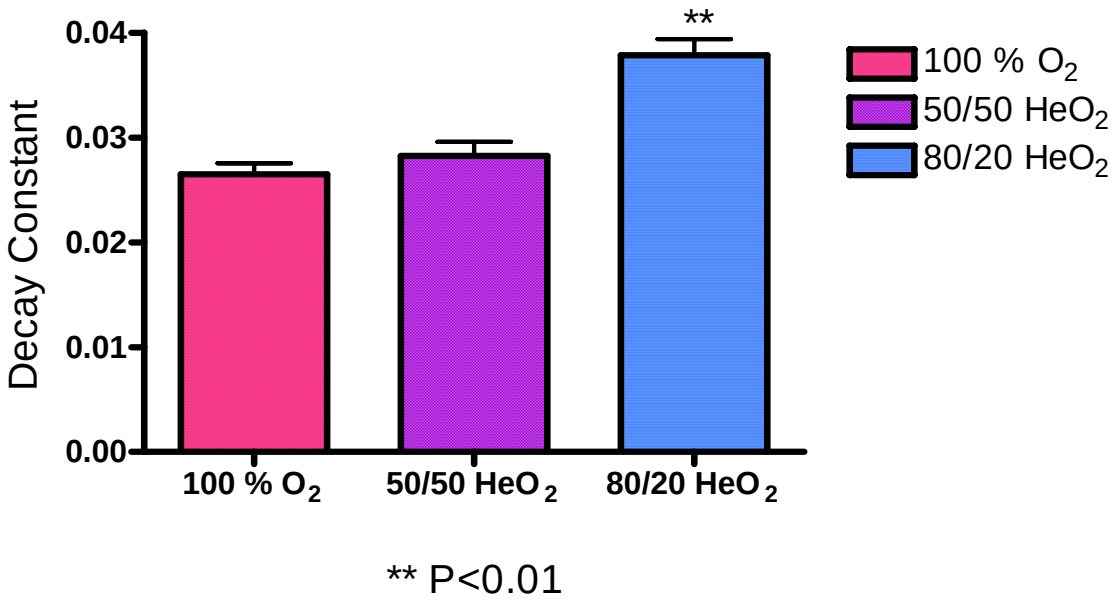


Figure 21: Comparison of the decay constants from the pooled breathing gas groups

The decay constants from the 50/50 HeO2 and 80/20 HeO2 breathing groups were compared to the decay constant of the 100% O_2 group using one-way ANOVA followed by Dunnett's test. It was found that the decay constant for the 80/20 HeO_2 group was significantly higher than that of the 100% O_2 group.

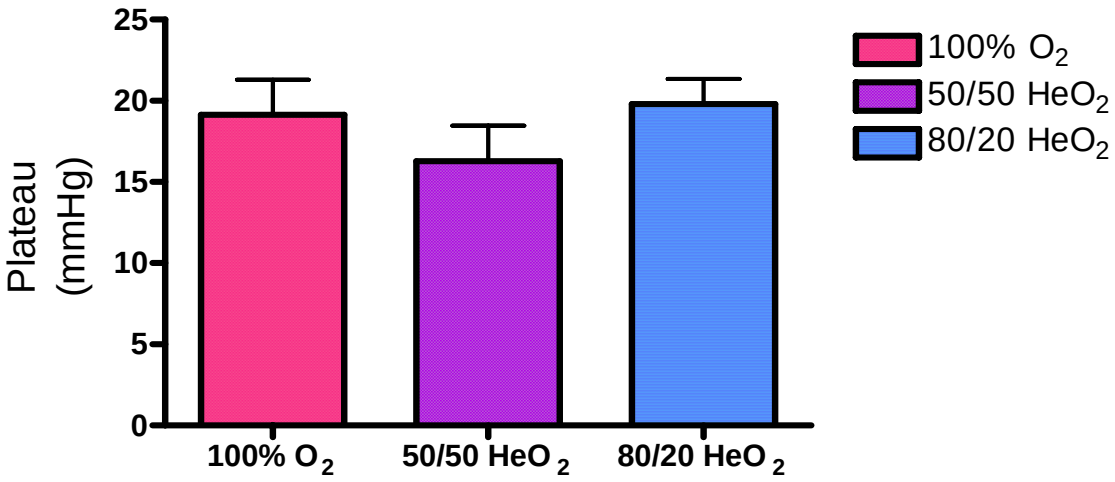


Figure 22: Comparison of the plateaus from the pooled breathing gas groups

The plateaus from the 50/50 HeO_2 and 80/20 HeO_2 breathing groups were compared to the decay constant of the 100% O_2 group using one-way ANOVA followed by Dunnett's test. It was found that there were no significant differences between the groups.

CHAPTER 4

GENERAL DISCUSSION AND CONCLUSIONS

The problem of DCS is unlikely to pass quietly into the night. In fact, it is more likely that the incidence of DCS will increase in the coming years. Although DCS is better understood now than it has ever been, the simple fact that it is largely a stochastic phenomenon all but guarantees that it will never go away. The probabilistic nature of DCS also means that its incidence is likely to increase as the number of people involved in diving increases. Current estimates place the number of recreational dives performed worldwide per year above 3 million, and the incidence of DCS somewhere between 0.1 and 9.8 cases of DCS per 100 divers, or between 1.0 and 28.1 cases of DCS per 10,000 dives(25, 167). Diving is also a sport which is increasing in popularity. A five-fold increase in the number of people participating in recreational diving was observed between in the 15 year period between 1985 and 2000(161).

The incidence of DCS in recreational diving alone would be sufficient to indicate that DCS will continue to be a problem for the foreseeable future, but recreational diving is not the only source of DCS. DCS is also encountered in the much more controlled realms of commercial and military diving. Although numbers are much harder to come by, DCS incidence in the commercial diving industry is believed to be between 1 case in 800 to 4000 dives, depending largely on the decompression tables being used(8). The military has fundamentally different objectives with its diving programs compared to either recreational

diving or commercial diving. Due to the nature of the work, a certain risk, and thus incidence of DCS, is considered 'acceptable' within the realm of military diving. Decompression table development for the US Navy is currently undertaken with the understanding that the acceptable incidence of DCS is 2.2%(163). The actual incidence of DCS within the realm of military diving is believe to be between 2 and 5 %, again, depending largely on which decompression tables are being used(163). Both commercial and military diving activities are unlikely to diminish in the short term, so even in these communities DCS is likely to be an ongoing problem for the foreseeable future. DCS also continues to be a problem in space exploration. When astronauts are operating outside the space vehicle their suits must be pressurized to approximately 0.25 ATA in order to allow them to operate the joints(168). Given that the crew spaces of both the space shuttles and International Space Station are pressurized to approximately 1.0 ATA with a gas miture much like room air, great time and care must be taken to denitrify astronauts prior to extravehicular activity in order to reduce the risk of decompression sickenss(68, 168).

Previous studies exploring the use of PFCs to treat and prevent DCS have demonstrated that the I.V. administration of PFC emulsions after decompression decreases morbidity and mortality in both a clear and consistent fashion(23, 31, 147-149, 151, 196). This prior work has all been done in conscious, spontaneously breathing, freely moving animals. This laboratory is the only laboratory conducting DCS research in anaesthetized, instrumented animal models. As such we are uniquely equipped to examine not only the survival benefits of PFC administration, but to elucidate the mechanism by which these benefits are provided. The studies described in this dissertation were the first studies

purposed to examine two potential mechanisms by which PFCs exert their protective effects – namely that they improve oxygenation and that they improve nitrogen (or any other inert gas) washout.

Study 1 in Chapter 2 above demonstrated that PFC administration improved whole-body oxygenation. Although it is rather intuitive that the administration of a therapy which is known to dissolve greater quantities of oxygen than blood plasma , such as PFC, should increase arterial oxygen content, this is the first study to demonstrate that this is, in fact, the case *in vivo*. That PFC is also able to increase both $\dot{V}O_2$ and $\dot{D}O_2$ demonstrates that this increased oxygen carrying capacity granted by PFC administration has functional benefits as well. Thus it is likely that at least a portion of the benefits previously observed with PFC administration are directly attributable to improved tissue oxygenation. Unfortunately this study was only able to make these measures at the whole-body level. Moving forward it would be important to examine the effects of PFC administration on C_aO_2, $\dot{D}O_2$ and $\dot{V}O_2$ in specific tissues, ideally in areas where blood flow is known to be obstructed by bubbles. Furthermore, in order to examine the mechanism by which PFC improves tissue oxygenation, it would be beneficial if some means to track the location of the PFC emulsion particles could be devised. While labeling the PFC itself is difficult due to their non-reactivity it might be possible to label the emulsifiers, and thus track the location of the emulsion particles. These studies could be used to determine if the PFC particles are able to flow past bubble obstructions, or perhaps move through them. Similarly, in order to examine how PFC improves oxygen delivery to cells and cellular organelles, labeled PFC emulsion particles could be used to determine if the PFC extravasates or remains within

the circulatory system. Much work remains to be done in order to fully understand exactly how PFC improves oxygenation.

Study 2 in Chapter 3 above demonstrated that PFC is able to increase the degree to which nitrogen is removed, but not the speed with which it is removed. This same study also demonstrated that high F_IHe was able to increase the speed with which nitrogen was removed, but not the degree to which it was removed. If nitrogen washout is to be studied in the future it would be desirable to carry such studies over longer periods of time, and to use other techniques in addition to real-time respiratory mass spectrometry to examine exhaled nitrogen. If exhaled gases were collected in a bag and the contents of the bag examined at various time points, data could be gathered with respect to not only the instantaneous partial pressure of nitrogen in the gas, but also the volume of gas expired.

The above conclusions from Study 2 suggest a potential beneficial effect of combining both PFC and high F_IHe for increasing the amount and rapidity of nitrogen washout. This study was, however, neither powered, nor designed to elucidate differences seen due to the combined effects of PFC administration and high F_IHe. As such this investigator does not believe that such effects would be substantive.

The beneficial effects of helium were only observed when the inspired breathing mix was 80% helium. With only 20% of the inspired gas consisting of oxygen it is unlikely that the oxygenation benefits of the PFC would be retained. This investigator also questions the wisdom of administering breathing mixes containing high F_IHe after decompression. Although the research done to date suggests that HeO_2 breathing with a high F_IHe after decompression results in faster bubble shrinkage and disappearance, the

results of these studies are of questionable relevance to clinical DCS(3, 10, 78-81). First, these studies were all conducted in rats, and it is well understood that rats do not develop DCS in the same way that humans and larger mammals do(39, 97). Their small body mass appears to offer a protective effect and more severe dive profiles are required to reliably generate DCS in rats than is required for larger mammals. Additionally these studies were not studying DCS *per se*, but rather were examining the growth, shrinkage, and disappearance of bubbles deliberately injected into adipose tissue prior to a hyperbaric exposure. The dive profile itself was not severe enough to result in *de novo* bubble formation(3, 10, 78-81). Although very interesting, this is a very different phenomenon from clinical DCS. It is unlikely that tissue gas tensions are as high in these models as they would be in clinical DCS. As such the growth and shrinkage of bubbles could not be expected to follow a similar time course in these models as would be the case in DCS.

Even if we were to interpret these results as representative of the growth and shrinkage of bubbles in circulation, the fastest shrinking bubbles required nearly 70 minutes to disappear(79-81). Although the removal of bubbles obstructing circulation and restoration of normal blood flow are important factors in tissue injury, the reason blood flow restoration is important is to restore oxygen delivery. It seems foolish to this investigator to hamper the ability of PFC to deliver oxygen to tissue by decreasing the F_IO_2 in order to decrease bubble dwell time from several hours to over 1 hour. It would appear to be of greater benefit to restore some oxygen delivery to tissue, even if it isn't a complete return to baseline, by administering PFC in combination with 100% oxygen breathing. This scenario seems more likely to maximize tissue survival in the case of DCS.

In future work studying the use of PFC for treating DCS, great care should be exercised in model choice. Previous work examining PFC administration to swine demonstrated clearly that pigs develop a pulmonary hypertensive response to PFC which is severe enough to result in decreased cardiac output(152). Findings from study 1 above demonstrate that a similar, though less severe, response occur in sheep as well. It is likely that this response will be observed in all cloven-hoofed animals. In order to get the clearest possible picture of how PFC effects oxygenation, it would be desirable to utilize a species in which this response does not occur.

Other issues in model selection include the use of conscious vs. anaesthetized animals, as well as spontaneously breathing vs. ventilated animals. These issues become important in the development and evolution of DCS. As discussed in the introduction above, one likely mechanism for the formation of bubbles during the development of DCS is cavitation nucleation. This occurs as a result of motion. Therefore the question of whether to use conscious vs. anaesthetized animals arises. Anaesthetized animals do not move spontaneously, and any movement imposed externally by investigators will not duplicate the normal muscle and joint movement as it would occur in a conscious, free-moving animals. Similarly, a spontaneously breathing animal is able to regulate its ventilatory drive in response to stimuli such as changing arterial pCO_2. A ventilator-dependant animal will not be able to alter its ventilatory pattern in response to such stimuli. As with all model choices, no one scenario is universally a better choice. Although a conscious, spontaneously breathing, free moving animal might be ideal for examining variables such as time to onset of symptoms, or mortality, it is very difficult to use such

models for the invasive monitoring of physiological parameters such as cardiac output. Models must be selected based on experimental objectives.

This work has demonstrated for the first time that the mechanism by which PFC exerts its protective effects includes both improved oxygenation and increased nitrogen removal. It is likely that the observed effects on oxygenation are more important in terms of explaining the previously observed beneficial effects of PFC in treating DCS than are the effects on nitrogen washout. Arterial oxygen content was increased 11% over saline control, while $\dot{D}O_2$ was increased by 10% and $\dot{V}O_2$ was increased by 22%. These are substantial increases in oxygenation. Meanwhile when the nitrogen washout curves were examined it was found that the plateau value for the PFC-treated group was 28% lower when compared to the saline control group. Although this seems like a large relative difference, in absolute terms the difference is only 6.24 mmHg.

Over the course of a long period of time this small difference may account for a substantial difference in the total volume of nitrogen washed out. It is also likely that the time required for the dissolution of bubbles is not decreased to a level which would eliminate the interruption of blood flow and resultant hypoxia induced by bubbles lodging in the circulation. The direct effect of PFC administration on the time required for bubble dissolution has never been studied, but the effects of other therapies such as HeO_2 breathing have been studied, as mentioned above. These studies indicated that when breathing air, the worst possible breathing gas to treat DCS, bubbles persisted longer than 220 minutes(79-81). The exact time is unknown because bubbles persisted beyond the period of observation. While breathing oxygen, the current gold-standard of DCS therapy,

bubbles persisted for approximately 150 minutes on average(79-81). Breathing 50/50 HeO_2, the gas which resulted in the shortest time required for bubble dissolution, reduced bubble persistence to approximately 100 minutes on average(79-81). Even if we were to suppose that the PFC is able to decrease bubble persistence by 100% over 50/50 HeO_2, bubble persistence times would still average 50 minutes. Obstructing circulation for 50 minutes is more than long enough to cause permanent injury in sensitive tissues such as neural tissue where it is generally accepted that interruption of blood flow for intervals greater than 6-10 minutes will result in permanent injury.

In conclusion, previous work has demonstrated clearly that PFC decreases DCS morbidity and mortality. The work described in this dissertation demonstrated that the most likely mechanism for the observed benefits is that PFC improves capacity of the circulatory system to carry and deliver oxygen to tissue, as well as increasing the ability of tissue to take up and use that oxygen. Although PFC does improve nitrogen washout, this is likely a minor contributor to its previously-observed efficacy in the treatment of DCS. I believe it is likely that PFC administration will one day become an adjunctive therapy in the treatment of DCS and perhaps even a definitive therapy in mild-to-moderate cases. This will require not only further animal study to demonstrate efficacy in less severe models of DCS, but also extensive human testing in order to demonstrate a lack of toxicity, appropriate dosing for humans, and will likely also require the development of new recompression tables for use in patients who still require recompression after PFC administration.

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APPENDIX A

The Two Faces of Eve: Gas Anaesthesia and Inert Gas Narcosis.

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Introduction

Gas anaesthesia is arguable one of the most important medical advances of the last 200 years. Introduced to the Americas some 160 years ago, these drugs form the foundation that all modern surgery is based on. Without surgical anaesthesia to render patients immobile, insensitive to pain, and to abolish, or at least limit their memories of the surgical experience, modern surgery would not be possible. Inhaled anaesthetics are also among the most dangerous and poorly understood drugs available in the modern arsenal – so much so that an entire specialty of medical practice has been developed in order to ensure that those using these drugs have the proper training and experience to do so safely. While gas anaesthesia has been a great boon for medicine, inert gas narcosis has been the scourge of deep sea diving. Inert gas narcosis has placed some fairly strict limits on the depths to which deep sea diving can be conducted safely, greatly limiting our ability to explore the 70% of our planet covered by water, except through indirect means such as the use of submersibles. In fact, it has been remarked that we know more about the surface of Mars than we do about the deep ocean. These two topics of discussion seem so far removed from one another that they have never really been explored in tandem, but what if they are not really separate topics after all? What if gas anaesthesia and inert gas narcosis are, in reality, different manifestations of the same phenomenon?

History

The phenomenon now known as nitrogen narcosis, or more broadly as inert gas narcosis, is a prime example of pharmacological activity of a so-called inert gas. From as

early as 1835 (19, 65, 75), it was understood that hyperbaric exposure resulted in symptoms such as sleepiness, impaired judgement, and hallucinations resembling intoxication or drunkenness (19, 55, 75). Over the years a succession of explanations for these observations has been postulated, including direct effects of pressure (19, 55, 75), high partial pressures of oxygen (19, 35, 75), underlying psychological factors such as latent claustrophobia (19, 63, 75), impurities in the breathing gas (19, 63, 75), and carbon dioxide. None of these proposed mechanisms were ultimately able to explain the mechanism of the observed symptoms without breaking down at some point. After a century of investigation into the mechanism of what came to be know as 'compressed air narcosis', in 1935 Behnke *et al.* first proposed what is now the commonly held theory that the raised partial pressure of nitrogen was responsible for the observed symptoms (13, 19). For several years there was a major rift in the emerging hyperbaric community between the proponents of the nitrogen hypothesis and the carbon dioxide hypothesis (11, 12, 19, 75). However, work in the early 1970's by Hesser *et al.* reconciled the nitrogen and CO_2 theories by demonstrating that the effects of nitrogen were the primary cause of narcosis, but CO_2 also had an additive effect on narcosis (19, 61, 62, 75).

Modern hypotheses

Inert Gas Theory

When Behnke *et al.* first proposed the currently held theory that the nitrogen (or more broadly, the inert gas) fraction of the breathing gas is primarily responsible for narcosis (13) in 1935, their assertion was based on what has come to be known as the Meyer-Overton hypothesis, which, in brief, states that the narcotic potency of an anaesthetic (or an inert gas) is related to its lipid solubility (78, 88). Lipid solubility is the

one physical property of inert gases that has been found to correlate consistently with their narcotic potency (19, 75). The Meyer-Overton hypothesis, with regards to inert gas narcosis, was found to be tenable by Carpenter. He showed that at their isonarcotic partial pressures, that is, the partial pressure at which each gas shows comparable pharmacologic effects, the inert gas concentration dissolved in the lipid phase is very similar across many gasses, but the partial pressure of gas required varies from 0.045 to 165 ATA (19, 29). Figure 1 also illustrates that lipid solubility correlates with relative narcotic potency better than other physical properties (75).

This assumes that the mechanism of action for the narcotic effect of inert gases is some sort of interaction with the lipid component of the central nervous system, which is currently one of the plausible sites of action for anaesthetics. This has not always been the case. The aqueous phase has also been suggested as a possible alternative (82, 89). The first aqueous phase hypothesis is based on the formation of hydrates (19). Pauling theorized that clathrates were formed in the aqueous phase such that the inert gas atoms, which he called 'guests', are held by Van der Waals forces in crystalline cages formed by the 'hosts', or molecules from within the cell, namely water molecules (89). Unfortunately these hydrates would be unstable under body conditions, so Pauling was forced to assume that there was some stabilizing factor present, such as the charged side-chains of proteins to make his theory workable (19, 89). Under this hypothesis the clathrates caused narcosis by increasing the impedance of nerve tissue, trapping ions involved in nerve conduction, and decreasing metabolism (19, 89). Miller proposed another aqueous-phase model which, although related, did not rely on hydrates (19, 82). Instead, Miller suggested that the presence of gaseous anaesthetics, such as inert gases,

resulted in an increase in the size of highly ordered shells of water molecules surrounding the dissolved gas molecule (19, 82). These 'icebergs' would decrease conductance in brain tissue, stiffen lipid membranes, and occlude ion channels (19, 82). In the mid-to-late 1960's work was done to settle the lipid phase vs. aqueous phase debate. This work demonstrated clearly that lipid solubility was a far better indicator of narcotic potency than hydrate dissociation pressure (43, 79, 80). No evidence was found in these studies to support the idea that the aqueous phase was the critical phase for the action of anaesthetic gasses.

Mechanisms

Once it was established that the site of action was, indeed, within the lipid phase rather than the aqueous phase, hypotheses began to emerge regarding what was actually taking place within the lipid phase that would result in narcosis with identical signs and symptoms being induced by a broad collection of gasses with no common structural features (75). Many hypotheses have evolved to explain the mechanism of inert gas narcosis and anesthesia, including hypoxia, depression of metabolism, cell membrane stabilization and stiffening causing decreased ion permeability, inhibition of the sodium extrusion pump, increased production of inhibitory neurotransmitters such as gamma aminobutyric acid (GABA) and interference with adenosine triphosphate (ATP) production (15, 25, 44, 70, 84, 91, 93). These fall into two broad categories, biochemical hypotheses or biophysical hypotheses. Biochemical theories imply some effect on respiratory enzyme systems, while biophysical theories imply some interaction with, or within part of the cell, such as the cell membrane (45, 59, 84, 101, 115). Until recently, no good evidence had been found to support biochemical changes at pressures relevant to

the clinical manifestations of inert gas narcosis (27, 28, 71, 74, 103, 111). This suggested that the narcotic action is more likely biophysical than biochemical and evolved into the 'unitary hypothesis of narcosis' – that the mechanism of narcosis is the same for all anaesthetic gasses (75).

By the late 1950's, the site of action of narcotic gasses had been attributed to synapses in the central nervous system. This was deduced largely from the work of Carpenter in the mid 1950's. He demonstrated that extremely high pressures (310-340 ATA) of argon, a gas with a narcotic potency more than twice that of nitrogen, were required to effect a block of conduction in isolated peripheral nerve preparations. Yet argon at a mere 18 ATA of pressure was sufficient to abolish any response to electrical stimulus applied to the foot pad of mice (29, 30). This suggests strongly that higher level functions in the brain are much more susceptible to inert gas narcosis than peripheral nerves. Later work examining reflex inhibition in the spinal cord demonstrated that inhibitory synaptic mechanisms were affected by inert gas narcosis before excitatory mechanisms (14, 15, 31), and that inert gas narcosis, like general anaesthetics, affects cells in the anterior horn of the spinal cord (7, 108, 109).

Lipid/Membrane Hypotheses

Physical hypotheses, based on the polarizability and the volume of inert gas molecules are generally much simpler and, according to some, seem more likely than biochemical hypotheses (19, 84). The critical volume hypothesis of general anaesthesia proposed that narcosis occurred when the anaesthetic agent entered the lipid part of the cell in sufficient quantity to cause the lipid portion of the cell, in particular the plasma membrane, to swell (72, 81). Accordingly, changes in lipid volume ought to differentiate

the anaesthetized from the unanaesthetized state. The critical volume hypothesis is supported by observations that anaesthetics and inert gases at increased ambient pressures expand the volume of lipid monolayers and bilayers, bulk phase solvents, oils, and even rubber (105). This hypothesis is further supported by the observation that gas anaesthesia can be reversed with the application of hydrostatic pressure, see figure 2 (72, 81, 99). The quantitative aspect of this hypothesis was developed which suggested that a 0.4% expansion of the membrane would be required to produce anaesthesia (73).

Although elegant in its simplicity, there are critical elements of this hypothesis that do not agree with observations. The critical volume hypothesis predicts that the percentage change in anaesthetic potency ought to be linearly related to pressure, and that the slope of this relationship ought to be the same for all anaesthetic agents (59). This has not been found to be true. It has been demonstrated that there is not a universal linear relationship between pressure and the anaesthetic dose required to maintain the same level of anaesthesia (59, 67, 107, 116). Quite the opposite, it has been shown that these pressure-anaesthetic interactions are curvilinear, and differ depending on the anaesthetic in question (59, 67, 107, 116). For instance, the amount of nitrogen required to maintain a given level of anaesthesia increases with pressure to ~50 ATA, at which point it plateaus and there is no further increase for pressures up to ~130 ATA (59, 67, 107, 116). Requirements for isoflurane, on the other hand, decrease up to pressures of ~8 ATA, after which they increase sharply, and continue to increase up to pressures of ~100 ATA (59, 67, 107, 116). Additionally, it has been observed that there is no appreciable increase in membrane thickness at narcotic concentrations of various agents (52, 106).

These observations led to the postulation of the multi-site expansion hypothesis (59). There are 5 key elements of this hypothesis (19, 59):

- General anaesthesia or narcosis can be produced by the expansion of more than one molecular site and these sites may have different physical properties.
- The physical properties of a molecular site may themselves be influenced by the presence of anaesthetics or pressure (i.e. compressibility)
- The molecular sites do not behave as if they were bulk solvents but have a finite size and a finite degree of occupancy
- Pressure need not necessarily act at the same site as the anaesthetic in order to reverse anaesthesia. Depending on the anaesthetic, one of the sites may predominate in determining the interaction between anaesthesia and pressure
- The molecular sites for anaesthesia are not perturbed by a decrease in temperature in a manner analogous to an increase in pressure

Although appreciable increases in membrane thickness have not been observed at clinically relevant doses of anaesthetic gases, it has been demonstrated that membranes do expand laterally in the presence of anaesthetic gases (32). This study found that narcotic agents such as nitrous oxide have an affinity for lipid monolayers, and that inert gases at partial pressures sufficient to bring about the observed clinical effects of inert gas narcosis should act at lipoprotein-water interfaces to cause a decrease in surface tension of 0.39 dynes/cm (19, 32). Work by Bennett *et al.* was able to confirm that inert gases do indeed penetrate lipid monolayers and affect surface tension as Clements and Wilson

suggested (18). Exactly how these changes could affect anaesthesia is not well understood, but it is believed that these changes in surface tension alter the function of the ion receptors and channels involved in synaptic transmission (18, 19, 32).

Other work on membrane model systems suggested that anaesthetic gases may also alter ion permeabilities. While examining cation permeability in membrane model systems, Bangham *et al.* found that n-alkyl alcohols, chloroform and ether result in a transient, reversible increase in membrane cation permeability (10). Inert gases behave the same way. Bennett and Hayward were able to demonstrate this by examining *in vivo* cerebrospinal fluid (CSF) levels of sodium, potassium and chloride while measuring auditory evoked potentials in cats. They found a significant decrease in CSF sodium and chloride, as well as the amplitude of cortical auditory evoked potentials, in animals compressed to 11 ATA breathing a mix of 80% nitrogen/20% oxygen or 80% argon/20% oxygen when compared to breathing 80% helium/20 % oxygen at 11 ATA or room air at ambient pressure (17). This is further supported by studies by Johnson and Miller as well as by Galey and van Nice (53, 64). Johnson and Miller found that when liposomes were exposed to butanol, ether and nitrogen in doses which would be just sufficient to abolish the righting reflex in newts, an increase in the permeability for potassium and rubidium was observed. An application of 152 ATA of pressure was required to counterbalance the permeability changes (64). Galey and van Nice found that pressures of nitrogen up to 88 ATA stimulate active sodium efflux and potassium influx across the red blood cell membrane, and that the effect is abolished by ouabain (53). They also showed that hyperbaric pressures of the non-narcotic gas helium, rather than nitrogen, tended to inhibit active sodium and potassium transport (53). Other work by this group using rat

brain synaptosomes showed that hyperbaric pressures of argon would stimulate potassium uptake by the synaptosomes while 69 ATA of helium or hydrostatic pressure inhibited the accumulation of potassium (19). This suggests that anaesthesia and narcosis may be the downstream product of gases dissolving into the membrane at various sites, altering ion conduction, and thus synaptic conduction, and ultimately consciousness.

More recently, Abraini modified this multi-site expansion model based on results obtained from human trials using hydrogen as a compression gas. He proposed a theoretical reconsideration of the interaction between inert gases at hyperbaric pressure, and the effects of pressure itself. According to this hypothesis, narcotic gas and pressure act at different hydrophobic sites and narcosis occurs when a critical level of expansion is reached at some cellular hydrophobic site in the central nervous system (1, 19). As far as light inert gases are concerned, all narcotic gases act at a common hydrophobic region through a non-specific mechanism (1, 19). This hypothesis suggests that the psychotic-like symptoms observed in humans at high pressure may be a paroxysmal symptom of narcosis, not simply a manifestation of the high pressure nervous syndrome (HPNS), and is a result of the sum of the individual narcotic potencies of the various inert gases in the breathing mix (1). This hypothesis was tested mathematically against various lipid solubility theories of inert gas narcosis and was found to be sound (1). This suggests that, depending on the environmental parameters (breathing mix, pressure), symptoms of inert gas narcosis or HPNS appear when a critical imbalance is reached between the narcotic actions of inert gas and the actions of pressure, which tend to reverse narcosis, on their respective hydrophobic sites (1, 19). Accordingly, inert gas narcosis and HPNS can antagonize each other or can occur simultaneously (1, 19).

Given the understanding that inert gas narcosis was somehow connected to changes in synaptic conduction in the CNS, some researchers began to investigate changes in neurotransmitters with hyperbaric exposure. Changes in levels of dopamine and norepinephrine have been observed by several groups, but whether an increase or decrease is observed seems to depend on what area of the brain is under investigation rather than the pressure applied (9, 77, 100). For instance, in the hypothalamus, dopamine and norepinephrine were shown to be decreased in response to 100 ATA trimix (helium/nitrogen/oxygen) and to 20 ATA nitrogen/oxygen mixtures, but were increased in the caudate nucleus (19, 77). Unfortunately these changes may not have anything to do with inert gas narcosis. Rostain and Forni were able to demonstrate a similar increase in striatal dopamine release in response to 90 ATA helium/oxygen mixture, 90 ATA helium/nitrogen/oxygen mixture (5% nitrogen), and 90 ATA helium/hydrogen/oxygen mixture (66% hydrogen) (19, 100). These mixes should have quite different narcotic potencies, but appeared to cause the same change in dopamine levels. These changes were attributed to, and are likely due to pressure alone, not narcosis (100). Balon *et al.*, while also looking at striatal dopamine release, found a 20% decrease in dopamine release in rats exposed to 30 ATA breathing a nitrogen/oxygen mix (9). This suggests that neurotransmitter release in response to hyperbaric exposure and inert gases is quite complex. It would appear that exposure to low pressures breathing a mixture of nitrogen/oxygen decreases striatal dopamine release, while exposure to high pressures breathing a helium/oxygen mixture increases dopamine release. Unfortunately it is very difficult to separate out the effects of the inert gas from the effects of pressure *per se* because gases with high narcotic potency will result in unconsciousness at high pressures.

Thus, in order to expose an *in vivo* preparation to high pressures, helium must be used as a diluent gas if any sort of behavioural observations are to be made. So long as gas pressure is used to generate a hyperbaric exposure it will remain near-impossible to distinguish the effects of high-pressure helium from those of pressure *per se*. Nonetheless it would appear that there are consistent, reproducible changes in dopamine release in response to hyperbaric exposure. This suggests that neurotransmission is likely altered under hyperbaric conditions and may offer a partial explanation of inert gas narcosis.

Other work has suggested that nitric oxide may play a role in narcosis. Vjotosh *et al*. found that when rats were compressed to 41 ATA breathing air, they showed alterations in motor activity at 5-12 ATA, ataxia at 10-34 ATA, and side body position at 26-41 ATA. These were taken as signs of nitrogen narcosis. When treated with the nitric oxide synthase inhibitors L-NAME or 7-NI, the above mentioned signs were abolished or attenuated (19, 114). While interesting, these results must be taken with a grain of salt. Air breathing at pressures greater than 10 ATA makes acute oxygen toxicity a serious risk. Since seizures are one of the symptoms of acute oxygen toxicity, the indicators that this group used as signs of narcosis may make narcosis and oxygen toxicity difficult to distinguish.

Protein/Metabolic Hypotheses

Research attention is now focussing on the possibility of direct interactions between inert and anaesthetic gases and proteins, lipoproteins, and other hydrophobic sites within the cell (2, 48-51). Much of this evidence comes from the anaesthesia community and the study of volatile, inhaled anaesthetics in general. If inert gas narcosis was solely a matter of gas dissolving in lipid membranes it would be expected that the

onset of narcotic effects would be linearly related to the rate of increase in pressure (19). The finding that this relationship was, in fact, sigmoidal suggests that the inert gas molecules are interacting with protein receptors directly and act as allosteric modulators of their activity (2, 19). The idea that the mechanism underlying anaesthesia involves an interaction with proteins is not new. This was first proposed by Claude Bernard in 1875 (20). He came to this conclusion based on the way some anaesthetic potencies deviated from that which would be predicted from their lipid solubility alone, combined with the understanding that many proteins contain small hydrophobic domains which would allow for interactions with small, hydrophobic compounds (20, 41). Unfortunately the interactions between proteins and narcotic compounds appear to be very short-lived, on the order of a millisecond or less (41). Conventional binding assays are simply unable to measure such low affinity binding (41).

Since direct measurements of binding are not possible, those interested in studying protein-based mechanisms of anaesthesia and narcosis have resorted to molecular pharmacology and assays of protein activity in various *in vitro* preparations (41). These techniques have evaluated possible protein/anaesthetic interactions based on two criteria: 'plausibility' and 'sensitivity'. Plausibility refers to the degree to which changes in protein activity observed in the preparation lines up with our preconceptions of anaesthetic mechanisms (41). For instance, it is believed that anaesthesia and narcosis are products of CNS depression; therefore the observation that an anaesthetic inhibits proteins involved in excitatory synaptic transmission, or activates proteins involved in inhibitory synaptic transmission would fit the 'plausibility' criterion. The sensitivity criterion would come in to play in order to evaluate the dose-dependence of the observed

changes in protein activity. This criterion would be satisfied if the observed *in vitro* EC_{50} is similar to the observed *clinical* EC_{50}. Plausibility, in this sense, is certainly a very fuzzy concept. Our understanding of the neurophysiology of consciousness is very limited. Consequently our understanding of altered states of consciousness is even more limited, so plausibility is very much open to the subjective interpretation of the investigator.

Inhalational anaesthetics including inert gases have been investigated in several different *in vitro* systems and have been found to alter the functions of many enzymes, receptors, transporters, ligand- and voltage-gated ion channels, as well as structural proteins (42). A growing body of evidence suggests that inhalational general anaesthetics work through interactions with proteins, particularly post-synaptic ligand-gated ion channels (6, 34, 37, 49, 51, 119). These interactions fit well, not only with the plausibility criterion, but with the sensitivity criterion; the doses observed to have appropriate *in vitro* effects are very similar to clinical doses used to produce general anaesthesia (60). The idea that inhaled anaesthetics exert their effects through modulation of inhibitory post-synaptic ligand-gated ion channels is interesting. When activated by the binding of the appropriate ligand (ex. GABA) these chloride channels open and chloride flows into the cell causing a hyperpolarization and decreasing the likelihood of action potential propagation (41). Clinically effective concentrations of several inhaled anaesthetics have been demonstrated to potentiate both GABA- and glycine-modulated chloride currents *in vitro* (85, 86).

Several problems still exist with the hypothesis that inhaled anaesthetics operate through the modulation of ligand-gated ion channels such as the GABA receptor. First, *in*

vitro, it has been shown that at high doses of anaesthetic drugs (above 1 mM), $GABA_A$ activity tends to be inhibited, yet clinically, increasing anaesthetic doses lead to deeper anaesthesia, not reversal (85, 86). Second, in neonatal rodents, chloride gradients are reversed, thus GABA acts as an excitatory neurotransmitter, but inhaled anaesthetics are still effective in these animals, although slightly higher doses are required (87). Third, if the anxiolytic effect of benzodiazepines is a result of potentiation of the $GABA_A$ receptor, inhaled anaesthetics must act through a different mechanism since their effect is decidedly non-anxiolytic (41). The early stages of general anaesthesia induced with inhaled anaesthetics produces an excitatory phase, which can produce seizure-like activity, whereas benzodiazepines prevent seizures (41). Fourth, chloride channel blockers and $GABA_A$ antagonists have only minimal effects on the potency of inhaled anaesthetics (57, 98). This evidence suggests that, although volatile anaesthetics can modulate ligand-gated chloride channel activity at clinically relevant concentrations *in vitro* (and possible *in vivo*), it is unclear how this effect is related to anaesthesia.

Anaesthesia may be a product, not of interaction with a single protein, but of interaction with multiple molecular targets. This is suggested by the observations that inhaled anaesthetic agents affect multiple proteins, as well as the fact that multiple anaesthetics with diverse molecular structures all produce the same end result: anaesthesia (41). There is evidence for unique binding sites for several inhaled anaesthetics on a single target, but it seems unlikely that a single molecular target would have specific binding sites for diverse molecular structures ranging from xenon to nitrous oxide to sevoflurine (40, 56). Other evidence suggests that there may be a single, selective target for each anaesthetic, but it seems unlikely that all these molecular targets

would lead to the same end result – anaesthesia (36, 95). This finding does, however, provide reasonable grounds to believe that interactions with multiple molecular targets may converge to produce the single effect of anaesthesia (41).

It should be remembered that specific interaction with a protein target and multiple sites of action are not mutually exclusive. It is well understood that ATP, oxygen, and calcium all bind selectively with multiple different targets. Volatile anaesthetics have also been shown to bind selectively with multiple different targets, including firefly luciferase, serum albumin, myoglobin, adenylate kinase, haloalkane dehalogenase and T4 lysozyme (21, 47, 94, 102, 104, 113). Even more compelling evidence comes from autoradiograms of rat brain slices probed with a radiolabelled halothane derivative. The radiographs revealed widespread binding throughout the brain whose distribution did not match that of any known receptor or channel (39), see Figure 3. Furthermore, the binding was reduced to background levels in the presence of a 10-fold excess of unlabelled halothane (39). When extracted and separated, multiple brain proteins were found to be specifically labelled in a saturable and stoichiometric manner with estimated affinities near the clinical EC_{50} (38).

The nature of the interaction between anaesthetics and proteins may lie in the structure of proteins themselves. It is understood that proteins fold into complex 3-dimensional structures which are not solid, but rather contain cavities. These cavities are believed to be critical structural elements in protein function as they introduce areas of instability which allow conformational changes to take place (24, 112). These cavities within the hydrophobic core of proteins provide plausible binding sites consistent with the observation that anaesthetic potency is correlated with lipid solubility. That potent

anaesthetics exhibit weak polarity is also consistent with the hypothesis that they bind in protein cavities as most cavities are also weakly polar (41). The elements of protein secondary structure which form the surface of these cavities could also provide an explanation for the weak stereoselectivity observed with some anaesthetics such as isoflurane (51, 76). Occupancy of these cavities by anaesthetic molecules could affect anaesthesia by limiting the motion that underlies protein activity (41). Studies have indicated that occupancy of cavities by small, hydrophobic molecules does reduce protein motion (24, 112). This is clearly a multiple target hypothesis, and nicely reconciles results form binding and functional studies (41).

Xenon

Thus far the terms 'inert gas narcosis' and 'anaesthesia' have been, to a degree, used interchangeably. This is due to both the hypothesis that all anaesthetics act through the same basic mechanism, as well as the observation that inert gas narcosis resembles early stages of anaesthesia and that inert gases can produce general anaesthesia if delivered at sufficient partial pressures. The inert gas xenon exemplifies much of this overlap and somewhat loose use of terminology. Xenon is an inert gas whose narcotic potency is so high that it can be used as a general anaesthetic at 1 ATA (92). If it can be assumed that inert gas narcosis exists as a single condition, regardless of the inert gas in question (to a large extent the research community has already done so in order to try to separate narcotic effects from pressure effects), what is understood about the mechanism of xenon anaesthesia can be extended to all inert gas narcosis and gas anesthesia (2, 9, 16, 53, 92, 99).

Investigations into the mechanism of action of xenon gas anaesthesia present a microcosm of investigations into the mechanism of action of the entire scope of inhalational anaesthesia. Both lipid (membrane) and direct interaction with protein hypotheses abound. In support of the membrane hypothesis, there is evidence that xenon can interact with lipid bilayers and change surface tension, bilayer volume, and pressure within the bilayer (110, 117). There is also evidence to suggest that the xenon dissolved in the membrane, despite its high lipid solubility, may not reside within the central core or tail region of the membrane. Rather, xenon may preferentially migrate to the amphiphilic region around the head groups after dissolving in the membrane (26, 117). This accumulation of xenon molecules in the membrane is believed to affect the structure and function of proteins embedded within the membrane (8, 41, 110). In particular, it is believed that changes in surface tension and pressure within the membrane result in conformational changes in ion channels within the synaptic terminals of neurons, resulting in decreased conduction, leading to anaesthesia (8, 26, 41, 110, 117).

The greatest criticism levelled against much of the work done on the basis of the lipid hypothesis of narcosis, be that *in vitro* or *in silico* work, is the fact that most of these studies are done using dipalmitoylphosphatidylcholine (DPPC) bilayers as a membrane system (41). DPPC is an easily obtained egg yolk phospholipid which contains no double bonds to oxidize and readily forms into a biologically relevant liquid crystalline bilayer (41). Unfortunately it is also far too simple a system to accurately model a biological membrane. Living plasma membranes, especially those of neurons, contain a highly heterogeneous mixture of lipids, some saturated, some unsaturated, some charged, some neutral, as well as cholesterol (41). Biological membranes also contain various proteins

which make up approximately 50% of the mass of the membrane (41). In addition, it is now understood that the inner and outer leaflets of biological membranes are composed of different phospholipids and that the membrane is arranged laterally into distinct areas known as lipid rafts, which are composed of distinct lipids and proteins (23, 41). For example, nicotinic acetylcholine receptors in nerve and muscle cells require the presence of cholesterol and anionic phospholipids in their immediate vicinity in order to function properly (22, 41). The simple membrane systems often used experimentally are a poor approximation of biological membranes and thus their findings for the mechanism of narcosis must be taken with a grain of salt, as it were. At the same time, they do demonstrate that it is plausible that the membrane is, at lease in part, responsible for the mechanism of narcosis.

There is also evidence that xenon anaesthesia/narcosis may be a product of interaction with proteins. Whereas most general anaesthetics have been shown to enhance the inhibitory activity of $GABA_A$ receptors, xenon seems to have little or no effect on them (46, 49). Instead it appears that xenon inhibits the excitatory action of *N*-methyl-D-aspartate (NMDA) receptors and, to lesser extents, neuronal nicotinic receptors and TREK-1 two-pore K^+ channels (34, 46, 58, 118). Interestingly, the NMDA receptor, a subtype of the glutamate receptor, is believed to be involved in learning, memory, and the perception of pain, which could explain xenon's attractive pharmacological properties (46, 96). This appears to be a non-competitive inhibition of the NMDA receptor, so xenon should strongly inhibit neuronal transmission even in the presence of high glutamate concentrations in the synaptic cleft (46). This inhibition of the NMDA receptor

by xenon goes a long way toward explaining the analgesic and amnesic effects of xenon (46).

X-ray crystallographic studies have also been performed in order to examine how and where xenon is interacting with various proteins. Unfortunately, membrane bound proteins such as the NMDA receptor are difficult to crystallize, so xenon has not been crystallized with the protein actually believed to be its target, but with soluble surrogate proteins. Xenon has been crystallized with urate oxidase, a prototype of various intracellular globular proteins, and with annexin V, a protein with structural and functional characteristics which allow it to be considered a prototype of the NMDA receptor (33, 34, 54). A single xenon molecule was found to bind to both of these proteins in a flexible, hydrophobic cavity (34). This is consistent with both the hydrophobicity of xenon and with the previous hypothesis that anaesthetic molecules exert their effect by binding proteins in these hydrophobic cavities (41). This suggests that it is plausible that xenon binds with the NMDA receptor, but that it is also capable of binding to a wide range of soluble intracellular proteins, consistent with the hypothesis that anaesthesia/narcosis is likely the ultimate product of multiple drug/protein interactions (41).

Other anaesthetic compounds which are believed to operate primarily by inhibiting NMDA receptor signalling, such as nitrous oxide and ketamine, have been observed to increase both global and regional cerebral metabolism in humans (66, 68, 69, 83, 90). Thus it would be expected that, if the anaesthetic action of xenon also operates primarily by inhibiting the NMDA receptor, that it should also increase both global and regional cerebral metabolism (97). Cerebral metabolic rates can be examined using

positron emission tomography (PET) and such studies have been performed (97). Because most general anaesthetics depress cerebral metabolism relative to conscious states, xenon anaesthesia was examined relative to propofol anaesthesia (3-5, 66). Contrary to expectations, it was found that xenon anaesthesia depressed cerebral metabolism both globally and regionally in the orbitofrontal, frontomedial, temporomedial, occipital, dorsolateral frontal, and lateral temporal cortices and thalamus (97). This suggests strongly that the mechanism of xenon anaesthesia/narcosis is not simply the inhibition of NMDA receptors.

Conclusion

Inert gasses clearly have physiological effects. To date, some work has been done to examine the effects of inert gasses on neurons, nerve conduction and consciousness, and a picture of how inert gases function as anaesthetics is beginning to emerge. The idea that inert gas narcosis can be reduced to a single cause is likely incorrect. The symptoms displayed vary widely, not only between different individuals, but also between different exposures for the same individual. The conditions required to bring about the onset of inert gas narcosis (ambient pressure, gas mix being breathed, temperature, psychological factors) also appear to vary widely. Hypotheses focusing exclusively on either cell membranes or protein interactions do not appear to tell the whole story. At this point in the research it would appear that there are elements of truth in both of these hypotheses. Continued research into both inert gas narcosis, and the mechanisms of general anaesthesia, particularly mechanisms pertaining to inhaled anaesthetics, are likely to further understanding of both conditions. The time may be nearing when, in order to truly understand the mechanisms of inert gas narcosis and general anaesthesia, hypotheses will

need to be able to bring together understanding which has been gained from both lipid- and protein-based models in order to construct a single model which can explain all the observations.

List of Figures

Gas	Molecular Weight	Volume	Lipid Solubility at 37°C	Oil:water partition coefficient	Relative narcotic potency
Helium	4	2.370	0.015	1.70	0.23
Neon	20	1.709	0.019	2.07	0.28
Hydrogen	2	2.661	0.040	3.10	0.55
Nitrogen	28	3.913	0.067	5.25	1.00
Argon	40	3.218	0.140	5.32	2.33
Krypton	83.7	3.978	0.430	9.60	7.14
Xenon	131.3	3.105	1.700	20.00	25.64

Figure 1. Narcotic potencies and physical properties of simple gasses. From (75).

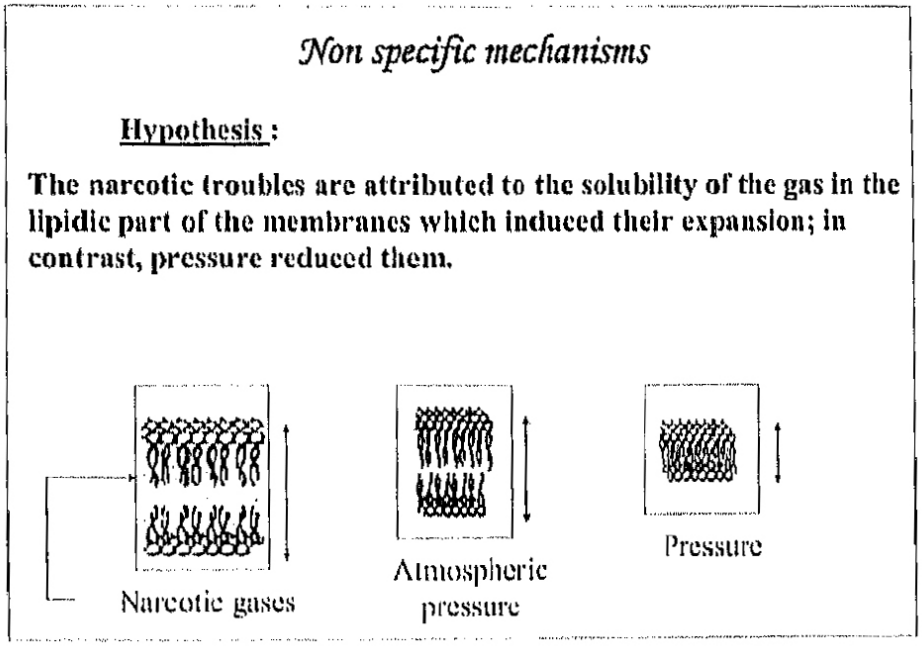

Figure 2. The critical volume hypothesis. Molecules of narcotic gases disolve in the cell membrane and cause it to swell. When the membrane reaches a certain volume narcosis is produced. Pressure reverses this narcotic effect by compressing the membrane. From (99).

Figure 3 **Autoradiogram of rat brain section photoaffinity labeled with radioactive halothane.** Degree of halothane binding is indicated by level of darkness; no other staining has been applied to the section. The binding shows little regional preference and is reduced to near-background levels in the presence of a tenfold excess of unlabeled halothane. The dramatic inhibition of labeling by non-radioactive halothane indicates that most halothane binding is saturable and specific, showing that many proteins could be involved in anesthetic action. From (39)

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VITA

Cameron Reid Smith was born on the 19th of August, 1976 in Ottawa, Ontario, and is a Canadian citizen. He graduated from the University of Guelph in June 2000 with a Bachelor of Science in Biomedical Science. Subsequently he attended the University of Toronto, graduating in August 2004 with a Master of Science in Pharmacology. In August of 2004 Cameron entered the Doctor of Philosophy program with the Department of Physiology (now Physiology and Biophysics) on the Medical College of Virginia campus of Virginia Commonwealth University. While attending the University of Guelph Cameron was heavily involved in the formation and training of what would become the University of Guelph First Response Team – an emergency medical services unit composed of students operating on the campus of the University of Guelph. While at Virginia Commonwealth University Cameron gained membership in the Honor Society of Phi Kappa Phi, the American Association for the Advancement of Science, the American Physiological Society, and the Undersea and Hyperbaric Medical Society. He also served as an observer on the MCV Honor Council. Through the course of his studies Cameron has received numerous awards fro academic achievement, including a University of Toronto Fellowship, the Robert W. Ramsey Award for most outstanding first-year Doctor of Philosophy student, the Charles C. Clayton Fellowship for outstanding scholarly achievement, and the Phi Kappa Phi graduate award for the School of Medicine. Cameron has presented his work at national meetings, including the U.S. Army's Advanced Technology Applications for Combat Casualty Care meeting, as well as the General Meeting of the Undersea and Hyperbaric Medical Society.