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Hyperbaric Oxygenation Papers

FORWARD

"Oxygen 2002" was a scientific symposium held in La Jolla California, July 1-2, 2002, to honor the life's work of Dr. Christian J. Lambertsen. The prime goal of the meeting was to bring together leading investigators in the fields of oxygen biochemistry, physiology, and toxicity, and diving and hyperbaric medicine, and to review the common themes and future directions of their work. This meeting was also the Tenth Symposium on Underwater and Hyperbaric Physiology. Indeed, Dr. Lambertsen chaired the first symposium, held in 1955.

Along with five other pioneers, Dr. Lambertsen founded the Undersea and Hyperbaric Medical Society. Therefore, it is fitting that the "Oxygen 2002" proceedings are being published in the Society's journal. With twenty-four invited speakers and a score of additional investigators who displayed posters on their discoveries, "Oxygen 2002" was a resounding success. We are proud of this International meeting, and delighted to have had the opportunity to honor Dr. Lambertsen. We are indebted to him for his lifelong vision and leadership in our field, and to the leadership of the Undersea and Hyperbaric Medical Society for providing structure to the symposium. We are also grateful to Dr. Claude A. Piantadosi, editor of *Undersea and Hyperbaric Medicine*, for facilitating publication of the symposium proceedings. We hope you will find fascination, as Dr. Lambertsen has throughout his career, in the wonderful topics of these papers.

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Co-Chairs of "Oxygen 2002"

Closed-circuit oxygen diving in the U.S. Navy.

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The pioneers of modern closed-circuit oxygen attack swimming were the Italians. In the early days of World War Two, the Italian Navy found itself lacking the surface warfare power of other nations. In an effort to overcome this deficiency, they developed a cadre of intrepid naval commandos who attacked ships riding on torpedoes and using closed-circuit oxygen underwater breathing apparatuses (UBAs). The Italians had been evaluating these methods of underwater sabotage as early as 1918 (1). Operating against overwhelming odds, these commandos damaged 2 British battleships in Alexandria Harbor in 1941, the HMS Queen Elizabeth and HMS Valiant (2, 3). Later, operating from an Italian tanker, the Olterra, that had been scuttled in 1940 but was later floated and moored in Algeciras harbor, Italian frogmen attacked allied shipping in Gibraltar in 1942/43 and damaged a number of ships (1).

British frogmen had a measure of success in this area as well, sinking six Italian ships in Sicily in 1943 and the Japanese cruiser Takao in the Johore Straights (Clark Presswood – personal communication). The biggest British success was the operation against the Tirpitz on 22 Sept 1943 (1). The Tirpitz was one of the most powerful vessels in the German fleet. It was anchored at the innermost end of the 20-mile Alten Fjord in Norway – a harbor protected by nets, mines, and listening posts. Three British mini-subs launched an attack on the harbor– two of the submersibles successfully penetrated the defenses and launched torpedoes. The Tirpitz was not sunk, but was incapacitated for six months. The crews of the midgets were captured.

At the start of World War II, the United States Navy had no combat swimmer capability. Diving was performed using the deep-sea hard-hat rig in which the divers were confined to the immediate vicinity of the support vessel. Combat swimming for the purpose of clearing obstacles for an amphibious landing was not a recognized need. At this time, a medical student at the University of Pennsylvania named Chris Lambertsen was designing and building the United States' first closed-circuit oxygen SCUBA rig. Dr. Lambertsen was a first-year medical student in 1939 when he completed the initial prototype of his Lambertsen Amphibious Respiratory Unit (LARU) (4). He first dove in his LARU prototype in 1940 in Lake Nokomis, near Minneapolis, Minnesota (5) to test the functioning of his new UBA. These were the first closed-circuit oxygen SCUBA dives in U.S. history. About 12 dives were accomplished, including one on which Dr. Lambertsen suffered an oxygen toxicity episode consisting of extremity and diaphragmatic twitching. Although he was tended from the surface, the line was improperly rigged and was dropped by the tender. Dr. Lambertsen managed to return to the surface under his own power (Chris Lambertsen – personal communication). Dr. Lambertsen had a reasonably finished product by the end of 1940. He demonstrated the LARU to the U.S. Navy Experimental Diving

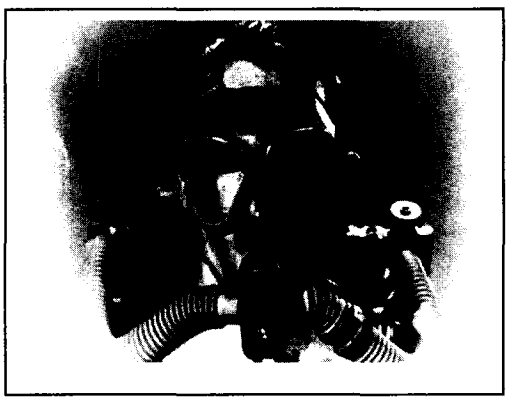
Unit (NEDU) in October of 1940 (Chris Lambertsen – personal communication), but there was little interest in this new type of diving apparatus.

Undaunted, in November of 1942, Dr. Lambertsen demonstrated the LARU MK II to individuals who were in the process of forming a maritime unit for the Office of Strategic Services. This classified demonstration took place in the swimming pool at the Shoreham Hotel in Washington DC. The LARU was a distinct improvement over the British and Italian UBAs used at the time in that it had a one-way, recirculating gas flow design that provided much more efficient carbon dioxide removal than the pendulum (or "counterlung") design used by the Italian and British UBAs. The Browne UBA was also evaluated, but the LARU was judged the best UBA for use by the Maritime Unit (5). Lambertsen's UBA was called simply the "Lambertsen Lung" by the OSS swimmers who used it during the war, (Tom Hawkins – personal communication) with the LARU MK III being the UBA used early in OSS operations and the MK 10 replacing it shortly thereafter. On 17 May 1943, Dr. Lambertsen conducted the first closed-circuit oxygen dive training in this country in the pool of the U.S. Naval Academy(5). The characteristics of the LARU used by these early OSS oxygen divers are shown in Table 1 (1).

Table 1.The LARU Closed-Circuit Oxygen UBA (1)

Closed-circuit rebreathing system
28 pounds
Full Face Mask
Oxygen cylinder - 2000 psi operating limit
Manual oxygen bypass
Over-the-shoulder breathing bag
Underwater gauge for oxygen bottle
Oxygen flow adjustable by needle valve

Fig. 1. The Lambertsen Amphibious Respiratory Unit LARU. (Photo Courtesy CJLambertsen)



The LARU is shown in Figure 1. Dr. Lambertsen graduated from medical school in 1943 and was commissioned as a second lieutenant in the Army Medical Corps. Also in May of 1943, the Chief of Naval Operations ordered the establishment of Naval Combat Demolition Units (NCDUs) made up of men trained as assault demolitioneers to blow up obstacles placed on enemy beaches to prevent boats from coming ashore (6). The Marine landing on Tarawa, where the landing boats were grounded on an offshore reef, resulted in a heavy loss of life and demonstrated the need for combat demolition swimmers in amphibious warfare(6). LCDR Draper Kauffman, who was the Commanding Officer of the Navy Bomb Disposal School, was chosen to lead this new enterprise and he set up training for the NCDUs in Fort Pierce, Florida. LCDR Kauffman had originally been denied entry into the Navy because he failed the entrance eye exam. He then volunteered to serve in the war, first with the French Army ambulance service, where he won the Croix de Guerre, and then with the British bomb disposal units. Training at Fort Pierce started in June 1943. Lambertsen demonstrated the LARU to LCDR Kauffman in October 1943, but was again informed that there was no place in combat beach reconnaissance and demolition operations for this radically new device (5).

Closed-circuit oxygen diving and combat swimming evolution in the United States continued, then, under the auspices of the OSS with Dr. Lambertsen leading the way. One of the

major factors that divers using 100% oxygen UBAs must contend with is central nervous system (CNS) oxygen toxicity. The French physiologist Paul Bert had demonstrated that breathing oxygen at increased pressure could lead to convulsions and death (7). Significant research had been done in this area by Dr. A.R. Behnke and his colleagues at NEDU as they explored the use of oxygen to accelerate decompression from long, deep dives and to treat decompression sickness. Following a series of chamber dives, Behnke and his colleagues published the first set of hyperbaric oxygen exposure guidelines in the U.S. military (2,8). (See Table 2.)

As we will see later on, had they been used as operational limits for OSS divers, the results might have been disastrous. Dr. Lambertsen displayed great insight in making the limits for OSS oxygen diving much more conservative than Behnke's chamber limits. Lambertsen's OSS oxygen exposure limits are shown in Table 3 (9). The increased conservatism in his tables at the deeper depths was not based on any personal observation that immersion and exercise increased the likelihood of oxygen toxicity, but from Lambertsen's realization that there would be a high probability of injury or death should a convulsion occur in a free-swimming diver (Chris Lambertsen – personal communication).

Table 2. Behnke Dry Chamber Oxygen Exposure Limits (2,8)

Sea Level	4 hours
33 FSW	3 hours
66 FSW	3 hours
99 FSW	45 min

Table 3. Lambertsen OSS Closed-Circuit Oxygen Exposure Limits (9)

Sea level	24 hours
40 FSW	2 hours
60 FSW	45 minutes
100 FSW	10 minutes

Dr. Lambertsen also realized that more definitive limits needed to be established and requested additional guidance from the Navy Experimental Diving Unit after the war (Chris Lambertsen – personal communication). This was a prescient action, because the OSS limits are still far less conservative than modern limits. The assertions made in the History of the OSS Maritime Unit (1) would find few subscribers today, "Diving with the Lambertsen Unit is possible to depths as great as 100 feet. No diver should remain at that depth longer than 15 minutes at one time, however.... A more workable depth is from 50 to 60 feet at which the unit functions perfectly and permits submergence for 45 minutes without danger" (1).

Despite these permissive deeper limits, the OSS had an excellent safety record using oxygen during World War II, with very few toxicity episodes and no fatalities (Chris Lambertsen – personal communication). This is probably due to Dr. Lambertsen's emphasis in training that the swimmers swim no deeper than required by the mission. Most of the diving was conducted between 20 and 25 feet. This chapter of U.S. closed-circuit oxygen diving history ended when the OSS was dissolved by President Truman on 1 October 1945 (10).

Table 4. 1946 NEDU Oxygen Exposure Limits (11)

Sea level	17 hours
30 FSW	2 hours
60 FSW	30 minutes

" The 60 FSW limit has been set by both the United States and British navies."

After the war, Dr. Lambertsen corresponded with CAPT O.K. O'Daniel to ask if NEDU had any guidance to provide on the subject of oxygen exposure limits for closed-circuit oxygen divers. CAPT O'Daniel responded on 30 September 1946 with the limits shown in Table 4 (11).

The pioneering work of Professor Ken Donald in the area of CNS oxygen toxicity in divers was done in the United Kingdom during World War II, but this work was not completed until the latter part of the war and was not published until after the war (12). Donald decided that the risk of CNS oxygen toxicity mandated a maximum depth of 25 feet for closed-circuit oxygen divers. He was also the first to describe the role of water temperature, immersion, and exercise in reducing a diver's tolerance to hyperbaric oxygen.

In 1946 and 1947, perhaps inspired by the work of Donald and the experiences of the OSS Maritime Unit, Yarborough and Behnke at NEDU undertook the first tests of oxygen tolerance using immersed, working divers done in the U.S. (3,13). They attempted 71 exposures to 2 hours at 40 feet. 19 of the 71 exposures were stopped because of toxicity episodes. The UBAs used for this study were the LARU and the Browne units and the water temperature was 90 degrees F. At 50 feet, 3 of the 5 divers developed signs or symptoms of CNS oxygen toxicity. At 30 feet, they found that there were no toxicity episodes in 35 one-hour exposures, but two divers had symptoms at 87 and 111 minutes(3,13). As a result of these investigations, the 1947 NEDU report stated that "For underwater work the safe inhalation of pure oxygen is limited to a depth of 30 feet." No time limit was imposed.

In an effort to preserve the diving capability developed in the OSS Maritime Unit, Dr. Lambertsen had arranged to have custody of an inventory of LARUs transferred to him so that he could introduce OSS diving techniques to other groups. During the post-war period, Dr. Lambertsen introduced his diving apparatus to U.S. Army Engineers, the U.S. Coast Guard, and U.S. Navy Underwater Demolition Team (UDT) personnel. In 1947, the UDTs, under the leadership of LCDR Doug Fane, saw the advantages of having their members trained in the use of SCUBA gear for combat operations. This decision may have been hastened by receipt of the now-famous letter that Fane received addressed to "Underground Demolition Unit Two." LCDR Fane proposed this training in 1947, but was informed by NEDU and the Navy Dive School that this type of diving gear was too dangerous(6). Undaunted, LCDR Fane invited Dr. Lambertsen to come to the Naval Amphibious Base in Little Creek, Virginia, in January 1948 to demonstrate the LARU to the UDT operators and conduct the first-ever training for Navy divers in SCUBA ("self-contained underwater breathing apparatus" - a term coined by Dr. Lambertsen)(4, 6,14).

Following this successful training, it was time to demonstrate for the Navy how closed-circuit oxygen diving might be employed by safely inserting combat swimmers using a submarine. On 22 February 1948, Fane and Lambertsen accomplished the first free-swimming, closed-circuit oxygen SCUBA lock-out and re-entry from an underway submarine (USS Grouper) operating off Saint Thomas, U.S. Virgin Islands. The sub was operating at periscope depth, so the lockout depth would have been approximately 30 feet. Although this would have been within the guidelines of the 1947 NEDU oxygen exposure limits, this was not a conscious factor in planning the dive, which lasted approximately 30 minutes (Chris Lambertsen – personal communication).

After the success of the USS Grouper closed-circuit oxygen SCUBA operations, Fane returned to the UDT base in Little Creek and established a "Submersible Operations" or SUBOPS platoon with men drawn from UDT 2 and 4(4). The activities of this group were extremely classified, even within their own organization. LTJG Bruce Dunning was the officer-in-charge of the SUBOPS platoon, which was the first unit with a SCUBA diving capability in the U.S. Navy.

Fane and Lambertsen next determined to do a study to better define the limits of safe oxygen exposures in free-swimming divers. They arranged a study at the Naval Submarine

Medical Research Laboratory escape training tower later that year. This study was conducted by Schaefer and Willmon and was the first time that fin-swimming, LARU oxygen divers were observed in a study under controlled conditions. The divers swam a circular path in the tower at a speed of 0.9 knots in 90-degree water temperature. They did fifty dives with a maximum exposure time of 90 minutes at various depths and had 14 toxicity episodes(4,6,15). This study also developed the Oxygen Tolerance Test, a 60 FSW for 30 minutes resting exposure in a dry chamber that was designed to test an individual's tolerance for hyperbaric oxygen. No specific new Navy-approved oxygen exposure limits were established after the trials, however, leaving the 1947 NEDU limit of 30 FSW for an unlimited time as the most authoritative Navy oxygen diving limit at this time.

In October of 1948, Lambertsen and Fane conducted the second operational demonstration of closed-circuit oxygen diving capabilities operating from the USS Quillback. The UDT divers were first trained to operate on the deck of the underway submarine. The dives were approximately 30 minutes in duration at a lockout depth of 30 FSW. Finally, the underway recovery of the British submersible canoe "Sleeping Beauty" aboard the Quillback was performed. Lambertsen positioned himself in the Sleeping Beauty ahead of the submarine on its course. He had a short bow line rigged from the bow to the cockpit of the Sleeping Beauty. After intercepting the submarine, which was towing a buoy, he threaded the Sleeping Beauty's bow line through a metal loop on the towed buoy and then drove the craft down the line with the submersible's power to the deck of the submarine. Losing only a stern plane to the sail of the sub, he maneuvered the Sleeping Beauty onto a cradle with the aid of the deck crew. The deck crew was out 30-40 minutes during the operation, at a depth of approximately 30 FSW. (Chris Lambertsen – personal communication) These operations demonstrated the feasibility of launching and recovering a free-flooding combatant submersible aboard an underway submarine and paved the way for modern-day SEAL Delivery Vehicle (SDV) and Dry Deck Shelter (DDS) operations. Figure 2 shows UDT members with their LARUs.

After the successful Quillback combat swimmer operations and training, Fane briefed VADM Jerraud Wright at Amphibious Forces, Atlantic Fleet. He was stunned to receive official correspondence directing UDT to confine itself to conventional hydrographic reconnaissance and beach clearance (4). Interpreting these orders somewhat creatively, Fane and Dunning continued to develop a submersible operations capability in the SUBOPS platoon. Perhaps appropriately, this training was headquartered in a building that had served recently as the base brig. The four UDTs on the East and West Coast used the LARU for submersible operations and training from 1948 into the early fifties. (Chris Lambertsen – personal communication)

Fig. 2. Underwater Demolition Team members with their LARUs. (Photo coutesy C.J. Lambertsen)

The next event in the evolution of SCUBA diving operations in the U.S. Navy was the introduction of the new French "aqualung" in (Jacques Cousteau and Emile Gagnan). The gas cylinders contained not oxygen, but compressed air. This apparatus was an open-circuit UBA, which meant that the diver's exhaled gas

did not re-enter the UBA so that carbon dioxide could be removed and the unused oxygen rebreathed, but rather escaped to sea. The key bit of technology needed to make this breakthrough was Gagnan's redesign of a car regulator such that it could sense the ambient pressure the diver was exposed to and provide him with inhaled gas at a pressure that was slightly higher than ambient. Cousteau and others in the French Navy had made many dives with this new apparatus in the years that followed. Fane met with Gagnan and arranged for him to come to Little Creek in the spring of 1949 to teach the UDTs how to dive with the aqualung(4). The aqualung had some advantages over closed-circuit oxygen UBAs: it was less complex, easier to set up, had a greater depth range, and reduced the possibility of diving accidents. These advantages led many UDT divers to have a strong preference for the new UBA in spite of the tactical advantages of the LARU. In one of the least distinguished chapters of UDT diving history, many of the remaining OSS World War II LARUs were consigned to a bonfire at a team beach party in 1953(4,5,16).

The early fifties saw the UDT thus introduce open-circuit SCUBA into use in the U.S. Navy. Fane allegedly made the first dive by an American using the new UBA, diving to 100 feet on his first dive(6). The aqualung was subsequently used by Fane to demolish a wreck in the Chesapeake Bay that was posing a hazard to navigation in December 1949. It was used by UDT in 1950 in Sleeping Beauty and other submersible trials. In 1952, a research program headed by Dr. Lambertsen conducted open-water trials in the waters off of Coronado, California with the aqualung and the LARU to study physiological limiting factors in underwater swimming (4). This series of research dives was also notable in that it was the first use of the newly developed wet suits by the U.S. Navy.

Another factor that contributed to the unfortunate but temporary demise of closed-circuit oxygen diving in UDT was the July 1952 publication of the first closed-circuit oxygen diving limits in the U.S. Navy Diving Manual, which stated:

"When diving with a mask, oxygen should not be used at depths greater than 30 feet. The time of dive should not exceed 30 minutes" (17). The 30-minute time limit was very restrictive and effectively put attack swimmers out of business, since most combat operations would entail much longer swims than this. This extremely restrictive limit was short-lived, however. Further research done at NEDU by Lanphier and his colleagues resulted in this overly conservative limit being changed(3,18). Lanphier did 51 dives using 19 immersed, working divers at a variety of depths. The divers were breathing oxygen (averaging 99.5% purity) from an open-circuit source and the water temperature was 80 degrees F. The limits recommended by Lanphier in Table 8-2 of NEDU report 11-54 are shown in Table 5 (18).

Table 5. Third NEDU Closed-Circuit Oxygen Limits - Lanphier 1954 (18)

10 ft for 120 minutes
15 ft for 90 minutes
20 ft for 65 minutes
25 ft for 45 minutes
30 ft for 30 minutes
35 ft for 20 minutes
40 ft for 15 minutes

After several years, the UDTs began to reconsider their infatuation with open-circuit SCUBA. Closed-circuit SCUBA offered the advantages of being much smaller and lighter than open-circuit UBAs that would last for equivalent dive depths and times. Another important advantage was that there is no stream of tell-tale bubbles cascading to the surface as the diver approached a hostile ship or pier, a decided tactical plus. Since the LARUs were no longer serviceable, the UDTs acquired the World War II-vintage Italian Pirelli UBA in the years after the Korean War (Norm Olson – personal communication).

The foremost individual credited with bringing closed-circuit tactical diving back to the teams was LCDR Frank Kaine during the time that he was Commanding Officer of UDT 21 in Little Creek, Virginia but the West Coast UDTs also acquired and used the Pirelli (Layton Bassett – personal communication).

Fig. 3. UDT diver with Italian Pirelli closed-circuit oxygen rebreather. (Photo courtesy C.J. Lambertsen)

As shown in Figure 3, the Pirelli was a pendulum-type rebreather, in which a single hose was used both to inhale from and exhale to the CO_2 scrubber and the breathing bag. The characteristics of the Pirelli are listed in Table 6(19). Pendulum rebreathers have an inherent design flaw. The gas flows to and from the scrubber and the breathing bag via the same hose, creating a "dead space" in the loop that contributes to CO_2 buildup. While this may not be a problem for divers at rest, it quickly becomes a problem for free-swimming divers. The Pirelli was given the nickname "The Black Death" because of the numerous CO_2 and oxygen toxicity episodes suffered by team members while diving with this rig. (Layton Bassett – personal communication) After a near-fatal accident with the Pirelli in 1956, UDT operator Harold Nething recalled, "...after some investigation, it was discovered that in the breathing bag where the scrubber canister attached to the breathing hose, there's a fitting that's sweat soldered on....it had parted and failed. Later, after testing all the Pirellis, about 85% failed." (Harold Nething –Internet site)

Table 6. Description of the Italian Pirelli (19)

LS-901
Pendulum system
Two 1.6 liter bottles of oxygen
Each oxygen bottle charged to 3000 psi
Constant flow regulator adjustable from 0.5 to 2 L/M flow
Lasted for up to 4 hours under normal working conditions
CO_2 absorbent canister inside the breathing bag

These problems were reported to the Bureau of Ships(20). Further investigation by NEDU resulted in a recommendation that no more Pirellis be procured (21). Use of the Pirelli decreased in 1956 and 1957 and this UBA was soon replaced by the initial German Draeger. The Draeger UBA is shown in Figure 4 and its characteristics in Table 7 (19).

Table 7. Description of Draeger LT Lund II (19)

Draegerwork, Lubeck, Germany
Two oxygen cylinders – 0.8 liters each
Charged to 2800 psi
Total gas supply of 320 liters (11.2 cu ft)
Constant flow regulator set to provide 0.9 L/min O_2
Also had manual oxygen bypass
CO_2 absorbent canister was inside breathing bag
Operating limit 90 minutes

The Draeger LT Lund II UBA had an excellent reputation in the UDTs (N. Olsen, and L. Bassett personal communications). Weber stated that "Most of our divers prefer the Draeger to other rebreathers due to simplicity of design and reliability of operation" (19). The Draeger was used only for several years (approximately 1957-1958) due to lack of replacement parts and subsequent maintenance problems. Subsequently, Scott Aviation reverse engineered and built a U.S. version of the Draeger. While

externally, it looked the same, it did not achieve the quality of the German version and was shelved shortly thereafter (N. Olson – personal communication).

Fig. 4. UDT diver with early German Draeger closed-circuit oxyen rebreather. (Photo courtesy T. Hawkins).

In the early 1950s, Dr. Lambertsen worked with the J.H. Emerson Company to develop the LARU MK 20 UBA. They introduced this updated UBA first to the Army. (Chris Lambertsen – personal communication) The LARU MK 20 was eventually modified and introduced into the Navy as the Emerson-Lambertsen UBA in about 1963(19). One major difference between this UBA, which came to be called simply the Emerson in the teams, and the LARU series was that the Emerson was typically used with a T-bit mouthpiece and a partial facemask that was isolated from the breathing loop. The LARU had been used with a full facemask by the OSS swimmers and the initial Army and Navy trainees. There are two primary advantages to using a full facemask with closed-circuit SCUBA: the airway is better protected in the event that the diver should become unconscious from oxygen toxicity or other diving disorder and the diver is better able to use underwater communication devices for operational or emergency communications. The Emerson-Lambertsen was also a recirculating system and had over-the-shoulder breathing bags, which have the advantage of being comfortable to breathe in both prone and sitting positions. One aspect of the rig that was not ideal was the 4-setting metered oxygen supply valve design. If the diver's oxygen consumption changed underwater, he might find himself with insufficient oxygen to breathe and would have to use the manual bypass valve. Conversely, if his oxygen consumption was lower than the add rate, his bags would overfill with oxygen and he would experience an undesired increase in buoyancy. (Don Crawford – personal communication) A description of the Emerson-Lambertsen UBA is provided in Table 8 (19) and a picture in Figure 5.

Fig. 5. The Emerson-Lambertsen UBA. (Photo courtesy U.S. Navy).

Table 8. The Emerson-Lambertsen Closed-Circuit Oxygen UBA (19)

Metered oxygen flow valve – 0.5, 0.9, 2.0, or 3.0 L/min
Cylinder charged to 2000 psi
Capacity 360 L (12.7 cu ft)
Normal duration of operation 120 min

The Emerson-Lambertsen served the teams well for almost 20 years, from 1963 until approximately 1981. By that time, replacement parts for the UBA had become very hard to obtain. Rigs were cannibalized for spare parts, maintenance was difficult, and increasing numbers of the rigs began to malfunction. By 1980, the problem had become severe and the Emerson was declared no longer usable by Naval

Special Warfare (Don Crawford – personal communication).

The demise of the Emerson meant that the UDT and now the SEA/Air/Land (SEAL) teams were in need of new closed-circuit oxygen UBA. NEDU evaluated both the German Draeger LAR III ("LAR" is an acronym for "Lung Automatic Regenerator") (22) and the LAR V (23) closed-circuit rebreathers as potential replacements for the Emerson-Lambertsen. The rig selected after NEDU testing was a modified version of the LAR V introduced into the Naval Special Warfare community in 1981, with the first 10 units going to SEAL Team Six (D. Crawford and R. Woolard – personal communications)

Fig. 6. SEAL diver with the Draeger LAR V/MK 25 closed-circuit oxygen rebreather. (Photo courtesy U.S. Navy).

The LAR V has several advantages over the Emerson-Lambertsen. At 25 pounds, it is significantly lighter. It is also smaller, simpler in design, and has (as did the LARU MK 20) a well-designed oxygen add system in which a second stage demand regulator opens any time that the diver empties all the air from his breathing bag. A description of the Draeger LAR V is provided in Table 9 (24) and a picture of this UBA is shown in Figure 6. This UBA is still the primary UBA used by Naval Special Warfare in 2002. Renamed the MK 25, it has been recently modified to include a larger oxygen bottle and a larger, better insulated CO_2 absorbent canister (MK 25 MOD 2) (24).

Table 9. The Draeger LAR V/MK 25 MOD 2 UBA (24)

Length: 18 inches
Width: 13 inches
Height: 7 inches
Weight:27 pounds
Buoyancy: Neutral
O2 Cylinder: 1.9 L at 3000 psig
Chest-mounted fiberglass housing
Bypass add rate: 60 liters/min
Oxygen addition by demand
No constant addition of oxygen

Although the Teams got their new UBA from the Germans, they took many of their combat swimming tactics from the French via SEAL Team TWO. Combat swimming had become an area of decreased emphasis during the Vietnam War, where the SEALs and UDT became jungle warriors. Many SEALs, including then-CDR Bob Gormly at SEAL Team Two, recognized the need to re-establish the SEAL's expertise in the water. LT Ryan McCombie, recently returned from Vietnam, was sent to France for an exchange tour with the French Commando Hubert in St Mandrier. There he was exposed to a totally different diving culture. During this period, the Commando Hubert were arguably the best combat swimmers in the world. Their training typically entailed 5-7 dives per week. The dives were complex, multi-dogleg and long duration. During the 6 month French basic training, Lt McCombie with Lt. Jean Francois Tardiveau as a swim buddy, completed a 7000 meter, 4hour 10 minute closed-circuit oxygen dive. This particular dive was remarkable, even for the Commando Hubert, and demonstrated what could be accomplished with the proper training and equipment. Now-LCDR McCombie returned from France with a clear vision of how Naval Special Warfare could enhance its combat swimming skills.

He was to have an opportunity to act on this vision. Several years later, CDR Rick Woolard assumed command of SEAL TeamTwo2 and LCDR McCombie was his Executive

Officer. CDR Woolard had been embarrassed by a poor showing of SEAL attack swimming abilities during a "Flintlock" combat exercise with German and Dutch counterparts in 1981. He also had great respect for French oxygen diving capabilities. While Executive Officer of SEAL Team TWO, McCombie had arranged for a Hubert officer to be assigned there. LT Francois d'Avout reported to SEAL Team TWO shortly after Woolard assumed command in 1982, and Woolard immediately directed McCombie and d'Avout to develop and conduct a course in attack swimming to correct the SEAL operational deficiency. The resulting eight-week Combat Swimmer Course stressed accurate underwater navigation, precise buoyancy control, long-distance underwater and surface swimming ("turtlebacking"), and full-mission profiles that realistically integrated the attack swim into stealthy air/land/water target approach and withdrawal scenarios. The instructors and students were carefully selected, and all graduates had to show they could approach, attack, and withdraw from targets miles from their dive point without surfacing despite multiple underwater course changes. In 1983, Woolard's efforts were rewarded when his SEALs successfully completed a long and very arduous attack swim during a major exercise in Germany...and their German counterparts did not. From then on, Combat Swimmer Course graduates routinely outperformed their European counterparts, and by the late 1980s they were teaching attack swimming to others. The course was eventually accepted by the Naval Special Warfare community as a major improvement in SEAL capabilities, and its primary lessons are still part of both the Basic Underwater Demolition/SEAL and SEAL Qualification Training courses that every SEAL must complete." (R. McCombie and R. Woolard – personal communications) Figure 7 shows SEAL team members using the LAR V.

Fig. 7. SEALs with Draeger LAR V/MK25 closed-circuit oxygen rebreathers . (Photo courtesy U.S. Navy).

No experimental basis was identified for these changes. By 1981, the USN oxygen exposure limits had been modified further (Table 11).

Table 11. 1981 U.S. Navy Diving Manual Oxygen Exposure Limits (26)

20 ft for 110 minutes
25 ft for 75 minutes
30 ft for 45 minutes
35 ft for 25 minutes
40 ft for 10 minutes

The Navy oxygen exposure limits also evolved over time. New limits appeared in the 1959 Diving Manual (Table 10) that were modified from those proposed by Lanphier in 1954 (25).

Table 10. 1959 U.S. Navy Diving Manual Oxygen Exposure Limits (25)

10 ft for 240 minutes
15 ft for 150 minutes
20 ft for 110 minutes
25 ft for 75 minutes
30 ft for 45 minutes
35 ft for 25 minutes
40 ft for 10 minutes

Note that the 240-minute limit for dives 10 feet and shallower as well as the 150 minute limit for dives 15 feet and shallower had both been dropped. Again, the reasons for these changes from previous limits were not documented in the Diving Manual (26).

For the SEALs to utilize their new Draeger Vs and newfound combat swimming skills, the advance in oxygen diving required next was the establishment of less restrictive closed-circuit oxygen exposure limits. Increasing contact with combat swimmers in allied countries revealed that their oxygen exposure limits were less restrictive in those in the U.S. Navy. In 1981, Naval Special Warfare requested that NEDU re-evaluate the oxygen exposure limits to see if longer exposures might be safely accomplished. NSW also requested that NEDU evaluate the feasibility of making a brief downward excursion after a lengthy exposure at a shallow "transit" depth. This request resulted in 3 major dive series conducted by Butler and Thalmann at NEDU between 1982 and 1984 (27,28,29). Divers were immersed, exercising, and subjected to moderate cold stress in an attempt to create reasonable "worst-case" conditions for operational combat swimmers. Experimental divers used the same Draeger LAR V UBAs then in use by the SEAL teams. The UBA was purged to achieve a minimum oxygen fraction of 95% before the exposure was started and CO_2 levels were constantly monitored to ensure that there was no CO_2 build-up, which would make the divers more susceptible to oxygen toxicity. limit. The trials began by re-evaluating the 40-foot exposure. A 20-minute exposure at this depth produced 2 convulsions in 17 dives while a 15-minute exposure produced no convulsions or definite symptoms of CNS oxygen toxicity in 41 exposures (27). A 15-minute excursion was then attempted following a two- hour "transit" period at 25 feet. This profile produced 2 definite hits on the previously safe 40-foot excursion and one convulsion at 25 feet. After consultation with operational SEAL units, the transit depth was reduced to 20 feet and the testing resumed (28).

The second set of dive trials finished re-evaluating the single-depth oxygen exposure limits. The new single-depth oxygen exposure limits proposed after this series (28) and displayed in Table 12 were approved for use in Naval Special Warfare in 1983 and are still in effect in 2002 (30). The second set of dive trials also found that a 20-foot oxygen exposure for periods of up to 4 hours did not adversely affect the diver's ability to make a brief downward excursion (28). The current U.S. Navy Transit with Excursion limits are shown in Table 13; they were also approved for use in 1983 and are still in use in 2002 (30).

Table 12. Current USN Single Depth Oxygen Exposure Limits (30)

25 FSW or shallower	240 minutes
30 FSW	80 minutes
35 FSW	25 minutes
40 FSW	15 minutes
50 FSW	10 minutes

Table 13. Current USN Transit with Excursion Limits (30)

Transit portion of dive 20 FSW or shallower	
Single excursion allowed	
21-40 FSW	15 minutes
41-50 FSW	5 minutes
Total dive time 240 minutes or less	

A third NEDU oxygen dive series was conducted in November and December 1985 and was designed to evaluate the feasibility of making multiple downward excursions from 20 feet on a 4-hour dive(29). This series encountered an increased incidence of toxicity episodes in attempting multiple excursions on a single dive and no modification to the single-excursion rule was proposed(29). A total of 686 dives were accomplished during the three series with 67 episodes of in-water CNS oxygen toxicity, including 8 convulsions.

On the protocols above, a single toxicity episode was seen on the 25 and 30-foot depths within an exposure time that was completed by many other divers without incident. Both divers suffered multiple oxygen toxicity episodes during the dive series and were considered to be more

sensitive to the effects of hyperbaric oxygen than their fellow experimental divers. Since both divers had passed the oxygen tolerance test (OTT) as part of their screening for diver training, the sensitivity of this test in identifying individuals who are unusually susceptible to oxygen and its usefulness as a screening tool was questioned. This issue was addressed by Butler and Knafelc following the NEDU oxygen dive trials(31). They identified three divers that had had multiple episodes of oxygen toxicity on profiles other divers had performed without difficulty. They then performed multiple OTTs on these individuals to see if the test was sensitive enough to identify any of the divers as sensitive on multiple exposures. None of the divers had symptoms on any of the OTTs, leading the investigators to conclude that the failure of the OTT to elicit symptoms of CNS oxygen toxicity in these divers was reproducible. The next question addressed was how many individuals fail the OTT (have signs or symptoms of CNS toxicity within the 30 minutes at 60 feet). A review of the records from the Naval Safety Center revealed a 1.9% failure rate among diving candidates undergoing the OTT. Since the individuals identified as sensitive to oxygen during the NEDU dive trials had repeatedly passed the OTT, the 1.9% of individuals who failed the OTT on the first trial were considered to be perhaps even more sensitive to oxygen. The authors therefore recommended that the OTT be retained for any divers who would be using closed-circuit oxygen SCUBA because of the high probability of a fatality resulting from a convulsion that occurred while engaged in untethered diving (31).

The issue of approving oxygen exposure limits that had been shown to produce CNS oxygen toxicity was contentious, especially in light of one convulsion that occurred at 25 feet after only 72 minutes, when many other divers were able to tolerate 4 hours at this depth without incident. Professor Donald commented some years later, "The present author would strongly oppose the acceptance of the possibility of acute oxygen poisoning in the oxygen exposure time limits recommended for routine diving operations. Such an acceptance could impair the traditional and essential trust between divers and those responsible for their safety" (3).

The safety of the new limits was greatly enhanced by a SEAL corpsman who inquired in 1983 about the rationale for the purge procedure used at the time. (Master Chief Johnny Johnson – personal communication) The Draeger LAR V purge procedure in use in 1983 was to manually fill the breathing bag with oxygen and then empty it by inhaling through the mouth and exhaling through the nose three times on the surface before the dive. The UBA was also purged every 30 minutes during the dive to protect against dilutional hypoxia occurring as the tissues of the body off-gassed nitrogen. A review of NEDU reports and the Navy Diving Manual revealed no explanation of why that particular volume of purging had been chosen nor any measurement of the oxygen fraction produced in the UBA by the procedure. (32) The procedure used by Lambertsen in the OSS and in the initial training of Navy UDT and Army cadres prior to diving the LARU was as follows: "....sucking the bag completely flat and closing the mask shut-off valve. O_2 could be added to the bag or not. Then, when ready to dive (could be an hour or more later), a full exhalation of air from lungs, and switch to O_2 rebreathing. No O_2 flushing of the unit was done. Any later gas venting supplemented this by accident and not intent." (Chris Lambertsen – personal communication) This procedure was used throughout World War II and apparently served well. The origins of the three-cycle fill and empty and the every-30 minute purge during the dive procedure that came into Navy use later remain obscure.

In rethinking the purge procedure at NEDU following Master Chief Johnson's question, Butler and Thalmann determined that the purge should seek to achieve a level of oxygen in the UBA only high enough to prevent hypoxia. This level was determined to be 45% for a purge being done on the surface (32) and 55% for a purge being done at depth (33). A single fill/empty

cycle of purging prior to the dive was found to be sufficient to ensure this level of oxygen in the UBA and to produce a mean value of 71% FIO_2 (fraction of inspired oxygen) on the surface. Purging nitrogen out of the rig beyond this level serves only to increase the risk of CNS oxygen toxicity and consume gas from the UBA cylinder, thereby shortening the gas supply available for the mission. The mean oxygen percentage in the UBA was found to increase to a mean of 82% as the diver descends to a depth of 20 FSW to begin his swim. Additional purging conducted during the dive was found to be unnecessary for hypoxia prevention, to consume additional gas, and to potentially compromise the diver's position if his bubbles are observed by hostile forces on the surface(32).

This seemingly modest decrease in FIO_2 may be of great importance to the safety of the diver. Using a probabilistic model, Harabin and her colleagues at NMRI showed that the risk of developing CNS oxygen toxicity from breathing "nearly pure" oxygen at 30 feet for 80 minutes is about 4 %. If the recommended new purge procedure is followed, however, resulting in a lower FIO_2 of 0.74, the probability of toxicity after 80 minutes is less than 0.1% (34). How safe have the 1983 oxygen exposure limits proven in practice? Walters et al addressed this issue in their 2000 paper(35). A review of the records from the Naval Safety Center found 157,930 LAR V dives with only one reported episode of oxygen toxicity.

Many SEAL operations, including ones that entail closed-circuit oxygen diving, are carried out in secret and never become public knowledge. One exception to this rule is the ship attack that was carried out during Operation Just Cause in Panama in 1989 (16).

CDR Norm Carley, Commanding Officer of SEAL Team Two, was directed to attack three Panamanian Defense Force (PDF) gunboats prior to the larger assault. The planners for Just Cause wanted to avoid major damage to the vessels so that they could be used by the new Panamanian government, but CDR Carley convinced them that this would entail unacceptable risks to the SEAL operators involved. The operation was complicated by several additional factors. The patrol boats were made of aluminum, so limpet mines would not stick to the hulls. The attack was instead carried out with haversacks of C-4 plastic explosives.

Cutting across the Panama Canal, the two SEAL combat rubber raiding crafts (CRRCs) ran into unanticipated boat traffic north of Balboa Harbor, including some boats with spotlights. The CRRCs, running at low speeds so not to leave a wake, avoided detection. Arriving at the far shore early, the CRRCs hid in a mangrove tree line north of Balboa Harbor while waiting to insert the SEALs. Two boats left Balboa Harbor, but the Presidente Porres remained at the pier. After 15 minutes, CRRC #1 started its motor and began creeping out of the mangrove. CRRC #2's outboard motor had quit, and it was thus unable to follow. Carley, aboard CRRC #1, decided to proceed to the insertion point alone. The CRRC advanced out of the mangrove, headed a few hundred yards in a southeasterly direction, and quietly approached a position 150 yards north of Balboa Harbor's Pier 18. With a backdrop of the darkened mangrove, the CRRC approached without being detected. A pair of SEALs, LT Edward Coughlin and EN3 Timothy Eppley, slipped over the CRRC's side at 2330, went underwater, and started toward their target. CRRC #1 withdrew, returned to the hideout, slipped a tow line to its disabled sister, and headed out of the mangrove. Together they proceeded to the insertion point off pier 18, where the second swimmer pair, ET1 Randy Beausoleil and PH2 Christopher Dye, quietly left CRRC #2. Swimming underwater, the second pair was five minutes behind the other two swimmers. To destroy the target, each swimmer pair was equipped with a 20-pound Mark 138 Haversack explosive package with a MCS-1 clock, a Mark 39 Safety and Arming Device, and a Mark 96 detonator.

After the SEALs were inserted, Carley ordered that CRRC #2 be towed back to NS-Rodman so that its outboard motor could be changed. Although the CRRC had a spare outboard motor on board, Carley felt that it was too risky to attempt an engine change out on the water, given the CRRCs' proximity to the target and the level of activity around Balboa Harbor. Besides, the starting process of the outboard motor was loud and sure to alert the PDF. Avoiding a compromise of the SEALs swimming to the target was uppermost in Carley's mind. On the return to NS-Rodman, the CRRCs evaded two more craft going across Balboa Harbor. The remainder of the assault force arrived at NS-Rodman and began changing CRRC #2's outboard motor. Carley observed the target area for indications that the PDF might be alerted. Balboa Harbor appeared quiet. The pairs of SEALs, swimming underwater on a compass bearing, approached pier 18. It became apparent to the swimmers that the marine effect of bioluminescence was playing havoc with their ability to read watches, depth gauges, and compasses. Underwater navigation was difficult. Surfacing under the pier, the swimmer pairs used it as overhead cover as they alternated between surface and underwater swimming to reach the inner part of Balboa Harbor. As the SEALs reached toward the shore end of the pier, they saw the PDF patrol boat was moored by its stern to a nearby floating dock adjacent to a quay wall and its bow pointed out into Balboa Harbor. The SEALs dove and approached the target underwater. Swim pair #2, ET1 Beausoleil and PH2 Dye, swam underneath the target at 0011, 20 December. It took them two minutes to attach the haversack of explosives to the port propeller shaft just forward of where the "V" strut held the shaft. They then began swimming south to pier 17. The other pair of SEALs, LT Coughlin and EN3 Eppley, arrived on target a minute later and attached a haversack to the starboard propeller shaft near the "V" strut. These SEALs finished the arming sequence of the demolition charges--the detonator cord leads between the two charges were tied to ensure dual priming--and set the charges to explode at 0100. The SEALs had 45 minutes to exfiltrate a safe distance from the target.

Just as Coughlin and Eppley swam away, the patrol boat's engines started. The propellers were not engaged and the boat remained stationary. Tonight, unlike previous nights, some PDF crew were aboard the patrol boat. The second pair of SEALs also swam underwater to pier 17. Following the contour of the pier for concealment, the SEALs swam away from the target. With the advancing of H-Hour, battles had started in Panama City with the attack on the Comandancia. Shortly afterwards, the SEALs were subjected to two intense underwater explosions. The SEALs, afraid they were compromised and under an anti-swimmer grenade attack from PDF soldiers patrolling pier 17, surfaced and hid behind pilings to escape injury. Continuing to move under the pier, the SEALs alternated between surface and underwater swimming to conserve oxygen in their Draeger systems. A couple of hundred yards further, four more underwater explosions forced the SEALs to surface again and take refuge behind the pilings. Although firing was heard overhead and tracers were seen arcing toward the Panama Canal, it did not appear to be directed at the SEALs.

Both pairs of SEALs were behind pilings under pier 17 when at precisely 0100 the charges underneath the Presidente Porres detonated. "The boat reared up forward . . . it went straight up--the bow went up," recalled LT Michael Argo, who observed the explosion through high powered binoculars from Naval Station Rodman. The explosion blew a hole ten feet wide through the hull and deck, destroying the stern of the boat. The engine room was a complete loss. The boat flooded and sank within two minutes. The floating dock next to the patrol boat, its steel floats punctured, swamped with water the next day.

Shortly after the explosion, most boats in Balboa Harbor started their engines and turned

their propellers as an anti-swimmer attack measure. The SEALs were behind schedule to make the extraction point and rendezvous with the CRRCs to be taken back to NS-Rodman. The extraction point was located at the south end of pier six, a structure 500 yards south of Balboa Harbor, and a distance that the swimmer pairs could not arrive at by the previously planned time of a few minutes after H-Hour. Prior to H-Hour, the swimmers had tried to establish communications with TU-WHISKEY to say they were behind schedule. But the radios were malfunctioning. After the explosion, the SEALs pairs started moving again, heading to the end of pier 17, on a course toward the extraction point. Swimming a course to reach pier 6 took the SEALs near the main shipping channel of the Panama Channel. As the SEALs swam into the Panama Canal, a strong current of six knots running in the direction of the Pacific Ocean nearly swept them off course. Just then a deep-draft ship was making its way through the Panama Canal shipping channel. As Coughlin recalled, "You can't tell under the water exactly where a vessel is; you just hear it getting louder, and louder--it sounds like a freight train coming." With the ship approaching, the SEALs descended to 45 feet to avoid being drawn into the propellers. The increased toxicity of the pure oxygen in the Draeger system in deeper water was risky. Alternatives, however, were lacking. The SEALs remained at this depth for 10 to 15 minutes until the ship passed overhead. They then ascended to 20 feet, executed a turn, and swam on a bearing for pier 6. Reaching the pier separately, the swimmer pairs surfaced, used the pier as overhead cover to swim on the surface, and reached the extraction point at its southern end.

Meanwhile, as the swimmer pairs were making their way under pier 17, the CRRC crews replaced CRRC #2's outboard motor. The engine change took just a few minutes. At 0045, both CRRCs departed NS-Rodman and arrived at the extraction point ten minutes later. They hid under the pier, eight to nine feet above their heads, as firefights erupted in the vicinity between PDF and American forces. A few minutes later the harbor shook from the explosion under the patrol boat. The CRRCs waited but the swimmers did not appear at the designated time. CDR Carley sent CRRC #2 to search for the SEALs in case they had missed the extraction point. CRRC #2 returned reporting no sign of the SEALs. The CRRCs continued their vigil at the extraction point. An hour passed before the first SEAL pair, Coughlin and Eppley, arrived at 0200. The other pair, Beausoleil and Dye, made it to the extraction point five minutes later. The SEALs were recovered and the CRRCs headed back to NS-Rodman. As the assault force cleared the far shore and went across the Panama Canal to NS-Rodman, infrared strobes onboard the CRRCs were turned on to help U.S. forces recognize the CRRCs as a friendly unit. A message was transmitted to TF White stating that the SEALs had been recovered and Task Unit-WHISKEY had executed its mission without causalities. (Norm Carley – personal communication)

Butler and Knafelc suggested in their 1986 paper (31) that the reported incidence of OTT failures was suspicious based on a smaller than expected number of OTTs reported. Walters *et al* reviewed records from the primary chambers administering the OTT to Navy SEAL candidates and found that the incidence of failure of the OTT was only 0.096%, much lower than previously reported (35). The conclusions and recommendations of this paper were:

1) The failure rate for the OTT as it is currently administered in Naval Special Warfare is 0.096%. This number is approximately 5% of the previously reported incidence of 1.9%, which was based on data from the Naval Safety Center.
2) The logistic burden of administering the OTT had caused testing to be currently conducted after the SEAL students have completed the most rigorous 9 weeks of SEAL

training. Disqualification of a SEAL candidate at that point in training should be based on clear and compelling evidence that he is unfit to continue training. The OTT does not meet that standard.

3) Even if a more severe OTT were to be developed, intra-individual variability prevents any single screening test from being a reliable indicator of increased oxygen sensitivity.
4) Factors other than individual oxygen tolerance such as a high exercise rate, diver hypoventilation, canister failure, inadvertent depth excursions, inadequate thermal protection, or excessive purging of the UBA may contribute more to the risk of operational oxygen toxicity than individual sensitivity (36).
5) Naval Safety Center dive reporting procedures should be modified to document all suspected episodes of oxygen toxicity which occur on closed-circuit oxygen dives. This should include a reporting format that provides for the maximum capture of pertinent data to facilitate accurate and reliable determinations of the CNS oxygen toxicity incidence in operational diving.
6) In light of items 1 through 4 above, the authors recommend discontinuation of the OTT as a screening test for Navy Seal candidates. The OTT was discontinued as a screening test for NSW candidates in 1999 (37).

Another significant advance in oxygen diving in the Navy was the establishment of UBA and oxygen exposure limits for resting as opposed to swimming divers. This physiological situation applies primarily to SEALs who are piloting or riding in SDVs. These free-flooding submersibles, whose operating characteristics are classified, are capable of transporting SEALs over long distances underwater. Since these divers have a much lower rate of oxygen consumption and CO_2 production, their gas supply and canisters should both last longer and the risk of CNS oxygen toxicity should be lower. This realization in 1996 resulted in the Naval Special Warfare Command initiating tasking for NEDU to re-evaluate limits for both the Draeger LAR V and the SEAL operator at 20 feet in a mostly-resting scenario. Marino and Maurer tested 8-hour dives in 76-81 degree F. degree water and found that all canisters were still adequately removing CO_2 after 8 hours (38). Approximately 25% of the UBAs had to have oxygen bottles replaced before the end of this period. There were no episodes of CNS oxygen toxicity although a number of divers displayed early symptoms of pulmonary oxygen toxicity. New limits were subsequently established by NAVSEA for this UBA which remain classified, but are significantly longer than allowed by the previous limit (39). At shallow depths, pulmonary oxygen toxicity or UBA limits may be the limiting factor for exposures rather than CNS oxygen toxicity (38,39,40).

Current research in oxygen diving in the U.S. Navy has focused on the development of deep water Draeger LAR V lockout procedures for the Advanced SEAL Delivery System (ASDS). The ASDS is a new submersible that transports SEALs close to their intended target in a dry, one-atmosphere environment. At their lockout point, the SEALs exit the ASDS and begin a combat swim for their final approach. Operational units using this craft requested that a procedure be developed whereby missions requiring deep-water lockout could be accomplished using a Draeger LAR V rather than a bulkier and more complex closed-circuit mixed-gas UBA. A proposed procedure has been developed in which the divers breathe chamber air and vent the UBA rig to equalize pressure during compression. They then exit the lockout hatch breathing from the submersible's built-in hookah rigs until they reach the ascent line. Once ready to

ascend, the divers swim toward the surface inhaling through the mouth and exhaling through the nose. This serves both to vent the expanding gas from the UBA and to provide the diver with compressed air to breathe during the ascent. The oxygen valve is closed until the diver reaches 15 feet and carries out an underwater purge. He then begins his swim with a purged UBA. This procedure was proposed by the Naval Special Warfare Command in 2001 (41) and has been successfully tested by NEDU in controlled conditions. (42) Additional testing of these techniques is ongoing at NEDU.

It is a noteworthy observation that Dr. Lambertsen was a member of the ASDS Medical Advisory Panel that developed the procedure described above, thus resulting in his contributions to closed-circuit oxygen diving spanning the entire history of the U.S. Navy experience in this area. Another important observation is that the Naval Special Warfare community was responsible not only for the introduction of closed-circuit SCUBA diving to the Navy, but for the first employment of open-circuit air SCUBA as well.

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Lambertsen and O_2: Beginnings of operational physiology

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INTRODUCTION

Significant events in history do not occur at random. They occur because individuals confront problems and have the ingenuity and motivation to find solutions. This is the story of how Dr. Christian Lambertsen developed closed-circuit O_2 diving for the U.S. military. It was a small episode in World War II, but ultimately significant for environmental physiology and hyperbaric medicine. More importantly, it illustrates how practical problems can inspire fundamental understanding. Much of the military part of the story was classified until the mid-1990s, when Sergeant Brian Danis, U.S. Army, brought it to light through the Freedom of Information Act.

School

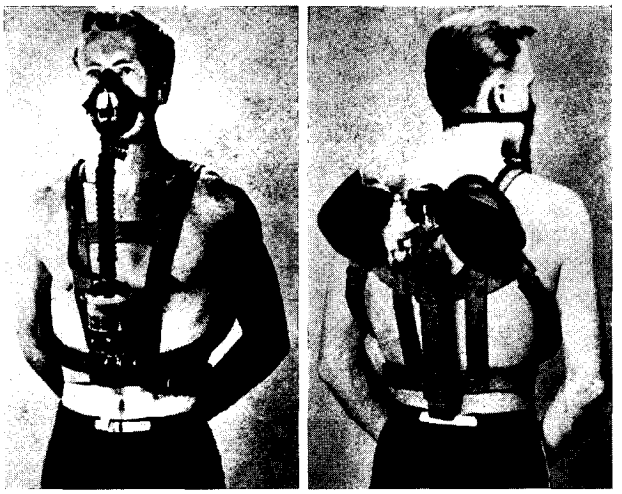
Chris Lambertsen grew up during the depression of the 1930s in Scotch Plains, New Jersey. He spent summers at the Jersey shore, building houses with an uncle. Before high school, he had decided on a medical career. After junior college and a two-year scholarship to Rutgers University, Chris entered medical school at the University of Pennsylvania in September 1939.

Classes began with respiratory physiology. To learn about oxygen (O_2) and carbon dioxide (CO_2), the students breathed hypoxic gas to unconsciousness, hyperventilated to become hypocapnic, and conducted breath-holding contests to feel the effects of hypoxia and hypercapnia. Given his experience at the shore, Chris was impressed by ten-minute breath-hold times after hyperventilation with O_2, and found O_2 and CO_2 particularly interesting for their control of ventilation.

His medical school lessons were useful during weekend underwater excursions at the Jersey shore. With the help of two cousins, he began by re-breathing from a bag, which was connected to a bicycle pump that provided fresh air from the surface. The cousins and pump were soon replaced with a cylinder of compressed O_2, but re-breathing was uncomfortable because CO_2 accumulated in the bag. When he learned of new anesthesia equipment that used a CO_2 absorbent, Chris solved his problem with a small CO_2 scrubber, fit between his mouth and the breathing bag.

Henry Bazett, his physiology professor, was enthusiastic and encouraging. Professor Bazett, an Englishman who had studied with John Scott Haldane, understood the problems of underwater respiration. To continue Chris' work, Bazett requested additional parts from the Ohio Chemical and Manufacturing Company, a maker of anesthesia equipment. Ohio Chemical responded with a job offer to Chris at thirty dollars a week for the summer of 1940. House painting on the Jersey shore stopped abruptly, and Chris took the train to Cleveland, where he and Mr. Sholes, the president of the company, decided that the summer's objective would be to develop an underwater breathing apparatus for use in lifesaving. The resulting apparatus was constructed over several weeks in Minneapolis, where Ohio Chemical made anesthesia equipment (Figure 1).

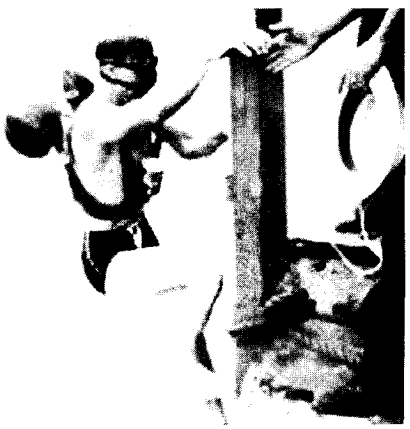
Fig. 1. The O_2 underwater breathing apparatus for lifesaving [1].



It was a semi-closed pendulum, or to-and-fro, rebreather, weighing 12 pounds with a 40-liter O_2 bottle. A regulator delivered O_2 at about two liters per minute, which was adequate for half an hour of light work. O_2 was re-breathed from an oronasal mask through a CO_2 scrubber into back-mounted breathing bags. Because the mask and hose were dead-space that retained CO_2, an exhaust valve was placed in the mask to release end-exhalation CO_2 into the water. O_2 added before the scrubber decreased the CO_2 in the first part of inhalation.

Chris tested his rebreather in Lake Nokomis, near Minneapolis, and in Lake Erie (Figure 2). One day, while at sixty feet, his eyes and one of his legs began to twitch, and he noted involuntary 'catches' in his respiration. Was this O_2 toxicity, about which so little was known? A fluttering diaphragm convinced him to abort the dive. In anticipation of such an event, he had attached to himself a line, which was tended by his companion in the boat, Mr. Sholes' son. The U.S. Navy Diving Manual instructed four pulls in the event of emergency, but when he pulled, the line came down on his head, as they had forgotten to tie it off in the boat.

Fig. 2. Testing the breathing apparatus in Lake Erie (C.J.L. photo).



Somehow, he surfaced safely, and with the tests mpleted successfully, returned to Cleveland, where he remarked to Mr. Sholes that the apparatus might be useful for miners trapped in hazardous atmospheres. Mr. Sholes thought this was a fine idea and had a gas-tight chamber constructed on the loading dock for a demonstration. The chamber was a sealed structure of wood and Plexiglas. It was large enough for a canary, a dog, and Chris, with his rebreather (Haldane had introduced canaries into British mines, for the birds were more sensitive than men to hazardous gases). The structure was flushed with CO_2 to remove the O_2, and filled with cyclopropane, a highly flammable anesthetic gas.

The demonstration was filmed, with the local press and fire department in attendance. The canary fell off its perch; the dog fell off its shelf. When Chris leaned over to check the dog, he, too, fell over. Something was wrong, and fire axes quickly dismantled the chamber where Chris was found unconscious. No one had realized that cyclopropane would penetrate the latex breathing bags and be inhaled. Mr. Sholes issued a stern reprimand, "Chris, you shouldn't have done that."

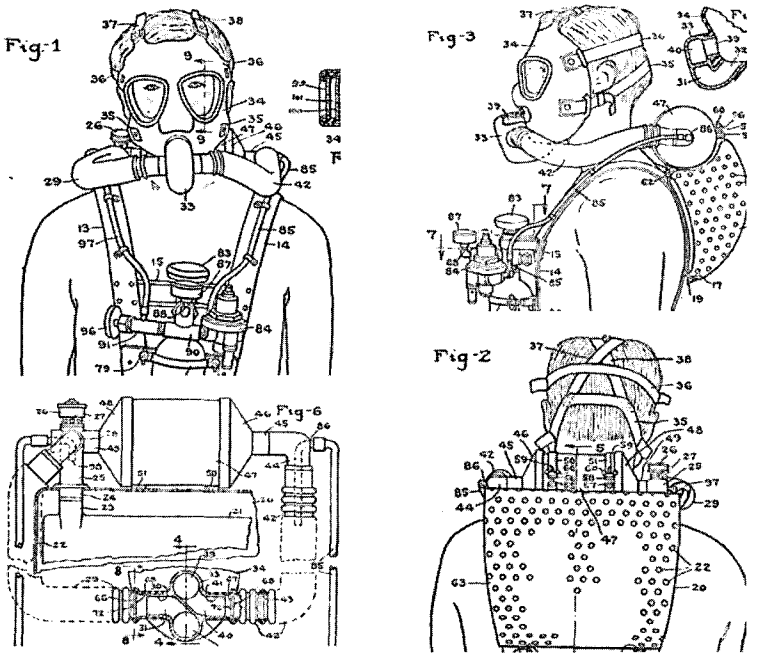
Chris returned to Philadelphia as a local celebrity at the University of Pennsylvania. His photograph (Figure 2) had been distributed nationally by the wire services because in 1940, it was news to spend twenty minutes underwater. Pleased as could be, Professor Bazett and the Dean of the medical school decided a description of the underwater breathing apparatus should be published in the *Journal of the American Medical Association* (1). Bazett submitted a letter of support to the journal that was descriptive and prophetic. He wrote, "The equipment was successful because it was designed by a man who studied the physiological principles carefully and is capable not only of testing it himself but training the users. It could not have been developed by a physiologist unfamiliar with the practical side nor by a swimmer without physiological training" (2).

A former British Army officer, Professor Bazett was concerned by England's precarious circumstances at the start of World War II, and saw that Chris's device could have military applications. He wrote to the British Admiralty, pointing out these potential uses: "The apparatus has advantages in lightness and freedom of movement in the water making it adaptable either for use of underwater troops of the trouble-making type or for night-raiding enemy defenses" (2).

Bazett also wrote to the U.S. Navy, and in January of 1941, he and Chris went to Washington, D.C., to visit the Navy Experimental Diving Unit (NEDU). An old professor and a young student, with a gym bag full of tubes and cans, were of little interest to the Navy, considering the challenges presented by the submarine force. In 1939, the Navy had rescued 33 sailors from the sunken submarine *USS Squalus*, using the new McCann Diving Bell (3). After the rescue, the Navy salvaged the *Squalus* with still-experimental helium-O_2 diving equipment and decompression schedules. The Navy had also developed the Momsen Lung, a lightweight underwater breathing apparatus for use in escapes from sunken submarines (3). Despite official Navy indifference, a junior medical officer at NEDU, Lieutenant Al Behnke, saw the potential military value of Chris' device, supported the publication of the journal article, and discussed with him the relative merits of the Momsen Lung.

The NEDU visit reoriented Chris's mind: lifesaving was out, military applications were in, and a major redesign was in order. Having just read Adriani's paper about CO_2 absorption in anesthesia machines (4), he realized that for effective CO_2 removal, exhaled gas must have adequate residence time near the absorbent granules. He reasoned that the residence time would increase if one-way valves were placed in the mask tubing so that gas would flow through the CO_2 scrubber in one direction only. This 'recirculating' design became the Lambertsen Amphibious Respiratory Unit (LARU) II (Figure 3), and the recirculating breathing loop is still used by most rebreathers. The scrubber was large enough to hold the tidal volume of a working diver so that CO_2 had additional time during inspiration to diffuse into the absorbent granules. A steel cage around the breathing bag made the unit less fragile.

Fig. 3. The Lambertsen Amphibious Respiratory Unit (LARU) II [5].



The LARU-II was almost ready for a demonstration at NEDU, but Chris wanted another test to ensure the system would work at pressure. He located a chamber on Welfare Island in New York City that its operators allowed him to use after five in the evening, with the understanding that "we will assume no responsibility for your well being during or as a result of your work here" (6). He and a physicist friend (Glen Millikan, inventor of the oximeter) took the train to New York in March 1942. Millikan operated the chamber from outside, while Chris conducted equipment tests inside. Recognizing the possibility of an O_2 seizure, Chris attached a line from his mask to an overhead pad-eye in the chamber, and stood during testing. Had he fallen down, the mask would have been pulled from his face. The test procedure was simple: increase the pressure, make notes (e.g. "my left eye itches") during a 15-minute wait period, and then go deeper.

The Welfare Island tests were satisfactory, and Chris wrote to now-Lieutenant Commander Behnke, "I will be able to go to Washington any time you suggest. As one of my professors put it, I do not intend to let my medical course interfere with my education" (7). But there were further delays until a former college professor, presently the chairman of the Medical Defense Research Council, encouraged the Surgeon General of the Navy to cut through the red tape. The next visit took place in April 1942, and Chris reported to Mr. Sholes, of Ohio Chemical, "My pressure tests went very well. CO_2 absorption was fine but O_2 poisoning came on at 80 feet. I was almost a goner" (8).

Underwater Troops of the Trouble-Making Type

Four people attended the NEDU demonstration from the British Special Operations Executive (SOE) and the recently formed U.S. Office of Strategic Services (OSS). These were covert agencies in search of an underwater breathing apparatus for reconnaissance and direct action missions. Chris had been rejected by the Navy for hay fever but was recruited by the OSS, and after graduation from medical school in June 1943, was assigned to the new Operational Swimmer Groups (OSG) as a First Lieutenant (1LT) in the Army Medical Corps. His duties were to perfect his underwater breathing apparatus and train the OSG swimmers to use it.

The British SOE was about six months ahead of the OSS in developing swimmers for its Sea Reconnaissance Unit (SRU) (9). The SRU began training in southern California in land warfare, demolitions, small boat handling, surface swimming, and breath-hold diving with the facemasks and swim fins used by California spear fishermen and abalone divers. In January of

1944, the SRU moved to Nassau, Bahamas, for dive training. Since the LARU was not yet readily available, the SRU trained with the British submarine escape apparatus.

While a medical student, Chris trained the first OSS swimmers in May 1943 in the swimming pool of the Naval Academy in Annapolis, Maryland. He conducted an abbreviated course using the LARU-III (Figure 4). Known as L-Units, these small swimmer cadres deployed to England with orders to attack the submarine pens in the Bay of Biscay on D-Day, but the operation was canceled because adequate thermal protection and transport across the English Channel were unavailable. Many of the L-Unit swimmers returned to the U.S. for further training.

Fig. 4. The LARU-III (C.J.L. photo).

In May 1944, the first of three full OSS Operational Swimmer Groups (OSG-1) followed the SOE's Sea Reconnaissance Unit to Nassau for dive training with the LARU-II (the LARU-II was identical to the LARU-III except for a smaller CO_2 scrubber and O_2 supply). 1LT Lambertsen remained in the States to finish production of the LARU-X. Figure 5 shows OSG-1 on the beach with the Duke of Windsor, then the Governor General and Commander-in-Chief (CINC) of the British West Indies.

Upon completion of dive training in July 1944, OSG-1 sailed for Hawaii and was offered by the OSS to Admiral Nimitz for employment. Admiral Nimitz had no need for divers but was pleased to have a group of skilled swimmers for the island-hopping invasion campaign in the Pacific.

Fig. 5. Operational Swimmer Group 1 (OSG-1) on the beach in Nassau (C.J.L. photo).

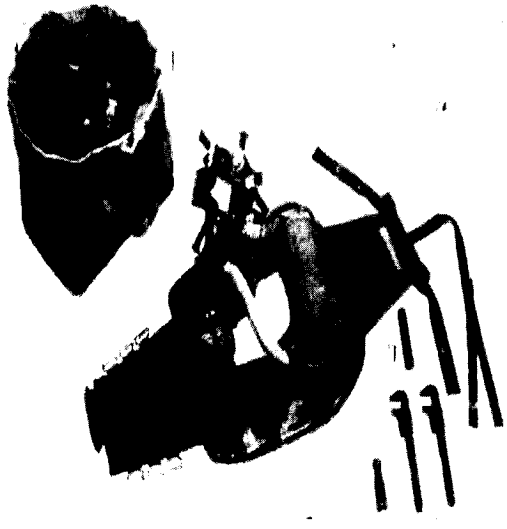
There had been too many U.S. Marine casualties during the amphibious landing at Tarawa in November 1943 because the topography of the underwater approaches to the invasion beaches was unknown. This led to the establishment of Navy Underwater Demolition Teams (UDT). Eventually, each UDT had 100 swimmers whose mission was to chart the water depths and search for obstacles off of the invasion beaches from the high water mark to a depth of 3.5 fathoms (7 m). Just before the invasion the next morning, under the cover of heavy bombardment from the fleet, the swimmers returned with demolitions and cleared the beaches of obstacles for the approaching landing craft. The OSS OSG-1 was immediately assigned to UDT-10, and taught the fledgling teams how to swim with fins and use facemasks. By the end of the

war, there were 20 UDTs with 2,000 swimmers, but OSG-1 never again used their LARU rebreathers.

Fig. 6. Prototype of the LARU-X (C.J.L. photo).

In July 1944, OSG-2 deployed to Nassau for dive training with the LARU-X, with 1LT Lambertsen as its instructor and physician. The LARU-X (Figure 6) was more robust than the LARU-II and LARU-III. Gone were the steel cage and other components incompatible with seawater. A complete field repair kit was also provided (Figure. 7).

Fig. 7. The LARU-X and toolkit (C.J.L. photo).

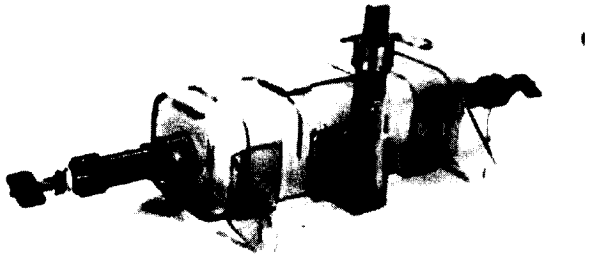


Lambertsen trained the OSS operational swimmers and observed each pair of students' performances. One day, he sank inertly to the bottom. Assuming this was part of the drill, the students continued to swim until, finally realizing something was wrong, they brought their unconscious instructor to the surface. He had taught them to purge their breathing bags with O_2 before diving to prevent dilution hypoxia, as O_2 was metabolically absorbed. This time, the instructor had neglected to purge his own bag, an object lesson that everyone remembered especially well. Upon regaining consciousness, Lambertsen went back into the water with the next pair of students to maintain their confidence and preserve the momentum of training.

The OSG-2 training activities not only taught diving but also tested equipment built by the OSS. In addition, it developed operational tactics for night reconnaissance and demolition. Figure 8 is a neutral buoyancy container for towing weapons or demolitions. Figure 9 is a limpet mine and firing device that a swimmer could attach to the hull of a ship.

Fig. 8. OSS neutral buoyancy container (CJL photo)

Fig. 9. OSS limpet mine/firing device (C.J.L. photo).



With OSG-2 well-trained and equipped, the OSS was asked to demonstrate its secret capabilities to the Navy in an exercise at Guantanamo Bay Naval Base, Cuba, in September 1944. Swimmers infiltrated the bay at night by submarine, and were inserted by rubber boat. The submarine torpedo nets around the harbor proved to be no obstacle (Figure 10). Swimmers went over (Figure 11) and under the nets (Figure 12), dropped them to the bottom with demolitions, and once inside the harbor, found it easy to put limpet mines on target ships.

Fig 10. A torpedo/submarine net used to protect harbors (C.J.L. photo).

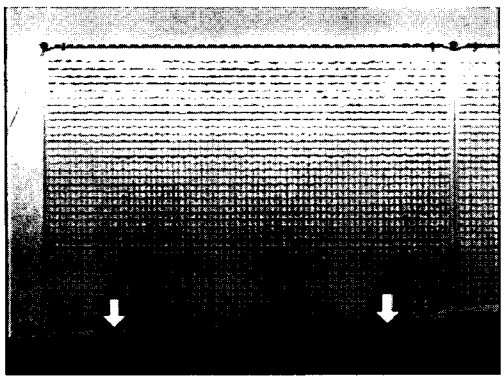


Fig. 11. An OSG-2 swimmer during the Guantanamo Bay exercise in September 1944 (C.J.L. photo).

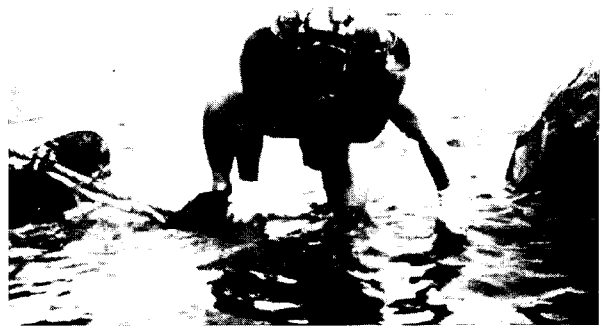
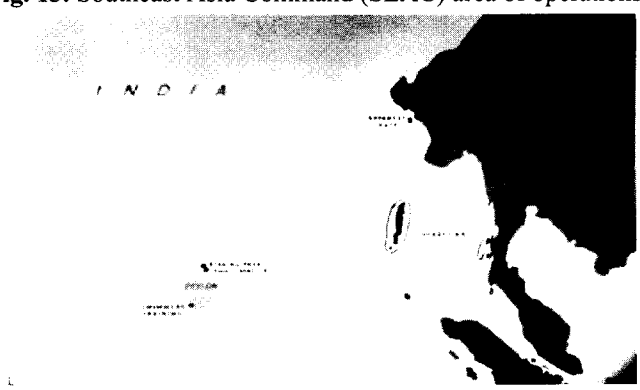


Fig. 12. OSG-2 swimmer going beneath a torpedo net (C.J.L. photo).

L-Unit personnel returned from England to train with OSG-3 at Nassau in October 1944. By this time, however, the war in Europe had moved to the continent, and there were no suitable targets for the U.S. OSG and British SRU. The Pacific theater offered more opportunities, but General MacArthur, CINC of the Pacific, had little use for 'special' units like the SRU and OSG, which he viewed as private armies. Admiral Mountbatten, CINC of the Southeast Asia Command (SEAC), felt differently, and the SRU and OSG were assigned to him. He referred to them as his pirates (9). OSG-2 operated with the SRU in the Burma campaign. Lambertsen, now a Captain (CPT), deployed with OSG-2 and then joined OSG-3 in Ceylon (today, Sri Lanka) to conduct training in swimmer delivery operations (**Fig. 13**).

Fig. 13. Southeast Asia Command (SEAC) area of operations (C.J.L. photo).



Sleeping Beauty

The Bay of Biscay operation for the L-Units during the Normandy invasion of Europe had been canceled, in part, because divers with fins could cover not much more than a mile while operational infiltrations required much lengthier transits. The SOE

had developed a number of experimental underwater craft, including a motorized submersible canoe known as the Sleeping Beauty (Figure 14).

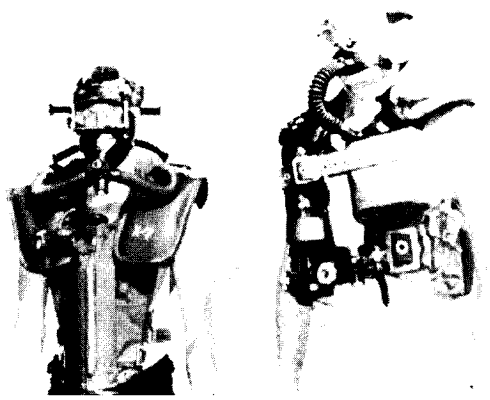
Fig. 14. Sleeping Beauty submersible canoe [11].

The Sleeping Beauty was 13 feet in length and accommodated a single pilot. Its range on the surface was thirty nautical miles at a speed of three knots, and it could dive to a depth of fifty feet (10). The SOE concept of operations for the Sleeping Beauty had the pilot approach a target vessel on the surface to a range of several hundred yards, submerge for the final approach, hang the vehicle from the target's bilge keel, and attach a limpet mine (Figure 15).

Fig. 15. Placement of a limpet mine by a Sleeping Beauty pilot [11].

The L-Units had returned to the United States in January of 1944 with a top secret Sleeping Beauty for testing and evaluation, and Lambertsen had an opportunity to study it in Washington. When he arrived in Ceylon in January 1945, he found two units available to him, and began an intensive program to train OSG-3 in the operation of the Sleeping Beauty. An SRU detachment on another part of the island was also training with the submersible canoe, and the two units got together to compare the LARU-X with the Amphibian-II breathing apparatus, which the Royal Navy had developed independently (Figure 16).

Fig 16. The LARU-X and the Amphibian Mark II (C.J.L. photo).



Throughout the war, British swimmers and divers occasionally became unconscious and were lost for no apparent reason, a phenomenon they called 'shallow water blackout.' From his own experience, Lambertsen knew that dilution hypoxia from inadequate purging of nitrogen in the breathing bag was one cause of unconsciousness. He demonstrated this to the SRU, using their officer in charge as an example (Figure 17). Other potential causes of shallow water blackout were CO_2 intoxication and O_2 toxicity, both of which could be brought on by CO_2 retention, as he would show in subsequent years (12). These were more likely with the Amphibian-II because its pendulum breathing circuit, as in the original LARU (Fig. 1), was predisposed to CO_2 retention. The British

Navy eventually restricted O_2 diving to 30 feet (10 m) and introduced rigorous nitrogen purge procedures (13), but the use of pendulum rebreathers continued in use until the 1980s.

Fig. 17. Demonstration of dilution hypoxia (C.J.L. photo).

CPT Lambertsen's training program for the Sleeping Beauty included the development of new tactical procedures that were based on its use to deliver operational swimmers. Instead of taking the submersible directly to a target, the pilot would navigate to within several hundred yards, park or moor the canoe on the bottom, swim to the target, place demolition charges on it, and swim back to the Sleeping Beauty. These procedures represented the birth of the swimmer delivery vehicle (SDV) concept. Before further development or operational employment, however, the war ended, and President Truman disbanded the OSS and sent home its skilled operational swimmers. SDV work did not begin again until 1948, with the Navy's Underwater Demolition Teams. It did not reach fruition until 1982, when the Navy's SEAL Delivery Vehicle Teams were commissioned.

Post-War

CPT Lambertsen was assigned to an Army hospital as a medical officer upon his return to the United States. Recognizing that the operational underwater swimmer program had abruptly ended with the demise of the OSS, he had the secret LARU-X declassified, and delivered two units each to the Navy, Army, and Coast Guard (being careful to obtain written receipts) with letters of explanation to senior officers. The commandant of the Coast Guard himself responded quickly, and Lambertsen was assigned to train a cadre of instructors for the possible use of O_2 rebreathers in rescue and recovery at sea. One day, after drifting deeper than intended, he found himself at 100 feet (30 m) with a fluttering diaphragm. His last action before an O_2 seizure was to inflate his breathing bags in order to become positively buoyant. He awoke with a buzzing in his head, staring into the eyes of a Navy chaplain.

Fig. 18. Lambertsen on assignment to the U.S. Army Corps of Engineers (C.J.L. photo).

An Army observer assigned during the Coast Guard training recommended that Lambertsen work with the Corps of Engineers to determine the feasibility of O_2 diving for river operations. During three months as a civilian, he tested British, Italian, and U.S. diving equipment, and developed river combat diving procedures. Figure 18 shows Lambertsen with a reel of telephone cable on his back, prior to a submerged passage across the Ohio River.

The U.S. Navy was the last service to seek advice. Actually, it was not the official Navy but Lieutenant Commander Douglas (Red Dog) Fane, U.S. Naval Reserve, the most senior UDT officer after the war. He was in charge of two small underwater demolition teams in Little Creek, Virginia. The Navy had proposed a change to the UDT name and mission. To prevent

this, Fane wanted to put the UDT underwater, as divers. In 1947, he invited Lambertsen, now a civilian instructor in pharmacology at the University of Pennsylvania Medical School, to Little Creek to train a selected group of UDT swimmers in tactical diving with the LARU and Sleeping Beauty (14). Butler describes this story in detail (15).

O_2 Research

When Lambertsen was released from active duty with the Army in 1946 as a Major, he returned to the Pharmacology Department at the University of Pennsylvania, where he had worked as a medical student. His four or more episodes of O_2 toxicity, including one seizure, had stimulated his curiosity concerning the underlying mechanisms of central nervous system (CNS) O_2 toxicity. This was a fortuitous time. Seymour Kety had recently developed a method for measuring cerebral blood flow and metabolism by using nitrous oxide uptake and washout from the brain (16). With a grant from the Office of Naval Research, Lambertsen turned the doors around in an altitude chamber to convert it into a pressure chamber. In the chamber, he used Kety's methods to study the effects of hyperbaric O_2 and inspired CO_2 on cerebral blood flow and O_2 consumption both in normal subjects and schizophrenic patients (12, 17, 18). Electroconvulsive therapy (ECT) was a common treatment for schizophrenia at the time. Using high-pressure O_2, rather than ECT, to induce convulsions was conceived as an alternative, if experimental, therapy.

These studies revealed that hemoglobin never de-saturated while subjects breathed high-pressure O_2. This interfered with the ability of the blood to transport CO_2 and caused the CO_2 tension in the brain to rise abnormally (17, 18). The elevated CO_2 tension stimulated ventilation, which reduced the CO_2 tension in the arterial blood. CO_2 was demonstrated to be a potent vasoactive signal to the cerebral circulation (12). Cerebral vasoconstriction and decreased blood flow induced by hyperventilation reduced O_2 delivery to the brain. A possible explanation for Lambertsen's observations while O_2 diving with the Navy and OSS was the idea that hyperventilation could abort the signs and symptoms of CNS O_2 toxicity. Conversely, the chamber studies with the schizophrenic patients showed that inspired CO_2 accelerated the onset of O_2 toxicity.

These were the beginnings of Chris Lambertsen's systematic study of the physiology of O_2 and its toxicity. Inspired by practical wartime experience, he developed a career of expanding interest and influence that continues to this day.

ACKNOWLEDGEMENT

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The Predictive Studies Series: Correlation of physiologic responses to extreme environmental stresses.

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INTRODUCTION

Periodically over the past four decades, investigators at the Institute for Environmental Medicine have carried out correlated physiologic experiments programs that were from the outset designated individually and collectively as 'Predictive Studies.' Because of my long and close association, I have been invited to summarize the scope of these studies designed to identify and quantitatively measure in human subjects the physiologic and pathophysiologic effects of extreme respiratory gas and ambient pressure environments that could limit or aid man's ability to live or work in those environments. Each of these eight broad studies was conceived, designed, and led by C. J. Lambertsen, and joined by selected collaborating participants from military, university, or corporate backgrounds. In most cases, the Predictive Studies employed a "dose-response" design, in which human subjects were exposed to a range of respiratory gases and pressures for durations that approached the limits of tolerance at both rest and during physical work. By measuring physiologic and/or toxic responses to each pressure-duration dose and then interpolating between doses, the intent was to "predict" responses to pressure-duration combinations over the ranges of stresses studied.

C.J. Lambertsen's earliest physiologic study of oxygen involved microtonometry to determine in-vivo relationships of PO_2, PCO_2, and hemoglobin O_2 saturation in the arterial blood of human subjects exposed to increasingly severe degrees of hypoxia (1). Ensuing analyses of arterial and brain venous blood at 3.5 ATA-inspired O_2, beyond full saturation of hemoglobin, demonstrated that human subjects are functional without the benefit of hemoglobin for O_2 transport to the brain or CO_2 transport from it (2). These early observations, superimposed on extreme personal exposures to hyperoxia and oxygen poisoning in development of practical self-contained diving (3, 4), led to seminal investigations of human respiration and brain circulation functions in hyperoxic states (5-7). Subsequently, the focus which emerged was on relations of evolving undersea and aerospace activity, in which Lambertsen played a special role as Chairman of the Man in Space Committee, Space Science Board, established by the National

Academy of Sciences-National Research Council. He served concurrently as Chairman of the National Research Council Panel on Underwater Swimmer Technology. These joint roles influenced the beginnings and evolution of the Predictive Studies Series, most of which have blended research in undersea, aerospace, and therapeutic aspects of unusual and useful atmospheric exposures, with an early emphasis on the expanding roles of diving.

Classical military and civilian diving operations were initially characterized by non-saturated, limited-duration diving from the surface, followed by decompression and a gradual return to normal ambient pressure. As the depth and duration of working dives increased, with increasing limitations by decompression requirements, the concept of inert gas 'saturation' diving was first proposed by Behnke (8) and later demonstrated to be practical in the Conshelf, Man-In-Sea, and Sealab programs (9). Such saturation exposures used helium. Whether they continued for one or many days, approximately one day of decompression was required for every 100 feet of depth. This led to the expansion of saturation-excursion diving, which involved prolonged exposure at one depth, with excursions to a greater depth followed by a no-decompression return to the saturation depth.

The prominent narcotic effects of nitrogen at shallow to intermediate depths necessitated the use of helium as the inert diluent gas at extreme depths. The absence of narcotic influences and relatively low respired gas density associated with helium diving allowed penetration to greater depths, but the adverse effects of compression and hydrostatic pressure *per se* became limiting (10-13). The evolutions of technical advances in diving exposed the diver to larger physiological stresses. These included decompression, oxygen toxicity, inert gas narcosis, hypothermia, increased work of breathing, and the adverse neurological effects of compression to extreme hydrostatic pressures. The Predictive Studies were designed to determine the limitations imposed individually and collectively by one or more of these stresses.

PREDICTIVE STUDIES OF TOLERANCE TO COMPRESSION AND INERT GAS ATMOSPHERES

The scope of the Predictive Studies to date is summarized in Table 1 (14). Available sources of additional details are cited in the text.

Table 1
THE PREDICTIVE STUDIES SERIES
DIVING, DECOMPRESSION, HYPEROXIA, HYPOXIA
Studies related to predictions of limiting physiologic effects in man of gases and pressure

PREDICTIVE STUDIES I (1969): TEKTITE I
A 60-day, open-sea exposure to normoxic N_2 at 43 FSW (2.3 ATA).
Collaborating sponsors: ONR, NASA, U. Penn, GE, Department of the Interior

PREDICTIVE STUDIES II (1970-1971)
A 14-day, continuous dry-chamber exposure to normoxic N_2 at 100 FSW (4 ATA).
Collaborating sponsors: U. Penn, NASA, Navy BUMED, ONR, NIH, Baylor U.

PREDICTIVE STUDIES III (1971-1973)
A 21-day, dry-chamber exposure to pressures ranging from 400 to 1200 FSW (12 - 37 ATA) while breathing He-O_2, N_2-O_2, Ne-O_2 mixtures.
Collaborating sponsors: U. Penn, Navy BUMED, ONR, NASA, NIH, Union Carbide, Ocean Systems

PREDICTIVE STUDIES IV (1975)
Saturation - excursion series in phases of 0, 400, 800, 1200, and 1600 FSW. Exposure to normoxic He. Physiologic studies and underwater work performance on oil wellhead.
Collaborating sponsors: U. Penn, Navy Medical R&D, ONR, NIH, NASA, Industry (offshore oil, gas, diving)

PREDICTIVE STUDIES V (1982-1987)
Definition of organ system O_2 tolerance - in relation to undersea activity, manned EVA, hyperoxic therapy, and therapy of undersea and aerospace decompression accidents.
Collaborating sponsors: U. Penn, Navy Medical R&D, NOAA, NASA, Industry (offshore oil, gas)

PREDICTIVE STUDIES VI (1988-1992)
Extension of organ tolerance at normal and increased ambient pressure. Optimized intermittency. Planning based on PS V - in relation to all oxygen uses.
Collaborating sponsors: U. Penn, Navy Medical R&D, NASA

PREDICTIVE STUDIES VII (1992-1997)
Interactions of hyperoxia, exercise, immersion, and CO_2 on brain oxygenation and neurological O_2 tolerance.
Collaborating sponsors: U. Penn, ONR

PREDICTIVE STUDIES VIII (1992-1997)
Influences of CO_2 on brain O_2 flow and respiratory control during hypoxia in work and at rest.
Collaborating sponsors: U. Penn, Navy Medical R&D, NASA

Predictive Studies I

Predictive Studies I, also called Tektite I, symbolic of a meteorite fallen to the ocean, was a 60-day, open-sea, collaborative saturation exposure to normoxic nitrogen in an underwater habitat at an effective depth of 38 feet, or about 2.2 ATA (15-17). Collaborating sponsors included U.S. Navy, NASA, U.S. Department of the Interior, General Electric Corporation, and the University of Pennsylvania. This investigation was done in relation to NASA interest in exploring nitrogen saturation diving as a study of prolonged physiologic entrapment. The subjects were marine scientists who did practical technical work during the long exposure underwater. Pulmonary function evaluations showed that inspiratory and expiratory maximal flow rates decreased significantly during exposure, and post-exposure results were consistent with an increased strength of respiratory muscles in response to the sustained increase in inspired gas density and work of breathing (17). A discrete event was the observation, post-decompression, of a gas bubble in the aqueous humor of the eye in one subject (Figure 1) (15,16). No limiting effects were found, but the experience gained was used in the design of subsequent exposures to higher pressures and gas densities.

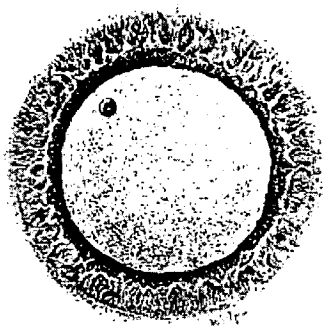

Fig. 1. Artist's drawing of a bubble in the aqueous humor of the eye as seen with an ophthalmoscope in a subject from Tektite I (15,16).

Predictive Studies II

Predictive Studies II, a 14-day, dry-chamber, continuous exposure to normoxic nitrogen at 100 fsw, or 4 ATA (18-25), was the first study to be done in a new environmental chamber system of the present Institute for Environmental Medicine at the University of Pennsylvania (18). The study was stimulated by the recognition that the potential benefits of manned undersea activity would be greatly enhanced by an ability to remain for extended periods at shallow and moderate depths, where much of the useful undersea work is performed. Areas of investigation considered likely to identify physiological responses or mechanisms that could potentially limit human tolerance or adaptation to increased atmospheric pressures included:

- Combined effects of increased work of breathing and nitrogen narcosis on respiratory control and pulmonary gas exchange.
- Effects of acute and sustained exposure to increased breathing resistance on pulmonary mechanical and other functions.
- Rates of adaptation to stresses imposed on pulmonary function and respiratory control, as well as rates of deterioration at rest and during exercise in the event that adaptations fail.
- Quantitative decrements, adaptations, and deteriorations in specific aspects of mental performance during acute and chronic exposure to nitrogen narcosis.
- Nitrogen influences on formation and destruction of blood cellular constituents.
- Patterns of chemical, endocrine, and metabolic adaptations during prolonged exposures to increased nitrogen pressure.

No serious, acute or chronic, toxic or nitrogen narcotic limiting effects developed during the 14-day exposure. Adaptation of respiratory control was manifested by a decreased ventilatory response to carbon dioxide, which did not interfere significantly with pulmonary gas exchange or progress with time (21). The respiratory muscles compensated for an increased work of breathing, thereby allowing exercise tolerance to remain high with adequate gas exchange. Cognitive function and technical competence remained more than adequate for the detailed experimental procedures carried out by and with the subjects. Quantitative measurements of mental function did not detect progressive deteriorations or adaptations to nitrogen narcosis (25). There were no alterations in blood cell formation or aging (22), or any limiting chemical, metabolic, or endocrine dysfunction (23). Plasma volume decreased concurrently with an increased urine output, but these changes were not functionally significant (24).

Predictive Studies III

Predictive Studies III comprised a multi-week series of dry-chamber exposures to normoxic nitrogen at pressures equivalent to 100, 200, and 300 fsw, culminating in a 21-day continuous exposure to simulated depths ranging from 400 to 1,200 fsw (13-37 ATA) while breathing nitrogen-oxygen, helium-oxygen, nitrogen-helium-oxygen, or neon-helium-oxygen gas mixtures (26). The gas-and pressure-related stresses experienced by each of the four subjects were, therefore, grossly more extreme than those studied in Predictive Studies II. The

experimental design was influenced by the awareness that actual exposure to hydrostatic pressures, which approached the limits of human tolerance, might involve an unacceptable level of risk. The danger was imposed by the unavoidable requirement for a slow decompression from prolonged or saturation exposure, thereby preventing rapid escape from dysfunctional or possibly harmful effects. In order to avoid this risk, the denser gas nitrogen, at relatively low ambient pressures, and neon, at intermediate pressures, were used to simulate the conditions and study the effects of increased gas density and the respiratory work expected to be associated with breathing helium-oxygen at extreme depths. The inhalation of a neon-oxygen gas mixture made it possible to study the potential (predictive) respiratory effects of breathing helium-oxygen at gas density-equivalent depths to 5000 fsw while remaining at an ambient pressure of 1200 fsw (26). Effects of extreme inert gas pressures on selected components of physiological function, as defined in Predictive Studies III (26), include the following:

- *Pulmonary Function*: As the density of respired gas and resistance to airflow progressively increased, pulmonary function indices, such as maximum voluntary ventilation and maximum expiratory flow rates, were progressively reduced, as previously described (27). However, the slope of flow rate reduction with respect to gas density appeared to approach an asymptote as density became extreme, an unexpected observation. This important characteristic facilitated the performance of high levels of exercise at extreme gas densities without incapacitating degrees of CO_2 retention.
- *Respiratory Control*: The slope of the ventilatory response to rebreathing metabolically produced CO_2 decreased progressively with increasing gas density. The associated reduction in ventilation was found to correlate with increased gas density and respiratory work, rather than with narcotic properties of the inert vehicle gases.
- *Respiratory Gas Exchange*: Blood gas measurements in arterial or arterialized venous blood detected no interference with O_2 or CO_2 exchange at rest or during exercise with nitrogen to 400 fsw, helium to 1200 fsw, or neon to 900 fsw.
- *Exercise Tolerance*: Two of the four subjects performed four sequential, six-minute periods of uninterrupted, incremental exercise on a bicycle ergometer during exposure to a range of increased gas densities and inert gas pressures. The highest exercise level was equivalent to about eighty percent of subject maximum work capacity at 1.0 ATA. Failure to complete this work profile occurred once in each of only two conditions, and for different reasons. In both cases, failure occurred during the last two minutes of the highest workloads. One cause of failure, which occurred while breathing N_2-O_2 at 400 fsw, was a severe degree of mental confusion and muscular incoordination induced by nitrogen narcosis. The second failure, observed while breathing crude neon at a gas density equivalent of breathing He-O_2 at 5000 fsw, was caused by high gas density and was associated with alveolar (end-tidal) CO_2 pressures of about 60 mm Hg. At the two lower workloads (300 and 600 kpm/min), ventilation was not subjectively difficult. Alveolar PCO_2 levels remained below 50 mm Hg.
- *Neurophysiological Changes*: There were no evident manifestations of a high-pressure nervous syndrome that was previously described (10-13) in association with rapid compression to extreme depths. This was attributed to the fact that the maximum 1200 fsw depth equivalent was intentionally achieved by a stepwise and slow adaptation to compression over several days.
- *Mental Function*: No detectable cognitive decrements were found with either helium or crude neon breathing at 1200 fsw. As expected, nitrogen produced a progressive central

nervous system depression that became prominent at pressures equivalent to 300 and 400 fsw (Figure 2).

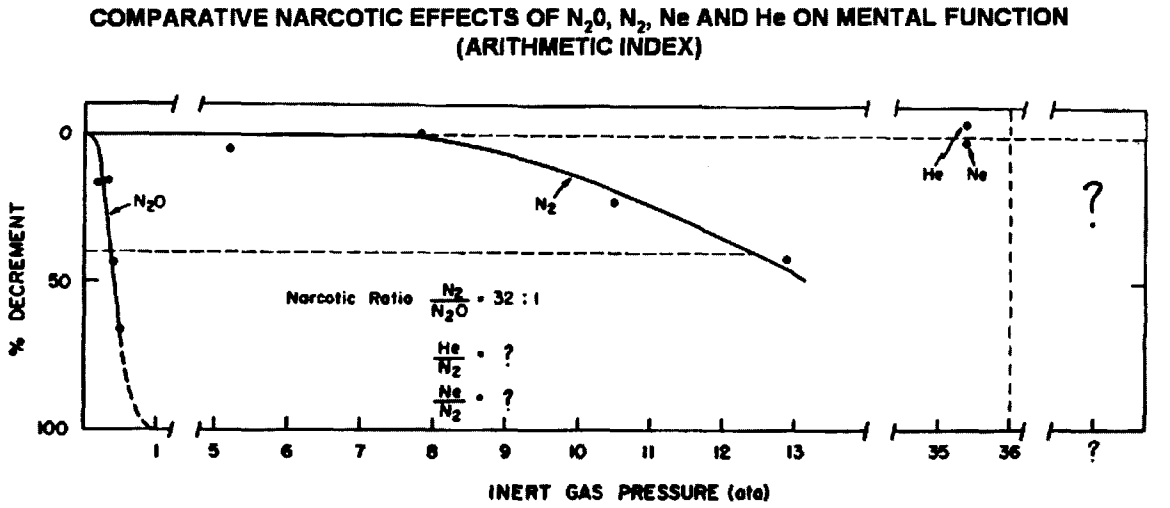

Fig. 2. Average decrements in paced arithmetic for two subjects during exposure to increased partial pressures of N_2, Ne, or He. Results are compared with average data from a previous study (28), in which eight subjects performed similar tests while breathing N_2O at inspired pressures to 0.5 ATA. Subjects remained extremely competent during N_2 breathing to nearly 8 ATA (about 230 fsw) and retained considerable mental function capacity to 13 ATA (500 fsw equivalent breathing air). The N_2:N_2O narcotic ratio was 32:1. Subjects were fully competent, without evident narcosis, during Ne or He breathing to 37 ATA (1200 fsw) (26).

- *Psychomotor Function*: Manual dexterity, coordination, and reaction time were impaired significantly only during nitrogen breathing at pressures equivalent to 300 fsw or greater.
- *Temperature Stress*: During exposure to helium at increased pressures, the subjects collectively negotiated a comfortable temperature. As helium density rose, the mean selected temperature was elevated with a narrowing of the comfort range.
- *Blood Chemical, Cellular, and Endocrine Characteristics*: With respect to pre-exposure controls, there were no physiologically important changes in blood electrolytes, blood cellular composition, catecholamine, or adrenal cortical hormone excretion.
- *Isobaric Inert Gas Counterdiffusion Syndrome*: An unexpected finding in Predictive Studies III was the occurrence of extreme pruritis, gas-filled skin lesions (Figure 3), and vestibular dysfunction when the subjects breathed N_2-O_2, N_2-He-O_2, or Ne-He-O_2 while their bodies were surrounded with He-O_2. Subsequent analyses and investigations of this phenomenon (29-31) led to its characterization as a new "gas lesion syndrome" (31), a gaseous supersaturation and venous gas embolism generated by unequal rates of inert gas counterdiffusion at stable (isobaric) ambient pressures (31). A resulting continuous evolution of subcutaneous and venous bubbles induced the potential of a possibly lethal embolization of gas from subcutaneous capillaries to the systemic circulation and heart (26, 29-31). As a possible cause of the vestibular symptoms, it was suggested that the inert gas counterdiffusion process might have occurred through the round window between the middle and inner ear, with the formation of bubbles in inner ear fluids (31). The detailed observations of isobaric inert gas counterdiffusion in Predictive Studies III,

Fig. 3. Gas-filled lesions in the skin of a subject breathing N_2-O_2 while his body is surrounded by He-O_2 at a pressure equivalent to 300 fsw (31).

and its studies in animals, provided the basis for its prevention in undersea operations and opened a new field of investigation that has been only partially exploited to date.

Predictive Studies IV

Predictive Studies IV was designed to investigate the physiological effects of staged rapid compressions in saturation-excursion diving on normoxic helium-oxygen to simulated depths of 400, 800, 1200, and 1600 fsw (32). There were two separate phases of investigation with four professional diver-subjects in each phase. Two divers participated in both phases. Phase One consisted of rapid compression to 800 fsw, a two-hour hold at that pressure, and subsequent rapid excursions to 1200 fsw while using 800 fsw as a saturation depth. Subjects were studied in pairs during the initial compression and successive excursions (Figures 4,5). Phase Two involved initial compression to 1200 fsw even more rapidly than before, a 22-hour hold at 1200 fsw, followed by repeated rapid and brief excursions to 1600 fsw with a return to saturation at 1200 fsw.

Fig. 4. Two diver-subjects after rapid compression to 800 fsw equivalent. Subject in foreground is having evaluation of bone conduction auditory thresholds. Subject in background is preparing to exercise on bicycle ergometer to measure ventilation and gas exchange (32).

Specific goals of Predictive Studies IV included the following:

- Determination of the character and time course of physiological and performance decrements during intentionally rapid compressions.
- Determination of rates of adaptation to compression effects upon reaching stable increased pressure.
- Develop markedly accelerated methods for decompression in deep saturation-excursion diving.
- Demonstrate work competence underwater at simulated depths of 1200 and 1600 fsw.

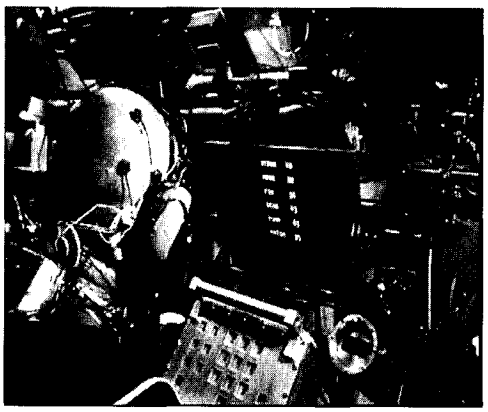

Fig 5. Resting subject station during computer-controlled and scored mental performance tests (32).

Previous investigators of high pressure nervous syndrome found that the rapid compression of human subjects to extreme pressures caused them to experience nausea, vomiting, dizziness, loss of alertness, indifference, disorientation, mental confusion, and somnolence (10-13, 33-35). Associated overt manifestations included coarse tremors, motor incoordination, impaired balance function, myofasciculations, and spasticity. Essentially all of the previously observed symptoms and overt

manifestations were also experienced by at least two of the diver-subjects at some time during the two phases of investigation. Detailed results of Predictive Studies IV are published in a comprehensive IFEM Report (32). Selected observations are summarized as follows:

- *Symptomatic and Overt Effects*: After rapid compression to 1200 fsw, symptoms were severe on arrival, with partial reversal over the next few hours. Only minor effects remained on the second day at 1200 fsw. Second-day excursions to 1600 fsw produced typical symptoms and signs, which were much less severe than on the previous day. Adverse effects continued to progressively ameliorate during successive daily excursions from 1200 to 1600 fsw. After the initial day of compression to 1200 fsw, subjects were capable of vigorous mental and physical activity, even at 1600 fsw, and skillfully performed precisely timed and coordinated technical maneuvers.
- *Vestibular Function and Balance*: The initial, rapid compression to 1200 fsw induced typical manifestations of vestibular dysfunction, such as dizziness, nausea, vomiting, and loss of alertness, usually accompanied by increased body sway. Eye muscle coordination was not affected, and there was no ocular dysmetria. Vestibular symptoms and signs decreased within one to two hours at 1200 fsw, were barely detectable by the morning of the second exposure day, and were absent by the third day.
- *Auditory Function*: No indications of hearing impairment were found during or after compression.
- *Visual Function*: Changes in visual acuity and accommodation were observed, but these were relatively small, transient, and functionally insignificant.
- *Speech Generation and Distortion*: Rapid compressions and acute exposures to 800, 1200, and 1600 fsw in He-O_2 did not interfere with neuromuscular or other functions in speech formulation or articulation.
- *Perceptual, Memory, Cognitive, and Performance Functions*: Susceptibility to compression and pressure effects varied widely in different subjects. During the initial compressions to 1200 fsw, mental slowness, delayed response and reaction times, increased errors, and occasional failures to follow test procedures were observed, usually in association with prominent discomfort in subjects. While remaining at stable pressure after the first day, subjects appeared close to full mental capacity, alertness, and manual dexterity in performing technical functions.
- *Sleep*: Neither sleep quality nor electroencephalographic activity during sleep was observably altered during saturation exposure at 1200 fsw.
- *Electroencephalographic Changes*: EEG changes, which were generally progressive with depth as pressure increased beyond 640-800 fsw, included disorganization of background activity and decreased frequency of background activity components, irregular low-frequency forms, and occasional paroxysmal lower-frequency activity. Most EEG changes did not correlate directly with symptoms or performance. The conduction latency of the secondary N2 component of the somatosensory-evoked cortical response was the only measured index of peripheral and central nerve conduction time that changed significantly. This change, which could have been a nonspecific effect, occurred only during the first few hours after initial compression to 1200 fsw. Visually evoked cortical

responses consistently had small amplitude decrements and latency increments as absolute pressure increased.

- *Tremor*: Integrated amplitudes of intentional and postural tremor doubled during rapid compression to 800 and 1200 fsw, reversing partially or completely within two to four hours at stable pressure. Despite early adaptation, tremor amplitudes again approximately doubled during 400-fsw excursions on exposure days two and three. However, the increased tremor amplitudes were nearly invisible and caused no detectable functional impairments.
- *Cardiac Electrical and Mechanical Function*: Cardiovascular functions did not appear to be affected by either compression or hydrostatic effects in combination with high helium pressures.
- *Pulmonary Mechanical Function*: Superimposed upon pulmonary function decrements caused by increased gas density alone, additional transient decrements in maximal ventilatory volumes and flow rates occurred during rapid compression to 1200 fsw. These changes, partially effort-dependent, were most evident when symptoms and other effects of compression and pressure were prominent. They also receded with time as symptoms diminished. Ventilatory responses were adequate to support a moderate level of exercise during and immediately after rapid compressions to simulated depths of 800, 1200, and 1600 fsw.
- *Breathholding Capacity*: Breathholding duration during stable exposure to a pressure equivalent to 1200 fsw was equal to that at one atmosphere.
- *Thermal Homeostasis*: As previously found (26), exposure to increased He-0_2 pressures was associated with an increase in comfort temperature and a narrowing of the comfort zone. Presence of a mild cardiovascular stimulus was reflected by measurable elevations in resting heart rate and cardiac output. Despite reasonable caloric intake and undetectable change in whole body oxygen uptake, body weight decreased slightly and progressively during exposure to high He-O_2 pressures.
- *Decompression from Excursion Exposures*: Using the results of previous U.S. Navy investigations of helium-oxygen, saturation-excursion diving (36) as a guide, excursion-decompression procedures were developed to allow decompression from a 1200 to 1600 fsw excursion in less than one tenth of the time required for decompression in a 400-foot excursion from sea level. Specifically, decompression to 1200 fsw after 55 minutes at 1600 fsw was accomplished in 90 minutes, as compared to the nearly 18-hour decompression usually employed after a 60-minute excursion to 400 fsw from the surface. An episode of vestibular symptoms occurred in one subject, with therapeutic resolution.
- *Underwater Work Performance*: The underwater work task consisted of a programmed sequence that involved the timed dismantling and reassembly of large valve flange and other oil wellhead components in the water-filled chamber compartment (Figure 6). Prior to the initial compression for Phase Two, training for the underwater work was performed at one atmosphere, both in dry conditions and under 10 to 12 feet of water. The average values of pulmonary ventilation and oxygen uptake for all four divers in air at 1.0 ATA were about 60 and 2.0 liters per minute, respectively. After establishing a range for the

four best time trials at 10 fsw, each diver performed the timed underwater work sequence at 1210 fsw during saturation exposure, at 1610 fsw on excursion, and at 1360 fsw during a stable phase of saturation-decompression. With the exception of one run by one diver at 1610 fsw, all of the time trials at simulated depths of 1210-1610 fsw were within or below the range for the four best trials at 10 fsw.

Fig. 6. Diver-subject performing underwater task sequence at 1610 fsw pressure equivalent, on excursion from 1200 fsw (32).

All of the time trials at extreme depths were performed after periods of adaptation, when effects of compression and pressure were minimal or absent, and diver-subjects demonstrated capabilities for practical underwater work at a simulated depth of 1600 fsw (32).

Hyperoxia Predictive Studies

Predictive Studies V, VI, and VII are referred to here collectively as the Hyperoxia Predictive Studies, extending from previous years of detailed study concerning the physiological effects of acute hyperoxia related to limits of closed-circuit oxygen diving (2, 5-7). The previous studies complemented earlier Navy-supported investigations by Behnke, et al (37, 38) at the Harvard School of Public Health. Those studies also accompanied extensive investigations of human oxygen tolerance by Donald (39) in the Royal Navy and Yarbrough, et al (40) in the U.S. Navy, which supported the initial use of closed-circuit oxygen diving for military covert operations during World War II (41, 42). Additional applications of hyperoxia in diving include its use in decompression (43) and in the therapy of decompression sickness (44, 45). Although Behnke and Shaw (44) proposed therapeutic administration of oxygen in decompression sickness in 1937, it was not until 1965 that oxygen treatment tables developed by Goodman and Workman (45) were formally accepted by the U.S. Navy. Concurrently, hyperbaric oxygenation was employed by Boerema (46) in surgical procedures and by Brummelkamp, et al (47) in the treatment of anaerobic infections. Subsequently, therapeutic applications of hyperbaric oxygenation were expanded to include several categories of ischemic wounds, compromised grafts and flaps, thermal burns, selected acute and chronic infections, and carbon monoxide poisoning (48, 49). Results of the Hyperoxia Predictive Studies have relevance to all of these applications.

Predictive Studies V

Predictive Studies V, performed from 1982 to 1987, involved continuous exposures to the different inspired-oxygen pressures of 3.0, 2.5, 2.0, and 1.5 ATA, to practical neurological and pulmonary limits of human oxygen tolerance (50). Early studies of pulmonary tolerance to continuous oxygen breathing at 2.0 ATA (51-53) provided a basis for later, more extensive studies of systemic oxygen poisoning with emphasis on visual and other neurological effects

(Figure 7). Concurrently, functional components of major organ systems were investigated (Table 2) in experiments designed to help define the consequences of exposures over a range of useful hyperoxic pressures (54, 55).

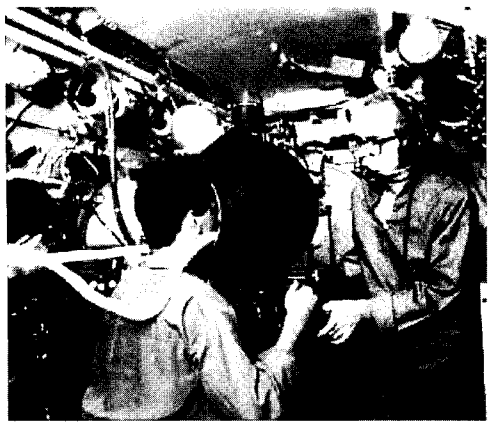

Fig.7. Measurement of retinal electrical activity (electroretinography) in response to a light flash while subject breathes oxygen at 3.0 ATA. Chamber lights are out for actual measurement.

The intended applications were related to manned undersea and aerospace activity, the expanding field of special forces self-contained diving operations, clinical hyperbaric oxygen therapy in general, and to the therapy of undersea and aerospace decompression accidents, specifically.

Table 2
HYPEROXIA PREDICTIVE STUDIES
Scope of Measurements

Electroencephalography	**Pulmonary Function**
Clinical interpretation	Flow-volume loops, spirometry
Response to phonics stimulation	Density dependence of flow
Visual Function	Pulmonary compliance
Peripheral visual fields	Airway resistance
Electroretinography	CO diffusing capacity
Visual evoked cortical response	Arterial Pco2, Po2, pH
Visual acuity	Closing volumes
Pupillary reaction	Ventilation uniformity
Accommodation	Peak respiratory pressures
Color vision	**Bronchoalveolar Lavage**
Auditory-Vestibular Function	Cellular composition
Audiometry (standard, high frequency)	Chemical composition
Brainstem auditory evoked response	**Respiration**
Caloric stimulation	Respiratory control
Eye tracking	Pulmonary gas exchange
Performance	**Muscle Power**
Perceptual, Cognitive, Psychomotor	Skeletal, respiratory
Cardiovascular Function	**Temperature**
Electrocardiography	**Hematologic Effects**
Cardiac output, rate, stroke volume	**Blood Chemistry**
Blood pressure, systemic vascular resistance	**Liver Blood Flow and Function**
Orthostatic reflex response	
Renal Function	

Predictive Studies VI

Predictive Studies VI, from 1988 to 1992, was designed to use the previous measurements of neurological and pulmonary responses to continuous oxygen exposure as baseline control data for the investigation of practical human oxygen tolerance extension by systematic alternation of hyperoxic and normoxic exposure periods. Extensive investigation in animals (56, 57) provided a basis for the selection of effective intermittent exposure patterns that could be evaluated in humans. Collaborating with those who had previously investigated human tolerance to continuous oxygen exposure at 2.0 ATA (51), Hendricks, et al (58), in an initial study, demonstrated that the progression of pulmonary oxygen poisoning at 2.0 ATA could be effectively delayed by alternating twenty-minute periods of oxygen breathing with five-minute periods of normoxia. Two additional patterns of intermittent

exposure at 2.0 ATA were evaluated as part of Predictive Studies VI, with comparable results (57).

Predictive Studies VII

Predictive Studies VII, performed from 1992 to 1997, complemented the baseline Predictive Studies V measurements by investigating conditions known to decrease human tolerance to the neurological effects of oxygen toxicity by causing them to occur more rapidly, or at lower oxygen pressures. These conditions include exercise during exposure to hyperoxia, underwater immersion, and accumulation of carbon dioxide from any of several possible sources. Occurring individually or in combination, at least some, if not all of these influences, exert their adverse effects on neurological oxygen tolerance by increasing brain blood flow and oxygen dose (57). Predictive Studies VII also demonstrated effective reduction of arterial PCO_2 and brain oxygen dose during hyperoxic exercise by voluntary hyperventilation (57).

Predictive Studies VIII

Predictive Studies VIII, in contrast to its hyperoxic predecessors, was designed to investigate the hypoxic end of the oxygen spectrum. The physiological effects of exposure to hypoxic, hypercarbic-inspired gas mixtures were studied with regards to NASA interests in the potential transient flooding of a spacecraft atmosphere with a hypoxic gas to suppress fire. Respiratory, cardiovascular and brain circulatory responses to hypoxia alone, and with concurrent hypercarbia, were studied at rest and during exercise. In this environmental extreme, the concurrent increments in brain blood flow and oxygenation associated with elevated partial pressures of carbon dioxide have a beneficial effect on human tolerance, rather than the adverse effects caused by the same physiological changes in a hyperoxic environment.

INSIGHTS FROM THE HYPEROXIA PREDICTIVE STUDIES

Major components of the results from the Hyperoxia Predictive Studies have been published in open literature (54, 55, 59-65). Other components, not yet published, are available in the Environmental Biomedical Stress Data Center at the University of Pennsylvania (57). Focussing on those that provide new information or insights that complement or extend earlier results, selected examples of specific observations from the Hyperoxia Predictive Studies are summarized in this presentation.

CNS Oxygen Poisoning.

Of 14 subjects who breathed oxygen at 3.0 ATA for up to 3.5 hours, one had a typical oxygen convulsion at 3.0 hours of exposure. As part of the emphasis on neurological effects of oxygen toxicity, brain electrical activity, ventilatory changes, and end tidal PCO_2 were monitored in each subject as potential manifestations of CNS oxygen poisoning.

In agreement with previous observations (66), there were no electroencephalographic changes prior to the actual seizure. However, there were previously unreported respiratory changes, characterized by prolonged expiration, decreased rate of breathing, decreased alveolar ventilation, and increased alveolar PCO_2 (50). These changes, which appeared to start about thirty minutes before the convulsion, produced a progressive PCO_2 elevation that peaked immediately before the time of seizure onset. It is likely that the associated increments in brain blood flow and oxygen dose accelerated the progression of CNS oxygen poisoning.

Visual Effects.

The most prominent visual effect was loss of peripheral vision, nearly complete in some subjects, as observed by Behnke, *et al* (38). Peripheral visual field changes in 14 subjects, who breathed oxygen at 3.0 ATA for up to 3.5 hours, are shown in Figure 8 (50). The visual field area was maintained in nearly all subjects for about two hours and then decreased rapidly.

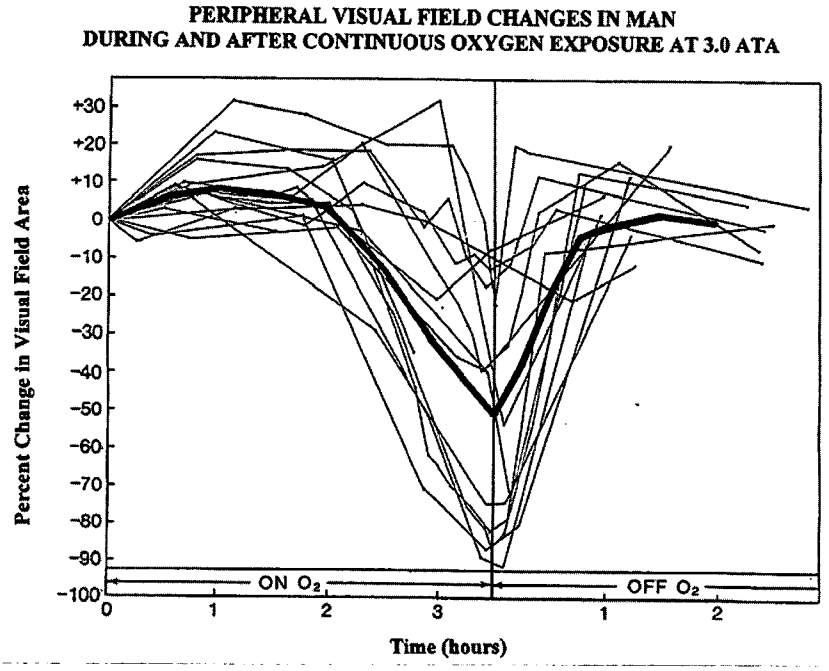

Fig. 8. Fine lines show changes in visual field area for individual subjects. Heavy line represents the average of 14 subjects (50).

About half of the subjects experienced nearly total loss of peripheral vision; the other half had less significant changes. In all subjects, essentially complete recovery occurred within about 45 minutes of air breathing. These measurements confirmed, and with the aid of better equipment, greatly extended the earlier observation of Behnke, et al in a single subject (38). Repeated measurements of visual field area in many additional subjects defined the rates of development and recovery, as well as the range of individual variability for this neurological manifestation of oxygen poisoning. Similar measurements of the decrease in visual field area at lower oxygen pressures revealed it to be of a progressively slower onset and smaller magnitude (57).

Syncope.

One of the subjects exposed to oxygen at 3.0 ATA had a transient syncopal episode at 2.5 hours (50, 67). Continuous recording of the electrocardiogram demonstrated a rapid onset of bradycardia, which culminated in a 13-second cardiac pause immediately before the loss of consciousness. The pause was ended by a sinus beat and gradual return to normal sinus rhythm. Consciousness was regained within one minute. In 1935, Behnke, et al reported the transient loss of consciousness associated with an absence of the radial pulse and fall in blood pressure (37). Similar episodes were later observed by Donald (39). Previous investigators have shown that the bradycardia that occurs during oxygen breathing, even at 1.0 ATA, is mediated by vagal action

on the heart (68). It is likely that the 13-second sinus pause in our subject represents an exaggerated example of vagal activity, which may be related to CNS oxygen toxicity.

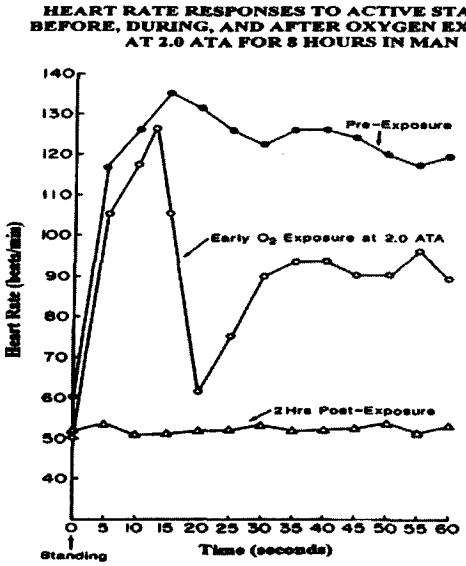

Fig. 9. Changes in heart rate during a 60 secon after standing upright from a resting, supine po

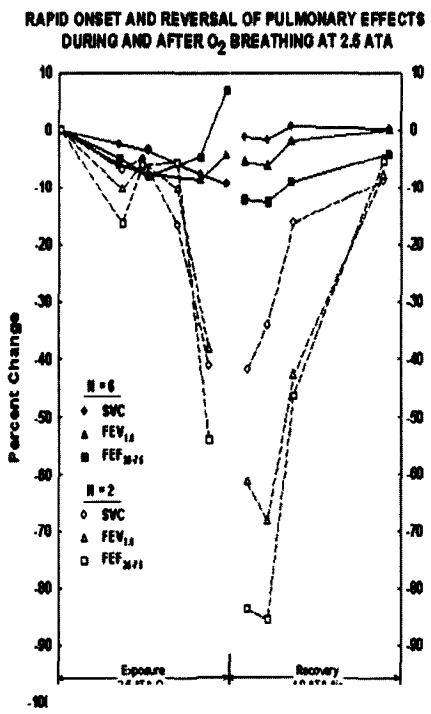

Fig. 10. Example of rapid onset and reversal of pulmonary effects during and after breathing xygen at 2.5 ATA for 5-6 hours (55). See text for discussion

Another example of exaggerated vagal activity is illustrated in Figure 9 (67). One of the cardiovascular functions monitored at regular intervals during exposure to oxygen was the reflex response to an abrupt change in posture, from the supine position to the standing position. The data shown in Figure 9 were obtained in a subject who breathed oxygen at 2.0 ATA for 8.0 hours. Changes in heart rate during the first sixty seconds after abruptly standing upright were measured before exposure, during early exposure, and two hours after the exposure. The normal response to standing upright is rapid acceleration of heart rate, followed by stabilization at a higher level. During early exposure, this response was modified in that the initial acceleration was not maintained, and the heart rate stabilized at a level only slightly higher than the resting level. The most unusual response was recorded two hours after the end of the exposure. At that time, heart rate remained absolutely constant for an entire minute after the subject stood upright. Despite the absence of an increase in heart rate, however, blood pressure was maintained and the subject did not lose consciousness. This may reflect an increase in systemic vascular resistance to compensate for the sustained period of bradycardia. While one component of the reflex response to standing was suppressed, another remained fully active.

Pulmonary Effects.

Figure 10 shows changes in pulmonary function measured during and after breathing oxygen for five to six hours at 2.5 ATA (55). The measurements included slow vital capacity (SVC), one second forced expired volume ($FEV_{1.0}$), and maximal mid-expiratory flow rate (FEF_{25-75}). Average values for six subjects are compared with those in two other subjects who had much larger pulmonary function decrements during and after the oxygen exposure.

Changes in the two subjects are characterized by an early onset during oxygen breathing, large magnitudes of effects, and rapid reversal upon the resumption of air breathing at 1.0 ATA. Subjectively, both subjects experienced a feeling of chest tightness and an inability to exhale rapidly despite exerting maximal effort. The rapid onset and reversal of these effects, as well as the large magnitudes, are all consistent with neurological interaction to magnify the direct pulmonary effects of

oxygen toxicity. Vagally-induced bronchoconstriction would provide a mechanism for such an interaction.

Exercise Effects.

As stated, a primary goal of Predictive Studies VII was the investigation of conditions known to decrease human tolerance to the neurological effects of oxygen toxicity. Early studies in both the U.S. Navy (40) and the British Royal Navy (39) showed that exercise during exposure to hyperoxia caused convulsions to occur more rapidly or at lower oxygen pressures than at rest. The data shown in Figure 11 are average arterial PCO_2 measurements in six men who performed incremental levels of bicycle ergometer exercise while breathing oxygen at 2.0 ATA (63). Prior to the start of exercise, arterial PCO_2 decreased from a normoxic value of 40.5 mm Hg to 34.3 mm Hg during oxygen breathing. This well-documented response to hyperoxia is caused by the modification of blood CO_2 transport with associated responses that include a PCO_2 elevation in central respiratory control centers, mild hyperventilation, and arterial hypocapnia (6). The related reduction in brain blood flow and oxygen dose (57) should have a protective influence. However, the ventilatory response to exercise during exposure to hyperoxia is such that the protective influence is removed by progressive elevation in arterial PCO_2. These responses provide a physiological basis for the adverse effects of exercise on CNS oxygen tolerance.

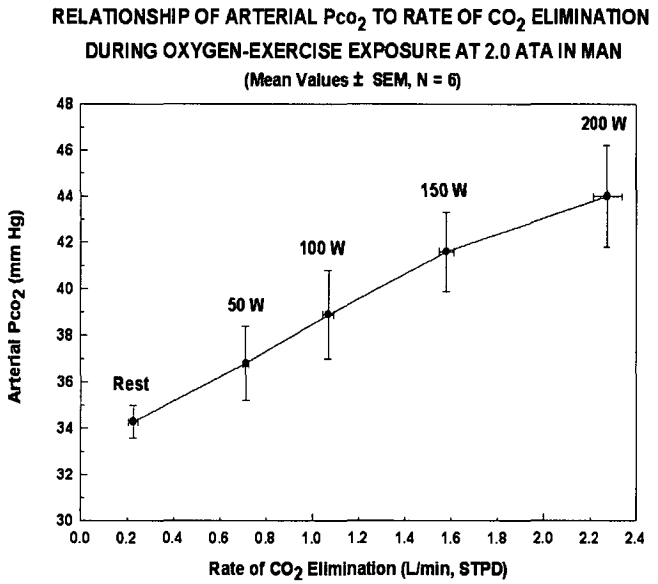

Fig. 11. Progressive rise in arterial PCO_2 during incremental exercise while breathing oxygen at 2.0 ATA. Rate of CO_2 elimination was measured as an index of work intensity (63).

CONTINUING RELEVANCE OF HYPEROXIA PREDICTIVE STUDIES

The results of the Hyperoxia Predictive Studies have relevance to diving and decompression, the therapy of decompression sickness, and the expanding applications of hyperbaric oxygen therapy. This relevance is likely to increase with improved understanding of current applications and the development of new uses. Although hyperoxia will always be toxic, its therapeutic properties can be exploited while remaining within the pressure-duration limitations imposed by concurrent toxicity. Effective extension of oxygen tolerance by systematically interrupted exposure or other methods will cause the limitations to be less restrictive. As basic mechanisms of oxygen toxicity are elucidated, it should be possible to ameliorate and/or further extend these constraints. Information and insights from the Hyperoxia Predictive Studies supplement the investigation of basic mechanisms by defining the functional deficits associated with early, reversible degrees of oxygen poisoning in specific organs and tissues. Further understanding of oxygen poisoning mechanisms requires identification of the many intermediate

actions and reactions that precede known functional derangements, as well as the reactive species and cellular responses that initiate them (62, 66).

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Erythropoietin production can be enhanced by normobaric oxygen breathing in healthy humans.

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INTRODUCTION

Erythropoietin (EPO) induces red blood cell production by activating red bone marrow progenitor cells, and is used therapeutically in chronic anemia. It is also used as an unauthorized adjunct to increase the oxygen transport capacity in the blood of athletes. Renal tissue hypoxia is the only widely accepted trigger for EPO production (1-3), even if new oxygen-sensitive sites have been recently proposed (4-6). This is well established in models of reduced-oxygen delivery during anemia (7), reduced renal perfusion (8), or hypobaric or normobaric hypoxia (9, 10) or hypoxemia. In one report, hemoconcentration, following sporting activities (11), has been reported to increase EPO secretion. There does not seem to be agreement on the existence of a circadian variation in EPO secretion (12, 13), although the exact timing and magnitude of the nadir and zenith are not unequivocally established, nor is the EPO plasma concentration.

Previous experiments in breath-hold divers have led us to hypothesize that another triggering mechanism might exist, independent from renal tissue hypoxia. After a series of deep breath-hold dives, two out of five divers showed a marked augmentation of serum EPO levels. During descent to depth, intra-alveolar oxygen tensions increase. During ascent from depth, oxygen tension falls to atmospheric values. During these (unpublished) experiments, no severe alveolar hypoxia was observed after surfacing, although EPO production seemed to increase. Recently, a Spanish study reported that short exposure to intermittent hypobaric hypoxia increased EPO production (14). We hypothesize that a sudden decrease in tissue-oxygen tension from hyperoxia back to normoxia might act as a trigger for EPO release.

MATERIALS AND METHODS

32 healthy subjects (23 males and 9 females), ranging in age from 22 to 47 years, participated in this study after written informed consent. They did not smoke for 24 hours before the test, nor did they take any medication or perform strenuous physical exercise. Sixteen of the participants were subjected to normobaric oxygen breathing (15 lpm) for two hours. In order to obtain more thorough tissue denitrogenation, they were asked to perform moderate physical exercise (twenty knee bends) every ten minutes (15, 16). Continuously monitoring the following clinical signs controlled oxygen breathing: mask fit, movement of the three one-way valves, movement of the reservoir bag, and moisture formation on the transparent mask during

expiration. Furthermore, transcutaneous oxygen tension ($PTcO_2$) was measured sequentially in all subjects using a Radiometer TCM3 (Radiometer, Copenhagen).

Blood samples were taken before the start and at the end of oxygen breathing, at time 0, then, at times 2, 4, 5, 7, 10, 24 and 36 hours after oxygen breathing. Total blood volume taken was less than 30 milliliters; this had no influence on hematocrit. The blood was immediately centrifuged (10 minutes at 3000 rpm), and the separated serum was frozen immediately to –80°C for a maximum of 24 hours before analysis. Serum EPO concentration was measured using a radioimmunoassay (EPO-Trac ^{125}I RIA, INCSTAR, Stillwater, USA). Hematocrit and hemoglobin concentrations were measured before and after oxygen breathing, as hemoconcentration is known to influence EPO production (11). Body impedance was measured before and after oxygen breathing. For this, a Tanita TBF-310-GS scale was used to test the eventual difference in body fat mass and the EPO response, as tissue denitrogenation rate can differ because of fat mass (17).

Without oxygen breathing, 16 healthy subjects were randomly submitted to the same blood sampling in order to establish a circadian EPO production rhythm, as reported in the literature (12).

Standard statistical analysis was performed, including mean, standard deviation, normality, median and the analysis of variance (ANOVA) as repeated measures to test the effects between and within subjects after Kolmogorov-Smirnov's test for normality. The post-test performed was Bonferroni or Dunnet comparison versus control values.

RESULTS

All subjects had baseline hematocrit, serum hemoglobin and impedance values within the normal range. These were not significantly altered after the oxygen-breathing period (Figures 1, 2).

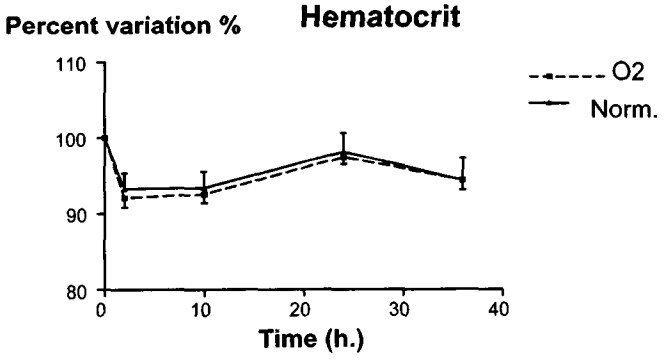


Fig. 1. Percent variation of hematocrit over 36 hours after two hours of normobaric oxygen breathing and the control group (Norm.).

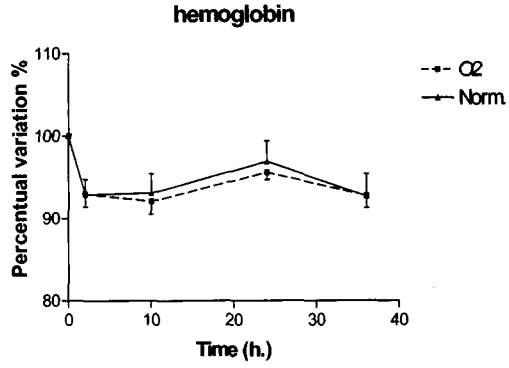


Fig. 2. Percent variation of hemoglobin over 36 hours after two hours of normobaric oxygen breathing and the control group .

In the control group of 16 divers who were not breathing oxygen, a circadian variation of serum EPO concentration was found. The nadir occurred at around 14.00 Hrs (8.96 mU/ml ± 2.1) and the zenith at about 22.00 Hrs (12.1 mU/ml ± 3.5) (Figure 3).

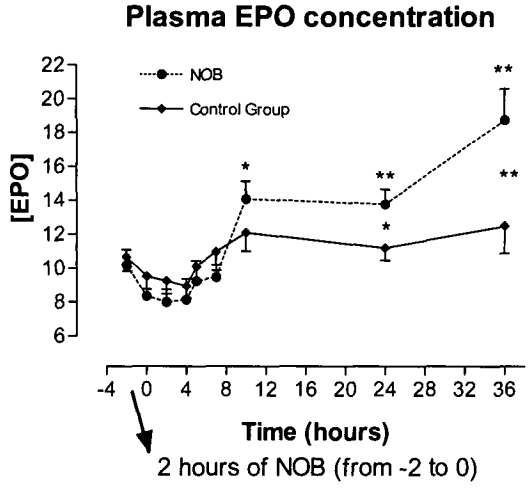
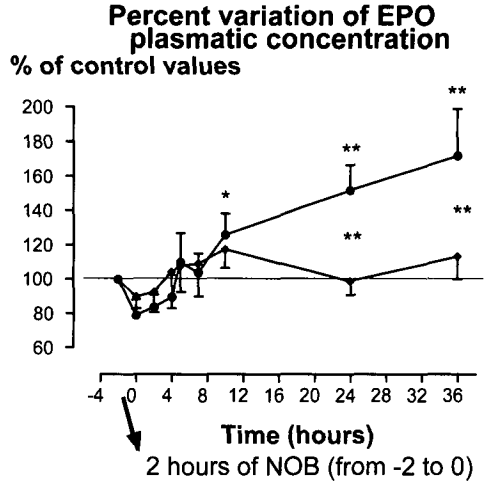


Fig. 3. Evolution of mean (± SEM) plasma EPO concentration (mU/ml) before and after two hours of 100% oxygen breathing over 36 hours (N=16); Time 0 = from end of two hours of oxygen breathing. (***: P<0.01). Circadian variation of serum EPO in control group (n=16) (not breathing oxygen) is also shown.

In the oxygen-breathing group, an initial decrease in serum EPO concentration after the oxygen breathing was followed by a significant increase at 24 and 36 hours (Figure 3). This increase was statistically significant, both with regard to the initial (pre-oxygen-breathing) value and to the mean variation of the control group (circadian rhythm group) (Figure 4). This increase seemed to start after a lag of about eight hours after cessation of oxygen breathing. This is consistent with the time lapse needed for message transcription and EPO protein synthesis by the renal peritubular cells (18).

Fig. 4. Percent variation of plasma EPO concentrations of the oxygen-breathing group (●) vs. the control group control group (♦)



DISCUSSION

A significant increase in serum EPO levels was measured after two hours of 100% oxygen-breathing. This increase was not related to a circadian EPO variation, nor could it be explained by changes in hematocrit level or whole body dehydration. By breathing 100% oxygen for two hours, a significant nitrogen washout was obtained, thereby increasing the relative oxygen content in all body tissues, including the kidneys. The cessation of oxygen breathing results in a rapid decline in oxygen partial pressure in the blood and tissues, followed by a gradual increase of the nitrogen content of these tissues. It has been reported that hypoxic periods of 45 minutes, but not of 30 minutes, trigger EPO production (13). A change in oxygen content induced by stopping the oxygen breathing, which could be called a "relative hypoxia," persists over a prolonged period . Since it is actually a return to the normal oxygenation state, it could constitute a sufficient trigger for increasing EPO transcription and secretion. It is not likely that an oxygen-induced arteriolar

vasoconstriction, via a decreased perfusion of the renal parenchyma, could be responsible for this increased EPO production. On one hand, hyperoxic vasoconstriction only attains significant levels in hyperbaric hyperoxia. On the other hand, renal oxygen supply would be balanced by the increase in arteriolar blood oxygen content.

CONCLUSION

These data demonstrate a previously unreported triggering mechanism, unrelated to an absolute decrease in oxygen delivery to the renal peritubular cells, for EPO production in healthy humans. Potentially, this mechanism could have clinical applications even if our results do not demonstrate significant hematocrit or reticulocyte increases (because of an insufficiently long follow-up). It may be that this mechanism induces an increase in red blood cell production. Moreover, recent studies demonstrate an important neuroprotective effect of EPO (19-23). This is a promising, multipurpose medical benefit for any pathology or sport susceptible to promoting adverse neurological effects as a result of environmental factors. Further studies are necessary to determine the duration and final magnitude of the increased EPO production, and the possibility of repeating the triggering mechanism over several consecutive days in order to get an additive effect on serum EPO levels. The administration of hyperbaric oxygen, instead of normobaric oxygen, should also be investigated (24, 25).

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Normobaric oxygen can enhance protein captation by the lymphatic system in healthy humans.

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INTRODUCTION

The use of normobaric oxygen (NBO) as a first aid tool for decompression sickness (DCS) has been advocated for a long time. Several beneficial effects of NBO have been demonstrated, one of which is the faster elimination of tissue nitrogen bubbles.

During DCS occurrence, a cascade of intravascular reactions has been demonstrated. These inflammatory reactions occur within minutes of the onset of DCS, and involve the precipitation of proteins on the gas-bubble interface, causing a stabilization of the intravascular and tissue bubbles. Little is known about the elimination of the protein-coated bubbles except that they are smaller than the obstructive ones, thus allowing them to pass through the circulation and probably enter the interstitium during the extravasation phase, subsequent to hypoxia.

Also known is that the proteins can denature. This may cause the accumulation of free fat globules found during decompression sickness (1, 2). Fat emboli have been observed in several studies of decompression sickness and can contribute to central nervous system damage(3). As interstitial proteins are evacuated by the lymphatic circulation, we wanted to investigate if NBO enhances lymphatic activity and thus, protein elimination (4, 5).

The rationale relations between edema and hypoxia are clear if we think of the augmented distance between capillaries and the presence of "oxidative burst" during inflammation involving bacterial activity (6). Furthermore, the presence of a large number of mitochondria in the lymphatic endothelium shows a marked oxidative metabolism for the activation of lymphatic contractile properties. It has been shown that reactive nitrogen species can reduce the contractile properties of the lymphatic vessel. Hence, denitrogenation has been proposed as a positive action on lymphatic contraction (7).

Oxygen, it seems, is involved in positive effects on lymphatic vessel metabolism and edema reduction. The potential interest in clinical use and a more in-depth understanding of NBO during in-field decompression first aid lead us to test the beneficial link between oxygen and lymphatic protein captation (8, 9).

METHODS

Seven healthy subjects (five males and two females) received an injection of 0.2 ml of Tc99-marked human albumin (HSA Nanocoll), diluted with 2.3ml of S.S.P.P., into the first interdigital space. This injection produced moderate subcutaneous edema. The saline preparation of the injected proteins had a protein size range between 50 and 100 nm, allowing the solution to be exclusively reabsorbed through the lymphatic bed. The proteins were first phagocyted by macrophages, then drained via the lymphatics (10). The lymphoscintigraphic technique was chosen because it is the usual clinical investigation for lymphatic activity, particularly on the upper limb for breast cancer patients.

Experimental lymphoscintigraphy sessions were performed while subjects (age range 19-27 years) were recumbent. Initial studies were performed while breathing air. On a separate day, the procedure was repeated, but the subjects breathed NBO from oronasal demand regulators (Life Support Product, Ltd.) immediately after injection and continued breathing for thirty minutes. The dynamics of the isotopic activity at the axillary ganglia was recorded to assess the speed and quantity of the lymphatic protein drainage. In parallel, $TcPO_2$ in the edema region was constantly monitored.

Exclusion criteria were diabetes, pregnancy, vascular disease, upper limb traumatic lesion, and some sports practice, such as volleyball and martial arts, since such activities can induce lesions of the lymphatic system.

RESULTS

Qualitative analysis

Since lymphoscintigraphy (Figure 1) is basically qualitative, we began with the classical approach. In all subjects, NBO produced a marked increase in the isotopic activity at the axillary level after one hour, starting thirty minutes after the beginning of oxygen breathing (the first thirty minutes were kept as the baseline level).

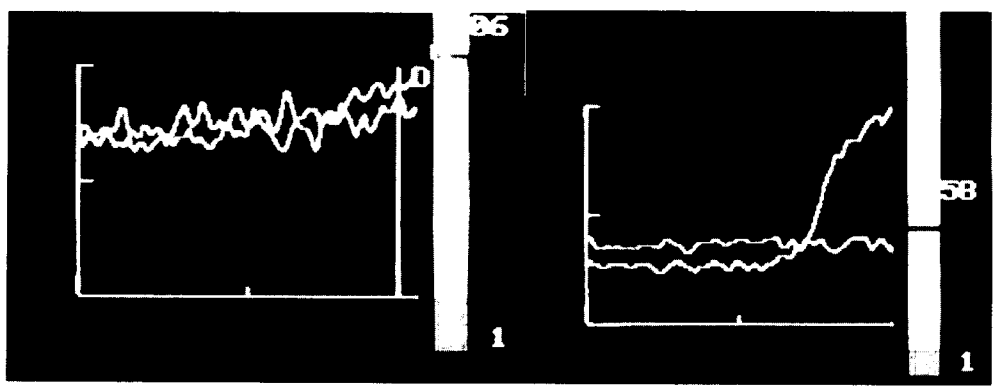

Fig. 1. The figure (left side) shows a typical lymphoscintigraphy without oxygen breathing. The pertinent trace is the white one because the other represents the other arm, without experimental edema. During oxygen breathing (right side), the trace of the injected arm rises drastically in all subjects. The Y-axis expresses the number of isotopic impacts per second; the X-axis expresses the number of frames stored. Each frame equals ten seconds.

In these subjects, $PTcO_2$ levels at the site of edema showed a marked increase during the first ten minutes. A plateau phase is then observed during the complete oxygen breathing time, followed by a rapid return to baseline after oxygen breathing cessation.

Quantitative analysis

This analysis is uncommon in lymphoscintigraphy. We analyzed the area under the trace (arbitrary units) to compare lymphatic protein elimination with and without oxygen breathing. The statistical analysis was accomplished using paired t-test after passing normality by the Kolmogorov-Smirnoff test. The area under the lymphoscintigraphic trace was significantly greater after oxygen breathing. The total amount of proteins eliminated was greater after 30 minutes of 100% oxygen breathing (P=0.0226).

The second quantitative analysis was the angular variation of the trace. We looked at the parameter that could best express the evacuation speed of labeled proteins. We chose the angular variation of the lymphoscintigraphic trace because the trace's slope expresses the speed of elimination. Figure 2 shows the mean angular variation of the lymphoscintigraphy trace. Control values were computed during the non-oxygen protocol. The pre-O_2 values were the angular values of the trace before oxygen breathing (baseline values). Increased absorption speed was noted after 30 minutes of 100% oxygen breathing.

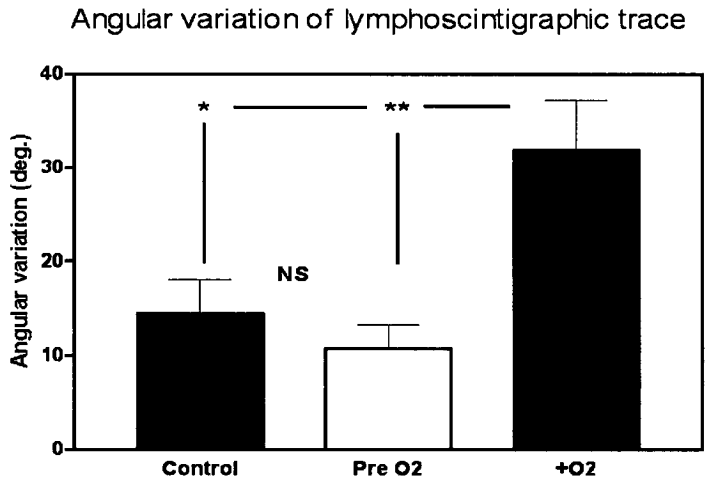

Fig. 2. Statistical comparison between the control absorption speed (angular variation) in the axillary region; the pre-oxygen situation and after 30 minutes of normobaric oxygen breathing.*=p>0,05; **=p>0,01

CONCLUSIONS

Breathing normobaric oxygen enhanced protein removal by the lymphatic system in all subjects (Figure 3). Our findings support an interest in giving oxygen for at least thirty minutes during on-site first aid in diving-related accidents, as this may be beneficial in increasing elimination of even protein-coated bubbles by the lymphatic bed. Other clinical interest may be in the treatment of lymphoedema, perhaps by administering normobaric 100% oxygen during manual drainage. In order to better understand our observations, new investigations, using different sizes of proteins, are planned to further clarify this phenomenon and explore possible macrophage activity influence.

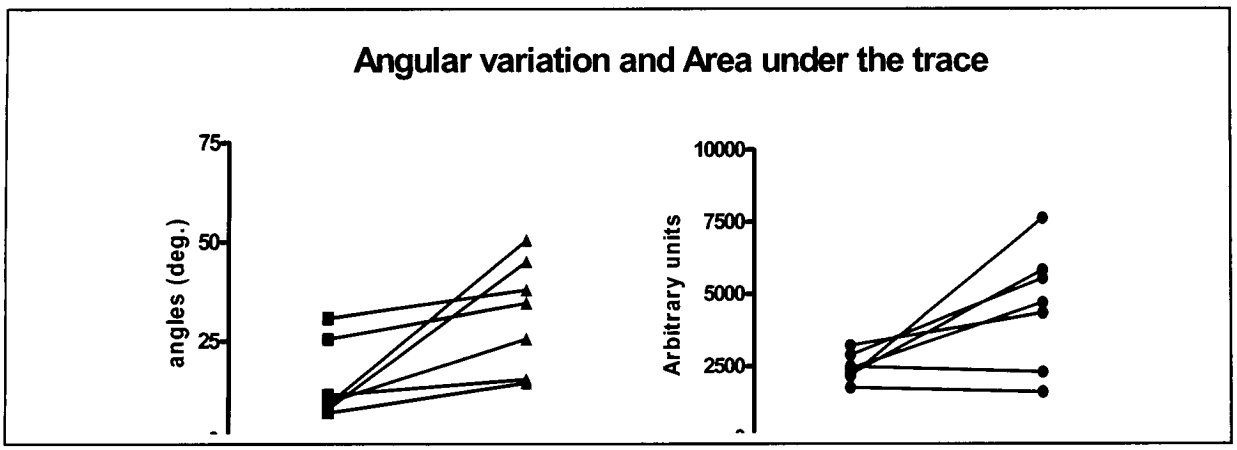

Fig. 3. Experimental data comparing (left side) angular variation of the lymphoscintigraphic trace after oxygen breathing, giving us an idea of the increased speed of resorption of marked albumin and the area under the curve, which expresses the amount of total marked proteins detected in the axillary area.

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CNS oxygen toxicity.

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INTRODUCTION

This short manuscript will briefly present our current knowledge about CNS oxygen toxicity: the clinical manifestations, descriptions and incidence of symptoms, and the time – duration relationship that defines the safety limits and risk factors leading to enhanced toxicity to hyperbaric oxygen. A very general outline will present suggested mechanisms underlying hyperoxia-induced seizures and strategies for protection against the seizures. Unfortunately, not much has been gained in our knowledge and understanding of CNS oxygen toxicity in recent years. Some concluding remarks will be given, as will some suggestions toward further research for increasing our knowledge about mechanisms, and better approaches for protection against the toxic effects of breathing oxygen at high partial pressures.

DESCRIPTION

There is no better description of CNS oxygen toxicity than the personal experience of Christian J. Lambertsen. "The convulsion is usually preceded but not always by the occurrence of localized muscular twitching, especially about the eyes, mouth and forehead. Small muscles of the hands may be involved and incoordination of diaphragm activity in respiration may occur. Eventually an abrupt spread of excitation occurs and the rigid "tonic" phase of the convulsions begins. Vigorous clonic contractions of the muscle groups of head and neck, trunk and limbs then occur becoming progressively less violent over 1 minute" (1). These convulsions are classified as generalized, tonic-clonic (grand mal) seizures (1). A typical electroencephalogram obtained during hyperbaric oxygen (HBO_2) seizures consists of spike and wave discharges (Figure 1) recorded in both hemispheres. They may start simultaneously or at random times in the cortical and subcortical areas (2, 3).

The hyperoxia-induced discharges are believed to be reversible, causing no residual neurological damage, and disappear upon reduction of the inspired oxygen partial pressure (1).

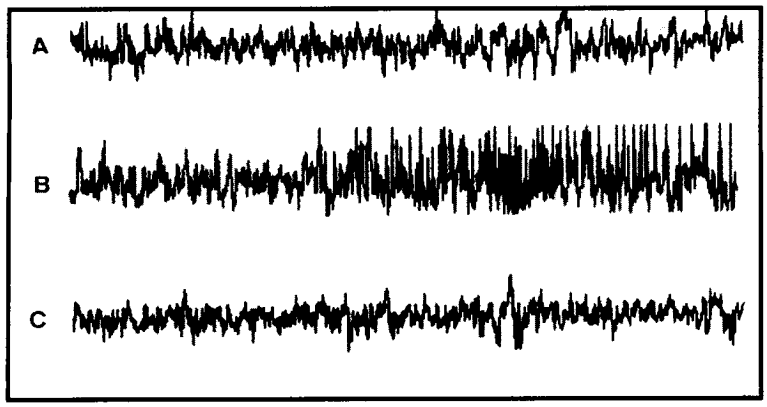


Fig. 1. Typical EEG from a rat exposed to 0.5 MPa oxygen. A) Air at atmospheric pressure. B) Appearance of typical spike and wave electrical discharges at 0.5 MPa oxygen. C) On return to atmospheric pressure. Calibration: 1 sec/50 V.

Hyperoxia-induced seizures are accepted as generalized, although several studies point toward local variations in subcortical brain areas. This might contradict the general nature of the seizure's development and indicate a more localized primary focus for the initiation of the epileptic activity. Several studies demonstrate variations in cerebral blood flow in different brain areas on exposure to HBO_2 (4, 5, 6); others present regional changes in amino acids and ammonia levels (7), variation in lipid peroxide distribution (8), and distribution of antioxidant enzymes (9).

Early changes in cortical electrical activity have been described on exposure to HBO_2 several minutes prior to the full development of the electrical discharges (10, 11). Unfortunately, almost thirty years later despite extensive research, we have no real-time correlate of pre-seizure EEG activity that could serve for the prediction of CNS oxygen toxicity (12, 13, 14).

Symptoms

Few large-scale human studies describing the symptoms of CNS oxygen toxicity are available in the literature (15, 16, 17, 18, 19, 20). The list includes a group of minor symptoms, such as nausea, dizziness, abnormal sensations, headache, disorientation, lightheadedness and apprehension, which are difficult to define. More unambiguous symptoms appearing on exposure to HBO_2 are blurred vision, tunnel vision, and tinnitus. Signs include respiratory disturbances, eye twitching, twitching of lips, mouth, and forehead, and convulsions. There is no consistency in the appearance of symptoms and signs prior to the development of the seizures. Despite our prolonged experience with experimental and accidental CNS oxygen toxicity, there is no consensus about the frequency and incidence of the different symptoms.

Table 1 compares symptoms from about 550 human exposures to hyperbaric oxygen, compiled from those performed by Donald in 1947 (16), Leitch in 1984 (17), and three series of experiments conducted in Panama City by Butler and Thalmann (18, 19, 20). The symptoms of CNS oxygen toxicity are presented in the declining order of their incidences of appearance. As can be seen from the table, there are inconsistencies in the frequency of symptoms' appearance in the different HBO_2 series. This symptom variability reflects the importance of environmental and personal factors on the development of CNS oxygen toxicity.

Dosing

The most famous relationship between inspired oxygen partial pressures and the time for the appearance of symptoms of pulmonary and CNS oxygen toxicity (latent period) is the classic work of Lambertsen and his colleagues at the University of Pennsylvania (Figure 2) with exposures to HBO_2 in a dry hyperbaric chamber (21).

TABLE 1

Donald	Leitch	Butler and Thalmann (NEDU)		
1947	1984	1984	1986	1986
Convulsion	Convulsion	Light – Headedness	Nausea	Muscle twitching
Twitching of lips	Unconsciousness	Convulsion	Muscle twitching	Dizziness
Vertigo	Cyanosis	Tinnitus	Dizziness	Blurred vision
Nausea	Limb shaking	Apprehension	Tinnitus	Dysphoria
Respiratory Disturbances	Dizziness	Dysphoria	Dysphoria	Convulsion
Twitching of parts other then lips	Strenuous breathing	Blurred vision	Confusion	Aphasiaia
Sensations of abnormality	Auditory aura	Tunnel vision	Convulsion	Dyspnea
Visual Disturbances	Breathing disturbance	Disorientation	Decreased auditory Acuity	Paresthesias
Acoustic Hallucinations	Nausea	Lethargy	Aphasia	Nausea
Paraesthesiae	Dissociation	Dysphasia	Tingling	Lightheadedness
	Apnoea	Aphasia	Numbness	Air hunger
	Loud cry/groan	Eye twitching	Choking sensation	Tinnitus
	Malaise	Nystagmus	Amnesia	Confusion
	Headache/pulsation	Incoordination	Muscular rigidity	Muscular rigidity
	Apprehension		Lightheadedness	Irritability
	Amnesia		Poor concentration	Hypoacusis
	Facial twitch		Visual disturbances	Hyperacusis
	Lip tremor		Decreased mental alertness	Poor concetration
	Disorientation		Increased respiratory rate	Tunnel vision
N=388	**N=35**	**N=28**	**N=33**	**N=59**

Table 1. Symptoms of CNS oxygen toxicity presented in declining order of incidence. Data from Donald, 1947 (16), Leitch, 1984 (17), & Butler and Thalmann (18,19, 20).

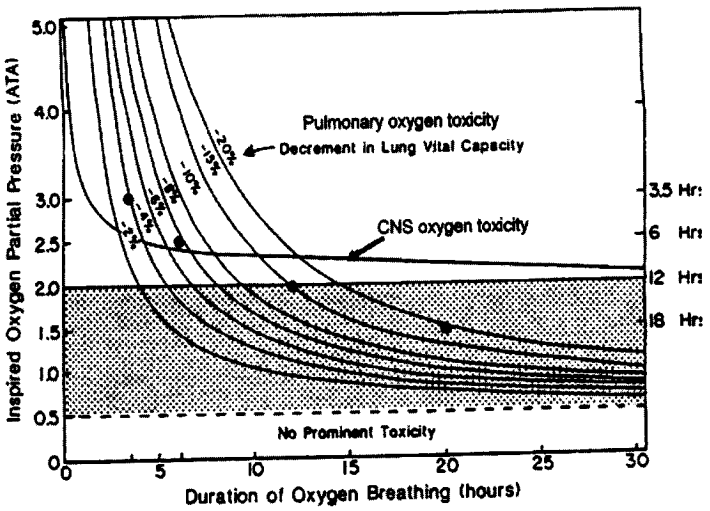


Fig. 2. Pulmonary and neurological oxygen tolerance curves for continuous exposures of normal men to hyperbaric oxygen. Adapted from Lambertsen CJ, Clark JM, Gelfand R, et al, 1987 (21).

Figure 3 displays the depth- time limits for wet (immersed) oxygen diving (19), setting the lower limit for oxygen diving at about 15 msw (50 fsw) for a period of 10 minutes and allowing safe prolonged oxygen diving for about 4 hours at a fairly shallow depth of around 7 meters (20 fsw) (threshold depth). Various environmental and personal factors may modify sensitivity to CNS oxygen toxicity, thus shortening the duration of the latent period and lowering the threshold pressure for the development of seizures.

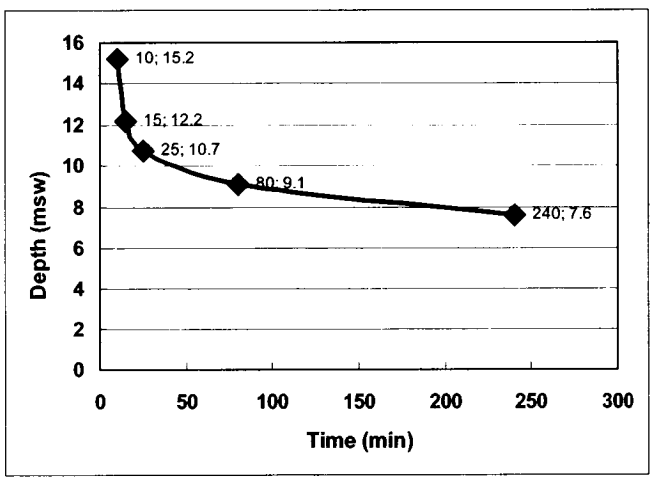
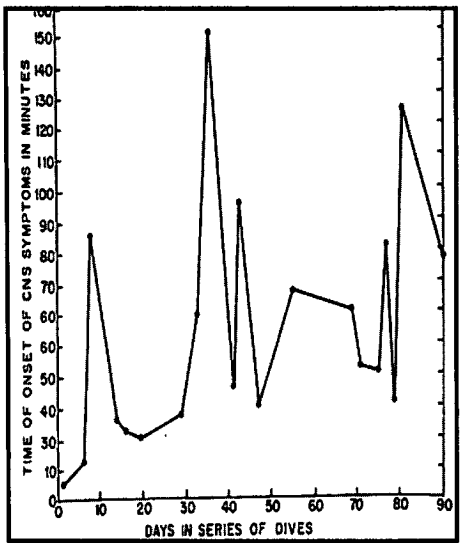


Fig. 3. Time–depth limits for oxygen diving. Data from Butler & Thalmann, 1986 (19).

Risk Factors

The exposure to hyperbaric oxygen in a wet environment increases sensitivity to CNS oxygen toxicity compared to exposure in a dry hyperbaric chamber (1, 16). Elevated concentrations of carbon dioxide (1, 14, 16, 22-25) and physical activity (exercise) dramatically decrease the duration of latent period for hyperoxia-induced seizures (1, 16, 23). The latent period for the appearance of electrical discharges in the EEG is significantly shorter in darkness than in light (26), suggesting the importance of visual input in the modulation of sensitivity to CNS oxygen toxicity. The risk for CNS oxygen toxicity is not determined solely by the partial pressure of the inspired oxygen, and even relatively low partial pressures of inert gases may contribute to hyperbaric-induced seizures (27). The increased sensitivity caused by inert gases could be explained by the involvement of free radical production (28). Circadian rhythm (29), various drugs, age (30), sex (31), interspecies differences and individual day to day variability (16) may contribute to the sensitivity to CNS oxygen toxicity (14). Figure 4 presents data from a unique study by Donald in 1947, in which he exposed a single diver to the same profile of HBO_2 for twenty times within a three month period, until the appearance of neurological symptoms of oxygen toxicity (16). As can be seen, there are large day to day



variations in time duration of symptoms, suggesting that there is no fixed, personal, predetermined threshold of tolerance to oxygen toxicity. This finding supports that using the oxygen test as a screening tool for oxygen diver selection is ineffective.

In spite of the known day to day individual variability in tolerance to hyperoxic seizures, and the evidence concerning modulation of environmental and personal factors on the sensitivity to high oxygen pressures, it is surprising that we are still using tables based on statistical methods and predictive models. A more optimal approach would be personal monitoring of oxygen toxicity using a real-time predictive system for detecting the first signs of toxicity.

Fig. 4. Day to day variations in time duration until onset of CNS oxygen toxicity symptoms of a single diver. From Donald, 1947 (16).

Mechanisms

Although hyperoxic-induced seizures have been well described, the effects of HBO_2 on neural elements remain poorly understood. Studies of vertebrate nerves demonstrated decreased excitability and blockage of impulse conduction (32, 33), while increased axonal excitability and an increase in the membrane time constant were demonstrated in the isolated nervous system of the cockroach (34). The synaptic mechanisms seem to play an important role in the development of the HBO_2-induced seizures. Among the main electrophysiological findings is an increase in spontaneous synaptic transmitter release (35), a reduction in the inhibitory transmission and enhancement of evoked excitability activity at the neuromuscular junction of the lobster (36).

HBO_2 is known to affect most of the neurotransmitters and the neurotransmitter enzymes, such as GABA, acetlycholine, glutamate, dopamine, ammonia, norepinepherine, and aspartate (37-44). It also affects neuromodulators, such as nitric oxide (NO) (45). Membrane-bound active transport systems are also impaired on exposure to hyperbaric oxygen (14, 38, 39, 46, 47), with implications on neural activity. These studies were performed in different nerve preparations and synapse models. Some used direct physiological techniques, while others used indirect biochemical methods. They indicate that HBO_2 affects almost all neural elements. However, the primary neural target responsible for development of hyperoxic seizures is still unknown.

It is well accepted that reactive oxygen species (ROS) are increased on exposure to HBO_2, and overwhelming the body's normal antioxidant defenses may mediate the hyperoxic insult (48). An increase in free radical generation in the brain precedes HBO_2-induced convulsions, as demonstrated in brain extracts (49). H_2O_2 is elevated in various brain areas on exposure to HBO_2 (9, 50, 51), and there is a rise in free radical levels in the blood of humans exposed to HBO_2 (52). Nevertheless, a recent study done with awake animals using a microdialysis probe failed to detect an increase in hydroxyl radicals prior to, or during, HBO_2-induced convulsions (53). Currently, the available experimental data does not allow for us to decide whether ROS are the primary cause for hyperoxic seizures activity, or alternatively, that ROS are acting indirectly on neural elements and via second messengers, such as small inorganic molecules or proteins, to elicit the epileptic activity. A study using blood-brain barrier integrity

as an index for injury in chronically EEG implanted rats revealed that CNS oxygen toxicity, at its early stages (onset of electrical discharges), is not associated with the altered permeability of the cerebral microvessels (54).

Protection

Intermittent exposure to hyperbaric oxygen breathing with air breaks at the same pressure is a technical approach for increasing the total time of exposure to hyperoxia (14). This procedure is routinely used in the clinical setting. Various pharmacological strategies have been tested for postponing hyperoxic-induced seizures:

1. Cerebral vascular modulation. Exposure to HBO_2 results in cerebral vasoconstriction, leading to a decrease in CBF (4, 5, 1), which is later followed by cerebral vasodilatation. It has been suggested that the breakpoint in cerebral vasoconstriction is correlative with the development of hyperoxia-induced seizures (4). Therefore, any pharmacological agent that induces cerebral vasoconstriction may have the potential to protect, or at least postpone, the development of the seizures. Caffeine, a well known cerebral vasoconstrictor postpones hyperoxic seizures in a dose-related manner (55). Two nitric oxide synthase inhibitors, L-NAME and 7-nitroindazole (7-NI), significantly prolong the latent period before onset of seizures on exposure to hyperbaric oxygen or to a hypercapnic-hyperoxic stress (56, 57). This supports the involvement of the L-arginine-NO pathway in the pathophysiology of hyperoxia-induced seizures.
2. Neural activity modulation. On the basis of the clinical manifestations of hyperoxic seizures, several anti-epileptic drugs have been studied. A significant prolongation of the latent period before seizures was demonstrated using carbamazepine (Tegretol) (58). Vigabatrin (Gama vinyl GABA), an irreversible inhibitor of GABA transaminase, successfully protected against hyperoxic seizures in a dose-related manner for prolonged periods of 24 hours or more (59).
3. Enhancement of the antioxidant state. Extensive research had been directed toward defining agents that will protect against oxidative stress by increasing the potential for neutralizing or scavenging the ROS. The results up until now are not very promising. It is possible to find contradictory reports on various antioxidants' abilities to protect against CNS oxygen toxicity. For example, Puglia and Loeb (60) found that two of the most studied antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), were protective, but both failed to exhibit protective effects in studies by Block, et al, (61). Others have shown that when entrapped in liposomes, SOD and CAT inhibit hyperoxia-induced seizures (62). No protection was found in transgenic animals over expressing SOD (63), and no effect was shown with knockout animals regarding pulmonary oxygen toxicity (64). Harabin, et al (65) have shown no correlation between the development of oxygen toxicity with intermittent exposures to hyperbaric oxygen and the levels of antioxidant enzymes.

Metal ion chelators, such as Deferoxamine, were not found to be protective against hyperoxic seizure (66). A variety of ROS scavengers and natural and synthetic antioxidants have been tested, showing different levels of protection in animal models (66-71). The Nitroxide spin traps Tempo and Tempol were found to be effective in preventing CNS oxygen toxicity and efficacy, correlated with their lypophilic properties (69, 70). Beta-carotene from natural origin (*Dunaliella bardawil*) was demonstrated to be among the most effective antioxidants after a one-week diet (71). The lack of dramatic protection against CNS oxygen toxicity by antioxidants is

consistent with an assertion by two ROS pioneers in a recent review article. Gutteridge and Halliwell write, "It was soon clear to many researchers that free radicals did not cause a plethora of diseases, neither were 'spoonfuls' of SOD or vitamins going to modify them, let alone cure them" (72). Deprivation of food or water prior to exposure to HBO_2 significantly prolonged the latent period to the onset of hyperoxia-induced seizures in a dose-related manner (73).

Further Research Areas

While trying to evaluate the current understanding of CNS oxygen toxicity, it is surprising that so much is unknown, or rather, that so little is known. Possible explanations for our limited knowledge in this field could be that HBO_2 is not a physiological signal, CNS oxygen toxicity is not a common disease, and for many years, HBO_2 diving was limited to combat military divers. Under such circumstances, not many research institutes or pharmaceutical companies undertake research or provide financial support for basic CNS oxygen toxicity studies. Many technical difficulties are imposed on experiments in hyperbaric conditions (74), whether simulated chamber exposures or deep-water diving (74). The candidate ROS that are suggested to mediate toxic effects have short half lives and are known to be site specific. This combination makes their real-time measurement a difficult task, especially in hyperbaric chambers. Oxygen toxicity is a multi-organ, multi-system toxicity. Therefore, it is difficult to separate the primary symptom from non-specific after effects. An additional reason for limited research may be the nature of the technological solutions available for avoiding CNS oxygen toxicity. They include using various mixtures with the precise control of oxygen partial pressure for diving and hyperbaric clinical use.

CONCLUDING REMARKS

1. An effort should be directed at establishing the importance and utility of hyperoxia-induced seizures as an experimental model of generalized (grand mal) epilepsy (75). Hyperoxia-induced seizures can serve as an excellent epileptic model since the epileptic agent disappears immediately on reduction of the oxygen pressure, and its toxic manifestations are believed to be reversible. Collaborations among scientists from the basic neurological research (especially the mechanisms of epilepsy) will increase our knowledge and improve our ability to use innovative strategies for the prevention of CNS oxygen toxicity.
2. We must continue to expand research efforts on the basic mechanism of oxidative stress and the role of second messengers in the pathophysiology of CNS oxygen toxicity. This approach will increase the need for more sophisticated methods of multimodal monitoring of physiological and biochemical parameters, such as mentioned by Rogatsky, Shifrin, and Mayevsky (76). A future protection strategy should consider the need for a continuous inflow of antioxidants or scavengers to neutralize the ROS or avoid its development in real time, during the entire period of exposure to HBO_2. That is, a patch that releases a continuous flow or an implanted biological generator of ROS scavengers.
3. An extensive effort must be given to having a personalized, real-time profile for any HBO_2 diver or patient, based on his personal, daily-specific sensitivity to HBO_2, instead of using tables of the time–duration relationship. In our technological era, this can be done with miniature sensors and wireless technology, which will follow the physiological parameters and pre-seizure modifications. The future vision for oxygen diving will be in a tailored CNS

oxygen toxicity monitor, which will assist in allowing each person to have an optimal level of hyperbaric oxygen without being exposed to its toxicity.

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Hyperbaric oxygen therapy: oxygen and bubbles.

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Recompression using oxygen is the accepted treatment for decompression injury. As early as 1877 Paul Bert stated that recompression using oxygen is the optimal treatment for decompression problems (2). He also pointed out that this treatment was very effective for getting rid of the gas from the vascular system but much less effective against paralysis and the serious types of decompression sickness.

A list of some of the treatments that are used presently is shown in Table 1. They vary quite significantly both in pressure and in oxygen content, going from 190 kPa (9 msw,30 fsw) on air to 780 kPa (68 msw, 204 fsw) using nitrox. Treatments to depth of relief or saturation tables are also used. Even if there is considerable clinical experience with other procedures than the standard US Navy Table 6 (280 kPa using oxygen), there is really little published data to support use of the other treatments. However, it is probably true that the available evidence suggests that treatment at depths shallower than 60 feet runs the risk of failure and that treatments deeper than 60 feet offers no particular benefit in the majority of cases (23).

Table 1. Treatments Used Today

- 190 kPa Air or O_2 (In-water recompression)
- 200 kPa O_2 (Boussuges et al)
- 220 kPa O_2 (Comex 12)
- 240 kPa O_2 (Ørnhagen)
- 280 kPa O_2 (USN 5 & 6, Kindwall)
- 400 kPa 50/50 Heliox of nitrox
- 600 kPa air or nitrox (USN 6A, RN 71)
- 780 kPa air and nitrox (USN 8, Hawaian)
- Depth of relief and saturation tables

It is commonly accepted that supplemental oxygen should be administered immediately after a decompression accident, and continued until the patient reaches the hyperbaric chamber. Generally, this will significantly reduce symptoms. A large number of patients will reach the chamber without any symptoms at all (14; 16). Still, there is general consensus that even if no symptoms are observable, the patient should be recompressed.

Studies have shown that while the majority of the commercial divers are treated within fifteen minutes after they develop symptoms, for amateur divers there is a considerably longer

time delay, many hours in most cases (15). Delay in starting treatment may influence results significantly (16), however, it seems that after some hours, a further holdup of treatment does not significantly influence outcome (14; 22). A study by McIver and McIver (15) showed that 61% of the divers who were treated within two hours were fit to dive after one treatment, while only 44% of those treated later were fit.

When gas is formed, the initial effect of the bubbles will be related to volume expansion and the mechanical effects of the bubble surface. Following this, bubbles will initiate a number of secondary biochemical effects, mostly related to inflammation. The secondary effects are not determined by bubbles as such but by how the body reacts to them.

The purpose of the initial treatment is primarily to diminish the mechanical effects of the bubbles, while the later treatments, which I have arbitrarily defined as those performed one to two hours after the insult, is HBO_2 treatment for both primary and secondary bubble effects. This critical time interval is difficult to define, and no one knows how long it is. One consequence of this approach is that it is beneficial for the final outcome to eliminate bubbles rapidly.

So what are these primary bubble effects? If we only consider the vascular bubbles (see later), my opinion is that the bubbles mainly damage the endothelium (18; 19) and only rarely do they lead to flow obstruction and ischemic effects. Total occlusion of flow is probably rare unless excessive amounts of gas are present

There are a number of secondary effects which I will not go through in detail, like activation of leukocytes, aggregation of thrombocytes, initiation of coagulation (21; 26). The body regards bubbles as foreign surfaces and responds to them some time after the gas bubbles have been formed. Rapid removal of the bubbles can perhaps prevent some of these secondary effects.

The question of the location of the decompression bubbles is of some significance. Behnke pointed out that the matter of bubble location was of the greatest importance since, if bubbles form extravascularly in the nervous tissue, any decompression poses the probability of serious consequences (1). It is well documented that bubbles can be observed in the venous system in nearly all decompressions (7; 17). Very little data actually show that there are many bubbles in other tissues, with the possible exception of fat (17) (1). Bubbles are not seen in flowing blood (10). Bubbles are usually formed at hydrophobic surfaces (27), where bubble precursors (nuclei) are stable. Following severe experimental decompressions, we did not observe bubbles in the muscles themselves, but on tendons and fascia (unpublished data).

Our hypothesis is that serious decompression problems, e.g. neurological decompression illness, are caused by the reaction of the body to intravascular bubbles. Bubbles in tissue may be involved and obviously are an issue in musculoskeletal DCS. The main role for tissue bubbles (or stationary vascular bubbles) is the effect on gas dynamics, as they slow down the elimination of inert gas (12). While of possible importance, there is little evidence that is contributes to disease process. One example comes from a study in goats done by Palmer published in 1998 (20). When staining for endothelium in the spinal cord he always saw endothelium surrounding the gas. He could never find gas bubbles that were not inside a vessel. Even if extravascular bubbles can not be wholly discounted as the source for serious decompression sickness, an hypothesis assuming that the main cause are vascular bubbles can be useful and testable.

Bubbles formed on the venous side of the circulation can go through the pulmonary vasculature (24) or through an open Foramen Ovale (25). There is also a potential for bubble formation in the arterial circulation. Several studies have shown that bubbles can be observed on the arterial side before they are seen on the venous side following decompression (1). As early as

1900 it was observed that if animals were killed under pressure and then decompressed, bubbles were found in equal amounts both in arteries and veins. If they were decompressed alive, the bubbles were mainly seen in the veins, indicating that the potential to form bubbles was just as great on the arterial side as on the venous side (11). If we assume that the mechanism of injury in DCI is similar to the one seen following ischemia and reperfusion, then the mechanism for the treatment effect of HBO_2 would be similar to what has been presented at this symposium.

When we use oxygen at increased pressure, it has effects that may influence the actual dosage of oxygen reaching the organs. We did a study in anesthetized pigs, demonstrating that there is a significant increase in shunt fraction in the lung even after five minutes of 100 kPa oxygen and that this fraction increased nearly three times following 200 kPa oxygen breathing (8). It is conceivable that the effect is considerably less in active man.

Another important factor is that oxygen has an effect on nitrogen elimination. In the same study, we looked at nitrogen elimination measured in the central venous blood. We were able to demonstrate that breathing 200 kPa oxygen significantly slowed down the washout of nitrogen by a factor of three (9).

We have performed a series of experimental studies where we have looked at the effect of pressure and oxygen on the elimination of gas bubbles in the pulmonary artery (13). In Figure 1, we see the effect of breathing oxygen compared to breathing air following a standard dive (500 kPa, 40 minutes breathing air, 200 kPa/minute decompression). The maximum amount of bubbles produced was set equal to 100%. Breathing air, the bubbles were eliminated rather slowly. Extrapolating this curve to zero will give an elimination time in the order of three to four hours. If 100 kPa oxygen was breathed, the bubbles were eliminated significantly faster. In this and the following studies, the treatment was started as soon as maximum bubble production was observed, usually 20 – 40 minutes after decompression.

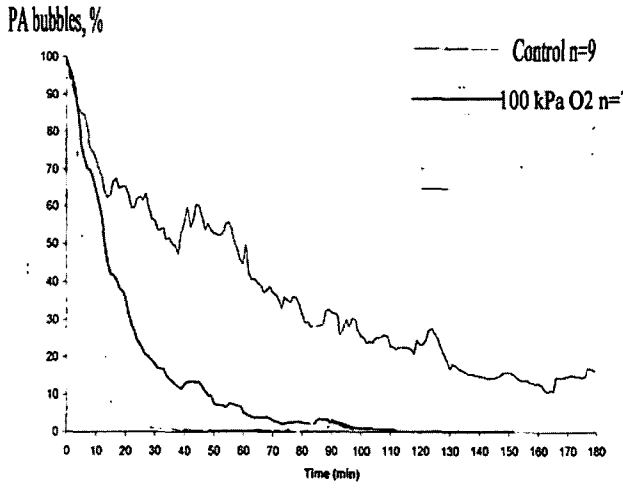
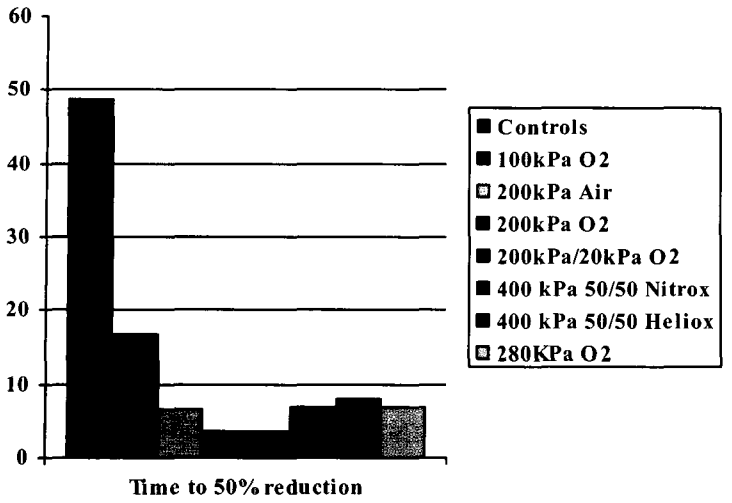


Fig. 1. The effect of air and 100 kPa oxygen on elimination time for gas bubbles in the pulmonary artery.

Following this treatment, only one animal in the air recompression group had any signs of central nervous injury during a one week follow up period (6).

In Figure 2 the effect of various combinations of pressure and oxygen content are compared. If pressure is increased to 200 kPa, the bubbles are removed significantly faster compared both to the control and to the use of 100 kPa oxygen. However, neither the addition of oxygen up to 280 kPa, nor the increase of pressure up to 400 kPa significantly influences the elimination time of bubbles.

Fig. 2. The effect of pressure and oxygen on the elimination of pulmonary artery bubbles following decompression.



In order to try to understand the relative importance of pressure and oxygen on bubble elimination, further experiments were performed. The results can be seen from Figure 3.The effect of compressing to 200 kPa breathing either 100 % oxygen or a nitrogen/oxygen mixture containing 20 kPa oxygen is shown. The time it takes to eliminate the gas is exactly the same.

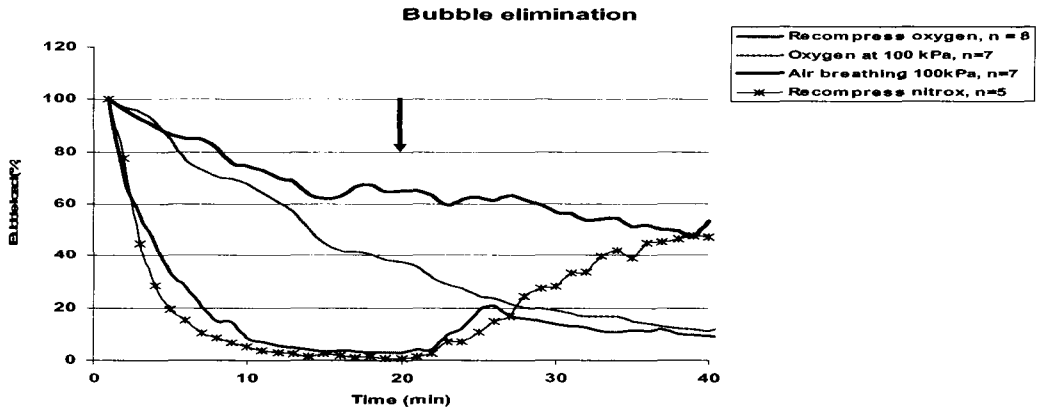
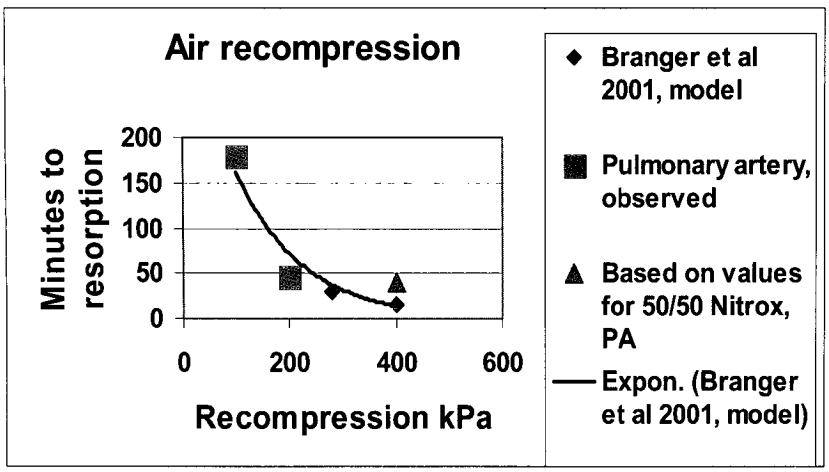


Fig. 3. The effect on pulmonary artery bubbles of recompression to 200 kPa breathing 200 kPa and 20 kPa oxygen. At the arrow, the animals are returned to 100 kPa breathing 100 kPa and 20 kPa oxygen respectively.

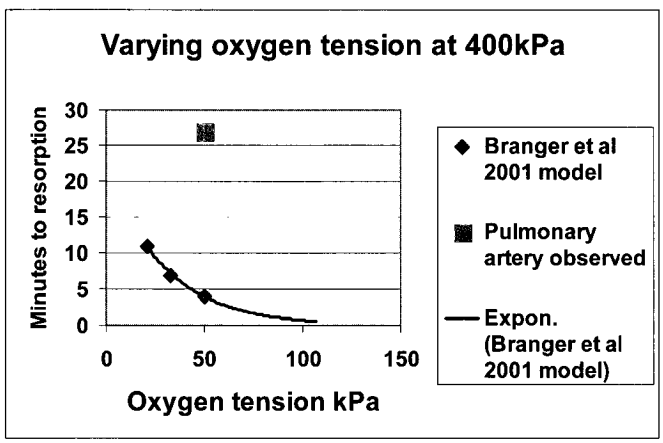
After 20 minutes (arrow) the animals were returned to pressure. Bubbles reappear and the number of bubbles increase until they reach the bubble elimination curve for air breathing and 100 kPa oxygen, respectively. This clearly indicates that it is the pressure that is the main determinant for the velocity whereby the bubbles disappear, while the oxygen increases the rate of elimination of the inert gas.(Brubakk et al, in manuscript)

Fig. 4. Bubble elimination following recompression breathing air. Own data and those of Branger et al.



Our results have been compared to those from Lambertsen's laboratory (3; 4). In Figure 4 we can see a comparison between our data and the ones from Branger et al when air recompression is performed. There is an excellent fit both for the data and their model. If oxygen is added, however, their model predicts considerably shorter elimination times for bubbles than what is actually observed experimentally as demonstrated in Figure 5. Similar results are seen at 280 kPa.

Fig. 5. Effect of oxygen on bubble elimination time. Comparison between own data and model of Branger et al.



These data indicate that increasing pressure to 200 kPa significantly decreased elimination time for bubbles. Additional pressure up to 400 kilopascals will not influence elimination time. Increasing oxygen tension during recompression will have no additional effect on elimination time. However, increasing oxygen tension will increase the rate of inert gas elimination.

Rapid "treatment" of bubbles is actually used commercially in a procedure called surface decompression using oxygen ($SurDO_2$). In this method, the diver is returned rapidly to the surface and then recompressed within 5 minutes to 220 kPa breathing oxygen in a chamber. This method is considered quite safe and effective. However, if considerable number of bubbles are produced during ascent to surface, even oxygen breathing for 68 minutes is not able to eliminate all gas and a considerable amount of bubbles are still present following final return to surface (5).

In 1978 Barnard stated, "It is to be hoped that in the near future we will have at our disposal a system of treatment based on sound theory, on firm experimental evidence, and extensive clinical trials which are flexible enough to suit the many different types of cases which will continue to occur and that this would be a result of our efforts to understand the etiology of the disease and achieve its prevention."

I think we still have not reached this stage and there are many things we do not know. While 100kPa oxygen and USN6 remains the standard method of treatment, I hope that some of

the thoughts and results presented here may initiate further studies and improvement in treatment procedures.

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Oxygen and the diving seal.

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INTRODUCTION

Diving seals are extraordinary animals. They are able to avoid hypoxia and the effects of oxygen deprivation far more efficiently than the vast majority of mammals. One of the kings of the diving world is the elephant seal (*Mirounga angustirostris*). These animals are capable of performing dives of up to two hours in duration (1) and have been recorded diving to depths of 1.5 kilometers (2). Perhaps more impressive are their routine diving behaviors exhibited during the 5 to 8 month migrations to the sea. During the biannual migrations between foraging grounds and the beaches where they moult and breed, these animals spend 80-95% of their time submerged (3). They follow a pattern of long, deep, continuous dives interspersed with brief surface intervals of 1-3 minutes (4).

It was probably in the early '30s and '40s that we really began to understand the physiology behind the impressive breath hold ability of these animals. Per Scholander, Lawrence Irving and their colleagues investigated the physiology of diving in a wide variety of organisms, subjecting them to forced diving protocols and facial immersion (5, 6). Their findings revealed that across species, there are three main physiological responses to facial immersion: 1) apnea; 2) bradycardia; and 3) peripheral vasoconstriction and hyperperfusion of the peripheral tissues. Over time, this triad of physiological events became known collectively as the mammalian diving response. The events that occur during diving are under the control of multiple reflexes, rather than the result of one single reflexive action. Experimentally, these physiological responses can be elicited through facial immersion. In marine mammals, the use of a diving helmet has been as effective as total body immersion in producing diving bradycardia (Figure 1).

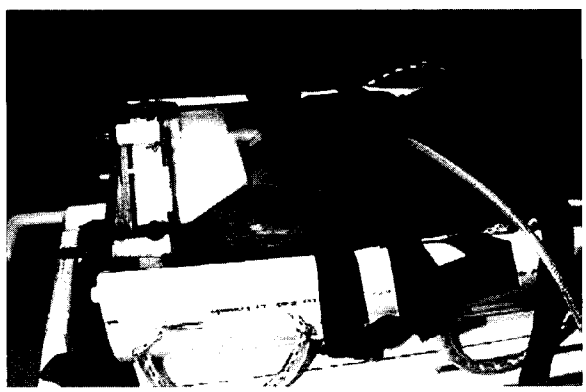

Fig. 1. Northern Elephant Seal in acclimation phase prior to imaging. The foam block situated in the upper margin of the helmet prevents the seal from raising its nostrils into the air pocket formed during exhalation. During acclimation, both valves are in the "open" position and a vacuum hose under the neck seal ensures adequate airflow through the helmet.

Bradycardia

Bradycardia and peripheral vasoconstriction act in concert to allow hypoxia-sensitive tissues such as the heart and brain to receive a constant delivery of oxygen. The dramatic onset

of bradycardia, illustrated in the ECG in Figure 2, was obtained from a captive harbor seal and indicates a 90% reduction in heart rate in the first 30 seconds of the dive.

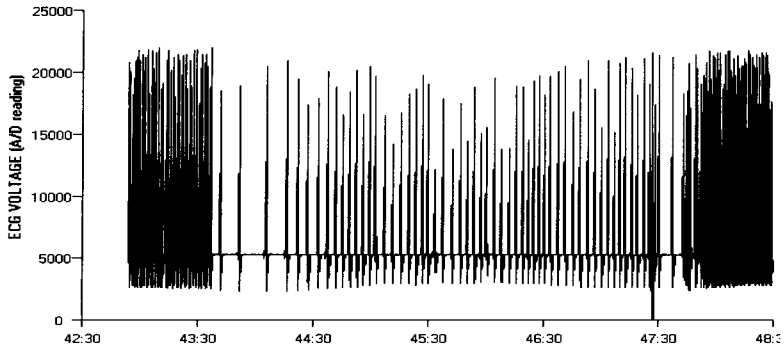

Fig. 2. Electrocardiogram from a experimentally dived harbour seal (***Phoca vitulina***). EKG from a experimentally dived harbour seal Diving heart rate averaged 12 beats per minute. Note the long interbeat intervals in the first minute of the dive (Thornton, unpublished data).

The benefits of bradycardia include reduced cardiac muscle workload (and thus reduced metabolic demand); a reduction in oxygen delivery to hypoxia-tolerant tissues, resulting in reduced oxygen consumption; and a reduction in cardiac output, which assists in maintaining blood pressure when the peripheral arteries are constricted.

Peripheral Vasoconstriction

In the periphery, tissues exhibit a reduced hypoxia sensitivity and are able to function partly or exclusively using localized oxygen reserves. Per Scholander first unraveled the concept of peripheral vasoconstriction by measuring circulating blood lactate levels. Scholander and Irving hypothesized that hypoperfused tissues will eventually have to rely on anaerobic metabolism. Initially, blood lactate levels appeared to tell a different story. Blood samples obtained during diving did not demonstrate an elevation in lactate. Instead, a striking increase in lactic acid production appeared during the *post dive* period. However, by obtaining muscle samples from animals in the predive, dive and post dive state, Scholander demonstrated that a marked increase in lactic acid formation occurs in the muscles during diving, but is not released into general circulation until the animal surfaced. He then hypothesized that a reduction in muscle perfusion during diving is behind the observed pattern of blood lactate (Figure 3).

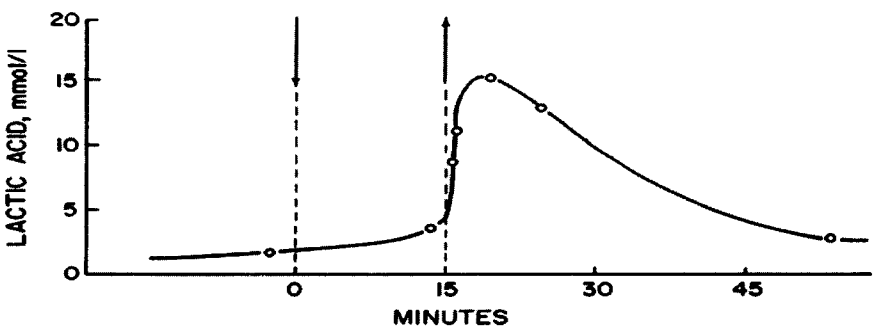

Fig. 3. Lactic acid concentration in the arterial blood of an experimentally dived grey seal *Halichoerus gryphus*. Dive indicated by arrows. (Redrawn from Scholander, 1940).

Hypometabolism

In addition to documenting the mobilization of lactic acid from the muscles of diving animals, Scholander calculated the contribution of anaerobic metabolism to the overall metabolic "debt" incurred during diving. The metabolic cost of diving is difficult to measure, but may be

estimated by evaluating the excess oxygen consumption in the post dive period. Scholander found that the aerobic contribution to diving is often less than the resting metabolic rate; therefore it was thought the balance of energy utilized during the dive would be supplied through anaerobic metabolism. However, the calculated total energy consumption (anaerobic and aerobic ATP production) for the duration of the dive was often below the level of a resting animal.

Studies on terrestrial animals have shown that there exists a linear relationship between blood flow and oxygen consumption at both the cellular and organism level (7, 8). This relationship holds true over a wide range of activity, suggesting that reduced perfusion results in an overall suppression of metabolism. It is likely, albeit difficult to demonstrate, that seals experience a significant reduction in overall metabolic rate related to peripheral vasoconstriction.

Morphology

The seal's physiological arsenal for the fight against hypoxia is supplemented by a number of morphological characteristics. Seal muscle is rich in myoglobin, containing 5-12 times the amount found in human muscle. Seals have a higher circulating blood volume and a higher resting hematocrit than terrestrial organisms. With a total blood volume in the range of 15% of body mass (human blood volume is ~5-7% of body mass), a considerable increase in oxygen storage is realized. During diving or periods of apnea, a significant and rapid rise in circulating red blood cells is observed (9, 10, 11; Figure 4). This variation in red cell mass indicates that seals have some method of sequestering red cells during non-apneic events. It was widely suspected that the source of these cells was the spleen.

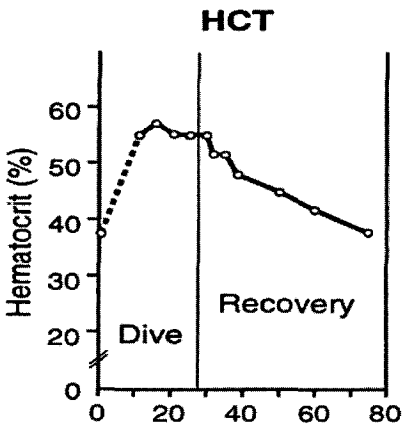

Fig. 4. Changes in hematocrit. Figure 4 illustrates changes in hematocrit during diving and recovery in a representative dive in the Weddell seal. After Hurford et al, 1995.

The Pinniped Spleen

Anatomical observations of seals dating back to the 1800s consistently remark on the size of the spleen. Autopsy data indicate that the spleen is approximately 1% body mass in the large seal species (Weddell, northern and southern elephant seal). As the spleen is composed of a smooth muscle capsule, which may contract at the time of death, these data most likely underestimate the working volume of this organ. Histological studies reveal that seal spleens are capable of sequestering significant quantities of red blood cells and possess contractile properties in both the smooth muscle capsule and the internal structural cells (12). In Weddell seals, epinephrine injection was followed by an increase in hematocrit and a decrease in splenic volume, as measured by ultrasound (13). As increased catecholamine levels are observed during diving in seals, a correlation between diving, splenic contraction and increased hematocrit seems likely. Although much evidence points toward the splenic role in diving, no measurements have been obtained during a dive.

At this point the field of diving physiology was dependent on indirect evidence. We began searching for methods that would allow for direct interrogation, and turned to magnetic resonance imaging (MRI). Now, if you've ever seen an elephant seal you will realize that this was a bit of a leap of faith, but through the collaborative efforts of this diverse group of individuals, we were, for the first time, able to view the physiological changes that occur during diving. In conjunction with the University of California Santa Cruz's elephant seal group, physicists from Stanford University's Center for MR Imaging, and physiologists from University of British Columbia, this project came to fruition (14). Five juvenile elephant seals were collected from Año Nuevo State Reserve (National Marine Fisheries Service Marine Mammal Permit # 786-1463) and were held at Long Marine Laboratory, UCSC for up to 8 days. The seals were released at the site of capture at the conclusion of the study. Images were obtained from 5 seals over 24 simulated dives. Facial immersion was achieved by slowly filling the helmet through the top valve and simultaneously closing the drain valve on the bottom of the helmet (Figure 5). Images were obtained before the dive (baseline splenic volume), sequentially during the dive (initiated as soon as the animal's nostrils were submerged) and continued until the helmet was drained and the animal took its first breath. Post dive times were recorded from the first breath and post dive imaging began 1 minute post dive. Images obtained between 15 and 20 minutes post dive were considered baseline.

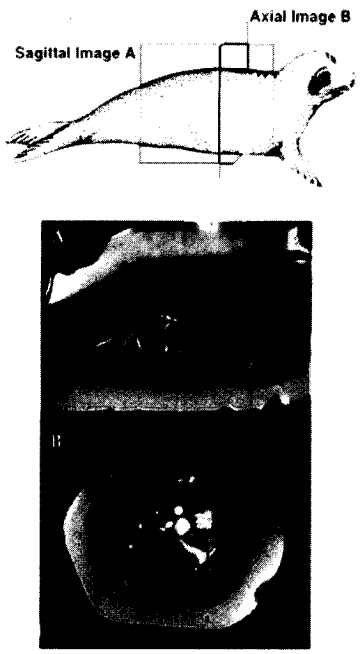

Fig. 5. Sagittal and axial images of the spleen of a northern elephant seal pup. Sagittal localizers were used to define the upper and lower image slice location and calculation of the number of axial slices required to image the total spleen. A series of 29-34 1.5 cm "slices" were used to image the organ completely, requiring less than a minute of scan time. Image A is from 5 cm left of the midline (spine) and image B is 8 cm below the diaphragm.

The most striking observation from these images is the rate at which the spleen contracted. By dive minute 3, the spleen had reduced to approximately one-fifth of resting volume and remained contracted for the duration of the dive (Figure 6).

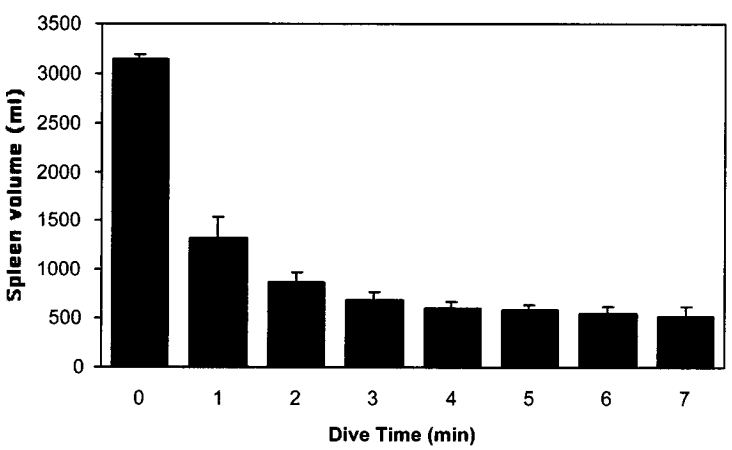

Fig. 6. Northern elephant seal spleen volume during rest. Northern elephant seal spleen volume during rest (Min 0) and diving (Min 1-7) was obtained using MR imaging techniques (n = 5, each individual's value is the average of four dives). Splenic volume does not decrease significantly after minute 2 (ANOVA, $F_{(6,28)}$= 33.94, P < 0.0001; Tukey Kramer HSD, P = 0.05). Error bars indicate SD. Thornton et al, 2001.

These data clearly support the existence of a diving-induced sympathetic contraction of the spleen and subsequent release of the stored erythrocytes; however, a discrepancy exists in the timing of splenic contraction and the rise in

circulating hematocrit. Complete splenic contraction occurs within 3 minutes of catecholamine stimulation, yet peak Hct is not observed until 15-25 minutes after the spleen has contracted (11, 13).

The second defining observation of this study was the appearance of a fluid-filled structure within the abdominal cavity: the hepatic sinus (Figure 7). Formed by the dilation of the hepatic veins, the thin walled sinus lies caudal to the diaphragm, draining from its midpoint through the diaphragm and into the thoracic portion of the posterior vena cava. The inferior vena cava and the hepatic sinus may contain up to one fifth of the animal's total blood volume and is a significant storage depot of oxygenated blood during dives.

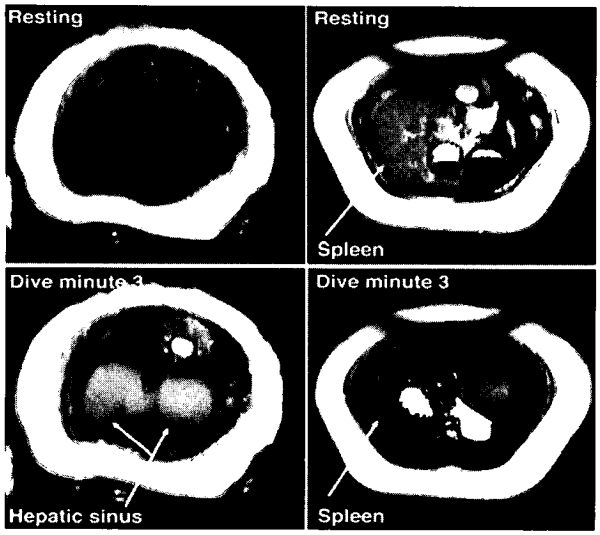

Fig. 7. Thoracic images of a northern elephant seal during rest and diving. Images on the left are from the region immediately caudal to the diaphragm; images on the right are 12 cm caudal to the diaphragm. Rapid contraction of the spleen and simultaneous filling of the hepatic sinus are observed. After Thornton et al, 2001.

Filling of the sinus is dependent on the closure of a muscular vena caval sphincter located on the cranial aspect of the diaphragm. In experimental dives using harp seals (*Pagophilus groenlandicus*), Hol et al (15) reported a marked constriction of the sphincter occurred 20 seconds after commencement of the dive, with dilation of the posterior caval vein and hepatic sinuses occurring before as well as during the 40 seconds following constriction. They also demonstrated a temporary relaxation of the caval sphincter during the dive and subsequent mixing of the blood in the sinus with that returning from the anterior part of the body.

The interplay between the spleen and hepatic sinus serves to explain a number of observed physiological events. Although the spleen has long been suspected as the source of the RBCs released during diving, the rate of splenic contraction has presented an apparent contradiction to the gradual diving-induced rise in Hct. In this study, maximal Hct occurred after the 7 minute dive had concluded, whereas the spleen had released the majority of its RBCs by Dive Min 2. The involvement of the sphincter-controlled sinus serves to delay the release of RBCs into general circulation and may abrogate the potentially deleterious effects of an acute rise in red cell mass. In northern elephant seal pups, contraction of the spleen in the first minute of the dive would result in an increase in vena caval blood volume at a rate of 23.6 ml/second (Min 0 to Min 1 decrease in splenic volume = 1417 ml/60 sec). Relocating the RBCs from the spleen into the sphincter-controlled venous sinus results in a gradual metering of oxygenated RBCs into the heart, protecting it from a drastic increase in right ventricular pressure at a time when diving bradycardia is most profound.

From the evidence presented herein, it appears that the system works as follows: facial immersion causes stimulation of the trigeminal nerve, leading to vagal stimulation, bradycardia, peripheral vasoconstriction and caval sphincter contraction. Circulating catecholamine levels rapidly increase, resulting in splenic contraction and the maintenance of peripheral vasoconstriction. The oxygenated RBCs of the spleen are then released into venous circulation. Venous blood returning to the heart is prevented from passing cranially through the diaphragm

by the occlusion of the sphincter, causing the hepatic sinus to fill. As the dive progresses, red blood cells are gradually metered out into general circulation via relaxation of the caval sphincter (Figure 8).

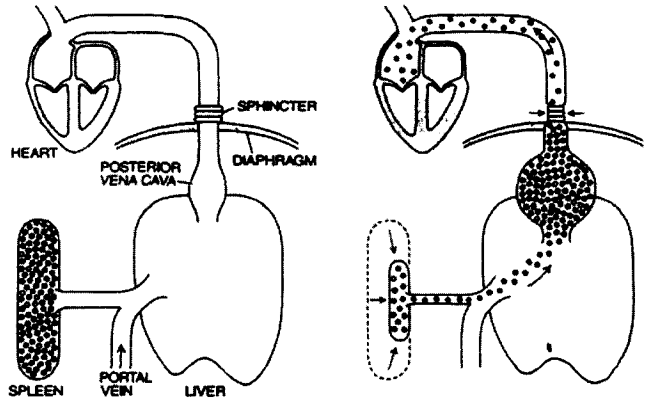

Fig. 8. Oxygen-rich blood cells released from the spleen. Oxygen-rich red blood cells (RBCs) are released from the spleen during contraction when the animal dives. The caval sphincter constricts the venous return to the heart, causing an expansion of the hepatic sinus. As the dive progresses, the oxygenated RBCs are slowly metered out into circulation via relaxation of the caval sphincter. After Zapol, 1987.

In 1987, Warren Zapol speculated on the interplay between the spleen and hepatic sinus, and these data essentially support his supposition (16). Zapol equated the seal spleen to a "SCUBA tank", providing the animal with continuous supply of oxygenated RBCs as the dive progressed. Based on this elegant system of storage, transfer, and metering of RBCs, it appears that the spleen does indeed function as a SCUBA tank, and increases the fitness of the species through elevated oxygen stores, increased dive time, and thus increased foraging success, predator avoidance and efficiency of locomotion.

Diving Adaptations

The field of comparative physiology was eager to label the spleen as a morphological SCUBA tank; a trait specifically adapted to an aquatic environment. Logically, we argue that you and I do not have a large spleen, nor do we possess a caval sphincter or hepatic sinus. And also obvious is our inability to perform substantial breath hold dives.

However, there are a number of caveats when labeling the spleen as an "adaptation to diving." The field of evolutionary physiology has become more rigorous in its definition of adaptation, requiring that the feature be a product of natural selection in the true Darwinian sense, provide an increase in the fitness of the bearer, and exhibit complexity and purpose. In order to evaluate whether the spleen is an adaptation to diving, we had to establish the purpose for which it was selected. An accepted means to establish evolution of a trait is to evaluate the structure and function of the trait in closely related species. In phocid seals, comparisons between species of varying diving ability should reveal traits that correlate with increased maximum dive time.

A fundamental problem exists when comparing related species: the closer the phylogenetic relationship, the more likely they are to exhibit similar traits. To remove the factor or relatedness and allow for the examination of each species as an independent data point, we use a phylogenetically independent contrast analysis. A study conducted by Mottishaw et al (17) examined a number of traits that have been traditionally referred to as "diving adaptations". This process allows us to take away the factor of "relatedness" and look at each species as an independent data point. The transformed data (standardized independent contrasts) may then be used in ordinary statistical procedures.

In order to stay submerged for a longer period of time, an air-breathing mammal must either increase the amount of oxygen carried within the body, or decrease the amount of oxygen

used. This study examined up to a maximum of 17 phocid and 15 otariid species, evaluating factors that would potentially extend diving time: blood volume, body mass, hematocrit, maximum bradycardia and splenic volume (Figure 9).

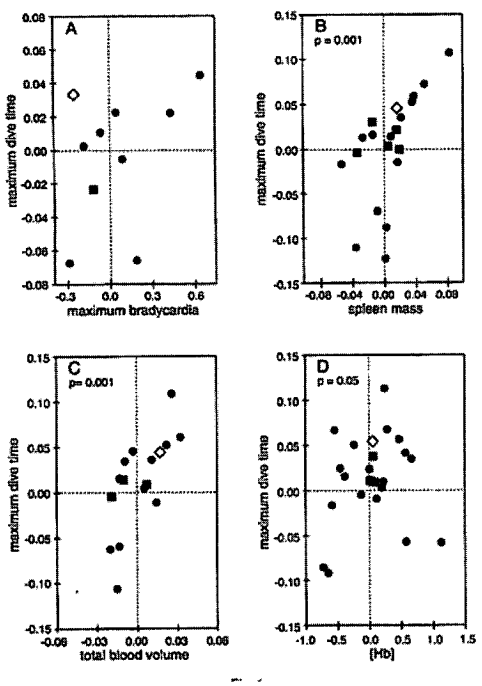

Fig. 9. Correlation of residuals. A. The correlation of residuals generated by regression of log maximum dive time contrasts and maximum bradycardia (not statistically significant; P=0.15). B. Significant positive correlation between residuals generated by regressions of log maximum dive contrasts and log spleen mass contrasts on body mass contrasts (r=0.69; P<0.001). C. Significant positive correlation between residuals generated by regressions of log maximum dive time contrasts and log total blood volume contrasts on log body mass contrasts (r=0.74, P<0.001). D. Significant positive correlation between residuals generated by regression of log maximum dive time contrasts and [Hb] contrasts on log body mass contrasts (r=0.46, P=0.05). In all graphs, circles (●) represent contrasts within the phocid species; squares (■) represent contrasts within the otariid species; and the diamond (◊) represents the root node, or contrasts between phocids and otariids. After Mottishaw et al, 1999.

As expected, body mass was positively correlated with diving ability, as the more oxygen you carry, the longer your expected dive duration. Also, the larger the organism, the lower the mass specific oxygen consumption; therefore, dive duration should be longer. Analysis of spleen size, blood volume and blood hemoglobin revealed similar positive correlations. In order to remove the influence of body size on each variable, residuals were generated and were then regressed on the residuals of each physiological or morphological variable; therefore, these correlations are independent of body mass. Again, the data seem to support the categorization of these characters as adaptations to diving.

About a year after we published this paper (17), I began to look at these correlations in a more rigorous light. The correlated evolution of these traits does not prove that they have been selected specifically for the task of increasing dive time. To evaluate the functional significance of a trait, the physiology must be examined in context with the life history of the animal. We have clearly demonstrated that splenic contraction accompanies facial immersion, and the larger the spleen, the greater the breath hold ability. In the wild, elephant seal pups leave the beaches of Año Nuevo in the spring and immediately enter into a continuous diving bout, averaging 20 minute dives and 1-3 minute surface intervals. The brevity of the surface interval illustrates a substantial paradox: although the spleen contracts and releases its contents into circulation within three minutes of submergence, in the post-dive situation the spleen takes approximately 20 minutes to passively refill. If the same physiology occurred in a free diving elephant seal pup off the coast of California (and there is no reason to believe it doesn't), a 1 to 3 minute surface interval is not sufficient to resequester the red blood cells and reduce hematocrit to resting levels. Therefore, if the spleen contracts at the beginning of a dive bout (which may last up to 8 months!), but is unable or unlikely to refill due to short surface intervals, is the spleen actually serving a useful purpose during diving? In fact, if we splenectomized a seal, would it affect the animal's diving ability?

The answer is *probably not*. The benefit of the spleen is its ability to release RBCs, which increase the oxygen carrying capacity within the vascular system during submergence. As it was previously thought that the spleen supplemented circulating blood with oxygenated RBCs during the dive, the inability to resequester RBCs during the surface interval brings this role into question. Elevated hematocrit throughout a diving bout has been observed in phocids both in the field (Weddell seals; (9, 11)) and the laboratory (northern elephant seal pups; (18)), lending further evidence to the lack of participation of the spleen during an extended diving bout. In fact, decreasing the circulating blood volume during the surface interval would delay replenishment of tissue oxygen stores and removing metabolic byproducts from the periphery, therefore any selective pressure for rapid resequestration of RBCs would be minimal. The question then becomes what is the selective pressure acting upon organogenesis and influencing spleen size in a diving pinniped? It has been suggested that these animals may utilize the spleen to sequester RBCs when the benefit of increased oxygen is offset by the cost of transporting blood of higher viscosity (19). In light of the time it takes to remove the supplemental RBCs from circulation and return them to the spleen, this alternate explanation has significant merit.

During diving, periods of high hematocrit are accompanied by vasoconstriction and bradycardia. The combined effect of these events would serve to decrease shear rate and result in elevated viscosity. Upon returning to land, these animals would experience a dramatic and prolonged increase in heart rate, leading to deleterious viscosity effects on the vascular system. Based on the data presented here, it appears that the spleen allows the seal to maintain a higher circulating hematocrit during periods of hypoxia, yet effectively reduce hematocrit and circulating blood volume when oxygen is not limiting, thus avoiding any deleterious effects of increased blood viscosity. In essence, the spleen is more likely to be an adaptation to the terrestrial portion of a pinniped's live history than a character that increases diving time.

Bradycardia Revisited

Although many of the morphological features related to oxygen storage correlated with diving ability, the PIC analysis revealed that bradycardia, often referred to as the key to the mammalian diving response, did *not* correlate with maximum dive time (Figure 10). In fact, the lowest heart rates found in the field show little variation within the pinniped species and are similar to those observed during forced dive studies.

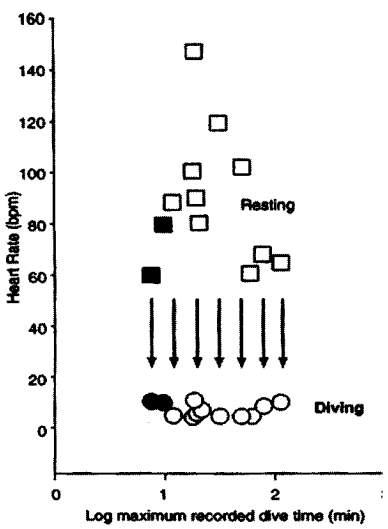

Fig. 10. Relationship between maximum diving duration and degree of bradycardia expressed in several species of sea lions and seals. It is evident that all of these species, both short and long duration divers, are able to activate bradycardia to the same extent (minimum heart rate 4-10 bpm). There is no statistically significant relationship between maximal bradycardia and dive duration. (After Mottishaw et al, 1999).

This surprising result contrasts with the prevalent view of bradycardia as an obvious adaptation for diving. To clarify, we agree that bradycardia is likely an integral component of a seal's diving ability; however, these results suggest that it is an ancestral and conserved trait, not specifically acted upon by the process of natural selection with the purpose of increasing breath hold ability. In other words, it is not an adaptation to diving, but is instead a hard-wired response found throughout

air-breathing vertebrates, possibly as an asphyxial defense mechanism. Indeed, within my own experience during 2nd year physiology labs, I have recorded students' heart rates in the 10 -12 bpm range during facial immersion, yet the maximum breath hold ability for humans (at least, for 2nd year physiology students!) averages less than one minute. Clearly, diving ability is more complicated than mere heart rate reduction.

A recent study by Elliot et al (20) from the University of British Columbia serves to further downplay the role of bradycardia in phocid diving ability. During voluntary diving in an 11 m deep tank, the cardiovascular responses to submergence of five harbour seals were manipulated using specific pharmacological antagonists, and the effects on diving behaviour were observed. Using a muscarinic blocker methoctramine, (diving bradycardia); the α-adrenergic blocker prazosin (diving vasoconstriction); and the β-adrenergic blocker metoprolol (post-dive tachycardia), they assessed the necessity of diving bradycardia, vasoconstriction and surface tachycardia in the performance of short dives and short surface intervals in harbour seals.

None of the pharmacological blockers had any effect on average dive or surface interval duration, and seals maintained a high percent dive time in all treatments (Figure 11). Thus, it appears that during short dives, harbor seals do not need to invoke bradycardia or peripheral vasoconstriction in order to maintain an efficient dive strategy.

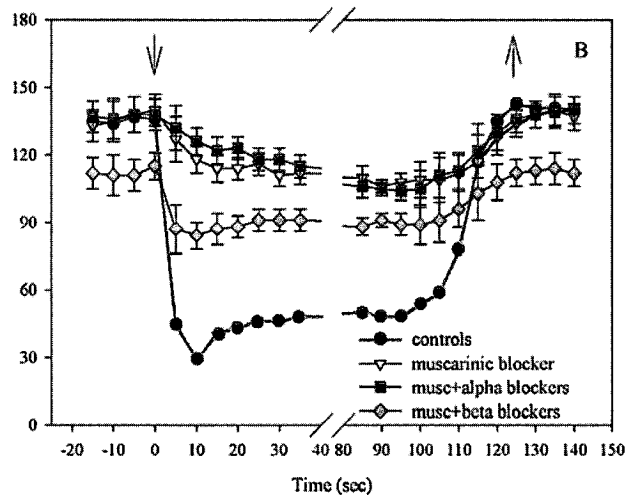

Fig. 11. Heart rate profiles.Heart rate profiles before, during and after voluntary dives in muscarinic-, muscarinic plus α-adrenergic-, and muscarinic plus β-adrenergic-blocked seals. Arrows denote the beginning and end of the dive. Each data point represents the mean heart rate for the preceding 5 s interval. For each treatment, mean heart rates during two dives (approximately 120 s) were averaged for each animal. Data from all seals (N=5) were then combined to give the means (±S.E.M) illustrated. The data were normalized so that dives of different lengths ended at the same time. (After Elliott et al, 2002).

Most of the original data on bradycardia was derived from forced diving experiments, complicating the physiological response to facial immersion with stress and fear response. Over the last 20 years we have witnessed significant advances in microprocessors and the scientific literature is rife with data from deployments on a vast array of diving animals. Measurements of velocity, swim speed, depth, and heart rate indicate that the classic mammalian diving response occurs in freely diving animals, but is more plastic and variable than what we have witnessed in the laboratory. Although the profound and dramatic response to facial immersion was somewhat abrogated in the field diving situation, the data from these animals were dramatic in their own right, documenting a life of underwater existence for months at a time, punctuated by surprisingly brief moments on the surface. For an air-breathing mammal to spend 4 to 8 months at sea, and 90% of this time submerged, one cannot fail to be impressed.

The plasticity of the bradycardic response brings into question how these animals modify their heart rate in response to various situations. This particular trace (Figure 12; (21)) shows a depth pattern of an animal diving at about 100 meters when it begins to ascend.

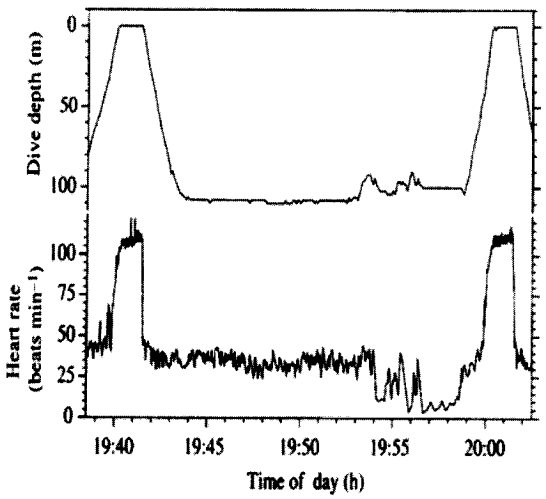

Fig. 12. Dive depth and instantaneous heart rate record from a northern elephant seal. This trace details a single dive. Note that, after ascending for one minute at 19:53:39h, the seal reversed direction. An abrupt decrease in heart rate accompanied the descent. This pattern was repeated 2.5 min later. After Andrews et al, 1997.

After a brief period of ascent, the depth meter indicated a reversal of direction and the animal began to descend, possibly in response to sighting a predator or school of fish. Correlated with that descent was a dramatic bradycardia. This animal's heart rate dropped from approximately 35 bpm down to 4 bpm. The pattern of ascent and descent continued, with concomitant changes in heart rate. Data such as these caused us to speculate on the animal's cognitive control over the diving response.

Cognitive Influence

To examine the cognitive influence on diving bradycardia, we raised 10 harbour seal pups from three weeks of age and using operant techniques and trained them to dive on command for various durations. Heart rates during one-minute apneic periods (slow wave sleep, awake, free dive, trained dive, and forced dive) were recorded and preliminary data are documented below. As expected, animals exhibited the highest heart rate during slow wave sleep, assumed to be the state of lowest cognitive input. Seals normally breathe in an apneustic pattern, pausing on the inhalation for minutes at a time, then exhibiting a rapid breathing pattern before initiating another period of apnea. Heart rates during 1 minute "awake" apneas (55 sec to 1 min 5 sec) demonstrated a greater degree of suppression over sleep apnea. The addition of facial immersion stimuli (i.e. apnea during diving) caused a further decrease in heart rate. Although quantification of "motivation" in a diving seal is impossible, the assumption is that a freely diving seal is less motivated to exhibit a profound bradycardia than a trained or forcibly dived seal. The data reflect this assumption, demonstrating an increase in bradycardia with decreasing control over the dive. In the forced dive situation, the animals were not exposed to any signals or visual cues as to the duration of the dive, therefore we hypothesized that a maximum bradycardic response would be exhibited to aid in survival of what was an unknown period of submergence.

In an attempt to quantify diving "motivation", the seals were trained to recognize two targets: a white circle indicating a short (1 min) dive, and a black square, signifying a long (>5 min) dive. The animals were also trained to surface into a respiratory dome at the completion of the dive. Catheters placed in the extradural intravertebral vein allowed for assessment of lactate. This system enabled the assessment of both the anaerobic and aerobic contribution to diving. In addition to physiological measurements, dives were recorded on video to assess differences in activity in response to the dive duration (Figure 13).

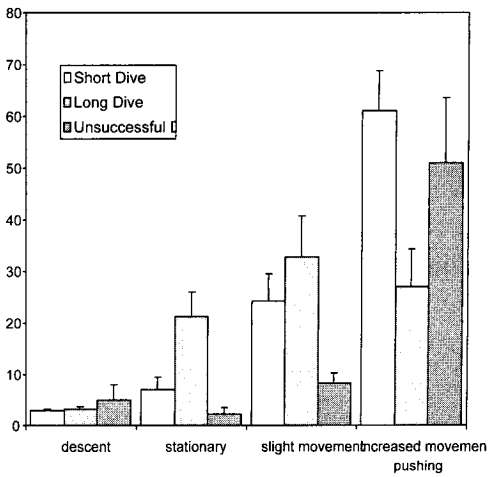

Fig. 13. Dive Assessments. Activity during short (1 min), long (>5 min) and unsuccessful (aborted long) trained dives (mean ± SEM; n=6). Short dives are not significantly different from unsuccessful dives in any category (ANOVA, $p<0.05$; Tukey Kramer HSD, a=0.05).

The proportion of time spent in each behavior category varied significantly with the type of dive. When the animal was presented with a short dive target, it would comply by placing its nose to the target and submerging with it to the bottom of the pool. Presentation of the long dive target often resulted in reduced compliance, departure from the pool or training area, or departure from the target once submerged. The behavior during a successful long dive was noticeably different, with a "settling in" period, followed by a reduction in activity for the duration of the dive. Only the first minute of each dive was used for analysis (i.e., short dive activity is compared with the first minute of a long dive).

One interpretation of these data is that in the first part of the dive, the animal makes a "decision" as to whether it will commit to the long dive. In an unsuccessful dive (a long dive aborted within two minutes of submergence), activity remains high and the animal never achieves the "settling in" that is so apparent from the videos of successful long dives. However, one may also hypothesize that the animal has expended too much energy in the first minute and is physically unable to perform a dive of long duration (unlikely, as the oxygen stores in these animals are sufficient to support aerobic dives of ~8-10 minutes).

Metabolically, these dives were aerobic in nature. In both long and short dives, diving and postdive blood lactate levels were not elevated over predive levels (as these dives are within the aerobic diving limit of this species, these data are consistent with our expectations). However, the post-dive oxygen consumption data were very interesting, suggesting that short dives resulted in diving metabolic rates that were in excess of resting metabolic rate. When the excess post-dive oxygen consumption is averaged over the duration of the dive (Figure 14), the data indicate that somewhere between a one minute and a five minute period of submergence, a dramatic suppression of metabolism was occurring. Although intriguing, these experiments were unable to shed further light on the mechanism of metabolic downregulation that occurs in a diving animal.

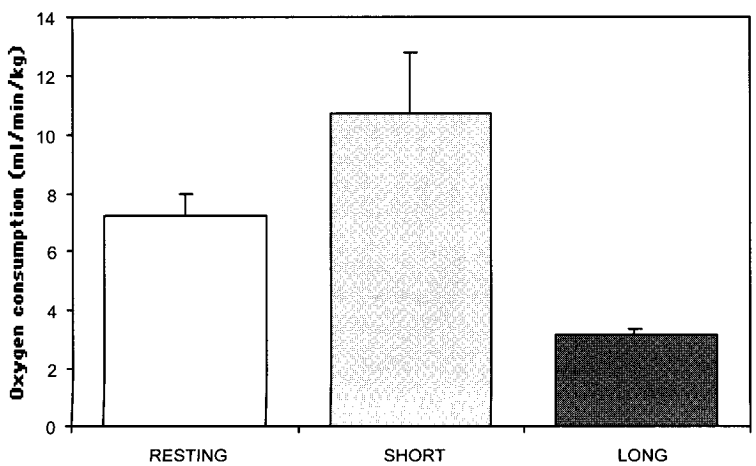

Fig. 14. Diving metabolic rate of harbor seals. Diving metabolic rate (ml O_2/kg/min) of harbor seals during resting (in water) and after long (>5 min) and short (1 min) trained dives (mean ± SEM; n=6). The quantity of post dive oxygen in excess of resting (predive) rate was assumed to be the aerobic cost of diving. Mass-specific oxygen consumption is significantly lower during long dives than short dives (t-test; P=0.05).

Phosphocreatine

For a more precise, real time analysis of diving metabolism, we turned again to the high-tech world of magnetic resonance and employed a technique called 31-phosphorous magnetic resonance spectroscopy (^{31}P MRS). This allowed us to interrogate high-energy phosphate flux within the muscle of northern elephant seal pups during a dive. Using the diving helmet described earlier, each seal was subjected to 3 to 5 dives of 8 minutes in duration. Data were acquired using a transmit/receive surface coil (diameters 20 cm and 10 cm respectively) placed on the dorsal surface of the animal. The *longissimus dorsi* muscle was evaluated, as it is the largest muscle involved in phocid locomotion.

The hydrolysis of phosphocreatine (PCr) is coupled to glycolysis through ADP and ATP, and through H^+ (formed during *in vivo* glycolysis) according to the equation,

$$H^+ + PCr + ADP \leftrightarrow ATP + Cr$$

PCr acts as an ATP buffer, maintaining cellular ATP levels when the rate of oxidative phosphorylation is insufficient to meet the short-term requirements. PCr hydrolysis may also be caused by an increased proton load, so if the tissue is affected by respiratory or metabolic acidosis, a decline in PCr may occur.

In diving seals, it has been assumed that muscle hypoperfusion causes a reliance on myoglobin-associated oxygen stores, with anaerobic compensation occurring when tissue oxygen stores are near exhaustion. We evaluated MRS-visible metabolite concentration changes that occur *prior* to tissue oxygen depletion (i.e. within the aerobic diving limit) to assess the hierarchy of metabolic pathways within ischaemic muscle tissue, therefore we did not expect to see significant hydrolysis of PCr in the quiescent muscle of a diving seal.

The results, however, were surprising. End dive PCr values from individual dives ranged from 36.27 – 116.63% of predive values. In 4 animals, PCr declined continuously from Dive Min 1 through 7. These animals should not be exhibiting elevated metabolic rates, as have sufficient myoglobin in their muscles for at least 12 minutes of aerobic metabolism in a completely ischaemic muscle. Yet if we assume that this PCr decline is driven by adenylates and calculate the muscle metabolic rate based on PCr hydrolysis, these seals are actually sprinting! I can assure you they were not sprinting when lying in the magnet; therefore we had to examine alternate explanations.

In the individuals which showed a decline of PCr (and not all animals did), there was a strong positive correlation with intracellular pH. Calculations involving potential sources of proton load indicated that a rise of this magnitude could only be caused by anaerobic metabolism and lactic acid formation. Now we have an animal which was assumed to be quiescent and aerobic, but the PCr data indicated a system that is either highly metabolic or producing copious amounts of lactate. Things were getting even more confusing.

When the data were considered in light of the pH values, a possible explanation emerged. If metabolism were to be inhibited at the mitochondrial level, an accumulation of the products of glycolysis would occur. Pyruvate, in the presence of lactate dehydrogenase, would be converted to lactic acid in the muscle. This mechanism operates under both aerobic and anaerobic glycolytic function (22, 23), would result in lactate accumulation in both tissue and blood, and could explain the elevated proton load in the muscle. However, blood samples obtained from five animals showed no significant increase in blood [La^-] during the dive or in the postdive period.

The presence of elevated [H^+] without a concurrent increase in blood [La^-] is possibly due to lactate recycling to glycogen or lactate oxidation to CO_2 and H_2O within the muscle. It has been assumed that the anaerobic contribution to energy production during diving would be

minimal until tissue oxygen stores are depleted. However, our data suggest that significant lactate production occurs *prior* to muscle oxygen depletion and must occur concurrently with oxidative phosphorylation. In an ischemic muscle, glycolytically generated H^+ would be trapped within the tissue and although buffered, would contribute to the observed pH drop and a shifting of the creatine kinase reaction to the right. In the muscle of a diving seal, the rate of lactate formation may be high enough to account for an elevated $[H^+]$, but low enough to allow for complete further metabolism of lactate to glycogen or to complete oxidation during the course of a dive.

Metabolic Downregulation: Future Directions

This led us to think about possible mechanisms of metabolic downregulation that may occur at the cellular level. To evaluate the mechanisms behind metabolic downregulation in a diving seal, my recent research has moved away from whole animal work and is now focussing on mitochondria.

Recent discoveries in the field of metabolic control indicate that mammalian tissues are not efficient consumers of oxygen. Oxygen is required in order to create the electrochemical gradient of protons across the mitochondrial inner membrane. These protons are then channelled through a chemiosmotic pump termed an ATPase, which is analogous to a hydroelectric dam. Following the same principle, if there are leaks in the dam, energy is lost and the system becomes less efficient. A futile cycle of outward proton pumping and inward proton leak occurs across the membrane, resulting in both heat production and the imperfect coupling of oxygen consumption to ATP synthesis. This proton leak is thought to account for 15-20% of standard metabolic rate (SMR) and represents a biochemical "inefficiency" in the system (24). There are many suppositions as to why this inefficiency exists, but at this time a definitive answer has not been achieved.

Mammals are also are quite inefficient at the cell membrane level. Tony Hulbert and Paul Else (25-28) investigated the cellular metabolic differences between ectotherms and endotherms and discovered that ectotherms are more metabolically efficient. They exhibit a reduction in ionic leak through their cells by maintaining "tight" cell membranes. Mammals, on the other hand, have a greater degree of leakiness in our cell membranes. In order to maintain membrane gradients, our pumps are constantly working overtime and as a consequence, energy (in the form of heat) is released. Hulbert and Else hypothesized that the reason why mammalian cells leak is for the purpose of heat production and the maintenance of endothermy. They found that the factor that correlated most strongly with the leakiness of the membranes is the degree of polyunsaturation in the lipid bilayer.

Up to 60 to 80% of the metabolic rate in a mammalian liver cell is due to maintaining the membrane gradient. The ability to decrease both cellular and mitochondrial leaks during times of reduced oxygen availability would provide a means of reducing oxygen consumption without sacrificing cellular performance. In perfused rat skeletal muscle, 50% of the resting respiration is attributed to proton leak (29). In a Weddell seal, muscle accounts for 35% of total body mass; therefore a reduction in proton leak could result in a significant decrease in whole animal oxygen consumption during diving.

To evaluate the effect of the diving environment on mitochondrial respiration, I am heading down to Antarctica to obtain muscle biopsies from Weddell seals. In a diving animal, vasoconstriction of blood vessels in the periphery, combined with conductive heat loss in water result in >10°C temperature drop in the muscle bed (30). In Weddell seals, muscle temperature

has not been measured, but the conductive heat loss caused by immersion in -1.8°C water, combined with significant peripheral vasoconstriction due to the mammalian diving response, are likely to result in dramatic reduction in temperature at the muscle-blubber interface (Figure 15). By subjecting seal muscle cells to reduced temperatures and increased atmospheric pressures, the effects of the diving environment on cellular respiration may be obtained. The second phase of this study involves measuring proton leak under similar conditions, thus enabling the quantification of mitochondrial efficiency at the cellular level.

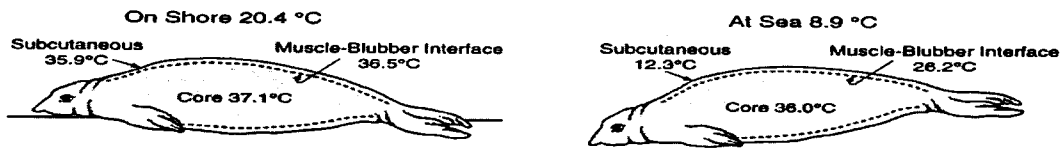

Fig. 15. Diagrammatic summary of temperature profiles of northern elephant seal. Diagrammatic summary of temperature profiles in the body of a northern elephant seal at rest in normoxia, normothermia (on shore) contrasted with regional heterothermy observed during diving at sea, assuming an average water temperature of 8.9°C (after Andrews, 1999).

A number of other factors may relate to mitochondrial efficiency, one being uncoupling proteins (UCPs), which bypass the ATPases and allow protons to leak through the membrane. Uncoupling protein 3 (UCP3) is found in skeletal muscle and may play a role in metabolic regulation at the cellular level. Quantification of UCP3 mRNA and protein levels in seal muscle will be conducted to reveal a possible role of UCPs in the metabolic plasticity of this species.

In closing I would just like to say that we're very pleased with how far the field has come and especially with our interaction between field physiology and technology and that we've been able to bring these two fairly diverse fields together to aid in our understanding of how oxygen is used in a diving animal.

ACKNOWLEDGEMENTS

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Reactive oxygen species and cell signaling with lung ischemia.

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INTRODUCTION AND OVERVIEW OF ISCHEMIA-REPERFUSION

This presentation concerns reactive oxygen species (ROS) and cell signaling in lung ischemia and during reperfusion. A number of years ago, McCord, Granger and others described the now-classic paradigm for ischemia-reperfusion injury: anoxia during ischemia results in the breakdown of ATP to xanthines and a protease activation that converts xanthine dehydrogenase to an oxidase. ROS are generated when oxygen is supplied during reperfusion, resulting in tissue injury (1, 2). Our contribution was the demonstration that ROS generation is initiated during the ischemic period and represents a response to altered mechanotransduction rather than a primary response to metabolic events.

We began the studies described in this report approximately fifteen years ago to investigate ischemia-reperfusion injury in the lung. Unlike other tissues, the lung parenchyma does not rely on its circulation for cellular oxygen requirements. Thus, the lung adds rather than removes oxygen from the pulmonary arterial blood. Ischemia in the lung, unlike that in other organs, does not result in tissue anoxia. Our initial goal was to use the isolated lung model in order to separate the effects of ischemia-reperfusion from those resulting from anoxia-reoxygenation. We called this model oxygenated ischemia because we continued to ventilate the lungs with air (plus 5% CO_2 to maintain pH balance) during the ischemia period. The ventilation of the lungs with nitrogen resulted in a marked decrease in lung ATP content, compatible with anoxia (3, 4). By contrast, measurement of tissue ATP after one hour of oxygenated ischemia showed no change compared with continuously perfused lungs. This confirmed the adequacy of oxygenation during the ischemic period (3, 5). Therefore, this isolated lung preparation can be used to study ischemia without the confounding effects of tissue anoxia.

Oxidative Injury in Lung Ischemia

Our initial observation was that oxygenated ischemia in the lung resulted in evidence of oxidative injury similar to that observed with anoxic ischemia followed by reperfusion in other tissues. We found that thiobarbituric acid reactive substances (TBARS) and conjugated dienes, indices of tissue lipid peroxidation, and protein carbonyls, an index of protein oxidation, increased progressively in the lung between 15 and 60 minutes of oxygenated ischemia (3, 6). Since oxidant production by the lung was unrelated to either anoxia or reoxygenation, the ischemia-mediated injury could not be explained by the classic paradigm for ROS production with ischemia-reperfusion. We considered potential mechanisms other than reoxygenation as

possible causes for the ischemic effect. Manipulation of glucose delivery and tissue pH showed that changes in these parameters were not responsible for the ischemia-mediated events (3).

Shear Stress in Lung Ischemia

At this stage of perplexity, we realized that endothelial cells are normally subjected to mechanical forces associated with blood flow and that an alteration in these forces with ischemia (i.e. no-flow) represents a major physiological change. Although the effects of increased shear stress on endothelial cells *in vitro* had been recognized (7), possible alteration of endothelial function with loss of shear stress *in vivo* had not been considered. The exposure of endothelial cells cultured under the usual static conditions to subsequent laminar shear stress elicits an adaptation response. The response is characterized by changes in ion channel activity during the first minute after imposition of shear, activation of signaling molecules during the subsequent hour, changes in cell surface proteins, including adhesion molecules over the next several hours, and finally, changes in cell shape with cellular realignment in the direction of flow (7). From this background, we reasoned that cells normally accustomed to shear stress might respond similarly when the shear is removed. The remainder of this presentation deals with the response to ischemia from the perspective of altered shear stress.

Endothelial Response *In Vivo* with Ischemia

We have now developed a scheme describing the changes in endothelial cells that we observe in the first minute following abrupt cessation of flow in the pulmonary microvasculature (Figure 1). The initial event is the rapid inactivation of cell membrane K_{ATP} channels, resulting in endothelial cell membrane depolarization. It is followed by the activation of endothelial membrane-localized NADPH oxidase. The NADPH oxidase enzyme complex has been well studied in polymorphonuclear leukocytes (PMN) and now, we and others have shown that the components of this oxidase are present in endothelial cells (8, 9). The depolarization of the endothelial cell also leads to the opening of voltage-dependent calcium channels. This results in calcium influx, activation of eNOS, and NO generation (Figure 1). While we can only speculate on the adaptive nature of these effects, we propose that they represent a physiological attempt to increase blood flow and perhaps initiate a signaling response, which may serve to generate or repair blood vessels.

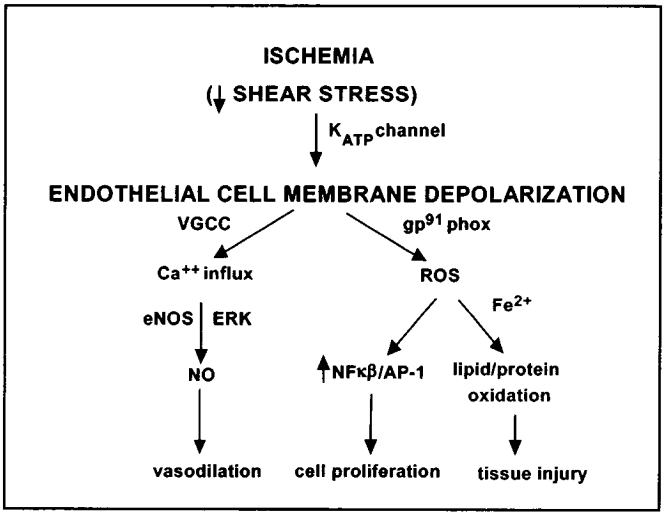

Fig. 1. Schematic demonstrating the response of the pulmonary vascular endothelium to oxygenated ischemia. See text for details.

Now, I will describe some of the experiments in greater detail. Studies with the isolated perfused lung utilized pleural surface fluorescence measurement as well as a subpleural imaging system in order to visualize these initial events with flow cessation. For the measurement of surface fluorescence, a light guide was placed against the pleural surface for fluorophore excitation and capture of fluorescence emission (10). For imaging, the lung was placed on the stage of an inverted epifluorescence microscope equipped with a

Metamorph imaging system or a confocal microscope, enabling us to visualize the endothelium of subpleural microvessels in the range of 20 to 50 μm in size (5). Imaging studies were carried out using a variety of fluorophores that were infused into the intact lung through the pulmonary circulation. Endothelial localization of fluorophores was confirmed by their co-localization with dil-acetylated LDL, an endothelial cell-specific marker (8, 11), and by a localization that was distinctly different from that observed with an epithelial cell marker instilled into the airway (5). Endothelial cell membrane potential was imaged with bis-oxonol or the more rapidly responding di-8-ANEPPS. We used a variety of probes to detect ROS: dichlorofluorescein (DCF), administered as the diacetate, is an intracellular detector not specific for ROS and might also detect reactive nitrogen species; hydroethidine, also intracellular, is oxidized by superoxide anion ; and amplex red, which is confined to the extracellular space, and in the presence of added peroxidase, detects H_2O_2. Fluo 3 was used to image intracellular calcium, and diaminofluorescein 2-T (DAF-2T) was used as a detector of NO.

Ischemia resulted in endothelial cell membrane depolarization, as indicated by increased bis-oxonol fluorescence (5, 12). This change was reversible with reperfusion and the cycle of depolarization-repolarization could be repeated for several cycles of ischemia and reperfusion (5). Ischemia also resulted in ROS generation (8, 10, 12) and increased intracellular Ca^{2+} (11). Our next goal was to couple video imaging to the microscopy system in order to determine timing for the observed changes with ischemia. Somewhat surprisingly, the increase in fluorescence compatible with membrane depolarization was extremely rapid and occurred within the initial one to two seconds after flow was suddenly stopped (13). The rapid increase within the first second raised the concern that this effect might represent a mechanical artifact, but a video scan at 18 frames per second demonstrated a gradual change in signal that rapidly reached a plateau value. Further evidence against an artifactual result was obtained by pre-treatment of lungs with lemakalim, a K_{ATP} channel agonist, which prevented the increase in fluorescence with ischemia (12, 13). Thus, lung ischemia resulted in rapid cell membrane depolarization that could be prevented with a K_{ATP} channel opener. Using a relatively crude calibration curve, we estimated that the membrane potential change with ischemia was about twenty millivolts (13).

Amplex red was used in video imaging studies as a probe for ROS (13). With ischemia, increased fluorescence was noted in approximately two to four seconds, progressively increasing during the subsequent several minutes of observation. ROS production was calcium-independent but was blocked with catalase, as expected. Fluo 3 was used as an indicator of intracellular calcium (13). An increased fluorescence in endothelial cells was observed at 10 to 15 seconds after the onset of ischemia. Based on results with thapsigargin, increased intracellular Ca^{2+} was due to an initial release from intracellular stores followed by influx from the extracellular space (13).

We also evaluated endothelial generation of NO using DAF-2T as the fluorescent indicator. This probe showed an increased fluorescence at 45 to 60 seconds after onset on ischemia (14). NO generation was blocked by the use of calcium-free perfusion medium or by a calmodulin inhibitor. Ca^{2+}/calmodulin-dependence is compatible with eNOS as the enzyme responsible for NO generation (14).

In summary, the use of fluorescent probes indicated a sequence of events associated with lung ischemia characterized by rapid membrane depolarization occurring in the initial one to two

seconds, ROS generation at two to four seconds, Ca^{2+} increase at 10 to 15 seconds, and NO generation at 45 to 60 seconds (Figure 2).

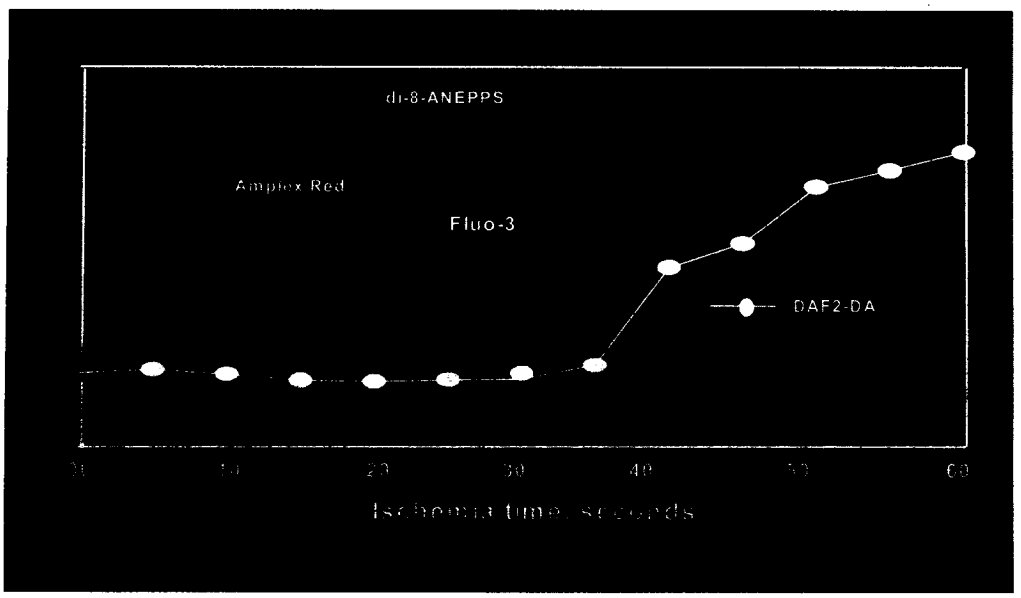

Fig 2. Temporal sequence of events in pulmonary microvascular endothelial cells, as indicated by fluorescent probes following the acute onset of lung ischemia. Di-8-ANEPPS, cell membrane potential; Amplex red, ROS production; Fluo-3, intracellular Ca^{2+}; DAF-2T, NO. Data from (13, 14).

Ischemia vs. Anoxia-Reoxygenation

We compared mechanisms for ROS generation during ischemia versus anoxia/reoxygenation (4). Ventilating the lungs with nitrogen for one hour, followed by one hour ventilation with oxygen produced anoxia/reoxygenation. DCF was used as an index of ROS generation in the absence or presence of inhibitors. Allopurinol was used as an inhibitor of xanthine oxidase. Diphenylene iodonium (DPI), an inhibitor of flavoproteins, has been used to inhibit NADPH oxidase activity in phagocytes, though it is not specific for that enzyme. DPI inhibits $gp91^{phox}$, the flavoprotein component of NADPH oxidase that transfers the electron to O_2 to generate superoxide anion. ROS production was observed with both ischemia and anoxia/reoxygenation, the former giving a somewhat greater level of DCF oxidation (4). However, the effects of the inhibitors were diametrically opposed (4). Allopurinol had no effect on ROS production during ischemia but as predicted, blocked ROS production with anoxia/reoxygenation. On the other hand, DPI blocked ROS production with ischemia but had no effect with anoxia/reoxygenation. These results suggest that NADPH oxidase is the enzyme responsible for ROS generation in lung endothelium with ischemia while xanthine oxidase is the ROS generator with anoxia/reoxygeneration.

NADPH Oxidase and ROS Generation

To study further the role of NADPH oxidase in lung ischemia, we utilized mice with "knockout" of $gp91^{phox}$ (kindly supplied by Mary Dinauer, University of Indiana). These mice were generated as a model of chronic granulomatous disease. Increased DCF fluorescence with ischemia in the wild-type mouse lung was similar to that described for the rat. However, there

was no increase in DCF fluorescence in lungs from $gp91^{phox}$ knockout mice, providing additional evidence that the source of ROS with ischemia is the NADPH oxidase (8).

In Vitro Models of Endothelial Ischemia

We extended our studies to *in vitro* models to further investigate the mechanisms for the response of pulmonary vascular endothelial cells to ischemia. Yefim Manevich designed a parallel plate laminar flow chamber with the dimensions of a standard curvette holder. It could be put into a fluorometer or spectrophotometer for direct and continuous read-out (15). Endothelial cells were grown under laminar flow conditions on an optically clear plastic slide. The initial studies utilized bovine pulmonary artery endothelial cells, but subsequently, rat pulmonary microvascular endothelial cells were evaluated when such preparation became available.

When cells were subjected to laminar flow for 24 hours, they reoriented in the direction of the flow, a well-known phenomenon, indicating flow adaptation (15). These flow-adapted cells were then studied with simulated ischemia by abruptly stopping the flow. With bis-oxonol as a probe of membrane potential, cessation of the flow resulted in a rapid increase in fluorescence compatible with membrane depolarization. This was followed by rapid repolarization when the flow was started again, reproducing the changes noted in perfused lungs (15). There was no change in bis-oxonol fluorescence with flow cessation in cells that had been subjected to flow for only thirty minutes before the experiment and therefore, were not flow-adapted. Thus, membrane depolarization in response to ischemia required flow-adapted cells which presumably is the state of the endothelium in the normal lung .

We next evaluated the generation of ROS in a spectrophotometer using cytochrome c added to the medium as a trap for extracellular superoxide anion (15). We postulated by analogy with PMN that the membrane-bound NADPH oxidase of endothelium would generate superoxide extracellularly. Reduction of cytochrome c and its inhibition by superoxide dismutase indicated the generation of superoxide anion with ischemia. The generation of superoxide reached a plateau after several minutes, likely because of the decreasing O_2 content of the medium. The time to plateau could be extended four to fivefold by gassing the medium with O_2 instead of air, though the initial rate of cytochrome c reduction was similar for the two conditions. Superoxide production was blocked by the addition of DPI to inhibit the NADPH oxidase. Cells that were not flow-adapted showed no change in superoxide production with simulated ischemia.

NO production by the cells was evaluated using DAF-2T as the fluorophore (15). Flow-adapted cells show NO production that was blocked by L-NAME, an inhibitor of NO synthase, and by the addition of EGTA to the medium, indicating calcium-dependence. Thus, simulated ischemia in this *in vitro* system reproduced the effects we observed with the perfused lung.

Cell Signaling with Ischemia

The above *in vitro* model permitted only short-term studies since O_2 availability became limiting and eventuated in anoxic cells. Therefore, we developed an *in vitro* model that permitted longer-term studies by using a commercially available artificial capillary system (16). This system consists of multiple porous capillaries that we pre-coat with fibronectin . During the cell attachment period, medium is perfused via abluminal ports in order to provide oxygen and substrate. Following attachment, we switch to luminal perfusion for flow adaptation. To impose ischemia, the perfusate is switched back to the abluminal ports so that the cells can be provided

with oxygen but do not experience shear stress. This protocol simulates our lung model of oxygenated ischemia. As with the isolated lung and parallel plate *in vitro* system, cells that were flow-adapted and then subjected to simulated ischemia showed DPI-inhibitable ROS generation, as indicated by DCF fluorescence (16) and NO generation, as detected with DAF-2T (17).

We used this *in vitro* model to investigate signaling mechanisms that might be initiated in response to ischemia. Simulated ischemia for ten minutes resulted in significant increase of phophorylated extracellular signal-regulated kinase (ERK) by immunoblot with no change in total ERK, indicating the activation of this kinase (17). Nuclear factor-kappa B (NF-kB) activation was demonstrated by electrophoretic mobility shift assay (EMSA) analysis of nuclear extracts from cells following one hour of ischemia (shorter time points were not studied) (16). There appeared to be activation of both the P65 homodimer and P50/P65 heterodimer. An increase also was seen by assay for activator protein-1 (AP-1) (16). Treatment with DPI to block ROS generation prevented the increases in ERK, NF-kB, and AP-1, as did ROS scavengers (catalase or N-acetyl cysteine) (16, 17).

These experiments indicate that the generation of superoxide with ischemia results in a signaling cascade, presumably with downstream effects. We evaluated cell proliferation as one of the possible responses to the ROS-induced signals. Ischemia resulted in a significant increase in ^{3}H-thymidine incorporation into DNA after 24 hours ischemia, indicating increased DNA synthesis or repair (16). The use of a fluorescence-activated cell sorter (FACS) showed a decrease in the percentage of cells in the G_0G_1 phase, and an increased percentage of cells in the S phase with ischemia, suggesting that ischemia did, in fact, activate cell division (16).

SUMMARY AND CONCLUSIONS

In summary, our studies, utilizing the intact lung and several *in vitro* models, have shown a characteristic response of flow-adapted endothelial cells to ischemia. We believe that this effect represents a response to decreased shear stress since it is unrelated to cellular oxygenation. The response is characterized by endothelial cell depolarization, followed by activation of the membrane-bound NADPH oxidase with generation of ROS, cell signaling, activation of transcription factors, and increased cell division. We postulate that the physiologic role of this response is an attempt to restore blood flow through vasodilation and the repair or genesis of blood vessels.

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The theory and application of intravascular microbubbles as an ultra-effective means of transporting oxygen and other gases.

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Theoretically, volume-stabilized microbubbles may effectively support gas exchange between lungs and tissues[1]. Subcapillary sized bubbles can be generated by i.v. injection of an emulsion of dodecafluoropentane (DDFP, boiling temp 29°C). At body temperature, the DDFP particles evolve into bubbles initially composed of DDFP gas and then equilibrate with O_2 and CO_2 tensions in surrounding tissues. Thus, O_2 is transported from the lungs and CO_2 from the tissues. The feasibility of this method for life-sustaining oxygen supply has now been demonstrated in erythrocyte deprived normovolemic rats both anesthetized[2] (100% mortality in controls) and awake[3], in erythrocyte depleted normoxic pigs[4] and in treatment of severe experimental right-to-left shunts in pigs[3]. Injecting hematologically normal rats with DDFP emulsion provides for markedly increased tissue (muscle) O_2 tensions. In a separate study, potentially fatal hemorrhagic shock was induced in anesthetized pigs (n=8) by removing 50% of the blood volume. After bleeding, the systolic blood pressure was 71±2 mm Hg. Treatment animals (n=4) received 0.3 ml/kg of the DDFP emulsion and controls (n=4) received 0.3 ml/kg of blank. All the controls exhibited falling blood pressure and died in 67±39 (SE) min. By contrast, the systolic blood pressure in the treatment animals increased to above 100 mm Hg and three of the animals were euthanized after 6 hrs of post-treatment observation. One treatment animal became hypotensive after 3 hrs and died. On autopsy, the kidneys of this animal showed a large number of cysts and minimal normal tissue. Thus, i.v. administration of a 2% DDFP emulsion, in extremely small doses, may provide effective first-line treatment of hemorrhagic shock. In another study, the emulsion greatly enhanced the elimination of tissue nitrogen in O_2 breathing pigs[5]. This may have implications for the prevention and treatment of decompression sickness.

Extrapolating from the studies reviewed above, it appears that less than 1 ml of emulsified DDFP can provide for the O_2 consumption of a resting O_2 breathing adult person. The efficiency of the microbubbles for O_2 transport is underscored by the extremely small doses (0.002-0.014 ml/kg body weight) of DDFP, in the form of a 2% emulsion, which were used in these experimental studies.

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Chemical oxidants acidify solitary complex (SC) neurons in the rat.

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INTRODUCTION

Breathing high levels of oxygen (*i.e.* hyperoxia) causes hyperventilation and unstable breathing in normal and carotid-deafferented animals (1,2). Prolonged exposure to hyperoxia can disrupt central nervous system (CNS) function and result in a condition termed CNS O_2 toxicity, the primary sign of which is convulsion (3,4). The mechanism by which hyperoxia stimulates ventilation and disrupts CNS function is unknown; however, these effects are thought to result from increased production of reactive oxygen species (ROS) during hyperoxia and subsequent oxidation of cellular components vital to normal function (4,5). At moderate levels, ROS, including superoxide and nitric oxide, as well as their reactive nonradical derivatives (*e.g.* peroxide, S-nitrosothiols), modulate many physiological processes (4,6), including the hypoxic ventilatory response (7). However, at higher concentrations, ROS can result in oxidative stress that damages cellular components and therefore, are toxic to most cells (4,6). For example, ROS have been implicated in central respiratory control disorders, such as central alveolar hypoventilation syndrome (*e.g.* sudden infant death syndrome) (8).

We have studied the electrophysiological effects of oxidative stress imposed by hyperoxia or by chemical oxidants on neurons from the dorsal medulla oblongata (9-11). In particular, we have studied the nucleus tractus solitarius and dorsal motor nucleus (*i.e.* solitary complex, SC). We showed that acute exposure to hyperoxia or chemical oxidants selectively stimulated firing rate of CO_2/H^+-chemosensitive SC neurons (4,11). The SC is an important cardio-respiratory control center and CO_2/H^+-chemosensitive neurons are thought to provide the primary stimulus for breathing (12). Therefore, our results may explain how oxidative stress causes hyperventilation, and with chronic exposure, possibly contributes to central cardio-respiratory control dysfunction. In addition, we showed that the antioxidant Trolox-C blocked the effects of hyperoxia, but not of hypercapnia, on neuronal excitability, thus suggesting that hyperoxia effects neuronal excitability by a ROS-dependent mechanism (11). We also showed

that high CO_2 (hypercapnia) stimulates CO_2/H^+-chemosensitive brainstem neurons by a decrease in intracellular pH (pH_i) (13). Together, these results indicate that CO_2/H^+ and redox signaling mechanisms are both present in the CO_2/H^+-chemosensitive population of SC neurons. These results, then, beg the following question: Is there an interaction between the CO_2/H^+ and redox signaling mechanisms at the level of the single neuron? The goal of this study was to test the hypothesis that oxidative stress in the form of chemical oxidants causes a decrease in pH_i.

METHODS

To test this possibility, we measured pH_i using ratiometric fluorescence imaging microscopy, utilizing the pH-sensitive fluorescent dye, 2'-7'-bis(2-carboxyethyl)-5-(and 6)-carboxyfluorescein (BCECF), in SC neurons in rat brainstem slices. BCECF is in a state that cannot be further oxidized. Therefore, we assumed that chemical oxidants do not directly affect the fluorescence of the dye. Details regarding the preparation of brain slices (9) and use of BCECF for pH imaging in brain slices have been previously described (14). Briefly, the brainstems from rat pups ranging in age from 2 to 15 days after birth were isolated and cut into 300 μm thick transverse slices. Slices were loaded in the dark with 20 μM BCECF (in the membrane permeable acetoxy methylester form) for 30-60 minutes at 37°C and washed at room temperature in artificial cerebral spinal fluid (aCSF) of the following composition (mM): 125 NaCl, 5.0 KCl, 1.3 $MgSO_4$, 26 $NaHCO_3$, 1.24 KH_2PO_4, 2.4 $CaCl_2$, 10 glucose and equilibrated with 95% O_2-5% CO_2, pH = 7.45. Experiments were performed on individual slices transferred to a superfusion chamber positioned on the stage of an inverted Nikon Diaphot microscope. The dye was excited every 60 seconds by brief alternating pulses of light with wavelengths of 500 nm (pH sensitive) and 440 nm (pH insensitive). pH_i is proportional to the ratio of emitted fluorescence (535 nm) at these two excitation wavelengths (F_{500}/F_{440}). This fluorescence ratio was normalized to the fluorescence ratio value at pH 7.2. Normalized fluorescence was converted to pH_i using the equation of Ritucci, *et al* (15). While measuring pH_i, oxidative stress was imposed by exposing a slice to the chemical oxidants chloramine-T (CT; Sigma-Aldrich) or N-chlorosuccinimide (NCS; Sigma-Aldrich) at concentrations previously shown to stimulate firing rates in 67% of SC neurons (500μM for CT and 1.0 mM for NCS) (11). pH_i responses to CT and NCS were compared to hypercapnic acidosis (15% CO_2), which is also known to stimulate firing rates of SC neurons (11-13). Paired-sample t-tests ($p \leq 0.05$) were used to determine when the mean population difference in pH_i at control and during oxidative stress differed significantly from zero.

RESULTS

Intracellular pH measured under control conditions was 7.42 ± 0.005 (N=31) and was similar to values previously reported for SC neurons (15). Exposure of SC neurons to CT and NCS caused a decrease in pH_i of -0.25 ± 0.02 pH units (N=29) and -0.24 ± 0.02 pH units (N=18), respectively (Figure 1). Interestingly, both CT- and NCS-induced acidification typically reached stable plateaus and showed no signs of pH_i regulation during oxidative stress. On return to control aCSF, pH_i typically showed some return to control levels; however, in most cases, pH_i, did not return to control levels even thirty minutes after removal of CT or NCS (not shown).

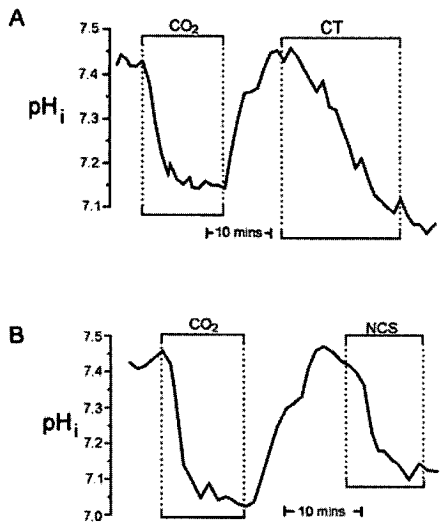

Fig. 1. Exposure to chemical oxidants decreased pH_i in 56% of SC neurons tested. A, representative pH_i trace from an individual SC neuron showed that 15% CO_2 decreased pH_i (~0.25 pH units) in a fashion typical for SC neurons. That is, pH_i remained acidified for the duration of the CO_2 exposure (*i.e.* no pH_i regulation; 13,15). Subsequent exposure to Chloramine T (CT, 500μM) decreased pH_i by an amount comparable to that of 15% CO_2 (~0.25 pH units). However, in most cases, pH_i failed to return to near-control levels when CT was removed (not shown). B, representative pH_i trace from one SC neuron showed that N-chlorosuccinimide (NCS, 1 mM) decreased pH_i by ~0.3 pH for the duration of the exposure (*i.e.* no pH_i regulation; 15). pH_i did not typically return to control levels when NCS was removed (not shown).

DISCUSSION

The observation that chemical oxidants decreased pH_i similarly to hypercapnic acidosis suggests that the response of CO_2/H^+-chemsensitive neurons to oxidative stress is partially mediated by decreased pH_i. We have previously shown that decreased pH_i plays a major role in the response of CO_2/H^+-chemosensitive brainstem neurons to hypercapnia (13). Also, we have shown that oxidative stress and hypercapnia have similar effects on the excitability of CO_2/H^+-chemosensitive neurons. That is, oxidative stress (hyperoxia, CT or NCS) and hypercapnia (15% CO_2) increase firing rate in conjunction with decreased membrane conductance (g_m), possibly by decreasing potassium channel conductance (11,13). Together, these results indicate that oxidative stress and CO_2 signaling mechanisms share a point of interaction (*i.e.* decreased pH_i) that may contribute to the response of CO_2/H^+-chemsensitive neurons to oxidative stress.

The mechanism by which chemical oxidants cause an intracellular acidification is unknown. However, there are several ways oxidative stress may decrease pH_i: i) oxidative stress may disrupt the citric acid cycle (16), resulting in increased lactic acid production (17); ii) oxidative stress may increase ATP hydrolysis, thereby releasing H^+ (19); and iii) oxidative stress may disrupt pH_i regulation by oxidizing the Na^+/H^+ exchanger (15). With regard to the first possibility, the buffering power of cells in this region is high, at 45 mM/pH unit (20,21). Therefore, to change pH_i by 0.25 pH units would require ~11 mM lactic acid (one H^+/lactic acid) to be produced during oxidative stress. This value is similar to transient increases in lactate levels reported to occur during focal brain activation, ~6 mM (22). It has further been shown that oxidative stress can increase lactate production in the CNS (17). Concerning the second possibility, for ATP breakdown to decrease pH_i by 0.25 pH units, hydrolysis of ~11 mM ATP (H^+/ATP) would be required. This value is well in excess of intracellular ATP levels in the brain, ~4.6 to 6.4 mM (23,24). With regard to the third possibility, our findings that pH_i did not recover during exposure to chemical oxidants suggests that the Na^+/H^+ exchanger, which is the major pH_i recovery mechanism in SC neurons (15), was inhibited by oxidative stress. However, Chambers-Kersh, *et al* (21) showed that the inhibition of the Na^+/H^+ exchanger under control conditions with amiloride did not acidify neurons in the nucleus tractus solitarius (*i.e.* dorsal SC). The possibilities listed above (i-iii) are not mutually exclusive and it is likely that the effects of oxidative stress involve a combination of these effects.

Oxidative stress-induced acidification may also feed back to exacerbate the effects of oxidative stress by increasing ROS production. For example, decreased pH_i may dissociate iron

from transferrin (5, 25) and possibly, ferritin (26), to facilitate iron catalysis of superoxide and hydrogen peroxide to the very reactive hydroxyl radical (*i.e.* Fenton reaction) (4) or increase the formation of reactive nitrogen species (25). Our current working model of the effects of oxidative stress and pH_i on the excitability of CO_2/H^+-chemosensitive neurons from the brainstem is summarized in Figure 2. This model illustrates that oxidative stress and CO_2 may both increase ventilation by stimulating CO_2/H^+-chemosensitive SC neurons. Possible interactions between oxidative stress, decreased pH_i and firing rate are also indicated.

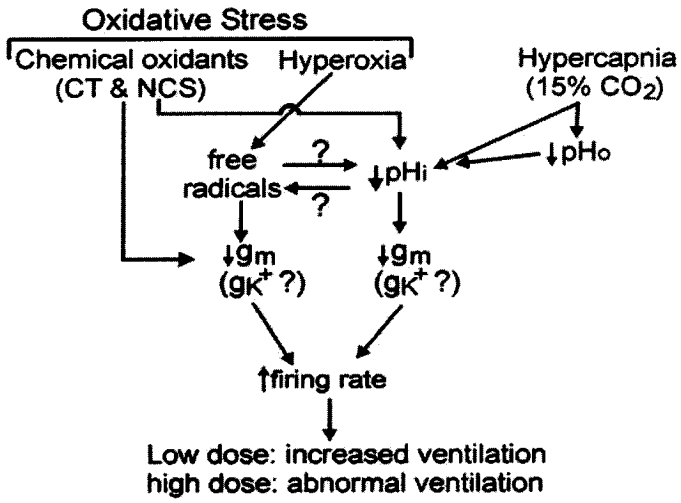

Fig. 2. Current model of the effects of oxidative stress on SC neurons. Oxidative stress in the form of chemical oxidants (CT & NCS) or hyperoxia via free radicals decreases membrane conductance (g_m), by possibly decreasing conductance of a K^+ channel, and increases the excitability of CO_2/H^+-chemosensitive neurons (11). Hypercapnia decreases pH_i, which causes decreased membrane conductance, possibly by decreasing K^+ channel conductance, and increases excitability of CO_2/H^+-chemosensitive neurons (13). Chemical oxidants also decrease pH_i (this study), suggesting that decreased pH_i plays a central role in the selective sensitivity of CO_2/H^+-chemosensitive neurons to oxidative stress. Previous research by others indicates that there is an interaction between free radicals and pH_i (18,24). This, however, has yet to be determined for neurons in the SC.

The observation that chemical oxidants decrease pH_i explains, in part, why acid-sensitive neurons (*i.e.* CO_2/H^+-chemoreceptors) are also highly sensitive to oxidative stress (11) and could thus help explain how oxidative stress causes hyperventilation (1,2). Likewise, it may explain how severe or chronic oxidative stress contributes to central cardio-respiratory control dysfunction (8).

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Combined oxygen and glucose sensing in the carotid body.

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INTRODUCTION

Oxygen sensing is essential for the adaptation of living organisms to variable habitats and physiologic situations. In mammals, survival in acute hypoxia requires fast respiratory and cardiocirculatory adjustments to guarantee proper O_2 supply to the most critical organs, such as the brain and heart (1). Reduction of arterial O_2 tension is detected by the carotid bodies, small ovoid organs situated in the carotid bifurcation, which contain afferent nerve fibers that activate the brainstem respiratory centers to produce hyperventilation. The O_2-sensitive elements within the carotid body are the ectodermal-derived glomus cells. These neurosecretory cells are electrically excitable (2, 3) and have O_2-sensitive potassium channels in their membranes (3, 4, 5). It is broadly accepted that inhibition of these channels by low pO_2 is a key step leading to membrane depolarization, external calcium influx and the activation of neurotransmitter release, which, in turn, stimulates the afferent sensory fibers. This model of chemotransduction, suggested by electrophysiological experiments, has been confirmed by monitoring cytosolic [Ca^{2+}] and quantal catecholamine secretion in single cells (6-11).

The mechanism of acute O_2 sensing based on the regulation of membrane potassium channels has been demonstrated to operate in other neurosecretory systems, such as cells in the neuroepithelial bodies of the lung (12), chromaffin cells of the adrenal medulla (13), or PC-12 cells (14). However, some investigators have argued that the O_2-sensitive membrane electrical events are not directly implicated in the chemotransduction because in their whole-carotid body preparations, the application of K^+ channel blockers do not increase the action potential firing frequency or secretory activity (15-17). It was also reported that tetraethylammonium (TEA), a blocker of the O_2-sensitive K^+ current in the carotid body, is unable to induce depolarization on dispersed rat glomus cells (18).

Given the discrepancies among observations in the carotid body reported by different authors, we have developed a slice preparation of the organ to study the O_2 sensitivity of glomus cells in the best possible physiological conditions (11, 19). The

carotid body thin slice has been found to be an excellent preparation to study the cellular bases for the chemosensitivity of glomus cells. We have found that carotid body cells are multimodal sensor organs that detect not only the reduction of the pO_2 but also the decrease of the extracellular glucose concentration.

RESULTS AND DISCUSSION

Electrophysiological Recording and the Responses of Glomus Cells to Hypoxia.

The procedures followed to make carotid body slices are described in detail elsewhere (11). Figure 1A shows the aspect of one glomerulus of glomus cells within a slice, with the arrow pointing to a well-identifiable cell, susceptible to being analyzed electrophysiologically. The distribution of the cells in glomeruli within the slice is consistent with the structure observed in the organ after sectioning and immunostaining for tyrosine hydroxilase (TH), the rate limiting enzyme in the synthesis of dopamine, a common catecholamine found in glomus cells (Figure 1B).

Figure 1. Electrophysiological recording on glomus cells within rat carotid body thin slices.

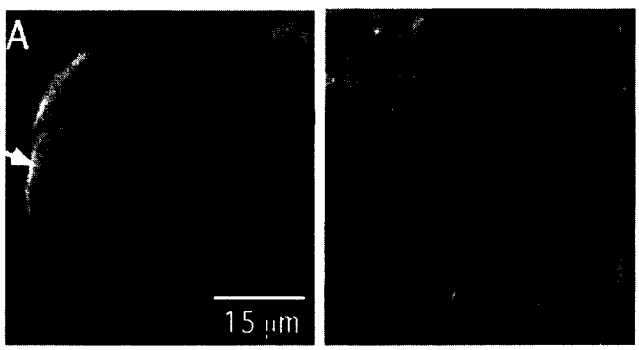

Fig. 1 A & B. Electrophysiological recording on glomus cells within rat carotid body thin slices. (A) Typical glomerulus in a slice with well-defined single glomus cells (arrow). (B) Carotid body slice immunostained with antibodies against tyrosine hydroxilase. Note the typical appearance of glomus cells with large nuclei and a thin layer of stained cytoplasm.

Stable recordings of membrane currents can easily be obtained from glomus cells in the slices by using the whole-cell configuration of the patch-clamp technique, as adapted in our laboratory (20, 21). The majority of the cells recorded in the slices (77% from a total number of 110) had inward and outward currents qualitatively similar to those described in enzymatically dispersed rat glomus, or type I, cells (22, 23). The remaining 23% of cells (n=26) had no measurable currents or presented a small transient outward current, thus suggesting that they were type II, or sustentacular cells, present in the carotid body (2, 20). The outward currents of glomus cells were highly sensitive to the application of external TEA (5 mM caused an inhibition of 67.5±6% at +20 mV, mean±standard deviation, n=4 cells) or the selective calcium-dependent K^+ channel blocker iberiotoxin (IbTX, 200 nM, caused an inhibition of 65% and 45% at +20 mV in two cells tested). Therefore, as described in isolated cells (22, 23), a large proportion of the outward current was due primarily to the activity of maxi-K^+ voltage- and calcium-dependent channels. As described in dispersed rabbit (3, 24-26) and rat (4, 22, 23, 27)

glomus cells, the amplitude of macroscopic voltage-dependent currents was reduced when exposed to low pO_2.

Typical recordings of O_2-sensitive currents are shown in Figure 1C. However, our work on slices has focused so far on the study of secretion rather than on the recording of ionic currents.

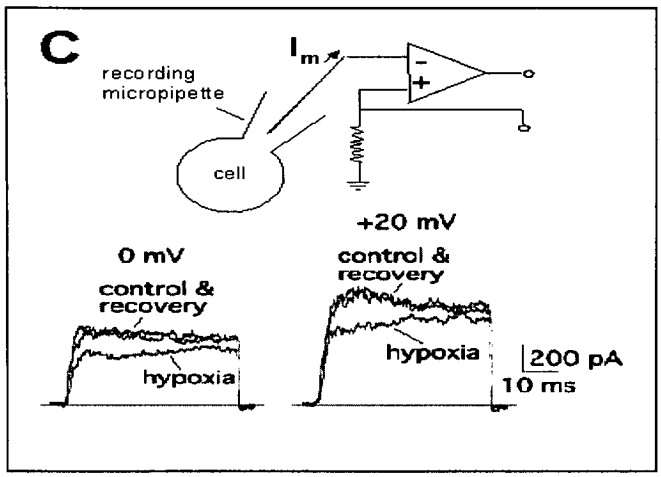

Fig 1C. Diagram of the whole-cell configuration of the patch clamp technique and superimposed macroscopic K^+ currents from a glomus cell elicited by depolarizing pulses from –80 mV to the indicated voltage in the three experimental conditions (control, low pO_2, and recovery).

We have found the slice preparation to be particularly convenient to study the responsiveness of intact glomus cells to hypoxia and other stimuli using the amperometric detection of catecholamines (7, 10, 11). Quantal transmitter release can be monitored with a polarized 8-12-μm carbon-fiber electrode positioned near the surface of a glomus cell (Figure 1D). The fiber is connected to a high-gain current-to-voltage converter and polarized to +750 mV, a value more positive than the redox potential of dopamine, the most abundant catecholamine in glomus cells. A representative secretory response of a glomus cell to depolarization by either a hypoxic solution or a high external K^+ solution is shown in Figure 1D.

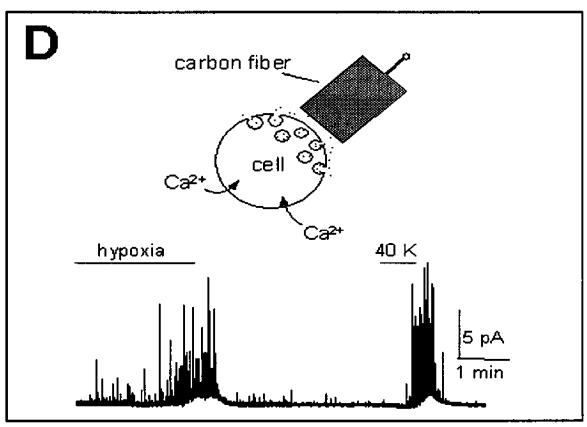

Fig. 1 D. Diagram of the amperometric detection of catecholamine release from a single glomus cell, and amperometric recording from an O_2-sensitive glomus cell illustrating the increase of secretory activity induced by hypoxia and high extracellular potassium. (Modified from Ref. 11 and 19.)

Spike-like signals resulted from the fusion of single vesicles. The area under individual spikes (quantal charge) yields an estimate of the number of catecholamine molecules released, assuming that two electron charges are transferred to the fiber during the oxidation of each catecholamine molecule. Average quantal charge estimated from high K^+-induced events was 40.3±17.6 fC (n=293 spikes in 7 cells), corresponding to ≈125,000±50,000 molecules per vesicle. These values, obtained from cells in slices, are comparable to those previously described in dispersed rat and rabbit glomus cells (7, 10, 11). The magnitude of the secretory responses of the cells was estimated either from the number of spikes in the minute after ninety seconds of exposure to the stimulus (frequency in events/min) or from the sum of all quantal charges measured in the same time period and expressed as femtocoulombs per minute (fC/min; secretion rate). This

last variable represents the total amount of catecholamine molecules released per minute at the peak of the response.

In slices with well-defined glomeruli, low pO_2 consistently induced a progressive increase in the frequency of secretory events (Fig. 1D), from almost rest in normoxia to a value of 48.6±19 spikes/min (n=24 cells) and a secretion rate of 1,710±270 fC/min (n=17 cells). At the peak of the response to hypoxia, the secretory events fused into a broad concentration envelope that quickly declined after switching to the control, normoxic solution . All the glomus cells that responded to hypoxia were also activated by solutions with high external K^+ (Fig. 1D), as expected from electrically excitable cells. Interestingly, we also observed glomus cells that were unresponsive to hypoxia but activated by depolarization with high external K^+. Cells insensitive to hypoxia were more frequently observed in slices that appeared somewhat unhealthy, possibly due to damage during the experimental protocol. One possible explanation is that these are cells with O_2 sensors uncoupled from the membrane ion channels. In all cells tested (n=10), the neurosecretory response to hypoxia was completely abolished by the addition of the voltage-dependent calcium channel blocker cadmium (Figure 2A) or the removal of extracellular calcium with EGTA (11). This observation confirmed the dependence of the response to hypoxia on the extracellular calcium influx through voltage-gated calcium channels, as previously shown on dispersed rabbit carotid body cells (7, 9).

Figure 2. Secretory responses of glomus cells in the slices to cadmium and K^+ channel blockers.

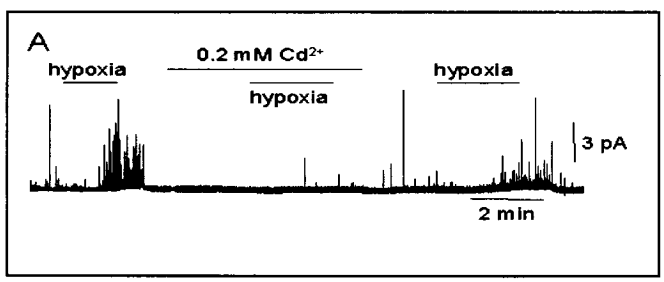

Fig 2A. Secretory activity recorded from an O_2-sensitive glomus cell to illustrate the reversible abolishment of the response to hypoxia during the blockade of Ca^{2+} channels by addition of 0.2 mM cadmium to the extracellular solution.

Secretory Responses of Glomus Cells to Potassium Channel Blockers

The major contributors to the O_2-sensitive macroscopic K^+ currents in rat glomus cells are voltage- and Ca^{2+}-dependent maxi-K^+ channels (4, 22, 23). Because TEA or iberiotoxin (IbTX) block these channels, we have studied whether, like hypoxia , addition of these agents to the external solution induces Ca^{2+} entry and secretion from glomus cells. In most cells studied (33 of 34), application of 5 mM TEA to the bath solution elicited an increase in the secretory activity similar to that triggered by hypoxia (Figure 2B).

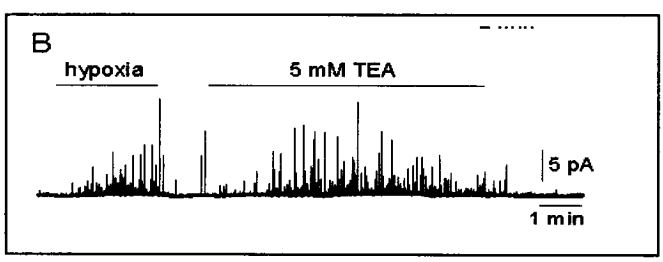

Fig 2 B. Amperometric recording from a glomus cell showing the similar effects elicited by low pO_2 and the application of 5 mM TEA.

The response to the blocker reached a frequency of

42±17 spikes/min (n=6 cells), with a secretion rate of 1,878±470 fC/min (n=6). These values are not significantly different from the respective ones obtained in low pO_2 (Student's t-test, p=0.05). The average quantal charge of events induced by TEA was 43±30 fC (n=275 spikes in 6 cells). This value is also similar to that estimated with events elicited by hypoxia (43±26 fC; n=576 spikes in 14 cells), suggesting that both stimuli trigger the release of vesicles from the same cellular pool. The effect of TEA was observed even in quiescent cells, without any measurable spontaneous quantal release, as well as in O_2-insensitive glomus cells. We have also tested the effect of IbTX, a selective blocker of Ca^{2+}- and voltage-activated maxi K^+ channels (28). Figure 2C illustrates the increase of secretory activity in a glomus cell exposed to 200 nM IbTX.

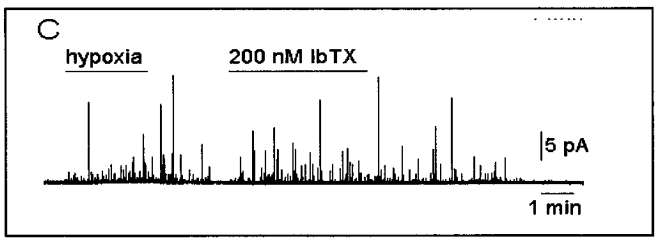

Figure 2 C. Secretory activity induced in a glomus cell by hypoxia and 200 nM IbTX (Modified from Ref. 11).

The response is similar to those obtained with TEA or hypoxia, although the recovery phase seems to be somewhat longer, possibly due to slower washout of IbTX. All these observations indicate that direct blockade of the O_2-sensitive K^+ channels with TEA or IbTX can elicit secretion from rat glomus cells in the slices. Our data make it difficult to understand why K^+ channel blockers do not activate whole carotid body preparations, in which the glomus cell-afferent fiber synapses are maintained intact (16, 17). As suggested before (11), a possible explanation is that the blockers do not diffuse at the appropriate concentration into the extracellular space of the carotid bodies either superfused by the bath solution or perfused through the carotid artery.

Sensitivity of Glomus Cells in the Slices to Hypoglycemia

It has been proposed that the carotid body participates in glucose homeostasis (29, 30), and recently, it has been shown that resection of the carotid bodies and surrounding tissues results in the impairment of the insulin-induced counter-regulatory responses to mild hypoglycemia (31). However, there is no evidence that any of the cellular elements in the carotid body can directly respond to changes in extracellular glucose concentration. Using the carotid body thin slice preparation, we studied the effect of external glucose removal on the secretory activity of the glomus cells (32). Figure 3 represents the response of intact glomus cells to low glucose.

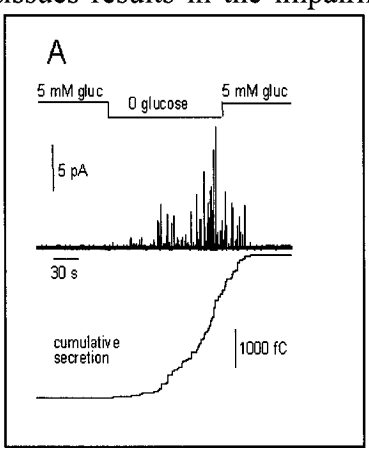

Fig 3 A. Top. Amperometric signal illustrating the increase of secretory activity in a glomus cell exposed to glucose-free solution. Bottom. Cumulative secretion signal (in femtocoulombs) resulting from the time integral of the amperometric recording.

In healthy preparations, exposure of a glomus cell in the slice to 0 glucose consistently produced a marked and reversible increase of cell secretory activity (Figure 3A). The average rate of secretion during the last minute of exposure to low glucose (1870±386 fC/min, n=14 cells) was over twenty times (Figure 3B) that of the control condition (88±45 fC/min, n=14).

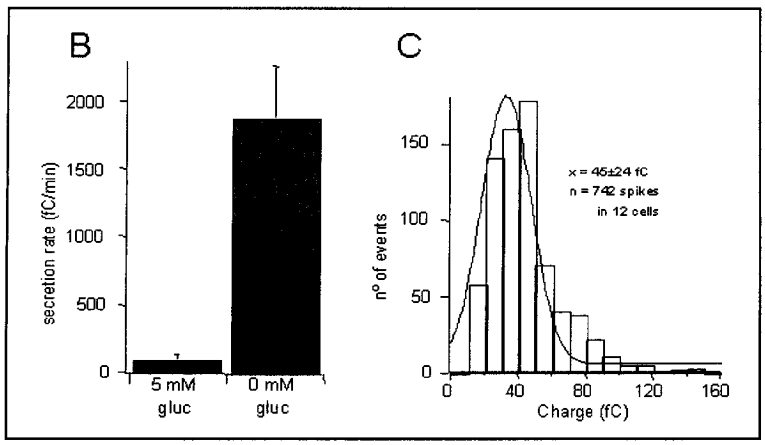

Fig 3 B and C. **(B)** Bar diagram quantifying the average response of glomus cells to 0 glucose. **(C)** Histogram representing the distribution of the area of exocytotic events in low glucose.

The size distribution and mean area of quantal events triggered by low glucose (Figure 3C) were similar to those previously observed in glomus cells activated by high K^+, hypoxia or TEA (see above), further suggesting that all these stimuli induce the release of a common vesicle pool. The effect of low glucose on glomus cells was concentration-dependent (Figure 3D) and additive with the effects of hypoxia.

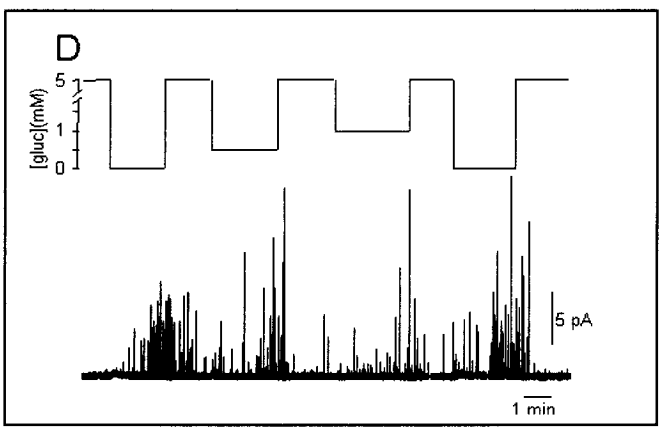

Fig. 3 D Secretory response of a glomus cell to different concentrations of glucose in the external solution.

At normal air O_2 tension (pO_2 ≈150 mmHg), secretion was evoked only when glucose decreased below 2 mM. However, at a pO_2 of 90 mmHg (a value close to the normal O_2 tension in arterial blood), glomus cell secretory activity was significantly modulated by glucose in the concentration range (2 to 5 mM) that includes the values observed in common hypoglycemic situations (32; Figure 3E).

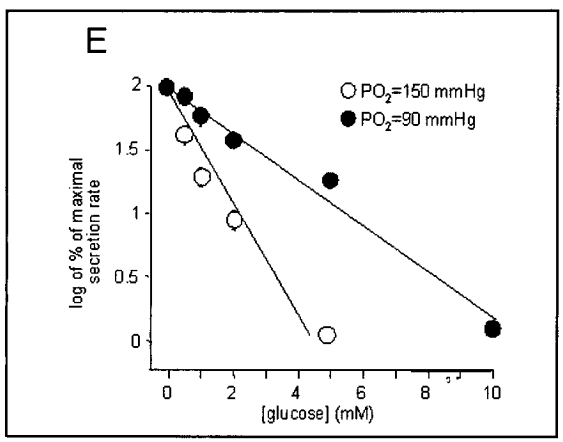

Fig. 3E. Logarithm of maximal secretion rate (ordinate) versus glucose concentration at two different pO_2 values. (Modified from Ref. 23.)

Catecholamine secretion induced by glucose-free solutions was totally suppressed by the addition of 0.2 mM Cd^{2+} to the extracellular solution in all cells

tested (n=3; Figure 4A). Because 0.2 mM extracellular Cd^{2+} completely blocks voltage-gated Ca^{2+} channels in glomus cells (7), this indicates that low glucose induces transmitter secretion when membrane electrical events result in depolarization and Ca^{2+} influx through voltage-dependent channels. Experiments on patch-clamped cells dialyzed with an internal solution containing 4 mM Mg-ATP confirmed this idea. Glucose deficiency produced a reversible reduction of peak outward K^+ current amplitude (Figures 4B and 4C) that at +20 mV, had an average value of 38±12% (n=7 cells). Low glucose appeared to act selectively on voltage-dependent K^+ channels since it had no effect on the small inward current characteristic of most rat glomus cells (data not shown).

Figure 4. Glomus cell membrane electrical events in the response to low glucose.

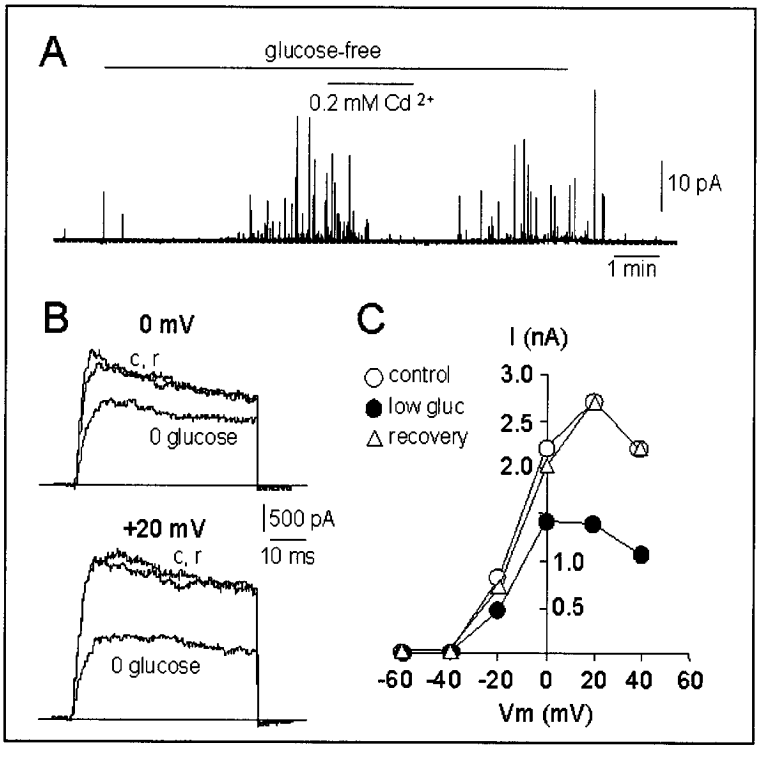

Fig. 4. Glomus cell membrane electrical events in the response to low glucose. (**A**) Reversible suppression of low glucose-evoked secretory activity by application of 0.2 mM cadmium to the extracellular solution. (**B**) Recordings of outward K^+ currents from a patch-clamped glomus cell depolarized to 0 and +20 mV and exposed to 0 mM glucose. The control (c) and recovery (r) external solutions contained 5 mM glucose. (**C**) Current-voltage relationship obtained measuring maximum current amplitudes from the experiment shown on B.

The electrophysiological and amperometric data obtained from glomus cells in carotid body slices strongly suggest that these cells are physiological low-glucose detectors capable of transducing glucose levels into variable rates of transmitter release. The low-glucose signaling pathways in glomus cells appear to be initiated by an inhibition of voltage-gated K^+ channel activity, which leads to membrane depolarization, Ca^{2+} influx through voltage-gated Ca^{2+} channels and transmitter release. Therefore, in glomus cells, low glucose and hypoxia converge to raise cytosolic [Ca^{2+}] and release transmitters, which stimulates afferent sensory fibers and evokes sympathoadrenal activation. These observations help explain previous reports of anesthetized animals exhibiting rapid increases in the output of hepatic glucose after the activation of the carotid body with sodium cyanide (29, 30), alterations of carbohydrate metabolism in acute hypoxia (33), or the impairment of an insulin-induced counter-regulatory response to mild hypoglycemia in carotid body resected dogs (31). Although the existence of peripheral glucosensors, presumably located in the liver or portal vein, has been proposed (34, 35), the

strategically located carotid bodies may be of special importance for brain homeostasis, as neurons are particularly vulnerable to the simultaneous lack of glucose and oxygen (36). The function of glomus cells as combined O_2 and glucose sensors, in which the two stimuli potentiate each other, is surely advantageous to facilitate the activation of counter-regulatory measures in response to small reductions of any of the regulated variables. Impairment of low-glucose sensing by carotid body glomus cells might contribute to the susceptibility of insulin-dependent diabetic patients to hypoglycemia.

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Effects of hyperoxia on neutrophil adhesion.

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INTRODUCTION

This presentation will focus on the effects of oxygen and some reactive chemical species on interactions between circulating neutrophils and the vascular endothelium. It will also outline how we believe these actions may be related to some beneficial effects of hyperbaric oxygen.

HBO_2 and Neutrophil Adhesion

There are a number of animal models that have been used to look at the effects of hyperbaric oxygen on disease processes. Listed in Table 1 are investigations using animal models where neutrophils, in particular, have been linked to the progression of pathology and where a beneficial effect of hyperbaric oxygen has been related to inhibition of one or more neutrophil responses. Zamboni and others (1,2) have shown an antagonism of neutrophil adherence following ischemia-reperfusion injury in skeletal muscle. Others have demonstrated similar effects following ischemia-reperfusion injury in the brain (3), and neutrophil adhesion in the lung after an intestinal ischemia-reperfusion injury (4,5). Neutrophil adhesion is a component of the pathological responses to carbon monoxide poisoning (6), decompression sickness (7), and to lung injury as a consequence of smoke inhalation (8). Studies with each of these disorders have shown that beneficial effect of hyperbaric oxygen is linked to inhibition of neutrophil adhesion. Therefore, based on a rather wide sampling of disease processes, inhibition of neutrophil attachment to blood vessel appears to be a common theme to beneficial effects of hyperbaric oxygen.

Table 1. Beneficial effects of hyperbaric oxygen associated with reduced neutrophil sequestration.

Skeletal muscle ischemia-reperfusion injury (1,2)
Brain ischemia-reperfusion injury (3)
Lung after intestinal ischemia-reperfusion injury (4, 5)
Brain after carbon monoxide poisoning (6)
Brain after decompression sickness (7)
Lung after smoke inhalation (8)

Cell surface molecules called β_2 integrins mediate irreversible adherence by activated neutrophils (9). Some β_2 integrins are always present on the neutrophil surface, and additional molecules are mobilized from pre-packaged granules within the neutrophil during the activation process. These molecules interact with intercellular adhesion molecules (ICAM) on the endothelial surface to cause irreversible cell-to-cell adhesion. Diapedesis of neutrophils through the vessel wall commonly follows this interaction. The process is part of normal physiology and occurs when neutrophils perform their normal function of protecting the body from infectious agents. The worry arises, however, when this process occurs after other insults, such as ischemia-reperfusion injury. In this scenario, the neutrophil will precipitate further pathological events.

Shown in Figure 1 is a summary of some of our observations with the rat CO poisoning model in which neutrophil adhesion to brain vasculature can be documented (6). Neutrophil adhesion *in vivo* was quantified as myeloperoxidase activity. A marked elevation of sequestered neutrophils occurs after carbon monoxide poisoning, and this is not seen in animals treated with hyperbaric oxygen after carbon monoxide poisoning. The point with this model is that hyperbaric oxygen will inhibit neutrophil adhesion in the brain and this will reduce the magnitude of brain damage based on a variety of different parameters.

The second panel of Figure 1 shows increased neutrophil sequestration in lungs 24 hours after smoke inhalation (8), and significant reduction if rats are treated with hyperbaric oxygen shortly after the smoke insult. The effects of hyperbaric oxygen can also be shown *in vitro*.

The third panel of Figure 1 shows results of studies performed by taking blood from rats and passing it through columns packed with nylon fiber. Neutrophil adherence to the nylon is approximately 25 % in control rats, and it is unchanged if rats are exposed to 100 % O_2 at 1 atmosphere for 45 minutes before blood is taken. If rats are first exposed to 3 atmospheres absolute (ATA) O_2 for 45 minutes, neutrophil adherence to nylon is less than 5 % (6).

Figure 1. Neutrophil adhesion and its inhibition by hyperbaric oxygen.

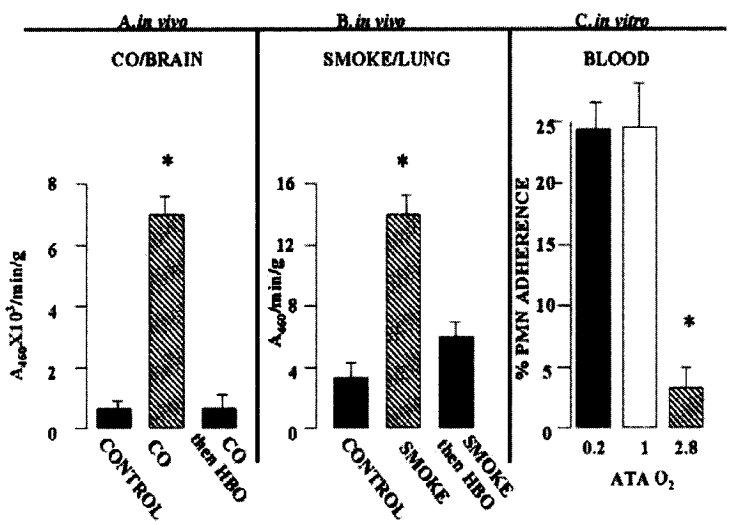

Fig. 1 Panel A: Myeloperoxidase activity in rat brain homogenates. Data are from (6) and reflect control values, values from rats killed 90 min after CO poisoning (CO), and those poisoned with CO, then exposed for 45 min 2.8 ATA O_2, so that they were killed at 90 min following the CO poisoning. **Panel B:** Myeloperoxidase activity in rat lungs. Data are from (8) and reflect control values, values from rats killed 24 h after smoke inhalation, and those exposed to smoke and to 2.8 ATA O_2 for 45 min, and then killed 24 h after the smoke inhalation. **Panel C:** Neutrophil adherence to nylon columns. Blood was removed from rats breathing air (0.2 ATA O_2), or after breathing pure oxygen at 1 or 2.8 ATA for 45 min. It was passed through columns following a technique to assess β_2 integrin-dependent neutrophil adherence (6). Columns reflect mean values $\pm$ SE (n=4 to 8 for all measurements),*$p<0.05$.

Once we demonstrated that inhibition of neutrophil adhesion could be monitored in blood drawn from animals exposed to hyperbaric oxygen, we extended our observations to humans. In

a study published in 1997 we showed that exposure to 2.8 or 3.0 ATA O_2 reduced β_2 integrin dependent adhesion to approximately 5 % of control and the effect persisted for approximately 12 hours (10). While our data indicate that there may be a benefit to hyperoxia with regard to temporary impairment of neutrophil adherence, we must be cognizant that more oxygen is not always good. Even with regard to neutrophil-endothelial interactions, there are instances when oxidative stress will actually increase cell adhesion. Superoxide and hydrogen peroxide have been shown to increase adhesion molecule expression and adherence between neutrophils and the endothelium in a cardiac ischemia reperfusion model (11-13). The point, therefore, is that oxidants can augment the inflammatory response. This issue must be reconciled with the other side, the antagonistic effects described above when determining fundamental mechanisms.

β_2 Integrin Adhesion Control Mechanisms

We have focused some effort on investigation of basic mechanisms for why hyperbaric oxygen inhibits β_2 integrin adhesion and how the beneficial versus harmful effects of reactive oxygen species may be reconciled. There are two pathways that have been demonstrated for how β_2 integrins are controlled in neutrophils. One pathway involves membrane receptors (14), and one was shown with membrane permeable agents that directly activate intracellular enzymes such as protein kinase C (PKC), to cause phosphorylation events leading to increased adhesiveness (15).

Shown in Figure 2 are the effects of various agonists on neutrophil adhesion (16). Neutrophil suspensions were prepared from control rats, and also those that had first been exposed to hyperbaric oxygen. We found that cells incubated with N-formyl-methionyl-leucine-phenylalanine (FMLP), which will activate cells via membrane surface receptors, exhibit a slight elevation in adhesion over control, but there was no response in cells first exposed to hyperbaric oxygen. In contrast, if cells were incubated with phorbol ester (phorbol 12-myristate 13-acetate, PMA), to mediate cell activation through PKC, adherence was increased whether cells were obtained from control (air-exposed) rats, or those first exposed to hyperbaric oxygen. These findings imply that much of the cell's internal mechanisms for controlling adherence are intact after hyperbaric oxygen and that the effect may be mediated by one or more membrane-associated process. In additional studies we demonstrated that incubation with the sulfhydryl-reducing agent, dithioerythritol (DTE), reversed the effect of hyperbaric oxygen but had no effect on control cells. A similar effect was observed after incubation with a membrane-permeable analog of cyclic GMP, 8 bromo-cGMP. These findings led to the idea that the effect of hyperbaric oxygen involved cyclic GMP, and may be mediated through oxidative "stress" of one or more membrane-associated sulfhydryl group. Moreover, based on the lack of effect of FMLP, versus the stimulatory action of PMA, hyperoxia was presumed to inhibit receptor-dependent cell activation.

We next examined cyclic GMP synthesis by neutrophils and found that this was increased when control cells were incubated with either FMLP of PMA (Figure 3). When the same studies were done with cells taken from rats first exposed hyperbaric oxygen, the cells did respond to PMA but not to FMLP (16).

Given that cyclic GMP appears to be involved with the effect of hyperbaric oxygen on neutrophil adherence, we next examined how a neutrophil makes cyclic GMP. There are two different pathways (17). Nearly ninety-eight percent of the cyclic GMP produced is synthesized by the cytosolic guanylate cyclase, a process presumably stimulated by nitric oxide.

Figure 2. Neutrophil adherence to nylon columns.

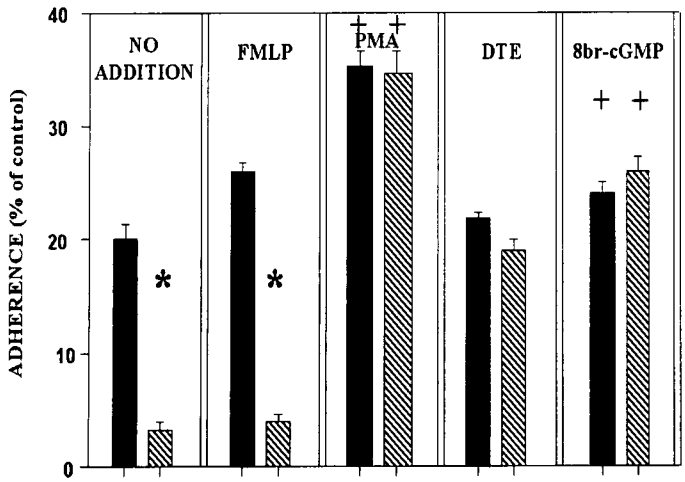

Fig. 2. Neutrophils obtained from peritoneal lavage of control rats or from rats first exposed to 2.8 ATA O_2 were incubated for 30 min with 0.1 μM FMLP or PMA, with 3 μM DTE, or 10 μM 8-bromo-cyclic GMP. A description of procedures and some data are from Chen et al. (16). Data represent percent of cells that adhered to nylon expressed as mean + SE (n=3 to 8 for each group), +P<0.05.

Figure 3. Neutrophil cyclic GMP (cGMP) content and effect of hyperbaric oxygen.

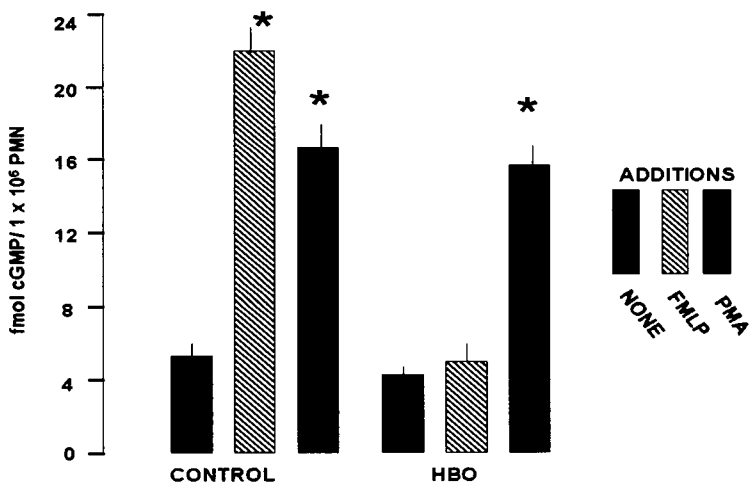

Fig. 3. Neutrophils were obtained by peritoneal lavage of rats exposed to air (control) or to 2.8 ATA O_2 for 45 min (HBO_2). They were plated onto plastic and exposed for 1 min to 0.1 μM FMLP or PMA. Data and procedures are from (16). Values are mean ± SE, n=7 to 34 for each group, *p<0.05.

Only about 2 percent of cellular cyclic GMP is produced by a membrane-bound guanylate cyclase. This is a very different protein from the cytosolic enzyme. It has no heme moiety and is not activated by nitric oxide. The enzyme can be activated by atrial natriuretic peptide, although whether this is physiologically relevant is unclear. If cells were incubated for 2 minutes in buffer containing 50 μM nitric oxide, cyclic GMP was increased by 18 ± 5% (SE, n=3), whether neutrophils were obtained from control animals or those first exposed to hyperbaric oxygen. This suggested that cytosolic guanylate cyclase was not inhibited by hyperoxia. In contrast, if neutrophil membranes were isolated to assay membrane guanylate cyclase a very different picture emerged.

Membrane fragments from control neutrophils exhibited significant production of cyclic GMP when incubated with FMLP, but this was not observed with membranes obtained after exposure to hyperbaric oxygen (Figure 4). Similarly, incubation with atrial natruretic peptide (plus ATP) stimulated cyclic GMP synthesis by membrane fragments from control cells but not those from animals exposed to hyperbaric oxygen. These data lead to the conclusion that membrane guanylate cyclase was inhibited by hyperbaric oxygen, and that impairment of membrane-associated cyclic GMP synthesis plays a role with inhibiting β_2 integrin adherence.

Figure 4. Guanylate cyclase activity of isolated membrane fragment from neutrophils obtained by peritoneal lavage of rats exposed to air (control) or to 2.8 ATA O_2 for 45 minutes (HBO_2).

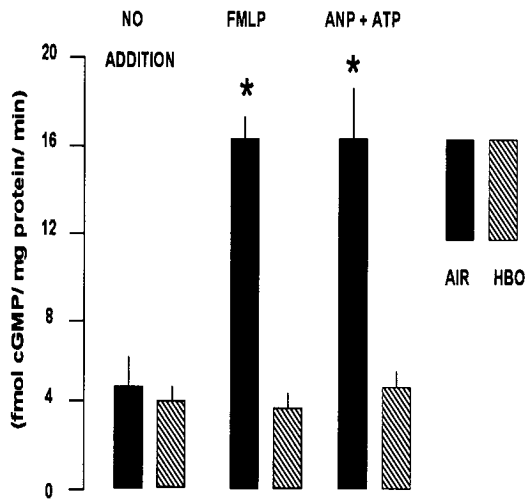

Fig. 4. Procedures are exactly as described in (25). Values reflect cyclic GMP (cGMP) after incubation for 10 minutes with 50 μM GTP (no addition of agonist), GTP and 0.1 μM FMLP, or GTP and 0.1 μM atrial natriuretic peptide (ANP) plus 330 (M ATP. Values are mean + SE, n= 4 to 17 for each sample, *p<0.05.

The studies outlined above were performed with rats, but similar findings have also been made using human neutrophils (10). Neutrophils were obtained from human volunteers before and after exposure to 2.8 ATA O_2 for 45 minutes. Control cells exhibited membrane guanylate cyclase activation by FMLP or ANP + ATP, but neither agonist had an effect on membranes from cells exposed to hyperbaric oxygen. Hyperbaric oxygen also inhibited ANP binding that was analyzed by Scatchard plot. There are approximately 7,300 ANP binding sites/cell, they form a single class, and they have a dissociation constant (Kd) of 450 pM (10). Following exposure to hyperbaric oxygen, however, no Scatchard analysis was possible since only a random pattern of points in the region of the plot origin was found.

In summary, these results lead to the conclusion that hyperbaric oxygen inhibits (2 integrin-dependent neutrophil adhesion by inhibiting membrane guanylate cyclase. How cyclic GMP synthesis regulates (2 integrin function is not yet clear. Neutrophil membrane receptors, such as the one binding FMLP, appear to be linked to membrane-bound guanylate cyclase via one or more G-proteins (16). Others have reported a cyclic GMP-dependent protein kinase that will phosphorylate cytoskeletal elements that may coordinate function of β_2 integrins (18).

NEW DIRECTIONS

These results were all published some years ago. So what is new? I'll share with you some of our more recent findings in the last few minutes. Starting from a slightly different angle, there are precedents in the literature that the free radical, nitric oxide (·NO) will inhibit neutrophil adhesion (19-21). In fact, there are several different adhesion molecules affected by ·NO, including P-selectin as well as β_2 integrins (22-25). Our interest was looking at how ·NO

inhibits β_2 integrins function. In a series of studies conducted in a manner similar to the hyperbaric oxygen experiments shown in Figure 3, neutrophils were exposed to a flux of ·NO to inhibit β_2 integrins -dependent neutrophil adhesion (26). If the ·NO-incubated cells were then exposed to FMLP, a membrane receptor-dependent activator, there was no effect. In contrast, if the cells were incubated with PMA to activate the intracellular kinase pathway, enhanced adhesion was seen. Similarly, when cells were incubated with a sulfhydryl reducing agent, this reversed the effect of ·NO. Quite surprisingly, cells could also be incubated with membrane-permeable 8-bromo cyclic GMP to reverse the effect of ·NO. The paradox here, of course, is that one might have argued that exposure to ·NO should elevate cyclic GMP, so how can supplying supplemental cyclic GMP reverse the anti-adhesion effect?

Figure 5 shows the cyclic GMP content of neutrophils exposed to a flux ·NO, allowed to adhere to plastic plates, and then stimulated with either FMLP or PMA (25). The point is that the pattern of effects with ·NO looks much the same as we saw using hyperbaric oxygen.

Figure 5. Neutrophil cyclic GMP (cGMP) content and effect of nitric oxide (·NO).

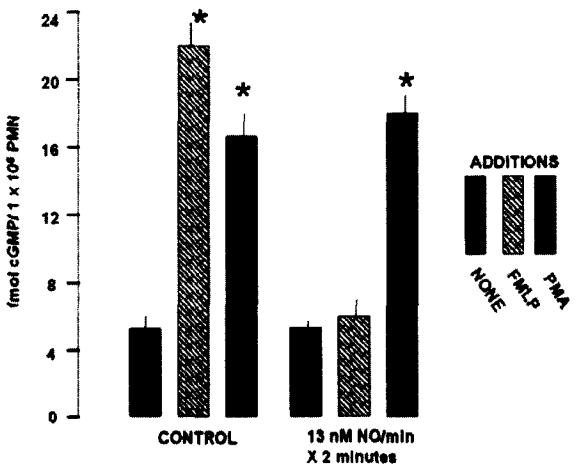

Fig. 5. Rat neutrophils were obtained by peritoneal lavage, plated onto plastic and, where indicated, exposed to 50 nM diethylamine NONOate to cause a 13 nM flux of nitric oxide/minute for 2 minutes. All cell samples were then rinsed and, where indicated, exposed to 0.1 μM FMLP or PMA for 1 minute. Data and procedures are from (25). Values are mean + SE, n=5 to 2 for each group, *p<0.05.

Looking directly at the membrane guanylate cyclase function of neutrophils before and after exposure to ·NO, we see that this inhibits enzyme activity whether membrane fragments are exposed to no agonist, as well as to FMLP or ANP/ATP (Figure 6).

Figure 6. Guanylate cyclase activity of isolated membrane fragment from rat neutrophils obtained by peritoneal lavage.

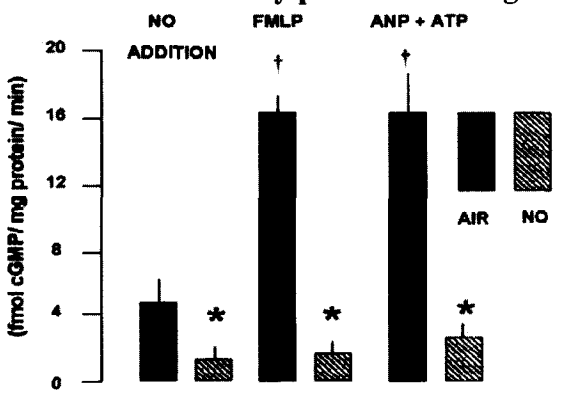

Fig. 6. Samples were obtained from control neutrophils (black bars) or from cells first exposed to 50 nM diethylamine NONOate to cause a 13 nM flux of nitric oxide/minute for 2 minutes (hatched bars). Procedures are exactly as described in (25). Values reflect cyclic GMP (cGMP) after membrane fragments were incubated for 10 minutes with 50 μM GTP (no addition of agonist), GTP and 0.1 μM FMLP (column marked FMLP), or GTP and 0.1 μM atrial natriuretic peptide (ANP) plus 330 μM ATP (column marked ANP + ATP). Values are mean ± SE, n= 5 to 17 for each sample, *p<0.05 versus air sample for each group, † p<0.05 versus Air, no addition.

The effect of ·NO on neutrophil adhesion is very much dose-dependent. Figure 7 shows neutrophil adhesion in control cells, and in cells exposed to differing fluxes of ·NO ranging from 2 to 133 μM/min. We think, based on work by others, that a level of approximately 20 to 30 μM may be close to that found in the perivascular zone of most vascular beds in the body. We think it notable that if cells are exposed to extreme concentrations of ·NO, such as those found in intense inflammatory zones like abscess cavities, ·NO does not inhibit neutrophil adhesion.

Figure 7. Dose effect of nitric oxide on neutrophil adherence.

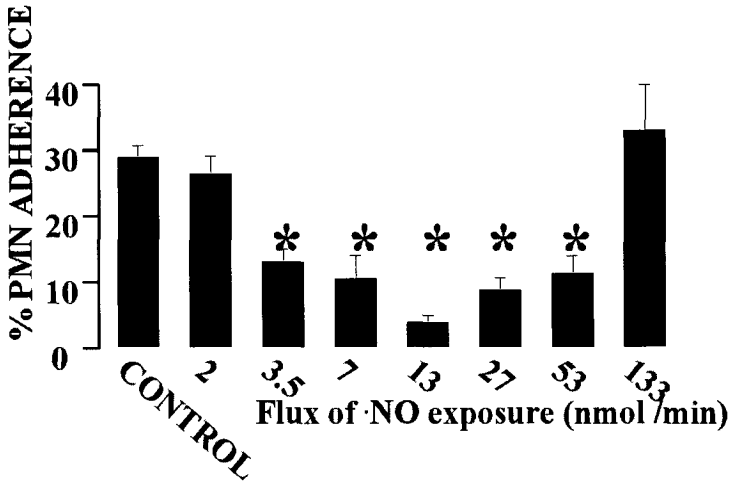

Fig. 7. Rat neutrophils were obtained by peritoneal lavage and exposed for 2 min to diethylamine NONOate at concentrations sufficient to cause a flux of nitric oxide ranging from 2 to 133 μM/min. After this exposure, cells were passed through nylon columns. Data represent percentage of cells that adhered to nylon expressed as mean ± SE (n=4 to 33 for each group), *p<0.05. Data and procedures are from (25).

Based on the previous work, the inhibition that we see is linked presumably to inhibition of the membrane bound guanylate cyclase. Even at a markedly elevated flux of ·NO the membrane guanylate cyclase will be inhibited, but we believe β_2 integrins function because there is adequate ·NO to diffuse into the cell and elevate intracellular cyclic GMP level by activating the cytosolic guanylate cyclase. This would negate the membrane-surface effects.

Because of the similarity in molecular mechanisms we have found between hyperbaric oxygen and ·NO, we have questioned whether the effect of hyperbaric oxygen may be mediated through ·NO. The way we tested this hypothesis was to look at neutrophil adhesion after rats were treated with an inhibitor of nitric oxide synthase, L-nitroarginine methyl ester (L-NAME). Figure 8 shows that whereas treatment with L-NAME had little impact on neutrophil adhesion by itself, pre-treated rats exposed to hyperbaric oxygen did not exhibit impaired neutrophil adhesion. Also shown are the effects of first incubating blood with antibodies that block β_2 integrin function. The inhibitory effect on control blood demonstrates the specificity of the assay for β_2 integrin-specific neutrophil adherence. The inhibitory effect on blood taken from rats first treated with L-NAME and then hyperbaric oxygen shows that the adherence seen in neutrophils from these rats was also due to β_2 integrins, and not to some alternative adhesion process.

Figure 8. Neutrophil adherence to nylon columns.

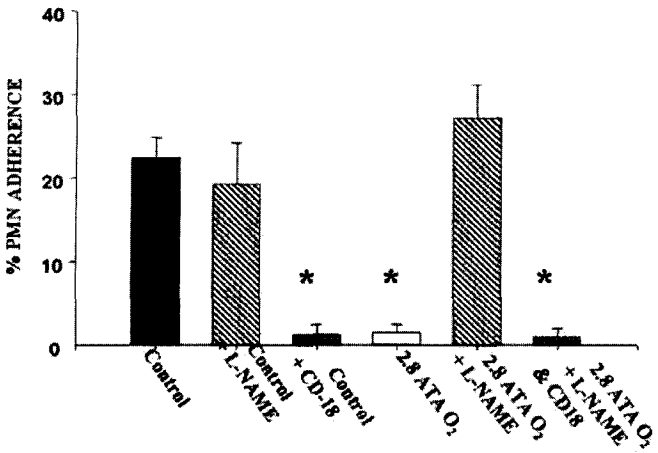

Fig. 8. Blood was obtained from control rats, or rats first exposed to 2.8 ATA O_2 and passed through nylon columns as described in (6). Where indicated, rats were injected with 40 mg/kg L-nitroarginine methyl ester (L-NAME) 2 hours and 45 minutes before sacrifice, or 2 hours before exposure to hyperoxia. Where indicated, anti-CD-18 antibodies (200 mg/ml) were added to blood prior to passage through columns to block adhesion dependent on β_2 integrins. Data represent percent of cells that adhered to nylon expressed as mean $\pm$ SE (n=4 to 11 for each group), *P<0.05.

CONCLUSIONS

We conclude from these findings that hyperbaric oxygen appears to act via NO to inhibit neutrophil β_2 integrin function. Additional studies are underway to elucidate the mechanism for augmented NO synthesis by hyperoxia.

We, and others, have demonstrated the ability of hyperbaric oxygen to inhibit neutrophil adhesion in humans (10, 26). The effect appears remarkably discrete, but there may be additional perturbations to cell function under some circumstances (26,27). We believe that the reason for the relatively discrete perturbations may relate to the localized effect of hyperoxia on membrane guanylate cyclase.

In closing, it should be acknowledged that there are still other effects of hyperbaric oxygen, and still more work to be done. Clearly, there are effects of hyperoxia on the endothelium that may have some effects with regard to inhibiting ischemia-reperfusion or other injuries (28). There is also a need for a tighter evaluation of the dose-response relationship with hyperbaric oxygen. It is well known that with extreme oxidative stress, neutrophil adhesion is increased (29).

ACKNOWLEDGMENTS

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Hyperbaric oxygen for delayed radiation injuries.

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INTRODUCTION

Based on distinctions in their pathophysiologies, radiation complications should be divided into acute and delayed. Acute complications are generally cellular and mucosal and are caused by direct damage to cellular DNA with resultant cell death. These are usually self-limited and generally not dose limiting. Late radiation damage is primarily vascular and stromal.[1] Late complications are the most dreaded and determine the limits of tolerable radiation doses. The tolerance to radiation varies considerably from organ system to organ system. For years radiation oncologists and pathologists have felt that the mechanisms of early and late damage are completely unrelated. Although there are distinctions and identifiable differences in the nature and site of injury, we are beginning to appreciate that late radiation damage actually begins at the initiation of treatment. An increase in various biochemical substances including fibrogenetic cytokines are identifiable from the onset of treatment.[2] These have been associated with late radiation damage and their serial assays may allow for prophylactic interventions. An elevation of those cytokines, which lead to damage or conversely a depression of protective cytokines may identify a group of patients at high risk for late radiation damage. This early identification of those at risk prior to manifestations of frank radiation damage may permit prophylactic intervention. An exciting potential area for further research will include the application of hyperbaric oxygen during this latent period after radiation but before expression of the clinically expressed damage. Certainly, if such a group of reliable predictive assays can be identified, other therapeutic strategies to prevent radiation damage will also undergo investigation.

To date hyperbaric oxygen (HBO_2) has been a very successful therapeutic modality for delayed radiation injury. It has also been shown to be useful in a prophylactic intervention in several circumstances. A recent systematic review by Feldmeier and Hampson, summarizes 74 publications that report the results of hyperbaric oxygen for a wide range of soft tissue and bony necrosis. This review was conducted in an evidence-based fashion. All but seven papers are positive in terms of their therapeutic effect. Most of the negative reports are in neurologic injury where, especially in the CNS, injuries have been refractory to virtually all interventions. The application of HBO_2 has had its earliest and best-studied application in mandibular osteonecrosis. HBO_2 is an effective treatment modality for radiation necrosis because, at least in part, the pathophysiology of this process is vascular and stromal secondary to obliterative endarteritis. HBO_2 has been shown to induce neovascularization in this hypoxic milieu and to reduce fibrosis in irradiated tissues.

Molecular oxygen available simultaneously with ionizing radiation is the most potent radiation sensitizer known. The first interactions of radiation and HBO_2 were based on this observation. Mostly during the 1960's and early 1970's, HBO_2 was used as a radiosensitizer for

external beam radiation, and numerous clinical trials were conducted whereby patients with malignancies were irradiated while at pressure in monoplace HBO_2 chambers. Although many of these trials showed improved local control of cancers, especially in head and neck cancers and cervical cancer, this improvement did not generally translate into improved cure rates, and the practice of HBO_2 radio-sensitization for external beam irradiation has been largely abandoned.[3]

The Etiology of Radiation Necrosis or Late Radiation Injury and the Rationale for HBO_2.

Late radiation changes are characterized by fibrosis and endarteritis. If nutrient blood vessels are significantly narrowed and if inadequate O_2 is available to meet metabolic demands frank aseptic necrosis occurs.[1]

The development of radiation-induced endarteritis requires time to become established. Clinically we see a latent period of months to years before these changes result in necrosis. The process can be set off by trauma to the tissues such as dental extraction or surgical incision.

HBO_2 has been shown to induce neovascularization and reduce fibrosis in radiated hypoxic tissues. Marx[4] has shown an increase in vascular density and cellularity in histologic specimens of tissues before hyperbaric oxygen compared to specimens taken from the same site after hyperbaric treatments just prior to mandibular reconstruction. Marx[5] has also shown an improvement in sequential transcutaneous oxygen measurements in patients undergoing hyperbaric oxygen for mandibular necrosis. Feldmeier[6-8] and his co-workers have shown decreased stromal fibrosis by morphometric collagen assay and functional compliance measurements in a murine model of radiation enteritis.

Site Specific Applications of Hyperbaric Oxygen to Delayed Radiation Injuries

Mandibular Necrosis

Prior to the 1970's, HBO_2 had been used with mixed success for mandibular radiation necrosis (ORN).

Beginning in the late 1970's Marx and Johnson and their co-workers while at Wilford Hall USAF Medical Center in collaboration with the USAF Hyperbaric Medicine Center at Brooks Air Force Base established a formal protocol for treatment and evaluation of mandibular necrosis.[9] The development of a "Staging" system led to a logical application of HBO_2 integrated with surgery. The specific recommendations for therapy followed quite logically from the assignment to a particular stage.

a. Stage I - Patients receive 30 HBO_2 treatments at 2.4 ATA for 90 minutes In these patients no more than minor debridement in the dental chair was felt to be necessary.
   (1) If response was good and exposed bone was covering, the patient continued for 10 more treatments.
b. Stage II - These were non-responders to the first 30 treatments in Stage I but in whom debridement rather than a discontinuity resection was felt to be appropriate. Debridement was not accomplished until 30 HBO_2 treatments are completed.
   (1) Post debridement ten additional treatments were given.
c. Stage III- These were non responders to Stage II treatment or those who present with cutaneous fistulae, pathologic fracture or resorption of the inferior cortical border of the mandible.

(1) 30 HBO_2 treatments given followed by resection with 10 post-op HBO_2 sessions.

(2) 10 weeks after resection reconstruction was accomplished followed by 10 post reconstructive HBO_2 sessions. Reconstruction involved the use of freeze-dried cadaveric bone used as a carrier tray for the patient's own corticocancellous bone harvested from the iliac crest.

(3) External jaw fixation was maintained for 8 weeks following the reconstruction.

With this protocol, success in mandibular reconstruction has been unexcelled even when compared to more surgically complex procedures such as microvascular anastomosis with free flaps. However, there is nothing to preclude the use of free flaps or myocutaneous flaps along with hyperbaric oxygen and it is always a good principle to combine optimal surgery with HBO_2 as an adjunct in improving the quality of tissues in the recipient bed.

Including the publications by Marx, a total of fourteen publications are available reviewing the experience of applying HBO_2 to mandibular necrosis[10-23]. One very small randomized controlled trial by Tobey et al[13] is positive. Only twelve patients were studied in this publication. These patients were randomized to 100% oxygen at 1.2 vs 2.0 ATA. The authors state that those patients treated at 2.0 ATA "experienced significant improvement" compared to the group receiving oxygen at 1.2 ATA. No details are given regarding randomization or outcome determination. In fact we cannot tell how many patients were assigned to each group. The study is randomized and doubly blinded in that neither the patient nor the clinician assessing the patient knew which therapy the patient was receiving

Other than the trial by Tobey, all of the rest of the publications present case series. Of the fourteen publications, only the report by Maier et al[21] fails to show an advantage for hyperbaric oxygen in the treatment of existing ORN. In this paper, none of the hyperbaric oxygen is given prior to surgery. Hyperbaric oxygen is part of the overall management only after an attempt is made at surgical correction. Marx has previously established the importance of pre-operative HBO_2 prior to surgical wounding in irradiated tissues. This principle has been widely accepted by those applying HBO_2 to the treatment of ORN.

If all of the cases are combined (excluding those reported by Tobey and noting that Marx's second report includes the fifty-eight patients reported earlier), we have a total of 371 reported cases of mandibular ORN. Improvement is reported in 310 cases or 83.6%. Resolution would certainly be a superior endpoint. However, especially in the earlier reports, hyperbaric oxygen was not combined with aggressive extirpation of necrotic bone or with surgical reconstruction of bony discontinuity. Certainly, Marx has reported the best results of any single author. Marx has identified the need for optimizing surgery and sequencing HBO_2 and surgery to include and emphasize the pre-surgical application of HBO_2. Marx reports 100% success, but his successful treatment includes mandibulectomy and reconstruction in 73% of his patients. Dr. Marx also sets high standards for successful intervention in those patients requiring mandibulectomy and reconstruction. Marx requires not only the successful re-establishment of bony continuity but also requires functional success in that these patients must be able to support a denture for cosmesis and mastication.

HBO_2 for Osteoradionecrosis Prophylaxis

A randomized trial by Marx et al.[24] compared penicillin to HBO_2 prior to dental extractions as prophylactic strategies to prevent mandibular radiation necrosis in heavily irradiated mandibles. With thirty-seven patients in each group, ORN occurred in 11/37 (29.9%) of penicillin group and only 2/37 (5.4%) in HBO_2 group.

This protocol randomized only patients with 6800 cGy or higher dose. The penicillin group received 1 million units of penicillin just prior to surgery followed by 500 mg penicillin p.o. Q.I.D. for 10 days. The HBO_2 group underwent 20 HBO_2 treatments prior to extractions and 10 HBO_2 treatments after extractions.

Two additional clinical series present their results in the prophylactic treatment of fifty-three additional patients. If we combine the patients from all three reports, we find an incidence of osteoradionecrosis (ORN) in 4.5% (4 of 90) of the HBO_2 prophylaxis group (2 of 37 Marx; 1 of 29 Vudiniabola[25]; and 1 of 24 David[23]). In Marx's control group, the incidence of osteoradionecrosis was 29.9% (11 of 37).

Laryngeal Necrosis

Fortunately, radiation-induced laryngeal necrosis is an uncommon complication. In a well designed radiation treatment course, its incidence should be less than 1%. Higher doses per treatment fraction, higher total doses and neutron irradiation increase the risk of laryngeal chondroradiation necrosis.

The effects of HBO_2 on chondroradiation necrosis of the larynx have been reported by three authors from three separate institutions.[26-28] The majority of these patients had severe (Chandler's Grade III or IV necrosis). Without hyperbaric oxygen treatment, the usual recommendation is laryngectomy for those patients in whom laryngeal edema is persistent. The rationale for this recommendation is that vast majority of patients with persistent edema have tumor, and even if they do not, salvage is not possible because no effective treatment had been known for laryngeal chondronecrosis. Symptoms of both laryngeal necrosis and tumor recurrence may include airway compromise, edema, fetid breath and production of necrotic debris. Biopsy in such patients may be subject to sampling error or may further exacerbate the necrotic process. Biopsy should be avoided or minimized if possible. Obviously, biopsy may be ultimately required to demonstrate or rule out tumor recurrence. If the results from these three trials are combined, only six of thirty-five patients treated with hyperbaric oxygen required laryngectomy. The rest maintained their larynx with most patients having good voice quality after HBO_2.

Other Soft Tissue Necrosis Injuries of the Head and Neck

Dr. Marx's chapter in the textbook, Hyperbaric Medicine Practice[29] reports his experience in a prospective controlled but not randomized study applying hyperbaric oxygen to soft tissue radionecrosis of the head and neck. Those patients who refused hyperbaric oxygen or for whom treatment was not practical due to having homes distant from a hyperbaric chamber or other financial reasons were assigned to the control group. These cohorts of patients were treated concurrently and all other aspects of their treatment were identical. In his report of 160 patients receiving hyperbaric oxygen in support of surgical resection or flap reconstruction in heavily irradiated patients comparing wound infection, wound dehiscence and delayed wound healing, Marx reports the incidence of complications in the HBO_2 group versus the control group in the following fashion: 1. Wound infection: 6% versus 24%; 2. Wound dehiscence: 11% versus 48%; and 3. Delayed wound healing: 11% versus 55%. Applying the Chi square test to these results we obtain P values of 0.004, less than 0.0001 and less than 0.0001 respectively for each of these outcome measures. These patients received twenty pre-operative HBO_2 treatments followed by ten post-operative treatments at 2.4 ATA.

In addition to the large controlled trial reported by Marx, there are three additional publications reporting case series in which hyperbaric oxygen has been applied to soft tissue radiation injuries of the head and neck (other than larynx).[30-32] These consistently report a

positive outcome in patients treated with HBO_2 for soft tissue radionecrosis of the head and neck. The case series by Davis et al[31] reports success in fifteen of sixteen patients treated for soft tissue radionecrosis of the head and neck. Many of these patients had large chronic soft tissue wounds as a result of their radiation injury. In 1997 Neovius[30] and colleagues reported a series of fifteen patients treated with hyperbaric oxygen for wound complications within irradiated tissues. They compared this group to a historical control group from the same institution. Twelve of the fifteen patients in the hyperbaric group healed completely with improvement in two and no benefit in one. In the control group only seven of fifteen patients healed. Two patients in the control group developed life-threatening hemorrhage and one of these did exsanguinate. Any practitioner experienced in caring for head and neck cancer patients has experienced at least one patient in his or her career that exsanguinated as the result of a soft tissue necrosis of the neck which progressed to erode into the carotid artery or other major vessel.

Chest Wall Necrosis

Radiation is a frequent modality applied to lung, breast, or esophageal cancers. Chest wall radiation necrosis occurs most frequently in breast cancer after mastectomy due to the need to treat skin and subcutaneous tissues to a relatively high dose since this tumor often recurs in the skin of the chest wall. HBO_2 in this setting has not been extensively reported.

Hart [11] has reported the use of hyperbaric oxygen as an adjunct to skin grafting in six patients with radiation injury of the chest wall with all patients experiencing graft take. Feldmeier and his colleagues[33] have reported a total of twenty-three cases of radiation necrosis of the chest wall: eight with soft tissue only necrosis and fifteen with a combination of bone and soft tissue necrosis. Resolution in those with soft tissue involvement only was 75% while those with a component of bone necrosis had resolution in 53%, and all of these patients required resection of necrotic bone. If bone is involved aggressive debridement to include resection of non-viable bone is required for good results

In a case report Carl and Hartmann[34] in 1998 published their results in treating a patient with long-standing symptomatic breast edema following lumpectomy and irradiation. The patient received fifteen, 90 minute HBO_2 treatments at 2.4 ATA. The patient had complete resolution of pain and edema.

Carl and his associates[35] in 2001 reported the outcome of 44 patients who suffered complications following lumpectomy and irradiation for early breast cancers. These patients were found to have pain, edema, fibrosis and telengectasias. Each patient experienced these complications in various combinations. The severity of symptoms was assessed a score for each patient based on a modified LENT-SOMA score. Only patients with at least grade 3 pain (persistent and intense) or a summed LENT-SOMA score of 8 were studied. Each patient was assessed a score from 1 to 4 in the severity of symptoms in the categories of pain, edema, fibrosis/ fat necrosis and telangectasia/erythema. Thirty-two patients agreed to undergo hyperbaric oxygen treatment while twelve women refused HBO_2 and constituted the control group. Hyperbaric oxygen treatments resulted in a statistically significant reduction in the post-treatment SOMA-LENT scores in women receiving hyperbaric oxygen compared to those who did not. Fibrosis and telangectasia were not reduced. Women in the control group continued to demonstrate symptoms at the completion of the trial with no improvement in pain or edema while seven women in the hyperbaric group had complete resolution of their symptoms at the end of the trial.

Radiation Cystitis

Several single institutional reports have shown efficacy in this rare disorder. Hemorrhagic cystitis secondary to radiation may require cystectomy if it does not respond to conservative measures such as instillation of formalin or alum. HBO_2 reports with serial cystoscopies and serial U.A.'s have shown resolution in a high percentage of cases. Success has varied from 60 to 95% and has been durable over time.

There are seventeen published reports detailing results of HBO_2 interventions in the treatment hemorrhagic radiation induced cystitis.[36-52] These publications are all case series. The report by Bevers et al[45], which includes the largest number of patients, was a prospective but not a controlled trial. In the final report by Weiss et al[47], the earlier patients reported by the same author were included. The second paper by Lee[42] reporting twenty-five patients includes the twenty patients previously reported by the same author. If we combine all those patients reported in these seventeen publications, we find a total of 190 patients treated with HBO_2 with 145 patients or 76.3% resolving when treated with hyperbaric oxygen.

Many of the patients reported in the hyperbaric experience had already failed conservative management including irrigation and the instillation of alum or formalin. Severe hemorragic radiation cystitis is unquestionably a life threatening and quality of life limiting disorder. Cheng and Foo[53] have reported their experience in managing nine serious refractory cases of hemorrhagic radiation cystitis without hyperbaric oxygen. Six patients were treated with bilateral percutaneous nephrostomies. Three patients required ileal loop diversions of their urinary stream. Four of nine (44%) patients ultimately died in spite of these aggressive treatments. Similarly, Sun and Chao[54] have reported a 3.7% mortality due to bladder injury in their review of 378 patients treated with radiation for cervical cancer.

A success rate of 76.3% with hyperbaric oxygen is all the more impressive when results with other more aggressive interventions are considered. It is also noteworthy that 16 of 17 publications are positive reports. These patients represent patients treated in several different countries on 3 different continents with consistent benefit seen in a large majority of patients in each study except that reported by Del Pizzo[46].

Radiation Enteritis /Proctitis

There are fourteen publications reporting experience in applying hyperbaric oxygen as treatment for radiation enteritis and proctitis.[55-68] The first paper is a case report detailing the successful treatment of a single patient with hemorrhagic proctitis. An additional eight case series reports have been published. Of the 114 cases reported in these nine publications, forty-one (36%) resolved and 68 (60%) improved after treatment with hyperbaric oxygen. In the report by Feldmeier et al, especially impressive was the resolution of fistulae in six of eight patients with only three requiring surgical closure. In the same report, only seven of twenty-six patients who healed required surgical debridement or surgical flaps or grafts.

The animal studies by Feldmeier[58,63] demonstrate a decrease in fibrosis and an improvement in compliance in the small bowel of animals receiving hyperbaric oxygen before frank necrosis was evident. In these studies enough time was allowed for the vascular changes and fibrosis associated with late radiation injury to be established prior to animal sacrifice. Characteristically, a latent period of several months to years is observed to occur between the completion of radiation and the clinical expression of radiation damage.

Miscellaneous Abdominal and Pelvic Injuries

Farmer and his colleagues[12] in 1978 as part of a report, which included radiation injuries to many sites, reported a single case of vaginal necrosis. This necrosis resolved with HBO_2. Williams and his associates[69] reported their results in treating 14 patients with vaginal radiation necrosis in 1992. Thirteen of fourteen patients had resolution of their necrosis with hyperbaric treatment. One patient required two courses of HBO_2. In 1996 Feldmeier and colleagues[59] reported a series of forty-four patients treated with various abdominal and pelvic injuries. The results from this report in treating small and large bowel injuries have already been discussed above. Twenty-six of thirty-one (84%) patients who experienced injuries to the abdominal wall, groin, perineum, vagina or pelvic bones and who received at least twenty hyperbaric treatments had complete resolution with treatment. This group included 6 patients with vaginal necrosis, all of whom experienced complete resolution with treatment. If we total the results reported in these three papers we find complete resolution in a variety of pelvic and abdominal soft tissue injuries in forty of forty-six patients (87%). All but one patient of the twenty-one reported in all three papers with soft tissue vaginal necrosis was treated successfully.

Extremities

Hyperbaric oxygen has also been reported as a useful therapy in radiation necrosis of the extremities. In the report previously discussed by Farmer and his colleagues[12] a single case of foot injury did not respond to hyperbaric oxygen. In a series published by Feldmeier and associates[70], seventeen patients with necrosis of the extremities treated with hyperbaric oxygen were reported. Sixteen of the seventeen patients had only soft tissue necrosis. Eleven of seventeen had resolution with HBO_2. If we restrict our analysis to those patients in whom follow-up was available and in whom there was no evidence of recurrent cancer, eleven of 13 (85%) had complete resolution.

Neurologic Radiation Injuries

There are fourteen publications wherein hyperbaric oxygen has been applied to radiation-induced neurologic injuries. [11, 71-83] These injuries include radiation myelitis of the spinal cord, radiation necrosis of the brain, optic nerve injury and brachial plexopathy.

Hart and Mainous[11] in 1976 reported five cases of myelitis treated with hyperbaric oxygen without significant improvement. Glassburn and Brady[71] have reported nine cases of myelitis in which patients received hyperbaric oxygen. Six of these nine patients improved, including improvement in motor function. In 2000 Calabro and Jinkins[79] reported a single case of transverse spinal myelitis in which the patient demonstrated progressive improvement including imaging documentation by MRI after treatment with hyperbaric oxygen. Feldmeier and his colleagues[75] have reported a statistically significant delay in the onset of transverse myelitis in mice who received hyperbaric oxygen after radiation but prior to the expression of spinal cord injury. There are no other known successful treatments for radiation induced transverse myelitis and the consequences of myelitis are permanent paralysis and loss of sensation below the level of involvement. The published experience reviewed above shows improvement in seven of fifteen patients treated with hyperbaric oxygen.

There are six published reports in which hyperbaric oxygen has been applied to the treatment of brain necrosis. In the publication by Hart and Mainous[11], a single case of brain necrosis is presented and this patient had improvement after treatment. In the paper by Chuba and associates[77], hyperbaric oxygen treatment led to temporary improvement initially in all ten pediatric patients treated. Ultimately, four of these patients died as a result of their malignancy. At the time of their publication, five of the surviving six patients had maintained the neurologic improvement. Leber and

colleagues[78]reported the use of hyperbaric oxygen in two patients with radiation necrosis after radiosurgery for arteriovenous malformations. After hyperbaric treatment, both patients had shrinkage of their lesions by imaging studies and one had complete resolution by serial MRI studies. The paper by Cirafisi and Verderamae[80] presents a case report of a single patient who failed to respond to hyperbaric oxygen. The patient had also failed to respond to steroids and anti-coagulants. Gesell and colleagues[82] have reported the largest experience to date in applying hyperbaric oxygen to the treatment of radiation-induced brain necrosis. These results were presented at the 2002 Annual Meeting of the Undersea and Hyperbaric Medical Society. After hyperbaric oxygen treatment seventeen of twenty-nine patients had an improved neurologic examination and in twenty of twenty-nine patients, it was possible to decrease steroid requirements. At the same meeting Dear and associates[83] presented their experience in treating twenty patients with radiation-induced brain necrosis. In eleven patients with Glioblastoma Multiforme, only one patient showed objective improvement. However, seven of these eleven patients were dead of tumor within a short time following hyperbaric oxygen and obviously had active tumor at the time of treatment. It is very likely that the presence of tumor contributed to the neurologic deficits manifested by these patients. It is a consistent observation that soft tissue necrosis lesions within previously irradiated fields will not heal if tumor is present in the wound or area of injury. In the other nine patients with other tumors reported by Dear, eight were subjectively improved and three had objective improvement. If we combine the results from these six publications, we find that twenty-nine of sixty-three (46%) patients reported had a positive therapeutic outcome with hyperbaric oxygen. No other treatments short of surgical resection of the necrotic focus have been effective.

There are four publications reporting the results in applying hyperbaric oxygen to the treatment of radiation induced optic neuritis. Again all four of these publications are case series including the single case report by Fontanesi.[74] Only four of the nineteen patients reported in these publications had visual improvement. Guy and Schatz[72] report in their series that hyperbaric oxygen must be initiated promptly. In their series, two patients had complete restoration of their sight when they began hyperbaric treatment within seventy-two hours of loss of their sight. The other two patients who had begun hyperbaric oxygen at two weeks and six weeks respectively after loss of vision had no improvement. In the paper by Borruat et al[76], a single patient with bilateral radiation-induced optic neuritis had complete resolution in the more recently affected eye and improvement but not resolution in the eye first affected. These results also suggest the importance of early intervention in order to obtain an optimal outcome. In this desperate circumstance where permanent blindness is likely to occur, a trial of hyperbaric oxygen intervention would appear to be justified based on humanitarian considerations. This therapy must be promptly initiated.

Finally, in regard to peripheral nerve injury treated by hyperbaric oxygen, we have the randomized controlled trial conducted by Pritchard and colleagues[81]. This study was designed to investigate the effect of HBO_2 in the treatment of radiation-induced brachial plexopathy. This trial is a negative study in that it fails to demonstrate a therapeutic ***improvement*** in neuropathy for patients receiving HBO_2. The median time from onset of symptoms until enrollment into the trial was eleven years. As noted above, in some neurologic disorders, a positive outcome for hyperbaric oxygen requires prompt intervention. Importantly and interestingly, patients in the hyperbaric arm did demonstrate less post study deterioration in neurologic function compared to the control group. This decrease in rate of deterioration was statistically significant. Six patients in the hyperbaric arm also experienced an unexpected reduction in symptomatic lymphedema of the affected arm.

The Issue of Carcinogenesis

An issue that frequently arises when considering a patient for hyperbaric oxygen who also carries a cancer diagnosis is what does HBO_2 do to growth or potential recurrence of the malignancy. In a publication from 1994, Feldmeier and his colleagues[84] reviewed the discoverable literature related to this issue. An overwhelming majority of both clinical reports and animal studies reviewed in this paper showed no enhancement of cancer growth. A small number of reports actually showed a decrease in growth or rates of metastases. In 2001 at the Consensus Conference jointly sponsored by the European Society of Therapeutic Radiology and Oncology (ESTRO) and the European Committee for Hyperbaric Medicine (ECHM), Feldmeier[85] updated this material. In this review, Feldmeier emphasized the differences known in tumor and wound healing angiogenesis. Each has similar but distinctly different processes operational. He showed that there are significant differences between tumors and wounds in the growth and inhibition factors, which modulate angiogenesis. He summarized the literature demonstrating that tumors, which are hypoxic, are less responsive to treatment, less subject to cellular death by apoptosis and more prone to aggressive growth and lethal metastases. Fears that hyperbaric oxygen may promote malignant growth are not supported by scientific evidence, and clinicians should not refuse to consider a patient for hyperbaric oxygen who has had a history of malignancy.

Additional Avenues for Research

As discussed in the Introduction, the identification of certain biochemical markers (mostly cytokines), which have been related to radiation injury, and their serial assay over time may allow for the identification of individuals at risk for radiation injury before the clinical expression of this injury. In this case therapeutic strategies could be investigated to determine whether the injury could be prevented. Hyperbaric oxygen would appear to have potentially broad application in this case.

Additional studies exploring methods by which hyperbaric oxygen can be utilized as a radiosensitizer are also justified by previous publications. The use of oxygen as a safe and effective radiosensitizer is based on sound radiobiologic principles. Some innovative methods to administer radiation during or just after a session of hyperbaric oxygen have been discussed by several authors. At the Children's Cancer Center at the University of Amsterdam researchers have pioneered a technique whereby children with advanced neuroblastoma are infused with a radioactive isotope (MIBG) and then treated with hyperbaric oxygen.[86] Response rates have been far superior to experience with the isotope treatment without HBO_2. Two different groups of investigators from Japan have published their experience in treating patients with high-grade primary brain tumors with radiation immediately after a hyperbaric oxygen exposure.[87-89] These results are also much more successful than historical experience without hyperbaric oxygen. One of these two groups has shown the importance of delivering the radiation within 15 minutes of the hyperbaric exposure.

SUMMARY

Hyperbaric oxygen has shown consistent benefit in treating patients with delayed radiation injury. It has also had success in preventing radiation injury in some instances. Additional study in identifying patients at risk for injury and delivering hyperbaric oxygen with prophylactic intent to prevent these injuries appears to be promising. Additional approaches to applying hyperbaric oxygen as a radiosensitizer also deserve further study. No convincing

evidence exists to support concerns that hyperbaric oxygen enhances or stimulates malignant growth.

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Oxygen 2002: Wounds

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INTRODUCTION

The years 2001 and 2002 were productive for oxygen research. During that time, we came to better understand the role of oxidants in the mechanism of healing, a large step. The list of known roles for oxygen lengthened. We found more proof that oxygen therapy is useful for wounds and wound infections. We learned better than before when hyperbaric oxygen therapy (HBO_2) will be effective and who will benefit from it.

We ended the twentieth century with the certainty that oxygen has its place in all major components of wound healing. In addition, we now know that those oxidants derived directly from oxygen, reactive oxygen species (ROS), are vital to the signaling process that transcribes collagen genes [1]). We learned that this effect applies to many cell types, including vascular endothelial cells. Oxygen, we realized, is absolutely required for the resistance to bacterial infection [2], but we now know that the bacteria-killing ROS are also fundamental to the entire process of healing, a rare theoretical simplification that we encountered in this area of research.

Going into the new millennium, we suspected that peroxide and other oxidants might regulate all of these events. The growing interest in nitric oxide (NO) upon the discovery that arginine accelerates wound healing was our first clue [3]). Since then, our suspicion has developed into fact, though the interest in NO as far as wounds are concerned, seems to have receded somewhat. The concept of increasing tissue oxygen tensions to promote angiogenesis and defeat infections is now defended by more than just a few qualitative clinical observations. Instead, we have a coherent theory, supported by known basic mechanisms.

To understand the advances, we must first disclaim the idea that reactive-oxygen species are usually harmful. In fact, the presence of ROS is a mainstay of life. They occur in countless, essentially chemical reactions, both enzymatic and non-enzymatic [4]. Oxidants become problematic only in excessive amounts [5]. Even though wounds are hypoxic by nature, it is clear they heal in an oxidative milieu. The H_2O_2 concentration in wound fluid is normally about 5 to 15 micromolar (unpublished). It is higher in neutrophils at the healing edge of the wound [2, 6]. The concentrations of other oxidants are unknown, but given the instability of most regulatory oxidants, their concentrations are probably not descriptive of their function.

The easiest and the least painful way to explain the new conception of wound healing, including the roles of ROS, is through the actions of oxygen in wound immunity. In this sense, wounds can be regarded as essentially inflammatory lesions.

Immunity

Wounds represent a severe break in immunity to bacterial infections. The more hypoxic a wound, the more vulnerable it is. Oxygen, through the production of ROS, is essential to resist infection (2, 7, 8). For instance, in the case of staphylococcus, the major wound pathogen, oxidants generated by leukocytes seem to be the only bactericidal mechanisms. Tissue hypoxia is probably the most significant background for vulnerability to wound infection (7).

The killing of bacteria within leukocytes has many mechanisms, usually divided into oxidative and non-oxidative. Non-oxidative mechanisms provide immunity to the run-of-the-mill bacteria that infest wounds only in the immune-suppressed patient. Oxidative killing, on the other hand, is responsible for eliminating the species that commonly infest wounds. Hence, we can conclude that hypoxia is the weak spot in the defensive barriers. Nevertheless, the 'spectrum' of oxidative killing is wide. Recent observations suggest that all kinds of leukocytes produce bactericidal oxidants and employ them as lethal agents to kill bacteria and even tumor cells. All wound cell types, fibroblasts, endothelial cells, neutrophils, and macrophages generate these oxidants (4). However, polymorphonuclear leukocytes seem to provide most of the action, at least in wounds in which these professional bactericidal cells kill almost all wound pathogens by engulfing them and exposing them to ROS. When oxygen tension is low, they lose this power.

The mechanism of bacterial killing is deceptively simple. Upon entry into the wounded area, leukocytes are 'primed' for greater activation by exposure to complement, antibody-opsonized bacteria, etc. When they ingest debris or bacteria and become fully activated, an enzyme complex, the NADPH or phagocytic oxidase (more precisely an 'oxygenase' and more easily called 'phox'), is assembled from its pre-existing components in the cell (9). It then begins a rapid increase in the cell's consumption of molecular oxygen, as much as fifty-fold, during which almost all consumed oxygen is converted to superoxide ($^{\cdot}O_2$). Through various means, $^{\cdot}O_2$ is thence converted to other oxidants, such as hydrogen peroxide, aldehydes, NO, etc. This is called the oxidative (or respiratory) burst. The genetic absence of phox causes a severe susceptibility to infection, mainly due to the types of bacteria that are common pathogens in wounds (10, 11).

The rough equation (the sum of several) for the oxidative burst is:

$$\text{Glucose} + \text{oxygen} \longrightarrow {}^{\cdot}O_2^- + \text{Lactate} + H^+$$

The activation of phox materially lowers the already jeopardized PO_2 in the wound, and raises the lactate and ROS concentrations. Most important is that the reaction proceeds at a rate proportional to the local concentration of oxygen (PO_2).

When assembled, phox is located in the phagosomal membrane. It adds an electron and injects the resulting $^{\cdot}O_2$ into the phagosome, where it, and other oxidants that derive from it, kill bacteria (12). This is the first function of oxidants. The degree to which it is performed is dependent on the oxygen and glucose that diffuse through the cell membrane. By supporting local PO_2 by providing oxygen and local warmth, wound infections that follow contaminated surgical procedures can be reduced by over sixty percent.

Lactate and the relatively long-lived, freely diffusible H_2O_2 escape into the cytoplasm and the extracellular space, where their next function is to act as extra- and intra- cellular 'messengers' in a process called redox signaling [13].

Redox Signaling

Among other functions, these longer-lived and diffusable oxidants are signals that one cell sends to itself or to another, directing the activities of the recipient cell. The paracrine mechanisms, most commonly employing H_2O_2, are best known.

By 2002 we already felt that hyperoxia is helpful to angiogenesis, despite conclusive data proving that vascular endothelial growth factor (VEGF) is a product of hypoxia. We have resolved this apparent paradox and can ascribe the solution to redox signaling, as well. Even in hypoxic circumstances, a low level of H_2O_2 instigates the production of angiogenic growth factors [14], while oxygen itself regulates vessel growth [15].

Oxygen, then, has several major roles in wounds. There is the production of energy, of course, but little respiration occurs in wounds, where energy is produced largely by glycolysis. Oxygen is important to structural protein synthesis, since hydroxylation of proline in procollagen gives collagen its tensile properties. The next, and perhaps last, function to be explored will be oxidants for signaling.

Lactate

Seen from a linear, rather than dynamic, point of view, the healing mechanism now briefly splits into two arms. Lactate enters, as well as leaves, cells. Intracellular and extracellular lactate is in equilibrium. When it exists in significant concentrations, it chelates iron (particularly intracellularly), thereby modifying Fenton chemistry and leading to more oxidant production, now intracellularly, and with an emphasis on hydroxyl radical. That is, the normal reaction between Fe^{++} and O_2 that releases H_2O_2 is changed somewhat to produce relatively more hydroxyl radicals (*OH) [16, 17]. This highly energized radical has a both a short life and a short path through which it can produce its effects. In the extracellular space, hydroxyl radical is scavenged by antioxidants. In the cell, it is released into or close to the nucleus and can exert many actions. For instance, oxidants are known to enhance the transcriptions of several pertinent genes, including those of collagen and vascular endothelial growth factor (VEGF) [18]. Loosely bound Fe^{++} is present intracellularly.

In a parallel step, lactate accumulation forces reduction of NAD+ to NADH via lactate dehydrogenase. This decreases the amount of NAD+ that is available for ADP-ribosylation [19]. This is known to enhance collagen gene transcription and control the activity of VEGF [19-22]. The details are not pertinent to oxygen, except that its existence allows NAD+ to be decreased, despite adequate oxygenation [23, 24]. Thus, the signaling for healing that resides in this reaction can persist, despite a relatively high PO_2.

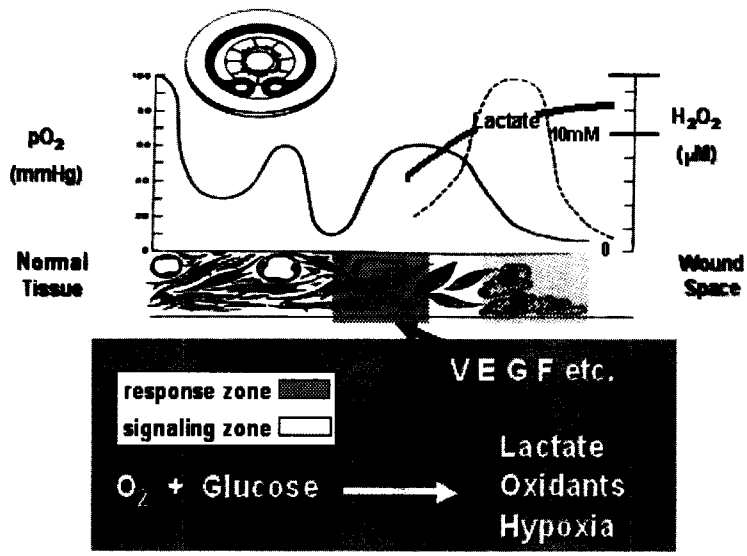
Adding lactate to closed wounds increases VEGF, TGF-β, and IL-1 several-fold (for a shorter time). It also raises collagen and angiogenesis at least forty percent. In the deposited tissue, collagen rises, while total DNA and total protein do not, suggesting that the lactate effect of ADP-ribosylation is particularly important to collagen [25].

From this discussion, it should be clear that the accumulation of lactate in wounds is not due to hypoxia [24]. Lactate is produced largely in the oxidative burst (above) and in the "Warburg phenomenon" [26], in which rapidly dividing cells rely heavily on aerobic glycolysis

—the metabolism of glucose to lactate in the presence of oxygen — as their prime energy source.

Growth Factors

Many of the growth factors/cytokines appear to be produced as a result of oxidant activity. Among these are VEGF, IL-1, TGF-β and IGF-1. A VEGF gene is known to respond to hydrogen peroxide alone [27]. The important growth factor, hypoxia inducible factor-1α (HIF-1α), which responds to hypoxia (a result of oxidant production) actually *requires* some sort of oxidant, in addition to hypoxia, for its transcription [28-30]. HIF-1α also goes on to stimulate VEGF. Also, lactate and peroxide lead to the expression of an early growth-response gene, one of the early responses to cell 'stress.' From this data, it appears as though the definition of wound could be, "a volume of tissue in which the microcirculation is injured, and at the same time, inflamed, oxygen-poor, oxidant- and lactate-rich" (Figure 1).



Fig, 1. Wound Model

Thus, *another function* of oxidants is to act as (intercellular and/or intracellular) messengers for the elicitation of growth factors/cytokines. This is not to say, however, that oxidants are the only path. IGF-1 also enters from blood and platelet derived growth factor (PDGF) from platelets. Oxidants also assist in the downstream effects. For instance, VEGF causes endothelial cells to migrate, multiply, and secrete proteases that clear out spaces in the pre-existing capillary membrane. The cells can then migrate toward the source of the VEGF. The function of at least one protease, MMP-9, is also redox-regulated. It is useful to visualize oxidant chemistry in a compartmentalized context.

Fibroblasts and Collagen

Procollagen gene transcription occurs upon exposure of fibroblasts to peroxide [1]. At this point, it is easiest to switch back to the subject of molecular oxygen. As noted, its concentration, (i.e. PO_2) is low within approximately a day of injury. This hypoxia becomes highly significant. In a twist that was almost unimaginable a year ago, our prior knowledge that oxygen is required at rather high PO_2 for fibroblasts and endothelial cells to produce collagen has been expanded. Adding oxygen at this point supports collagen deposition for mechanical support of tissue integrity as well as for the endothelial cells to align themselves into tubes and support

blood pressure [31], i.e., to support angiogenesis. In short, wound healing (and signaling) seems to require lactate, as well as sufficient oxygen, to support collagen deposition and oxidant production. These two processes compete for oxygen as clinical behavior predicts. Both excessive and insufficient inflammation retard healing. Therefore, the trick is to get it just right. This is where hyperbaric oxygen enters the scene.

Hyperbaric Oxygen

One might ask if the administration of more oxygen would lower lactate levels. In short, this does not happen [32, 33]. Lactate has other sources in wounds. First, rapidly dividing cells release large amounts [of lactate] (Warburg Effect) by aerobic glycolysis. Literally, all cancer cells do this. Second, as noted above, leukocytes provide the energy for oxidant production from aerobic glycolysis. That is, the production of lactate actually amplified as oxygen availability increases. This means that lactate production may increase with *rising oxygen* and release more lactate as a consequence. This occurs between PO_2 zero and about 800 mm Hg. What lactate comes from hypoxia itself, if any, is not known.

The success of periodic high-oxygen concentrations in the breathing mixture depends on its transmission to the wound. If it is not transmitted, there is no hope of increasing healing. When excess oxygen arrives, oxidant production increases. In particular, H_2O_2 is increased, leading to more VEGF, additional collagen, and increased bacterial killing. In some wounds, the H_2O_2 may briefly reach toxic levels, but prompt cessation of added oxygen allows the antioxidant defense mechanisms to operate. Then, there may be hypoxia with a second stimulation of VEGF that is translated into blood vessel growth in the next cycle. In all of this, the lactate levels remain high [33]. Thus, the very nature of HBO_2 therapy, cycles of added oxygen, may account for some of its success.

CONCLUSION

In order to accept the new data, we must modify our attitudes about oxidants. Even if one is addicted to anti-oxidants, like vitamin E, ascorbate (ascorbate, a necessary cofactor for deposition of collagen, is another pertinent and tangential subject to the oxygen story), etc., one must also admit that oxidants are essential to life. Most reactions that use molecular oxygen, of which there are many, involve the formation of a transient oxidant radical. This is exaggerated in the normal inflammation of wound healing. Thus, the architecture of wounds, the oxygen gradients that cross the inflamed zone, is the setting for their sensitivities to hypoxia and responses to oxygen [34].

The interjection of ROS has so far provided such a cohesive and continuous set of mechanisms that they might well be thought of as the core of repair. Many parts of healing, lysis of matrix, integrins, and mitogens, we suspect, will be hung on this core when the full explanation is finally disclosed.

Life seems to be perched on a narrow path between too little oxygen, or asphyxia, and oxidation (rust). However, this does not mean that we cannot supply more oxygen and more oxidants to our useful advantage for short periods of need. Important defenses, such as inflammation and wound healing, depend on them. The basic rule is that health requires short and/or gentle exposures, while long and extreme exposures are generally toxic.

Where these realizations will take us is anyone's guess, but I predict that the advances of the last two years will lead to practical application. Perhaps one might not have to think any

further than of adjusting the redox potential of wounds in order to adjust healing — whether we want less or more. It would be nice to have some agent that can act as a potent means of preventing scarring. That may be the next challenge. As of 2002, with the discovery of oxidant signaling, it seems feasible.

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Hyperbaric oxygen therapy in orthopedic conditions.

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BACKGROUND

Since 1972, Japan and the United States have operated a cooperative program focusing on the research of diving and physiology. I have attended these meetings since 1973. The US-Japan Cooperative Program in Natural Resources (UNJR) meeting is held every two years, in both countries on an alternating basis. At each meeting Dr. Christian J. Lambertsen has given me very valuable advice about how to design our research and present our papers (Figure 1).

Fig. 1. UNJR Meeting – Yokosuka 1987

I have also attended the Undersea and Hyperbaric Medical Society annual meetings of the since 1975. He has told me many things about research and the presentations of papers. I am extremely grateful to him for his help. Today, I will present my paper on hyperbaric oxygen therapy in orthopedic conditions.

From 1972 to 1981, I worked in Kyushu Rosai Hospital in Kitakyushu City. My primary research involved dysbaric osteonecrosis in divers. We treated more than 400 shellfish divers and performed radiological investigations on the divers in Ohura. Dysbaric osteonecrosis was found in 268 of 450, or 59.5%, of the divers (1, 2). Within that group, 73.1% were known to have been treated for bends. Thus, bends were significantly related to the occurrence dysbaric osteonecrosis.

We have also undertaken cooperative research with Dr. Charles E. Lehner, from the University of Wisconsin in Madison. We sent our diver's diving profile to Dr. Lehner who succeeded in producing experimental dysbaric osteonecrosis in sheep (3). We have taken part in productive, cooperative research with scientists in the US through the UJNR program.

At Kyushu Rosai Hospital at that time, the indications for HBO_2 therapy were very limited. HBO_2 therapy was approved only for carbon monoxide poisoning, decompression illness and gas gangrene. Had we treated other diseases, our insurance system would have cut all reimbursement for HBO_2. This was our most significant problem with HBO_2 therapy. However, the Japanese Society for Hyperbaric Medicine gained political power through the efforts of Dr. Sakakibara and others. We can now treat many kinds of diseases with HBO_2 therapy.

Fig 2. Prof. Juro Wada President of the 4th International Congress on Hyperbaric Oxygenation in Sapporo (1969)

The first diving bell was imported from Holland in 1834 and placed at the Mitsubishi Heavy Industry Nagasaki Ship Product Factory. Mankichi Masuda used helmet diving to collect abalone in the Chiba Prefecture in 1877. Diving fishermen in Oura, Ariake Sea, continue the same style of helmet diving.

In 1958, Saito reported HBO_2 therapy for stroke (4). An HBO_2 chamber was built in Kyushu Rosai Hospital for Decompression Illness (DCI) and carbon monoxide (CO) poisoning in 1961. In 1965, Professor Juro Wada reported HBO_2 therapy for carbon monoxide poisoning and severe burns caused by the fire from a 1965 coal mine gas explosion (5). He organized the fourth International Congress on Hyperbaric Medicine in Sapporo in 1969 (Figure 2).

In 1966, the Japanese Society for Hyperbaric Medicine was established. In 1994, Mahito Kawashima organized the 29th Annual Meeting in Nakatsu. In 2002, Sugiyama held the 37th Annual Meeting, in Tokyo. As of June 30, 2001, there were 903 monoplace HBO_2 chambers and 54 multiplace chambers in Japan. The indications for the emergency use of HBO_2 therapy of the Japanese Society of Hyperbaric Medicine are as follows: CO poisoning, gas gangrene, decompression illness, air embolism, crush injuries, severe burns, shock, myocardial infarction, ileus, cerebral embolism, cerebral edema, brain ischemia, acute retinal artery occlusion, and spinal cord injuries. Indications for non-emergency use include malignant tumor treated with chemotherapy or radiotherapy, refractory ulcer, ischemic skin flap after skin graft, SMON (Kinohorm Poisoning), one week after the onset of emergency cases of cerebral ischemia, refractory osteomyelitis, and sudden deafness.

HBO_2 Therapy in Kawashima Orthopedic Hospital

The history of HBO_2 therapy at Kawashima Orthopaedic Hospital in Nakatsu is as follows: In 1981, an HBO_2 chamber was built and now two multiplace chambers are used for HBO_2 therapy (Figure 3).

Fig. 3. Chamber for HBO_2 at Kawashima Orthopaedic Hospital

In 1987, the tenth meeting of Japanese Bone and Joint Infection Society was held in Nakatsu. In 1990, the third Kyushu-Okinawa Society for Hyperbaric Medicine was held in Nakatsu. In 1994, The 29th Meeting of Japanese Society for Hyperbaric Medicine was held in Nakatsu. The 2001 International Seminar was also held in Nakatsu (Figure 4).

In terms of therapy from 1981 to 2001, the hospital performed 145,700 treatments in 4035 patients (Figures 5, 6 and 7). In an orthopedic hospital, infectious diseases are an important indication.

Fig. 4. 2001 International Seminar in Nakatsu

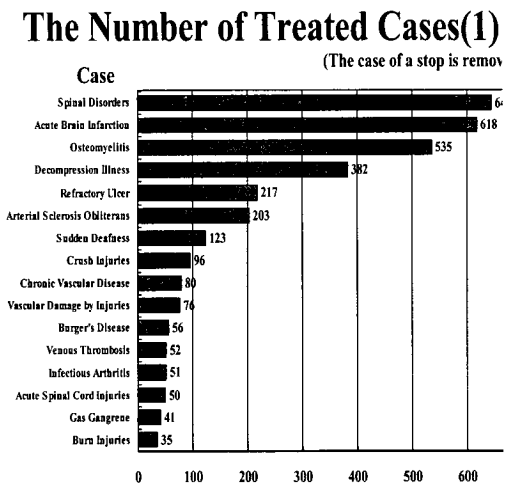

Fig. 5. The number of treated cases (1)

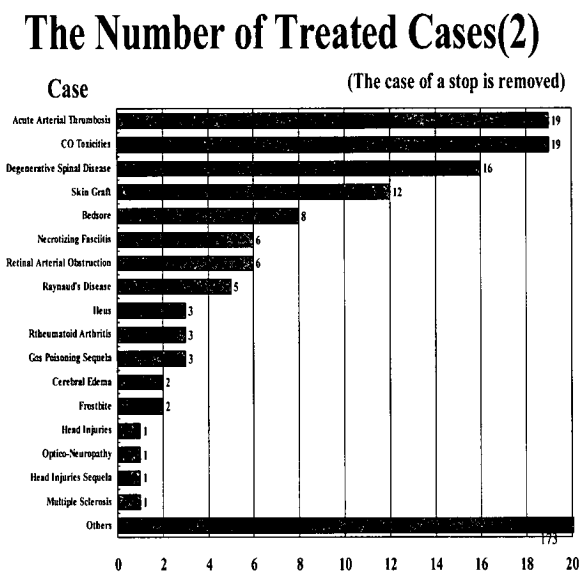

Fig. 6. The number of treated cases (2).

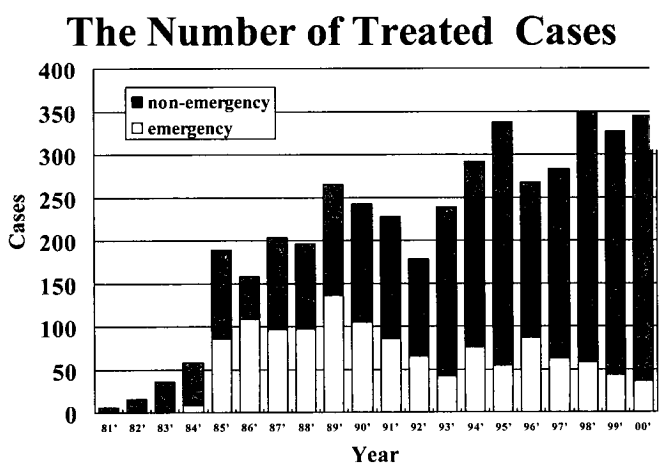

Fig. 7. The number of treated cases (3)

Crush Injuries

Crush injuries are traumatic ischemia that cause such severe damage to tissues from the energy transfer that tissue survival is in question (6). Usually, two or more tissues are injured severely enough that their survival is unsure. Edema due to tissue hypoxia has detrimental effects on wound healing and infection control. It interferes with oxygen availability for cells that already

have increased oxygen needs. A second harmful effect of edema is the collapse of capillaries. The edema fluid increases the interstitial pressure around the capillaries. Once the interstitial fluid pressure exceeds the capillary perfusion pressure in a closed space, the capillary bed collapses, and flow in the microcirculation ceases.

Bacteria grow almost without restraint if circulation is disrupted at the site of injury. With the disruptions of the blood supply, antibiotics can no longer reach the injury or infection site. In the hypoxic environment, neutrophils lose the ability to generate the reactive oxygen species that kill bacteria. As tissue oxygen is much reduced in acute traumatic peripheral ischemia and decreases a local site's ability to handle infection, impaired wound healing, and wound contracture are additional secondary problems. A wound will not heal unless there are sufficient oxygen tensions for fibroblasts to function (8). A tissue oxygen tension of 30mmHg is required for fibroblasts to mobilize and produce the collagen matrix needed for neovascularisation and wound repair.

Vasoconstriction is a secondary effect of HBO_2. This leads to edema reduction. Hyperbaric oxygen exposure causes a 20% reduction in blood flow. With decreased blood flow, extravasation of fluid in the area of injury is decreased. Since capillary resorption of extracellular fluid continues, the net effect is edema reduction. Edema reduction of 20% or more has been observed in laboratory studies (9). Increased oxygen content in the blood from HBO_2 compensates for the decreased flow. Moreover, flow in the microcirculation is improved as edema decreases and reduces external pressure around the microcirculation. HBO_2 reduces the amount of skeletal muscle necrosis. It also reduces post-injury muscle necrosis and edema. HBO_2 for crush injuries should be started as soon as feasible. It is also used as an adjunct for the management of compartment syndromes and other acute traumatic peripheral ischemias. We have treated many crush injuries with severe swelling, edema and infections, and the wounds completely healed.

Osteomyelitis

The oxygen tension in osteomyelitic bone is low, rarely exceeding 25mmHg of oxygen. Using animals, Mader, *et al.* have shown that oxygen tensions in normal, as well as infected, tissue are increased by HBO_2 (10). In the Mader studies, the therapy increased the oxygen tensions in both normal and osteomyelitic bones (10). Under ambient conditions, the oxygen tension in the osteomyelitic bone was 23mmHg, whereas oxygen tension in the normal bone was 45mmHg. HBO_2 increased the oxygen tension to 104mmHg in osteomyelitic bone, and to 322 mmHg in normal bone.

The polymorphonuclear leukocyte (PMN) is primarily responsible for fighting bacterial infection. Using an *S. aureus* model, Mader showed a proportional relationship between oxygen tensions and phgocytic killing ability. Increasing the oxygen to 150mmHg and 760mmHg killed the greatest number of *S. aureus* (10). The study showed improved treatment of experimental staphylococcal osteomyelitis with the adjunctive use of HBO_2, probably as the result of enhanced oxygen-dependent killing mechanisms.

Fibroblasts cannot synthesize collagen or migrate to the affected area when oxygen tensions are less than 20mmHg. Elevating oxygen tensions to levels greater than 200mmHg allows a return to normal function (8). Increasing oxygen tensions with HBO_2 therapy is a means of returning fibroblastic activity to normal. Following differentiation from fibroblast-like mesenchymal cells, osteoblasts deposit a layer of coarse immature fibrillar bone. This immature

bone is then replaced by mature lamellar bone, which is functionally reconstructed by resorption and deposition by osteoclasts and osteoblasts (11).

Barth, et al demonstrated the beneficial effects of HBO_2 therapy on bone healing by showing that the metaphyseal defects in the cortex of rat femurs were healed by primary ossification when rats were treated once-a-day for 90 minutes with HBO_2 at two atmospheres (12). Vancomycin, quinolones, sulfomides, and the aminoglycoside class of antibiotics have been shown to be far less active in the hypoxic environment (13). Mader has shown that with HBO_2 therapy, the bactericidal activity of aminoglycosides is enhanced (10).

We have treated 256 cases of osteomyelitis without HBO_2 therapy by debriding the infected area with closed irrigation. We achieved good results in 226 cases (88.3%), fair results in 7 cases (2.7%), and poor results in 23 cases (9.0%). We treated 433 cases of osteomyelitis with HBO_2. Good results were obtained in 398 cases (91.9%), fair results in 10 cases (2.3%), and poor results in 25 cases (5.8%). The results of the treatment with HBO_2, therefore, were better than the treatment with non-HBO_2 ($p<0.01$) (Table 1).

The result of the Treatment for 668 Cases of Osteomyelitis

	Non-HBO (Closed Irrigation only)	HBO
Good	226(88.3%)	398(91.9%)
Fair	7(2.7%)	10(2.3%)
Poor	23(9.0%)	25(5.8%)
Total	256	433

($P<0.01$)

Table 1

In the case of H.M., a consistent discharge was present from the tibia area. A skin and bone defect near knee joint was readily observable. We treated the condition with closed irrigation suction treatment and a skin muscle graft. Then, we started HBO_2 therapy. Two months later, the wound was completely healed.

The case of F.H., the victim of a traffic accident, was very serious. One hospital treated him, but delayed union, consistent discharge, and a defect of the tibia were seen. He was sent to our hospital, but he refused admission and operation. We treated him with HBO_2 therapy only. Three months later, the discharge stopped and the bone was healed. After six months, an almost-fusion of the tibia bone was observed, without any kind of the bone graft or other operation.

Gas Gangrene

Gas gangrene is a fulminating myonecrotic infection caused by clostridial species of bacteria. Untreated, this characteristically has a rapidly fatal outcome. Brummelkamp and associates first reported the use of HBO_2 in the treatment of gas gangrene in 1961. Demello, et al reported greater survival in dogs with experimental gas gangrene when three treatment modalities were used together compared with the use of one treatment modality or in combinations of two (14). Of more than twenty exotoxins produced by six species of clostridial organisms 7 are capable of producing lethal gas gangrene in man (15). When *C. perfringens* is the offending organism, one of these, alpha toxin, is of chief clinical significance. Alpha toxin is a lecithinase C that hydrolyzes the intact lecithin molecule to produce phosphoryl chlorine and a water insoluble diglyceride. The progressive nature of gas gangrene depends on the continuous production of alpha toxin by the organism.

Demello *et al.* did a comparative study on gas gangrene in dogs of the outcomes of all combinations of HBO_2, antibiotics, and surgery. They found that when all three treatment modalities were used together, the mortality was significantly lower than when only one or two were used (16). Hill and Osterhout saw a significantly increased survival rate in mice treated with HBO_2, when compared with controls not so treated (17). Stevens found that oxygen tensions of 40mmHg suppress clostridial growth, and oxygen tensions of 80mmHg suppress toxin synthesis. The cumulative mortality of their series was approximately 25%, while the disease specific fatality rate approximates fifteen percent. When patients were started in treatment within 24 hours of the presumptive diagnosis of gas gangrene, the disease-specific fatality rate was reported as five percent (18).

We have treated 32 cases of gas gangrene. Twenty-nine (90.6%) patients had good results and 3 (9.4%) had poor outcomes. We had one amputation and two cases were fatal.

Necrotizing Fasciitis

Necrotizing fasciitis, originally called hemolytic streptococcal gangrene, Meleny's ulcer or acute dermal gangrene, is a progressive, generally rapid spreading, inflammatory process in the deep fascia with secondary necrosis of subcutaneous tissues and skin. Skin necrosis occurs due to thrombosis of subcutaneous blood vessels. The whole area may become anesthetic by necrosis of nerve fibers. Riesman reported a mortality rate of 66% in necrotizing fasciitis in those not treated with HBO_2 and 23% in HBO_2-treated cases (19). Primary and aggressive surgical debridement is the cornerstone in the management of this disease. Early and extensive incision of the skin and subcutaneous tissue, wide into healthy tissue, followed by the excision of all necrotic fasciae, nonviable skin, and subcutaneous tissue, is necessary. Antibiotic treatment has an important place in the combined management of necrotizing fasciitis, although it is adjunctive to surgery. Mader and Thom have extensively outlined the rationale for the use of adjunctive hyperbaric oxygen and the mechanisms (20, 21). The main goals are a) the improvement of tissue pO_2, b) the improvement of phagocytic function through stimulating the oxygen-dependent killing mechanisms, either directly or indirectly, and c) the diminishing of edema and improvement of circulation in affected areas. This can be roughly summarized as the stimulation of the host defense and repair mechanisms (22).

Diabetic Foot

One of the most difficult complications of diabetes is foot infection. It has been reported that of the total diabetic population requiring hospitalization, 20% are admitted for foot problems and 30% have evidence of peripheral vascular disease (23). The peripheral neuropathy associated with diabetes leads to hypesthesia, which allows the unperceived development of traumatic pedal wounds. In addition, diminished pedal pain perception allows for the development of severe infections before patient become aware of them. Ulceration can develop and become secondarily infected. Even without the development of infection, these atrophic ulcerations are difficult to heal because of continued weight bearing. The accelerated atherosclerosis of infra-inguinal arteries observed in diabetics can produce significant, asymptomatic ischemia of the foot (24).

In a large clinical series of 168 patients with grades three and four diabetic foot lesions, Davis (25) reported a 70% success rate with the combined management protocol of daily debridement, metabolic control, and daily HBO_2 for 30 to 60 days. Oriani, et al (26) reported on the effect of the HBO_2 group which consisted of 62 patients while the matched control group had eighteen patients. In the HBO_2 group, 96% of the patients healed, while 4% underwent

amputation. In the control group, 66% achieved primary healing, and 33% required amputation ($p < 0.001$). We have treated 51 cases. Primary healing occurred in 44 cases (86.3%).

Other Indications

Oxygen is necessary for bone viability, healing, and remodeling. Osteocytes have the lowest requirement for oxygen. Osteoblasts, the bone-forming cell, have an intermediate oxygen requirement. This is reflected in an eight-fold or greater increase in blood flow in healing fractures. Osteoclasts, the bone-resorbing cells, have the highest oxygen requirement. Their metabolic activity may be a hundred times as great as that of the osteocytes (27). Basset reported that multi-potential cell precursors of fibroblastic origin form bone when exposed to high oxygen tensions and compressive forces. However, when oxygen tensions were low, cartridge was formed instead (28). Many studies have shown beneficial effects of HBO_2 on the mobilization of bone precursors, osteoid formation, and fracture healing. Increased oxygen enhances bone resorption and remodeling through stimulation of the osteoclasts. HBO_2 treatment is used for delayed union of fracture, radionecrosis, and idiopathic femoral head necrosis. Hyperbaric oxygen may be beneficial, especially when used in conjunction with other orthopedic interventions, such as core decompression, bone grafting, and electrical stimulation (29).

Hyperbaric oxygen was also used for various kinds of brain and spinal cord neurosurgical pathology. The main mechanisms of the effectiveness of HBO_2 in neurological disorders are the relief of hypoxia, improvement of microcirculation, relief of cerebral edema by vasoconstrictive effect, preservation of partially damaged tissue, prevention of further progression of secondary effects of cerebral lesions, and improvement of cerebral metabolism.

Lumbar spinal stenosis is a disorder of the spinal cord caused mainly by compression. Clinical results estimated by "criteria of the result of treatment lumbar part disease" (Japanese Orthopedic Association) for 143 cases treated by HBO_2 therapy from 1995 to 1999 showed an improvement in 125 cases (87.4%).

SUMMARY

As is well known, the origins and development of hyperbaric medicine are closely tied to the history of diving medicine. Our HBO_2 studies stemming from diving medicine date back to 1972. We concentrated our early basic research on dysbaric osteonecrosis. There are now good indications that HBO_2 is helpful in a variety of orthopedic conditions. However, hyperbaric medicine in orthopedics is still relatively new and some aspects of it remain controversial.

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Brain "Implications for HBO_2."

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I am a neurosurgeon and will discuss the clinical use of hyperbaric oxygen therapy (HBO_2) on the central nervous system, especially the brain. Is there a role for HBO_2 in brain injury? This is not an easy question to answer. I shall focus on traumatic brain injury. To date, "Brain Injury" is not among the approved indications of the UHMS.

I have been working in Fukuoka, Japan. Fukuoka is located in the north of Kyushu Island and is the site of pioneering work in HBO_2. My colleague, Dr. H. Yagi, is one such pioneer, as is Dr. M. Kawashima. We have a sub-society in the Kyushu district under the Japanese Society of Hyperbaric Medicine (JSHM). In Kyushu, we have now 168 monoplace and 17 multiplace chambers. We treat various kinds of diseases using these chambers. Last year, we treated 182 patients in the hospital using HBO_2. About half of vascular disease cases, both in the central nervous system and peripheral regions, were indications for HBO_2. Others had many kinds of diseases, including head injury. Last year, only two patients were given HBO_2 to treat head injuries. Because it is such a small number, my talk will be about a pilot study. We are estimating the effect of HBO_2 on brain injury and going to the next step. The treatment pressure commonly used in Japan is 2 to 2.8 ATA, depending on the severity of the disease. The treatment time is sixty minutes. The patients breathe 100 percent oxygen once each day.

Table 1

Classification of Head Injury (Gennarelli)

1. Skull injuries
   1) Vault fracture
   2) Basilar fracture
2. Focal injuries
   1) Epidural hematoma
   2) Subdural hematoma
   3) Contusion
   4) Intracerebral hematoma
3. Diffuse brain injuries
   1) Mild concussion
   2) Classical cerebral concussion
   3) Prolonged coma (Diffuse axonal injury: DAI)
      a. Mild DAI
      b. Moderate DAI
      c. Severe DAI

Skull fracture itself is not an indication for HBO_2, because the brain injury itself is crucial. It is important to determine whether or not the brain injury is focal or diffuse. Focal injuries include several types of hematomas and contusions.

A head injury classification is shown in **Table I** (1).

Hematomas include epidural, subdural and intracerebral. Diffuse brain injury can occur due to concussion or diffuse axonal injury (DAI), which typically causes coma. DAI is divided into three categories: mild, moderate and severe. The candidates for HBO_2 are focal injury and DAI. Evacuation of acute epidural hematoma (AEH) should be done promptly, but in pure acute epidural hematoma, HBO_2 is not indicated.

The prognostic outcome of severe head injuries is still poor, especially in traumatic acute subdural hematoma (ASH). In comatose patients with ASH, severe cerebral contusion or edema accompanying hemorrhage which is initially undetectable by CT may be progressive.

Fig. 1. Representative case of ASH with a poor outcome.

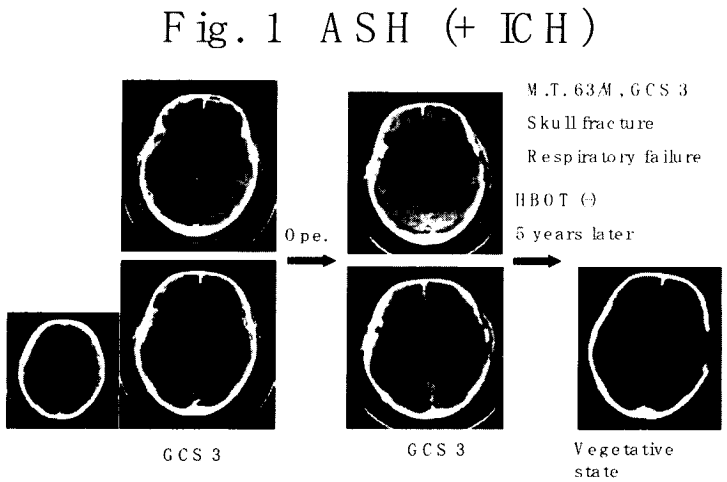
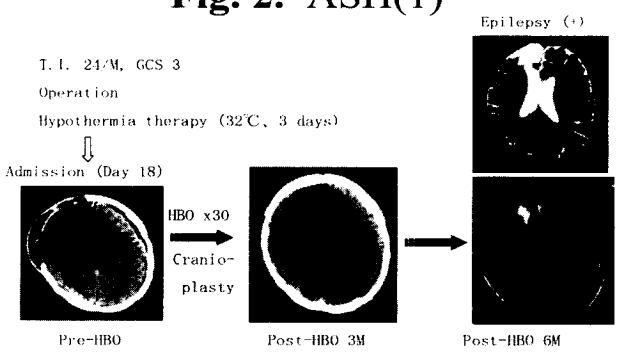


Figure 1 shows the head CT of a 63-year-old man who sustained his injury in a traffic accident. He suffered coma (Glasgow coma scale: GCS 3) and acute respiratory failure. Intratracheal intubation was done and CT was checked. The head CT showed a skull fracture and bilateral high-density subdural spaces due to subdural hematoma and contusional intracerebral hematoma in the left temporal lobe. An emergency operation was done. The hematomas of the left side, both subdural and intracerebral, were evacuated and external decompression (removal of the bone flap) was performed. Four days later, he was still comatose and the CT was checked again. The middle portion of Figure 1 shows low-density areas consistent with bilateral brain edema. A new hemorrhage also appeared.

Fig. 2. ASH(1)



Neither hypothermia nor HBO_2 was performed in this case because his injuries were very severe. Although the patient survived, he has never recovered consciousness and has remained in a vegetative state. An MRI taken five years later (Figure 1) showed marked ventricular dilatation due to severe brain atrophy. This suggests the existence of the late type of cell death. When the brain is injured, primary and secondary injuries occur. The primary injury is inevitable but preventing or minimizing secondary injuries is the key point of treatment. Hypothermia therapy has been attempted, but satisfactory results have not been obtained. Therefore, a combination of hypothermia and HBO_2 has been considered in severe ASH patients. A second case of ASH is shown in Figure 2. This patient underwent surgery and hypothermia therapy at the university hospital for three days. He was then transferred to us, 18 days after his accident. His CT shows a low-density area in the right frontal lobe and a defective skull (Figure 2, left image). After thirty sessions of HBO_2 and cranioplasty, he recovered well and with no neurological deficits. An MRI taken six months later showed a minimal lesion in the right frontal lobe.

The third case had a similar injury, though slightly more severe. The patient underwent cranial surgery and hypothermia therapy for four days and was transferred to us 23 days after head injury. At the time, head CT showed low density areas in bifrontal and left temporal lobes. HBO_2 was started and he underwent cranioplasty. After thirty sessions of HBO_2, he recovered. The head MRI (FLAIR image) showed minimal lesions in both bifrontal and left temporal lobes. The patient returned to work.

Table 2. Case Summary of Post-operative HBOT in Focal Injuries

No.	Age/Sex	Dx	Duration of Hypothermia	HBOT Started on	HBOT Total	GOS
1	24/M	ASH	3d	18d	30	GR
2	38/M	ASH	4d	23d	30	GR
3	21/M	ASH	9d	28d	50	MD
4	42/M	ASH	8d	51d	30	VS
5	19/M	ASH	-	47d	69	SD
6	60/F	ASH	-	13d	30	SD
7	73/F	ASH, ICH	-	1d	50	VS
8	49/M	AEH, ICH	-	5d	15	D
9	57/M	ICH	-	17d	20	SD
10	63/M	ICH	-	30d	30	GR

(32-33°C)

Table 2. A list of ten patients who underwent operations for focal brain injuries and had HBO_2.

The overall outcome is bad. However, in cases one through four, all of whom received hypothermia therapy (cases one through three are presented here), three of four recovered well; 50% is good recovery (GR) and 25% is moderate disability (MD). The combination of hypothermia and HBO_2 is expected to be an effective method to treat comatose, post-operative patients suffering from ASH.

Next, I will discuss diffuse-type injury, or the so-called diffuse axonal injury (DAI). DAI is caused by a shearing mechanism where there is usually no indication for surgery.

Fig. 3 Mild DAI (1)

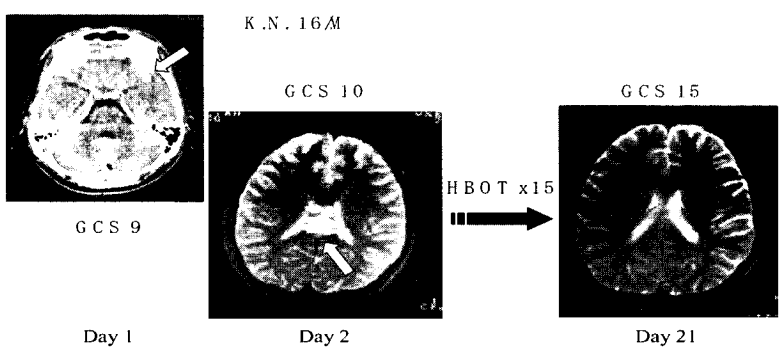


Figure 3 illustrates the case of a 16 year-old boy that had a traffic accident and was transported to us immediately. Head CT shows a small, high-density area at the tip of the left temporal lobe (arrow). In spite of only a small lesion, his consciousness was disturbed (GCS9). The MRI was checked the next day and revealed a white hyperintensity lesion on T-2 weighted images between the bodies of lateral ventricles, that is, corpus callosum (Day 2, arrow shown). This is a characteristic of DAI. The patient underwent 15 sessions

of HBO_2. The lesion disappeared and he was completely recovered.

A summary of DAI patients treated with HBO_2 is shown in Table 3. Mild to moderate DAI patients recovered well, but for those classified as severe, the outcomes were poor. Case number five had an additional fifty sessions of HBO_2 at another hospital after discharge. She was getting better and is now in MD.

According to an interesting paper published last year in *Journal of Neurosurgery* (2), the increased cerebral metabolic rate of oxygen ($CMRO_2$) and decreased CSF lactate levels after HBO_2 indicate that treatment may have improved aerobic metabolism in severely brain injured patients. It was also reported that HBO_2 reduced mortality by fifty percent in a prospective, randomized trial of severely brain-injured patients. Their functional recovery, however, was not improved [3]. Our cases showed functional recovery. We speculate that HBO_2 may have a promoting effect on some kinds of nerve growth factor (NGF). To conclude, it is time to answer the question: "Is there a role for HBO_2 in brain injury?" At this time, my answer is yes. We believe the indications for HBO_2 in brain injury are as follows:

Table 3. Case Summary of HBO-treated DAI

No.	Age/Sex	DAI	HBOT		GOS
			Started on	Total	
1	16/M	Mild	3d	15	GR
2	38/M	Mod.	12d	20	GR
3	24/M	Mod.	6d	20	GR
4	32/M	Severe	20d	60	SD
5	19/F	Severe	33d	50	SD→MD
6	30/F	Severe	73d	30	SD→MD

(1) Focal injuries, including acute subdural hematoma, contusion and intracerebral hematoma. These frequently coexist. The combination of hypothermia and HBO_2 is expected to be an effective method to treat comatose patients with severe focal injuries, especially those with acute subdural hematomas, after surgery.

(2) DAI; mild, moderate and severe. HBO_2 is effective in DAI when the disturbance of consciousness is severe, in spite of mild or lack of abnormal CT/MRI findings. The HBO_2 indications are not definite. Randomized control studies should be done to prove the efficacy of HBO_2 on severely brain-injured patients.

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Carbon Monoxide Poisoning.

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INTRODUCTION

Carbon monoxide (CO) is an odorless, colorless gas and stable product of incomplete hydrocarbon combustion. CO is toxic, and the topic of CO poisoning is timely because it is still a common accidental poisoning in the United States. There are many ways to be poisoned, particularly by inhaling exhaust fumes from internal combustion engines. CO's high chemical stability at physiological temperatures is a major point of emphasis because this property determines its biochemical activity and toxicity in the body (1). And recently, the apparent capacity of CO to serve as a signaling molecule in basic cellular processes has renewed scientific interest in the gas. This presentation, however, focuses primarily on the toxic effects of CO in the brain, because the brain is the major organ in which lasting effects of CO do occur. If one examines the brains of people who die from CO poisoning, a diverse neuropathology is found. Different brain regions are also affected differently, but all types of structures in the brain, including the basal ganglia, hippocampus, white matter, and cortex are susceptible to injury by CO. This complicated neuropathology suggests that CO poisoning produces a multifaceted mechanism of brain injury.

History of CO in Biology

It is useful to digress to the history of CO in the study of heme proteins because heme protein binding is a key to understanding the mechanisms underlying the nature of CO's pathology (2). The history of CO in biology reaches back to Claude Barnard at the Sorbonne in the 1860's who discovered that the gas causes asphyxia by chemically combining with hemoglobin. At the turn of the last century J.S. Haldane proposed the use of canaries in mines to detect CO in settings where coal gas poisoning was a problem. Small birds are very sensitive to CO because they have a rapid circulation time and a small hemoglobin volume. Otto Warburg, the German biochemist of the 1920's discovered that CO reversibly inhibits cell respiration. Warburg also found that he could reverse the effects of CO on cells by illuminating them with specific wavelengths of light that turned out to correspond to the absorption peaks of cytochrome *c* oxidase (see 1).

During and after World War II, CO-hemoglobin binding interactions were worked out, including its chemical ability to shift the oxygen dissociation curve of hemoglobin to the left. In 1950 cytochrome P_{450} was discovered, a family of proteins named after their CO absorption peak, which appears in the UV region at 450 nanometers. Not long

thereafter, it was discovered that CO was made endogenously in the body (2)—during heme catabolism—subsequently shown to be an effect of heme oxygenases (3). Hyperbaric oxygen (HBO_2) was first proposed by Pace *et al.* in 1950 (4) as a therapy for poisoning some 85 years after Claude Bernard's original description of the classical asphyxia mechanism. Pace reported that the rate of CO elimination from hemoglobin could be greatly accelerated by HBO_2 administration. This idea was then put to use ten years later by Smith and Sharp (5).

Two mechanistic observations of notable mention involve the work of Ronald Coburn at the University of Pennsylvania (6). Coburn found that CO bound myoglobin in skeletal and cardiac muscle *in vivo* and that this binding occurred in proportion to the CO to O_2 ratio in the cell. This demonstrated Otto Warburg's important principle governing the uptake of CO by living tissues and was even used by Coburn to predict the cellular PO_2. Then in the late 1970s, Caughey and Young (7) discovered that mitochondria actually oxygenate CO to CO_2 and that this involves cytochrome *c* oxidase (8). Caughey and Young thus explain the 1930s observations of Fenn and Cobb that living muscles actually slowly burn CO (9). Thus, when this speaker came to study CO in 1980 there was already a great deal known about the cellular and biochemical activities of this important gas (see 10).

Mechanism of Action of CO

In the 1980's the prevailing opinion about the mechanism of CO poisoning was that it was entirely due to cellular hypoxia. Today we recognize that there is at least a dual poisoning mechanism, and perhaps more subtle effects of the gas related to interference with cell signaling processes. However, Bernard's chemical asphyxia mechanism, known as CO hypoxia, is a key initiator of the process. Carboxyhemoglobin (COHb) does not carry oxygen and the O_2-binding sites on the hemoglobin molecule that are not occupied by CO show an increased oxygen affinity. This is the allosteric mechanism responsible for the shift of the oxyhemoglobin dissociation curve to the left (Figure 1). Thus, CO binding to hemoglobin causes both an anemia-like effect and an increase in the O_2 affinity of hemoglobin.

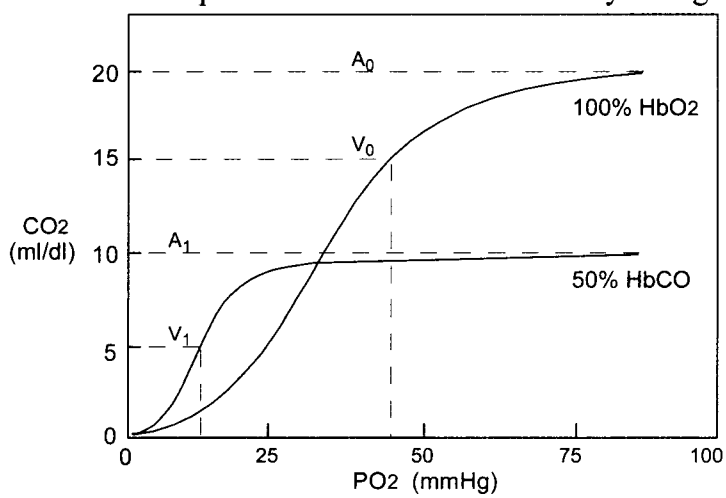

Fig. 1. Effect of carboxyhemoglobin formation on PO_2. Curves show the COHb-related decrease in the oxygen content of blood and left shift of the position of the oxyhemoglobin dissociation curve, which lower tissue PO_2 (see text for details).

The relationship of the equilibrium CO binding to hemoglobin dates to Haldane in the late 19th century (11). This so-called Haldane relationship is states that the steady-state

carboxyhemoglobin to oxyhemoglobin ratio is M times the ratio of the partial pressures of CO and O_2. M is a binding constant which for human hemoglobin has a value of about 220. Thus, hemoglobin has a much greater affinity for CO than O_2:

$$\frac{HbCO}{HbO_2} = M \times \frac{PCO}{PO_2}$$

This biochemical mechanism has physiological importance because it causes asphyxia or tissue hypoxia. In the presence of COHb tissue, PO_2 must fall unless O_2 delivery (cardiac output) increases or metabolism (O_2 consumption) declines. This CO-related fall in tissue PO_2 was first measured experimentally in animals more than 30 years ago.

The familiar oxyhemoglobin dissociation curve of Figure 1 plots PO_2 on the abscissa and the oxygen content of blood (CO_2) on the ordinate. The top curve shows the normal oxyhemoglobin curve for 100 percent HBO_2 and the difference between the arterial and venous points shows that about a quarter of the oxygen is extracted from the blood at a normal cardiac output and oxygen uptake rate. The effect of CO is illustrated at 50 percent COHb, where the anemia-like effect reduces the arterial oxygen content by one half. This means that a normal O_2 extraction lowers venous O_2 content, and hence tissue PO_2 is considerably reduced relative to normal conditions (same blood flow and oxygen uptake rate).

The presence of tissue hypoxia clearly produces many direct cellular effects, but hypoxia also increases cellular CO uptake. This was first appreciated by Warburg when he was studying CO effects in yeast. Warburg discovered that he could relate the uptake of CO to a constant (Warburg constant) which is simply the fraction (n) bound to CO, divided by [1-n] times the ratio of gas partial pressures. Thus both uptake mechanisms, the hemoglobin binding mechanism and the cellular gas uptake mechanism, depend on the ratio of the partial pressures of CO to O_2.

When tissue hypoxia occurs during CO poisoning deviations from the effect of simple hypoxia appear in part because the CO moving slowly into cells has inhibitory effects on cellular heme proteins such as myoglobin. CO's chemical stability means its main important biochemical effect is to bind to reduced transition metals. The body's most abundant transition metal, iron, is the main target and it binds CO only while in the ferrous (Fe^{2+}) state. Tissue hypoxia enhances CO uptake both by decreasing PO_2 relative to PCO and increasing the Fe^{2+} content of the cell. Thus, hypoxic conditions favor the binding of CO to heme proteins (6, 10). The heme proteins shown in Table 1 have been found to take up CO in living systems. In work done by Steven Brown in my laboratory about 12 years ago, cytochrome *a, a_3*-CO binding was shown to occur *in vivo* in the brain in the presence of a normal hemoglobin circulation (12). In principle, and as shown by experimental

Table 1. CO Interferes with Cell Function by Binding to Fe(II)

Hypoxia enhances CO uptake by heme proteins
Guanylate cyclase
myoglobin
cytochrome *a,a_3*
cytochrome P450
Intracellular uptake of CO alters heme protein function and causes oxidative and nitrosative stress.
Impaired heme protein function causes cell death; the mechanisms are complex and necrosis and apoptosis have been observed simultaneously.

measurement, intracellular CO alters heme protein function. In doing this, CO re-routes reducing equivalents (electrons) and creates oxidative and nitrosative stess (13-15), which will be discussed below.

The combined stress of hypoxia and too much intracellular CO leads to cell death, which derives from multiple factors, some of which are still unclear. In the brain, cell death of different types occurs at different times, and we have detected neuronal cell death of necrotic and apoptotic types (16). The precise cell death mechanisms are therefore complicated and not yet worked out at a molecular level, and they are a topic of an entirely separate discussion. It is sufficient to say that in brain and muscle, lower PO_2 will promote greater tissue uptake of CO *in vivo*, CO binding to heme proteins is observable, and on re-oxygenation, the presence of bound CO prolongs the period of energy deficit, increases the oxidative stress in the cell, and increases the probability of cell death (17).

One mechanism of oxidant production in CO poisoning is by the CO binding to cytochrome a,a_3 in mitochondria, which not only interferes with respiration but increases the rate of reactive oxygen species generation during the re-oxygenation period because it reverses only slowly after the cell PO_2 is restored. Measurements in my laboratory in the 1990s showed that this effect was associated with an increase in mitochondrial hydrogen peroxide leakage and significant mitochondrial glutathione depletion (14).

An important implication of PO_2 dependence on tissue CO uptake has to do with how CO is distributed throughout the body tissues. Inhaled CO rapidly crosses the alveolar capillary membrane and enters the intravascular space where it binds primarily to hemoglobin. According to the Haldane relationship, most of the CO is bound to the red blood cell at steady state, but there is equilibration with tissues, mainly involving CO binding to myoglobin and heme protein enzymes. At equilibrium for the body, this settles out normally with about 80 percent of the body store of CO in the intravascular space and about 20 percent in the extravascular space. In addition, endogenous CO production a normal adult is about 12 milliliters per day. Some of this CO is bound to heme protein enzymes, some is oxygenated by mitochondria to CO_2 and the rest enters the blood and escapes from the lungs.

When the concentration of inspired CO increases, the apparent volume of distribution of CO compartments increases; there is more CO in the vascular space and more CO in the extravascular space (6). The body burden of CO thus increases, and the amount of CO in the tissue expands further as the PO_2 falls. The more that tissue PO_2 falls, the greater the CO burden will be in the extravascular compartment, particularly in heart and skeletal muscle. After CO poisoning, if O_2 is breathed, the PO_2 in the intravascular compartment increases first because it is easier to raise the blood PO_2 than it is to raise the tissue PO_2. Thus, the amount of CO in the vascular compartment (COHb) declines before the CO in the tissues dissipates.

It is under these circumstances that we often encounter our CO poisoned patients. They frequently present with little or no elevation of COHb level. If they've been breathing oxygen the COHb is low, yet the clinical examination is abnormal, and there is likely an appreciable body store of CO, although this still needs experimental confirmation. Thus, further oxygen breathing or hyperbaric oxygen therapy should in theory clear the tissues of CO, and the clinical status can be restored to normal.

At this point, a few more words about oxidant mechanisms are useful because it provides special insight into one of the mechanisms of injury. In particular, new injury mechanisms have been identified related to the relationships between CO, reactive nitrogen species (RNS), and reactive oxygen species (ROS). Again, a key to understanding these principles is by their relationship to molecular iron (Figure 2).

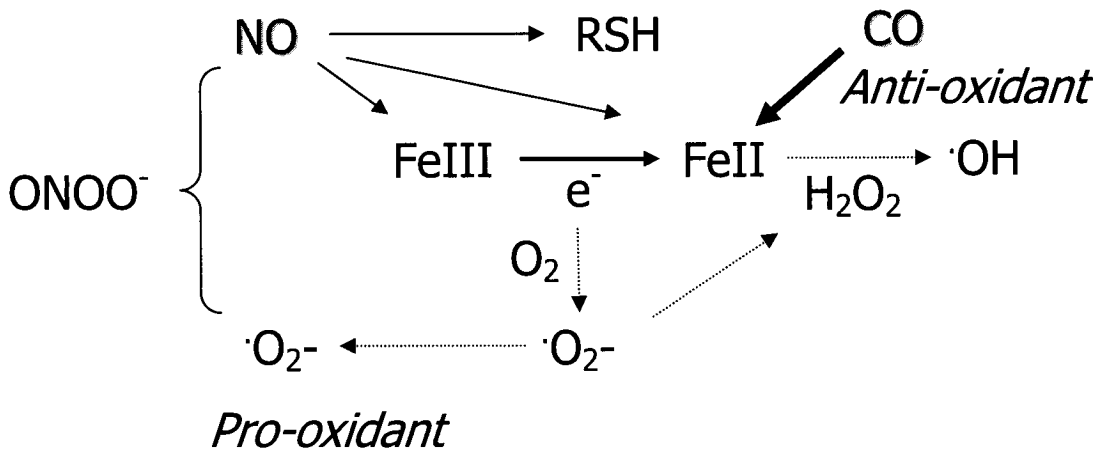

Fig. 2. Interactions between carbon monoxide (CO) and nitric oxide (NO) lead to changes in oxidative and nitrosative stress, which are are based primarily on the redox state of iron in the cell.

In vivo, Fe^{2+} is generally well-sequestered; non-sequestered Fe^{2+} is harmful because it increases the generation of hydroxyl radical (·OH) in the presence of hydrogen peroxide (Fenton reaction). Because CO binds only Fe^{2+} it been proposed by some to have an antioxidant effect by preventing free Fe^{2+} from participating in Fenton reactions. However, the body's mechanisms for sequestering Fe^{2+} are highly effective; this then is a hypothetical mechanism for which the evidence is not yet great. However, in the presence of nitric oxide (NO) the chemistry of CO changes because NO has possible alternative fates. First, NO is capable of binding either Fe^{2+} or Fe^{3+}. And though NO binds Fe^{2+} it is slowly displaced from the iron by CO. Thus, CO binding to Fe^{2+} supervenes over NO binding to Fe^{2+}. Second, reduced protein or peptide thiols are excellent NO-ligands (nitrosothiols, SNO), and these SNO compounds have both positive and negative effects on cell function. In situations where both CO and NO are present in the cell, what happens chemically depends to a great degree on the O_2 concentration. For example, re-oxygenation of a tissue after CO poisoning may allow electron transport systems that have been blocked to re-route electrons directly to O_2 forming superoxide anion, which then via enzymatic or spontaneous dismutation, leads to H_2O_2 production. Superoxide may also interact with NO to produce the very strong oxidant peroxynitrite (15). These are clearly pro-oxidant effects that damage constitutive cellular macromolecules. Furthermore, they may disrupt cell signaling processes in the brain that rely on endogenous CO production (18).

Clinical aspects of CO poisoning

The combined effects of cellular hypoxia, CO itself, and oxidative and nitrosative stress produce the pathology of CO poisoning and its clinical manifestations as well as the poor correlation between tissue damage and blood COHb. Normal individuals breathing clean air have COHb levels of 1 to 2% from endogenous CO production. If one lives in a large city like San Diego or Los Angeles, the COHb level is about 2%. If one rides on the freeway to work every day COHb may reach 3 to 5%. In smokers, for each pack of cigarettes consumed each day, the COHb level rises roughly 5%. However, in the absence of heart or lung disease, symptoms of CO poisoning do not generally appear until the COHb level reaches about 15%.

In severe CO poisoning, most patients have reached levels of more than 20% COHb; they have had significant tissue hypoxia and a significant increase in tissue CO burden. Therefore, the signs and symptoms of CO poisoning do not correlate with COHb level. They are nonspecific and variable. The most common symptoms are headache, nausea, vomiting, confusion, and flu-like illness. There are no reliable physical signs. It is notable that cherry red skin is rare. Also, in older adults, cardiac damage is common and may be overlooked easily. During the wintertime it has been estimated that 5 to 20% of emergency department patients with flu-like illnesses have occult CO poisoning. Thus, a high index of suspicion for the poisoning always should be maintained. The diagnosis is confirmed by Co-oximetry, performed equally well on an arterial sample or a venous sample of blood.

A major concern of treating victims of CO poisoning is the delayed neuropsychiatric syndrome (DNS). This interesting syndrome is characterized by a variable lucid interval followed by new neurological signs or symptoms that develop some days to weeks after acute poisoning. DNS is seen in 3 to 20% of acute CO poisoning victims, most often in older or more severely poisoned patients. Loss of consciousness has appeared as an independent risk factor for DNS in many of the reports in the literature. The long-term cognitive manifestations of the delayed syndrome can be very troubling and even disabling. Depression and memory loss are most common but dementia, Parkinson-like syndromes, seizures, and blindness have all been reported.

The prognosis of DNS patients has been hard to determine as will be discussed in the section on clinical trials. However, a few prognostic factors are clear. A poor outcome is predicted by advanced age, loss of consciousness, lengthy CO exposures, and metabolic acidosis. Independently, hypotension and cardiac arrest are poor prognostic factors and predict permanent disability and death. The long-term neurological effects of untreated CO poisoning are appreciable. The first to point this out were Smith and Brandon in 1973 (19). These authors, however, did not discriminate between the DNS and patients that had permanent sequelae from a severe initial poisoning. What is interesting about this series is that a significant number of patients, 13%, had gross neuropsychiatric abnormalities, about 30% had deterioration of personality and more than 40% had memory problems. This also was the first work to point out the relationship between loss of consciousness and persistent neurological sequelae.

Therapy of CO Poisoning

The mainstay of therapy for CO poisoning has traditionally been the administration of normbaric oxygen (NBO_2). The original rationale for HBO_2 was to

hasten elimination of COHb, and this rationale still holds today. But today the goals of HBO_2 therapy are more comprehensive because HBO_2 is intended to reverse the ongoing cellular energy deficit and prevent late cell death by a range of mechanisms; this has been the modern promise of HBO_2.

The potential benefits of HBO_2 are as follows: 1- eliminate COHb rapidly, 2- maintain adequate cerebral oxygen delivery, 3- eliminate CO from tissue heme proteins and restore their functions, e.g. improve energy metabolism, 4 - decrease cerebral edema, 5 - decrease leukocyte adherence, and 6 - decrease oxidative stress, e.g. interrupt lipid peroxidation and glutathione depletion. Most of these potential salutary effects of HBO_2 cannot be achieved with NBO_2.

The current UHMS treatment recommendations are simple and based on clinical empiricism. The UHMS has recommended HBO_2 for loss of consciousness or any other neuropsychiatric signs or symptoms (not headache alone) or evidence of cardiovascular compromise (20). Recommended treatment pressures have been between 2.4 and 3.0 ATA for 90 to 120 minutes. Residual neurological effects are treated for up to a maximum of 5 sessions, after which by peer review is recommended. The UHMS treatment guidelines are also practical because HBO_2 is relatively expensive, access to it is limited, and potential side effects such as O_2 toxicity have sometimes made it controversial. Since 1989 the question of efficacy of HBO_2 in acute CO poisoning has been addressed in six randomized control trials (RCT), which vary greatly in quality, cogency of study design, endpoint selection and outcomes.

The first RCT was that of Raphael *et al.* from Paris in 1989, who randomized 343 patients without loss of consciousness to receive either six hours in NBO_2 or two hours of HBO_2 at 2 ATA plus four hours of NBO_2 (21). In a second arm, 286 patients with loss of consciousness were randomized to one or two HBO_2 sessions at 2 atmospheres. Raphael *et al.* found no difference in outcome in either arm of the study. But they found very high residual neurological effects in all groups, 32 to 34% without loss of consciousness, which is similar to what Smith and Brandon reported in 1973, and 46 to 48% in patients with loss of consciousness (19). These high residual effect rates raised a number of criticisms including overly broad entry criteria, adequacy of the 2 ATA schedule for HBO_2, effect of treatment delays of up to 12 hours, and weak outcome measurements. But the study was a catalyst for better designed RCTs trials, which began appearing over the next few years.

The second RCT was a trial of 26 patients by Ducasse, also from France (22). Two-thirds of patients had loss of consciousness and surrogate outcome measurements were used. In other words no measurements of cognitive function were done; the investigators simply assessed symptoms, EEG and cerebral blood flow responses to acetazolamide. Ducasse reported significant positive effects of the HBO_2 treatment at three weeks but this result was not widely accepted. The work was published only as an abstract, it was a small study and the follow-up period was quite short. There was no blinding and the validity of the surrogate outcome measurements was questioned.

The third RCT of Thom *et al.* at Pennsylvania included 60 patients with moderate CO poisoning, excluding loss of consciousness and cardiac dysfunction (23). Thom et al used oxygen at 2.8 atmospheres versus NBO_2 and performed follow-up evaluations with serial, neuropsychological tests. The study was stopped early due to detection of benefit

in the patients who received HBO_2 therapy. These results fit with the clinical wisdom of the time, and with results of case series of HBO_2 treatment of CO poisoning.

The next study by Scheinkestel *et al.* from Australia was highly controversial, and in a sense, opened Pandora's Box (24). Scheinkestel randomized 191 patients of different poisoning severities to receive either daily HBO_2 at 3 atmospheres for 60 minutes and then three to six days of high-flow NBO_2 or high-flow NBO_2 for three to six days. Outcome was assessed by neuropsychological testing after the treatment course and at one month after poisoning. No significant HBO_2 treatment effects were detected.

The problems of design and implementation of the Scheinkestel study are so serious as to call into question any "findings". These design flaws, however fatal, were initially overlooked by many clinicians, and the study was quoted as "evidence" that HBO_2 was not effective. In addition to patient enrollment problems, the O_2 doses did not meet clinical standards; the difference in O_2 dose between study arms was negligible and only 46% of the patients were followed-up. Although the study fails to provide clinically useful information it points out one of the problems of negative clinical trials; the trial can be negative for a host of the wrong reasons including poor design.

The fifth RCT, Daniel Matthieu's study in France, is still ongoing (25). At an interim analysis 575 patients had been randomized to a single HBO_2 treatment (2.5 ATA for 90 minutes) versus 12 hours of NBO_2. The patients are being followed serially for a year. At the three month follow-up there was a significant statistical effect of HBO_2 of about twofold with a strong p- value. The difference diminished at six months, a trend was present but without statistical significance. The trend was lost by one year, when outcome in both groups was the same. Matthieu has continued this trial to try to identify subgroups of patients that are most likely to benefit from HBO_2. The results of Matthieu raise some interesting points that will be covered below after discussing Weaver's study.

Weaver *et al.* from Salt Lake City have published a large RCT, where patients were stratified by age, exposure time, treatment delay, and history of loss of consciousness in which they detected a significant benefit of HBO_2 therapy (26). The Weaver study was double-blind, randomized and placebo controlled; patients were treated in a monoplace chamber three times at 6- to 12-hour intervals with HBO_2 or sea level O_2 (NBO_2). Weaver operated on an intention to enroll 200 patients; 152 were actually enrolled, with one-to-one randomization. The trial was interrupted at the third interim analysis because of a difference in favor of HBO_2.

The poisonings in Weaver's study patients were fairly severe, mean COHb of 25% and half of the patients had suffered loss of consciousness. The HBO_2 therapeutic advantage held up after adjusting for pretreatment differences, i.e. cerebellar dysfunction, and for stratification. In patients with complete follow-up data (94%), 24% of the HBO_2 group had cognitive sequelae compared to 43% of the NBO_2 group. It is worth noting again that 43% typifies the literature reports of residual effects in people who don't receive HBO_2; thus 24% is a significant decrease in cognitive sequelae.

The Weaver trial has a number of great strengths. The investigators preserved the double blind design, defined their endpoints *a priori*, and corrected the neuropsychiatric tests for age, gender, and education. The patients were treated as soon as possible after CO poisoning, the follow-up rate, 94% is exceptional, and the analysis was done by intention to treat.

Despite the excellence of the Weaver study and the positive results, there are still some unresolved treatment issues. These are put forward now (Table 2) for later consideration. In short, clinical research on CO poisoning still suffers from the lack of objective criteria or tests to identify high-risk patients or to predict risk of both delayed and permanent neurological sequelae.

Table 2. Unresolved Issues in Treatment of CO Poisoning

1) Are 3 HBO_2 treatments in 24 h necessary?
2) If 1 treatment is used, should the O_2 dose be greater than 2.5 ATA or longer than 90 min?
3) Should patients with milder CO poisoning receive HBO_2? If so, what criteria are appropriate?
4) Should patients be given HBO_2 more than 12 or 24 hours after poisoning?
5) Are O_2 toxicity and other side effects of HBO_2 significantly greater with multiple treatments?
6) How should cost/benefit of multiple treatments be assessed?

This problem has three parts. First, at the basic level, no one yet understands the exact mechanisms of cell death or the etiology of the delayed neurological syndrome. Second, no one yet knows the optimal dose of HBO_2, for example, number of treatments or best treatment pressure. Third, no one knows the time after which HBO_2 is no longer effective. Most of the trials have treated as soon as possible based on the six-hour window of opportunity proposed in Goulon's 1969 retrospective study (27).

What follows is a synopsis of clinical issues that have arisen primarily since Weaver's study data became available (28): 1) Are three HBO treatments in 24 hours necessary? Most of the benefit in the Weaver study was found after the first treatment. 2) If one or more treatments are used, must the oxygen dose be greater than 2.5 ATA or longer than 90 minutes? This point is specifically in reference to our practice at Duke in which our treatment outcomes were good before the results of Weaver were published. 3) Should patients with mild CO poisoning receive HBO_2 and if so, what treatment selection criteria should be used? 4) What treatment should be given to patients who are not selected for HBO_2? 5) Should patients be given HBO_2 more than 12 to 24 hours after the discovery of the poisoning and is the cost-benefit of HBO_2 reasonable after such delays? This issue becomes a notable problem when a patient has to be transported a long distance. 6) Is the cost-benefit of multiple HBO_2 treatments worthwhile? In other words are side effects of multiple HBO_2 sessions like O_2 toxicity important problems? These are questions that need to be addressed and may require one or more future randomized control trials.

A validated definition of "severity of poisoning" has been lacking, which if defined, certainly could be incorporated usefully into a future study design. Also, treatment protocols that are implemented should be clinically reasonable and commonly available; one could logically argue three treatments in 24 hours as clinically unnecessary for the majority of CO poisoned patients.

The nature and timing of exit evaluations are important considerations that need to be defined *a priori* because lack of appropriate long-term follow-up has been a limiting problem in a number of studies. A strategy being discussed for a multi-center RCT of HBO_2 among investigators at several large centers is one that would consider stratification by a valid definition of severity of poisoning, (e.g. high versus low risk), randomization of the patients to either one, two, or three treatments; stratification by

treatment delay, (e.g. less than or equal to 6, 6 to 12 or 12 to 24 hours), and rigorous follow up at multiple time points, including a one-year analysis.

A summary of this discussion can be made in three fairly straightforward points: First, the basic science studies of CO poisoning demonstrate multiple toxicity mechanisms involving the brain. This is by no means a simple problem but fortunately many of the toxicity mechanisms appear to be amenable to timely HBO_2; this conclusion is based both on a sound biochemical rationale and on rigorous experimental data. Second, well designed clinical trials now strongly support the use of HBO_2 therapy in selected patients. Third, there are significant unresolved treatment issues, including how to identify patients at high risk for DNS, determining the optimal number of treatments, and defining the effect of treatment delay on the patient's clinical outcome.

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Hyperbaric Oxygen as adjunctive therapy in *Vibrio vulnificus* septicemia and cellulitis.

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BACKGROUND

One of the most common bacteria in tropical to subtropical seawater, *Vibrio vulnificus* is an invasive, gram-negative bacillus and recognized as a cause of fulminant primary septicemia, wound infections and gastrointestinal disorders[1]. In the United States, it is found predominantly along the coastlines of those states that border the Gulf of Mexico, but has also been isolated from areas in New England and the northern Pacific Coast[2]. *Vibrio vulnificus* infections can occur in patients with underlying liver disease, who have acquired the organism through the gastrointestinal tract after recent consumption of raw shellfish, or through inoculation of bacterium through contamination of a wound with seawater.

Table 1. Laboratory Values on Admission

Variable	Value
White blood cell count (/μl)	3.3
Differential count (%)	
Granulocytes	79.8
Lymphotes	16.3
Monocytes	3.0
Esinophils	0.3
Platelets	101
Na	143
K	4.6
CL	104
CO2	25
BUN (mg/dl)	23
Glucose (mg/dl)	102

Liver Function Test

Total protein (g/l)	6.3
Albumin (g/l)	3.3
Total Bilirubin (mg/dl)	0.6
ALK (U/l)	39
ALT (U/l)	65
AST (U/l)	60
GGT (U/l)	200
LDH (U/l)	605
CK (U/l)	300

CASE REPORT

A 69 year-old male with alcoholic liver disease presented to the University of Texas' Medical Branch Hospital with a day-old fever, chills, and intense pain in his right arm. He had prepared raw fish for dinner one day prior to admission. In the next few hours, extensive erythema, multiple blisters, and bullous lesions developed over the right shoulder, forearm and right hand. He subsequently became hypotensive, with diminishing mental status. The results of laboratory studies upon his admission are shown in Table 1. *Vibrio vulnificus* infection was suspected. The patient was started with ceftazidime, levofloxacin, and doxycycline intravenously and closely followed by a surgical team. The patient continued to exhibit a spiking fever despite being administered three intravenous antibiotics. Two areas of the skin and subcutaneous tissue in the right arm and hand became progressively necrotic. An x-ray of the arm showed no subcutaneous gas

or osteomyelityis changes. *V. vulnificus* was identified from the blood culture and necrotic wounds in his arm and hand. In consideration of the positive effects of hyperbaric oxygen (HBO_2) on treatment of severe infections, it was initiated on hospital day three, with a daily exposure for a total of twenty sessions. The patient's blood pressure and temperature became normal; he eventually recovered fully and his ulcers were healed completely and without the need of grafting.

DISCUSSION

The clinical features of *Vibrio vulnificus* infections are shown in Table 2. The severity of *V. vulnificus* infections is related to both hosts and bacterial factors shown in Table 3.

Table 2. Clinical features of *Vibrio vulnificus* infections

Septicemia
Shock
DIC
Endocarditis
Wound Infection
Necrotizing fasiciitis
Compartment sygrom
Cellulitis
Gastrointerstinal Illness

Table 3. Predisposition conditions and risk factors of *Vibrio vulnificus* infections

Host	**Associated Conditions**
Liver Cirrhosis	Consuming raw sea food
Alcohol Abuse	Contact sea water
Hemochromatosis	
Sideroblastic Anemia	
Iron overload	
Chronic renal insufficiency	
Cancer Immunodeficiency	

The invasiveness of the *V. vulnificus* is associated with the presence of the polysaccharide capsule and cytotoxin. The production of protease, lipase, cytolysin, hemolyysin, hyaluronidase, mucinase, DNase, bradykinin, sulfatase, and tumor necrosis factor was implicated as contributing to the virulence of *V. vulnificus*[3]. The pathogenecity of *V. vulnificus* is due, in part, to its ability to cause transmural vasculitis in subcutaneous tissue. Thrombosis of the blood vessels may play an important role in the necrotizing process, which is characterized by fevers, chills, and bullous skin lesions. *V. vulnificus* septicemia is associated with a mortality greater than 50%; with septic shock the mortality approaches 100%. Patients with underlying chronic illnesses, such as cirrhosis or immunosuppression, have been noted to be at high risk for the rapid progression of this infection[4]. The early microbiologic diagnosis and a combined therapeutic approach, including aggressive antibiotics and hyperbaric oxygen therapy, may have contributed to the successful management of this patient.

Oxygen tensions play an important role in the outcome of infections . Hyperbaric oxygen exerts antimicrobial effects by increasing the intracellular flux of reactive oxygen species. Hyperbaric oxygen also potentiates the activity of many antimicrobial drugs by increasing the transportation or direct synergistic effects. With regard to host defenses, hyperbaric oxygen therapy augments tissue oxygen partial pressure, allowing increased bacterial killing by providing a substrate for the formation of oxygen-free radicals and augmenting respiratory burst. During the healing process, hyperbaric oxygen increases the formation of capillaries for oxygen, nutrients, and antibiotic delivery, leading to more rapid overall wound healing[5,6].

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Acupuncture-HBO_2 combined therapy in a persistent left hemiface hyperalgesia: A case report.

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This case report describes successful treatment of refractory hyperalgesia by a combination of acupuncture and hyperbaric oxygen therapy (HBO_2). A 41 year-old female patient showed the sudden appearance of intense and persistent left hemifacial hyperalgesia resistant to all therapies. This happened immediately after several dental surgery procedures. In the absence of absolute contraindications, and with the local approved indication for "severe inflammation of soft tissues", the patient underwent 20 sessions of HBO_2 at 2.5 ATA (25' O_2 + 5' Air x 3 each). There was no clinical response, and the patient describes no change in pain or general conditions. The patient had already undergone some acupuncture treatments by this time, as well, but with no effect. With the patient's consent, we decided to try a combined Acupuncture-HBO_2 therapy. After a seven day pause in HBO_2 treatment, the patient underwent a second series of 20 sessions of HBO_2, 1 session a day (Mon – Sat), and 8 acupuncture sessions while receiving HBO_2. We treated particular wrist point #2-4-5 (wrists/ankles micro-system), M-HN-18, ST-36, BL-67.

RESULTS AND CONCLUSION:

After combined therapy, the patient reported only a mild persistent hyperesthesia in the zygomatic area. Additional drug treatments were abandoned. Ultrasonography documented total resolution of inflamed areas and pain assessment (McGill Pain Questionnaire – MMPI – BDS) was consistent with an almost totally resolved problem. In spite of the obvious limits of a single observation, we consider it possible that combined Acupuncture-HBO_2 therapy may be benefit some chronic inflammatory conditions.

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Thorsen E, Risberg J, Segadal K, Hope A. Effects of venous gas microemboli on pulmonary gas transfer function. Undersea Hyperbaric Med 1995; 22:347–353.

Hempleman HV. History of decompression procedures. In: Bennett PB, Elliott EH, eds. The physiology and medicine of diving. London: WB Saunders, 1993:324–375.

Kindwall EP, Goldmann RW. Hyperbaric medicine procedures. Milwaukee, WI: St. Luke's Medical Center, 1970.

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2. Clinical research should be conducted only by scientifically qualified persons and under the supervision of a qualified medical man.

3. Clinical research cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

4. Every clinical research project should be preceded by careful assessment of inherent risks in comparison to foreseeable benefits to the subject or to others.

5. Special caution should be exercised by the doctor in performing clinical research to which the personality of the subject is liable to be altered by drugs or experimental procedures.

CLINICAL RESEARCH COMBINED WITH PROFESSIONAL CARE

1. In the treatment of the sick person, the doctor must be free to use a new therapeutic measure, if in his judgment it offers hope of saving life, reestablishing health, or alleviating suffering.

If at all possible, consistent with patient psychology, the doctor should obtain the patient's freely given consent after the patient has been given a full explanation. In case of legal incapacity, consent should also be procured from the legal guardian; in case of physical incapacity the permission of the legal guardian replaces that of the patient.

2. The doctor can combine clinical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that clinical research is justified by its therapeutic value for the patient

NON-THERAPEUTIC CLINICAL RESEARCH

1. In the purely scientific application of clinical research carried out on human beings, it is the duty of the doctor to remain the protector of the life and health of that person on whom clinical research is being carried out.

2. The nature, the purpose, and the risk of clinical research must be explained to the subject by the doctor.

3a. Clinical research on a human being canot be undertaken without his free consent after he has been informed; if he is legally incompetent, the consent of the legal guardian should be procured.

3b. The subject of clinical research should be in such a mental, physical, and legal state as to be able to exercise fully his power of choice.

3c. Consent should, as a rule, be obtained in writing. However, the responsibility for clinical research always remains with the research worker; it never falls on the subject even after consent is obtained.

4a. The investigator must respect the right of each individual to safeguard his personal integrity, especially if the subject is in a dependent relationship to the investigator.

4b. At any time during the course of clinical research the subject or his guardian should be free to withdraw permission for research to be continued.

The investigator or the investigating team should discontinue research if in his or their judgment, it may, if continued, be harmful to the individual

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Atmospheres absolute is a modified unit of pressure due to the appendage "absolute"; the symbol "atm abs" is preferred over "ATA" for the modified unit.

1 atm 1 atm 1 atm 1 atm 1 bar 1 bar 1 bar 1 bar 1 MPa	=	1.013250 bar	1 atm	=====	33.08 fsw	1 atm	= 10.13 msw
	=	101.3250 kPa	1 bar		32.646 fsw[a,b]	1 bar	= 10.00 msw
1 psi	=	14.6959 psi	1 fsw		3.063 kPa	1 msw	= 10.000
1 psi 1 torr	=	760.00 torr[d]	1 fsw		22.98 torr	1 msw	= 1.450 psi
	=	100.000 kPa	1 psi		2.251 fsw	1 msw	= 75.01 torr
	=	100,000 Pa[d]					
	=	14.50377 psi					
	=	750.064 torr					
	=	10.000 bar					
	=	6,894.76 Pa[d]					
	=	51.7151 torr					
	=	133.322 Pa[d]					

[a]Primary definition for fsw; assumes a density for seawater of 1.02480 at 4°C (the value often used for depth gauge cali-bration).

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1. Standard Practice for Use of the International System of Units (SI). Document E380-89a. Philadelphia, PA: American Society for Testing and 1989.