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The Use of Non-Explosive mixtures of Hydrogen and Oxygen For Diving

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HYDROGEN AND OXYGEN FOR DIVING

by

William P. Fife

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ABSTRACT

The purpose of this report is to place under one cover a summary of hydrogen-oxygen (hydrox) diving to date, and a detailed description of the techniques which have been developed to conduct such diving in this laboratory.

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Although the development and testing of man decompression tables was not a part of the U.S. Navy-supported program, it is included here to provide a broader picture of hydrox research.

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THE USE OF NON-EXPLOSIVE MIXTURES
OF HYDROGEN AND OXYGEN FOR DEEP DIVING

HISTORICAL BACKGROUND:

The effects of hydrogen-oxygen breathing mixtures on animals initially was examined in 1789 by Lavoisier (1) who placed guinea pigs in bell jars containing a mixture of hydrogen and oxygen. The initial concentration of oxygen was "in nearly the same proportion in volume which exists between life-giving air and nitrogen gas in the atmosphere." The animals apparently survived 8 to 10 hours. Although at the time it was felt that they succumbed to ammonia, it is probable that they died from CO₂ buildup since it is well known that under similar experimental conditions animals will survive longer if CO₂ is absorbed.

One hundred forty-eight years elapsed before a hydrogen-oxygen breathing mixture again was examined, this time by Case and Haldane (2). These workers breathed a mixture of hydrogen, nitrogen and oxygen in a hyperbaric chamber at an ambient pressure of 10 atmospheres for up to 6 minutes. They reported no adverse reaction. Even at that early date, work was cited which indicated that combustibility studies already has been carried out by a Professor A. C. G. Egerton, who assured Case and Haldane that 4% oxygen in 96% hydrogen was non-explosive.

In 1943, in part because of the anticipated difficulty in obtaining helium, Arne Zetterstrom of the Swedish Navy suggested the use of hydrogen-oxygen breathing mixtures as an alternative to helium-oxygen for diving (3). Zetterstrom developed an ingenious

method of cracking ammonia catalytically, resulting in a gas mixture containing 75% hydrogen, 25% nitrogen and unchanged ammonia. By removing the ammonia and adding 4% oxygen he arrived at a breathable mixture containing 72% hydrogen, 24% nitrogen and 4% oxygen. He compressed this mixture into standard cylinders onboard ship. In 1943 he conducted 2 successful open ocean dives on this mixture to 40 and 100 meters. He also conducted 2 additional dives to 70 and 160 meters respectively on a 2-gas mixture containing 96% hydrogen and 4% oxygen (4). On the 160-meter dive he lost his life when one of the two winch operators raising his platform hauled him directly to the surface without observing decompression stops. Since upon reaching the surface he still was breathing 4% oxygen he succumbed to hypoxia and probably air embolism.

The Swedish Navy continued for a short time after that accident to study the feasibility of hydrogen-oxygen diving under the encouragement of Engineer Commodore Ture Zetterstorm, the father of Arne. A laboratory explosion related to testing the safe limits of hydrogen-oxygen mixtures apparently greatly influences the Swedish Navy's decision to discontinue further work in this area (5).

After the rather unfortunate experiences in Sweden, interest in the use of hydrogen-oxygen as a breathing gas lay dormant until 1966 when Brauer (6) began to study the possible effects of hydrogen on various mammals. His work, principally on mice and monkeys, suggested that hydrogen might have a less narcotic or convulsive effect than helium at the same pressure (7) (8) (9) (10) (11) (12), but the full scope of biological effects were not precisely determined. His work did, however, lead him to conclude that molecular hydrogen is not toxic and is less narcotic than helium.

As a result of these favorable results from short exposures with small animals, Brauer and his French co-workers attempted to carry out a human dive to 300 meters. Because of a number of problems on this dive they did not shift to the use of hydrogen-oxygen. They did, however, raise further question as to the possible narcotic effect of helium which Bennett (13) and others (14) had reported occurred below 800 feet. The terms "Helium Tremors," and later "High Pressure Nervous Syndrome" (HPNS), were applied to this phenomenon.

From these observations, in 1967 it appeared that at depths of 1000 feet divers might be approaching the limits at which helium could be used. In view of the clearly anesthetic properties of Xenon at one atmospheres (14), and the narcotic effect of nitrogen at 6 to 7 atmospheres, it came as little surprise to many workers that helium might cause a similar response.

The appearance of the HPNS caused several workers to develop the capability to work with hydrogen-oxygen mixtures involving animals studies in the laboratory. Edel, supported by a commercial diving company began to study the possible use of hydrogen as a replacement for helium in diving. The diving company financed several early man dives, beginning with hydrogen-oxygen exposures of 10 minutes at a simulated depth of 200 feet. Independently, Fife also began to develop the capability to work with hydrogen, using dogs under the support of Texas A&M University funds for Organized Research.

In 1968, Fife and Edel joined efforts to conduct several extended deep saturation dives on 2 dogs using hydrogen-oxygen and helium-oxygen breathing mixtures, first at a depth of 300 FSW and then 1000 FSW. Work also continued with man dives to gradually increase hydrogen exposures. This series ended in 1969 with exposures of up

to 2 hours duration (15). One of us (W.P.F.) was exposed to a 1½-hour 200-foot hydrogen dive, followed 8 days later by the 2-hour dive to the same depth. No ill effects were noted from the hydrogen, although in some of the 9 shorter dives several of the divers experienced mild bends.

In 1969, French workers (16) exposed a number of rabbits to a simulated depth of 944 feet (29 bars). As a precaution they accomplished this by lowering the animal chamber from pontoons to a depth of 6 meters under the ocean using external heating strips to maintain chamber temperature. A television camera observed the animals while their EEG's were monitored from the surface. The EEG's of these animals began to show abnormal trends within 2-3 hours and within 7½ hours there was electrical silence. All animals were dead within 20 hours.

Further work by Fructus and Naquet was carried out on baboons (*Papio papio*) at simulated depths of between 300-675 meters. Of the 8 baboons used, all demonstrated abnormal EEG patterns and 4 died. Most troubling of all was the reported presence of brain abscesses in several of the animals. These abnormal EEG's and brain abscesses will be discussed presently.

Upon completion of their initial work on dogs, Edel and Fife again began to work independently, Fife emphasizing dives to 1000 FSW with dogs, rats and mice; while Edel carried out another series of man studies.

In 1976, Fife began a series of studies to develop man decompression tables for hydrogen-oxygen diving under support of the Texas A&M Sea Grant Program. Working jointly with Mezzino, tables were developed and tested to a depth of 300 feet, with bottom times of up to 30 minutes. Interim 300-foot tables of up to 45 minutes bottom time now exist.

The U.S. and Swedish Navies have retained an interest in hydrox diving although neither at present apparently has an in-house hydrox capability. There also are informal remarks to indicate that the Soviet Union may be examining hydrox diving but we know of nothing definitive since the earlier work of Lazarev (17).

POTENTIAL VALUE OF HYDROX DIVING:

Many divers refuse to consider the possible use of hydrox for diving, believing that it is too dangerous. Most do not realize that at sufficiently low levels of oxygen the mixture is not explosive. Although hydrogen is not now being suggested as a complete replacement for helium the merits of its use should be carefully reviewed.

Firstly, it is well known that in some countries there is no indigenous source of helium while in other countries the helium is found in such low concentrations that its recovery and purification is very costly. The most abundant sources of helium are found in the United States, Africa, Netherlands, and Canada. This dearth of helium causes concern, not only because of its natural scarcity but because in some countries political instability or reduced governmental support of reclamation programs places the continued availability of helium in some doubt.

In this country, although most users are not now concerned over potential shortage of helium, one estimate suggests that this country wastes over 1 billion cubic feet of helium per year, and that our present rich sources will be exhausted by the year 2000 (18).

This does not mean that this country would find itself without helium, but that it would be necessary to revert to sources of low concentrations, resulting in the need for more expensive methods of extraction. Based on the laws of supply and demand, it should be expected that the availability will decline and the cost will rise considerably above its present level. It would seem desirable to search for another breathing gas.

In the past, the cost of hydrogen-oxygen gas mixtures has been about twice the cost of commercially available heliox, and, at this

time to our knowledge is being mixed by only one commercial company. Thus, from the stand-point of cost it would not be economically feasible if procured in this way. However, the hydrox mixing technique newly developed in our laboratory appears to be both safe and brings the cost below that of heliox. We now can produce 3% hydrox for less than \$20 per cylinder at current prices.

Secondly, hydrogen has not yet been studied sufficiently to determine if it can improve the speed or safety of decompression. It seems clear that with dogs, decompression from 1000-foot saturation dives is different with hydrogen than with helium. Optimum hydrox decompression tables have not yet been worked out, but it would seem intuitively possible that the judicious use of another gas in the decompression profile could capitalize on the potential for a higher inert gas gradient. It should be noted here that none of the animal decompression tables employed pure oxygen. The addition of oxygen in these decompression profiles would enhance the rates of decompression. It should be noted that in commercial operations a slight increase or decrease in the decompression rate after extended saturation probably would have little economic significance and would provide little incentive for switching to hydrox.

A third possible value of hydrox is that it has a lower viscosity and density than heliox. This becomes significant at greater depths as demonstrated by Dougherty (19). His work showed that the maximum breathing capacity of a diver at 200 feet breathing hydrox is improved approximately 40% over heliox, and 141% over compressed air. While a diver at shallow depth (400 to 600 fsw) performing at a modest work level may not find the 40% improvement particularly important, it may become very important at high work levels. Further, as the diver

moves to greater depths it seems clear that the increased breathing resistance will become work limiting. The precise depth at which this would become serious is not known. Lambersten and his workers (20) exposed divers to a simulated depth of 1200 feet breathing a mixture of helium, neon, and oxygen in which the breathing resistance was equivalent to breathing helium-oxygen at a depth of 5000 feet. This would suggest that man may be able to perform at low to medium work levels at these depths using heliox. However, at these depths the 40% increase in breathing capacity afforded by hydrox probably would become important. It seems clear that breathing resistance will limit the maximum diving depth long before absolute pressure causes damage at the tissue level, it may be that hydrox will be essential if man is to function effectively at deeper depths (below 4000-5000 feet), and may increase work capacity at a much shallower depth.

Finally, consideration must be given to the possibility that hydrox may reduce the High Pressure Nervous Syndrome (HPNS). This was first suggested that Brauer who showed the monkeys did not develop HPNS on hydrox until they had reached a depth of about 1200 FSW (10). With helium, HPNS can be expected to appear between 800-1000 FSW. Our own work failed to show gross symptoms of HPNS in either dogs or mice at a depth of 1000 FSW, although it should be noted that our compression rates were slow.

METHODS

HYDROX EXPOSURE:

In the laboratory, there are two general types of hydrogen diving, each calling for different techniques. In one type, the chamber is flooded with hydrox and the diver simply breathes the ambient gas mixture. In the second method, the chamber is compressed with an inert gas containing no oxygen while the diver breathes a hydrox mixture by mask. In this mode, since the chamber still contains its original oxygen, it remains a normoxic mixture and the subject can breathe the ambient gas mixture in an emergency in event of mask failure. In the second method, to reduce the build-up of hydrogen in the chamber, the exhaled gas is conducted to the outside and is dumped into the air through a flash-back arrestor. It should be noted that venting hydrox gas into the chamber from the mask does not create an explosive environment since the oxygen levels cannot be raised above 3% in this way. However, such a build-up would require the chamber to be flushed with nitrogen or helium containing 3% oxygen to remove the hydrogen. While the second method usually is more economical, its use on conscious unrestrained animals is not practical since they must be intubated or taught to wear a mask. Thus, animal dives have used the first method while man dives so far have used the second method.

A. TECHNIQUE FOR HYDROX DIVING WITH ANIMALS, AND SATURATION DIVING WITH MAN.

The detailed step-by-step procedure and the specific rationale for each step is described in Appendix 1. Briefly, the procedure is to compress the chamber with an inert gas (helium or nitrogen) to a depth of 200 FSW (7 ata), at which time the level of oxygen will be

reduced to 3%. Since 3% oxygen is non-explosive and at the same time is sufficient to sustain life at that depth, it then is possible to flush the chamber with 3% hydrox (3% oxygen in 97% hydrogen) without danger. After replacing helium and nitrogen with hydrox, the chamber can be compressed to the desired depth with pure hydrogen. During the dive, metabolized oxygen is replaced by adding 3% hydrox.

During decompression, the oxygen concentration is raised to the appropriate level by flushing in 3% hydrox. In this way, hydrogen never is allowed to come in contact with oxygen at an oxygen level above 3%. Upon returning to 200 FSW, the chamber usually is flushed with 3% oxygen in 97% helium (heliox). A 3% oxygen-nitrogen mixture may be used but this may result in rather severe nitrogen narcosis if it follows an extended absence of nitrogen. After removal of the hydrogen, decompression can proceed in the usual manner either with helium or compressed air.

B. TECHNIQUE FOR NON-SATURATED HYDROX DIVING WITH MAN.

A detailed description of the step-by-step procedure for conducting hydrox chamber dives with man can be found in Appendix 2.

This procedure differs from that in which the chamber is flooded with hydrox since the hydrox can be delivered by mask and the exhaled gas removed by an overboard dumping system.

In developing this method, an effort has been made to reduce the number of gas mixtures required, and to employ techniques compatible with those which would be used in manned operational dive.

The chamber first is compressed to a depth of 100 FSW with nitrogen while the diver breathes compressed air. He then begins to breathe 10% oxygen in helium (10% heliox) by mask while the

chamber is further compressed to 200 FSW with nitrogen. Upon reaching 200 FSW (7 ata) the breathing mixture is shifted to 3% heliox to wash out the 10% oxygen; and then shifted to 3% hydrox. The chamber then may be compressed with nitrogen to the desired depth.

Decompression is just the reverse of compression. The chamber pressure is reduced in accordance with the decompression schedule until arriving at 200 FSW. The breathing mixture then may be shifted to 3% heliox to wash out the hydrox and then shifted to 10% heliox after which ascent is begun. Care must be exercised to assure that the chamber oxygen levels are raised sufficiently to sustain life as the diver ascends to provide a safety back-up in event of mask failure. Decompression may proceed normally, using the same mask for oxygen breathing which often is called for at a depth of 40 or 60 FSW.

The disadvantage of this system is that on long dives the diver must wear a mask for an extended period of time. This disadvantage is overcome if the chamber is flooded with hydrox as in the method first described.

The use of the overboard dump system is further described in the discussion of safety precaution.

EXPERIMENTAL PROTOCOL:

This work has encompassed the use of 4 different species:

1. Dog
2. Mouse
3. Rat
4. Man

A. Protocol: Dog

Most of the major deep dive studies in our laboratory were conducted on the mongrel dog. These dogs were chosen for their good disposition and were held in the laboratory long enough for laboratory personnel to become familiar with each personality. With two exceptions all dogs were in excellent health at the time of the dive. One dog (Lurch) was found later to have heart worms but responded to treatment. Another dog (Zithy) had cellulitis in one leg which was missed on her pre-dive physical examination. Two dogs (Pattycake and Ginger) were about 1 month pregnant at the time of their dives.

An effort was made to avoid as much medication as possible. Cortisone especially was avoided because of its possible association with aseptic bone necrosis. All dogs did, however, receive regular rabies and distemper inoculations as well as periodic worming and dipping.

All animals were fed a standard dry dog food ad lib, supplemented by a daily ration of corn oil. They usually were allowed to roam freely over a one acre field, and slept in an open shelter.

Prior to each dive the subject was given a brief physical examination. This included drawing blood, and usually lung and liver biopsies, both conducted under a thorazine tranquilizer and xylocane

local anesthesia. Since none of the dogs showed evidence of a pneumothorax after the lung biopsies, none of the dives were delayed from this cause.

The animals were placed in the pre-heated chamber and compression begun (See Appendix 2 for compression techniques). The rate of compression was held to 20-40 feet per minute to reduce the noise level in the chamber. Further, upon arrival at each additional 100 FSW, compression was stopped for a minimum of 30 minutes to allow leak checks to be made of the chamber, and to minimize possible HPNS.

All dives with dogs, except for the initial one were planned for a depth of 1000 FSW. When this depth was reached the chamber temperature was stabilized to an ambient temperature of between 32°C and 35°C with occasional adjustments made for the animals comfort. The hydrox bleed-in rate was established to match the metabolic requirements of the particular animal and to hold the chamber at oxygen levels of between 0.6% and 1.3%. The effect of these oxygen levels are discussed below: The carbon dioxide scrubber motor was set at a flow rate which would hold CO₂ levels to no more than 0.003%. Both oxygen and CO₂ levels were continuously measured by an on-line system.

During the dive, the water tray occasionally was flushed to assure fresh drinking water, while the bottom of the chamber was flushed after each urination if it was noticed, or at least once each 6 hours. Since the dog usually reserved one spot for defecation, this was left in the chamber. It appeared to pose no serious problem. Also during the dive the attendant frequently talked to the dog via intercom. The ability of the dog to see the attend-

ant and to hear the attendant's voice appeared to be reassuring to some animals.

Several different decompression tables were used on the dogs in an effort to establish borderline profiles both for hydrox and heliox, and in this way provide a basis of comparison between these two gases.

Initially, an effort was made to standardize on a decompression table which called for 15-minute decompression stops to a depth of 800 FSW, and 30-minute stops throughout the remainder of the ascent with longer stops at each 100-foot mark. It was found, however, that while this profile was satisfactory for heliox (Figure 1) it produced decompression sickness when used with hydrox. This is illustrated in Figure 2. Further, with hydrox, decompression sickness usually occurred in the 500 to 700-foot range.

Treatment of decompression sickness depended upon the depth at which it occurred. If it occurred deeper than 100 FSW, it was found that the most satisfactory results were achieved if the chamber was recompressed 100 FSW below the depth at which it first was observed. However, if it occurred above 100 FSW or after reaching the surface, the animal was treated on a modified U.S. Navy Table 5. The single instance of decompression-induced CNS bends occurring on the surface after a hydrox dive was treated on a modified U.S.N. Table 6.

It will be noted in Figure 2 that in this particular instance the dog was recompressed 200 feet rather than 100 feet after bends were recognized. These bends involved the spinal cord and resulted in loss of hind limb coordination. Because the animal had just awakened it could not be precisely determined at what depth the symptoms actually occurred. Since she had been seen using her hind limbs

normally at the 800-foot depth, she was recompressed to 100 FSW deeper than the last asymptomatic depth, with several short pauses during recompression to evaluate symptoms. Shortly after reaching 900 FSW she regained full use of her hind limbs. The second recompression at 700 FSW was due to a subjective judgment by the observer that the rear legs again showed weakness. This was shown to be due to sore foot pads and not decompression sickness.

As a result of these and other studies, it seems clear that recompression to depths less than 100 feet deeper than the depth of bends onset could result in only partial or temporary relief. Rarely did the 100-foot rule fail to provide adequate relief although in 2 instances the animals retained minor residual CNS symptoms which receded over the next 2 months.

Since one objective of these studies was to compare the physiological effects between heliox and hydrox diving, the decompression profile shown in Figure 4 was employed on both heliox and hydrox dives. Further, bottom times were standardized at 48 hours for both gas mixtures. In this way, decompression sickness was avoided in all subsequent studies. Although it is probable that when used with helium this table has a greater margin of safety against bends than for the same dive employing hydrogen, there is no evidence that this affected the comparative physiological studies.

Post-dive brain biopsies were carried out on two animals for electron microscopic evaluation. Brain necropsies were carried out on four animals in a search for brain lesions. Pre- and post-dive kidney biopsies for electron microscopic studies were carried out on two animals.

A study of core temperature during 100-foot dives was conducted. To accomplish this a small sub-carrier oscillator with FM transmit-

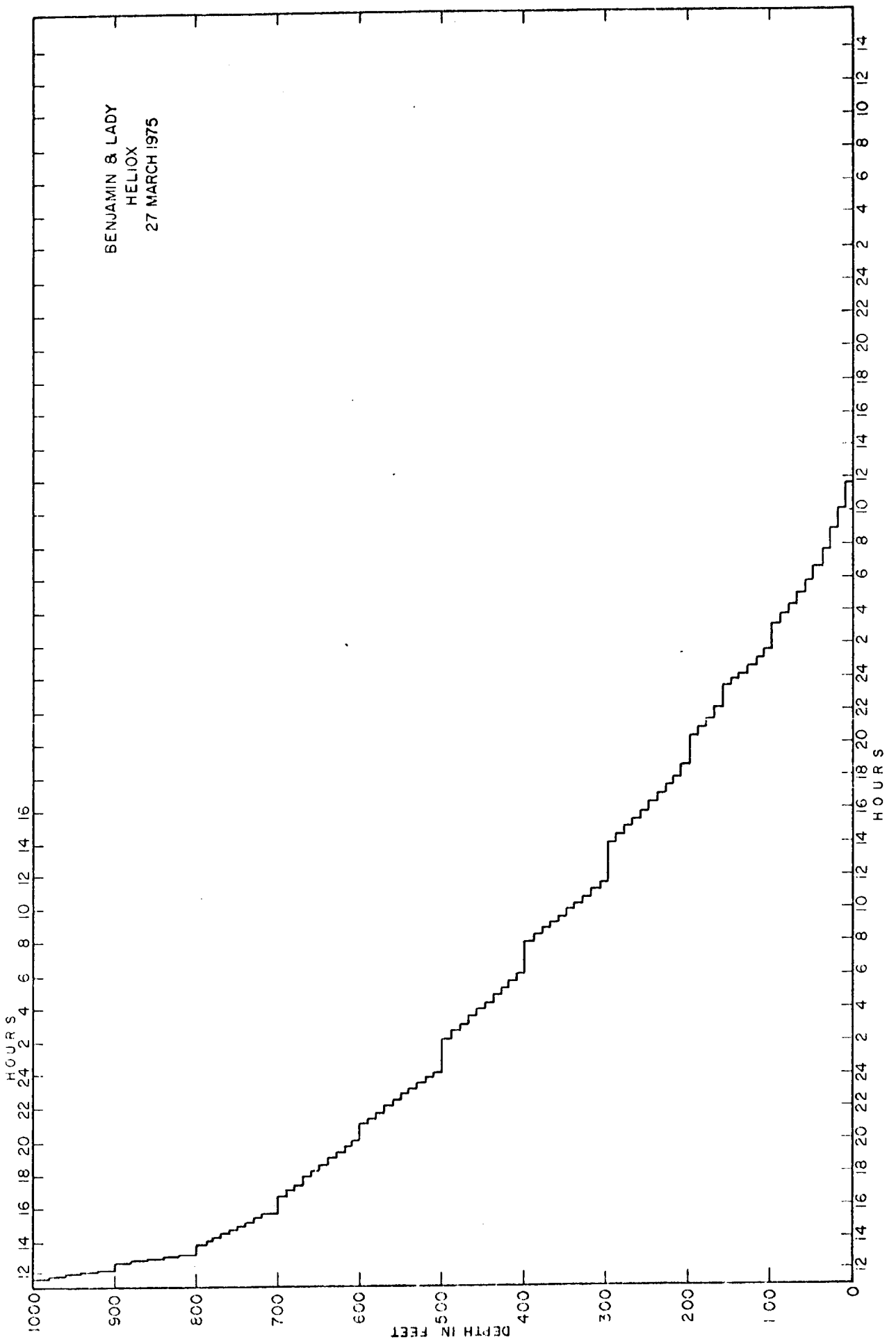


Figure 1.

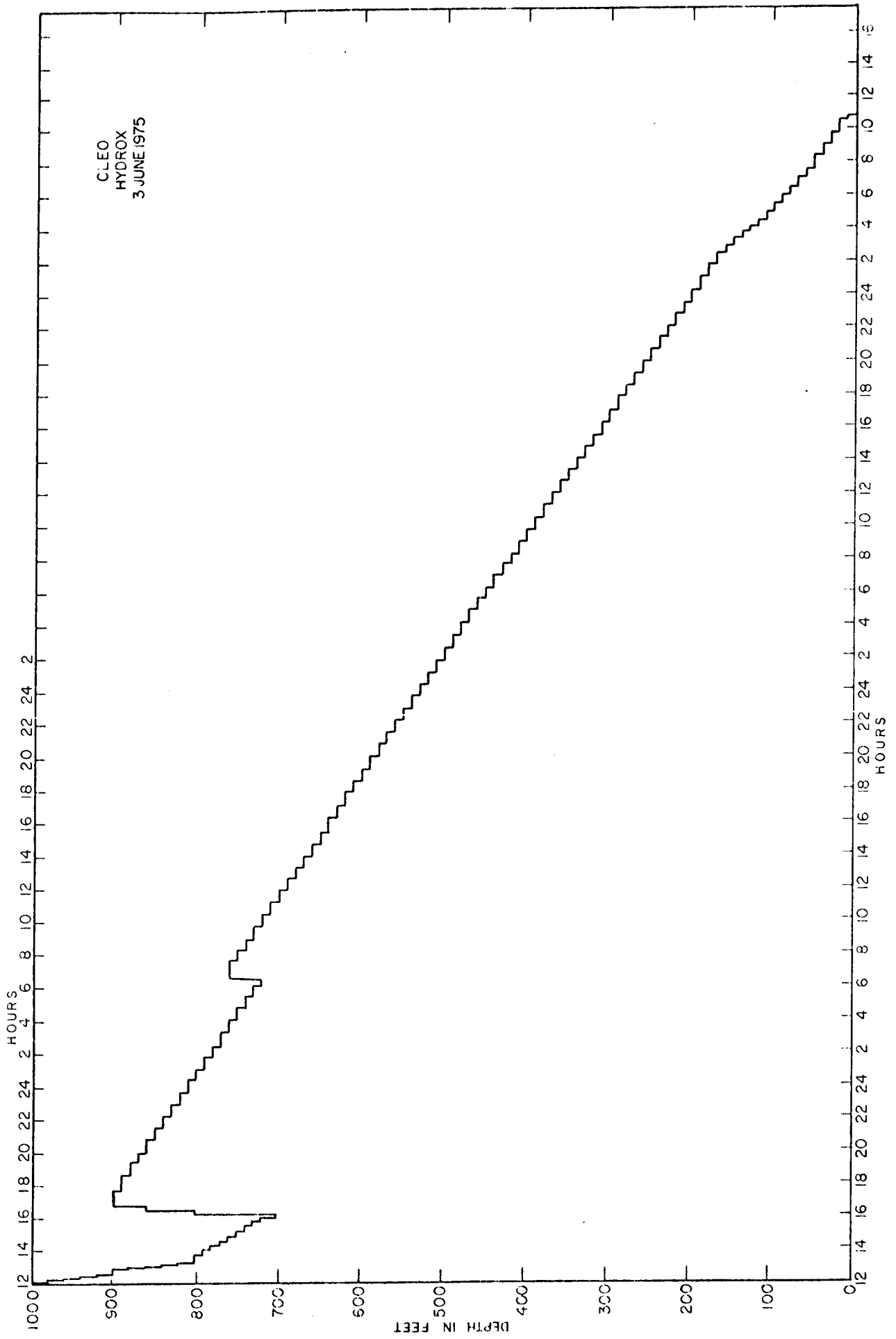


Figure 2.

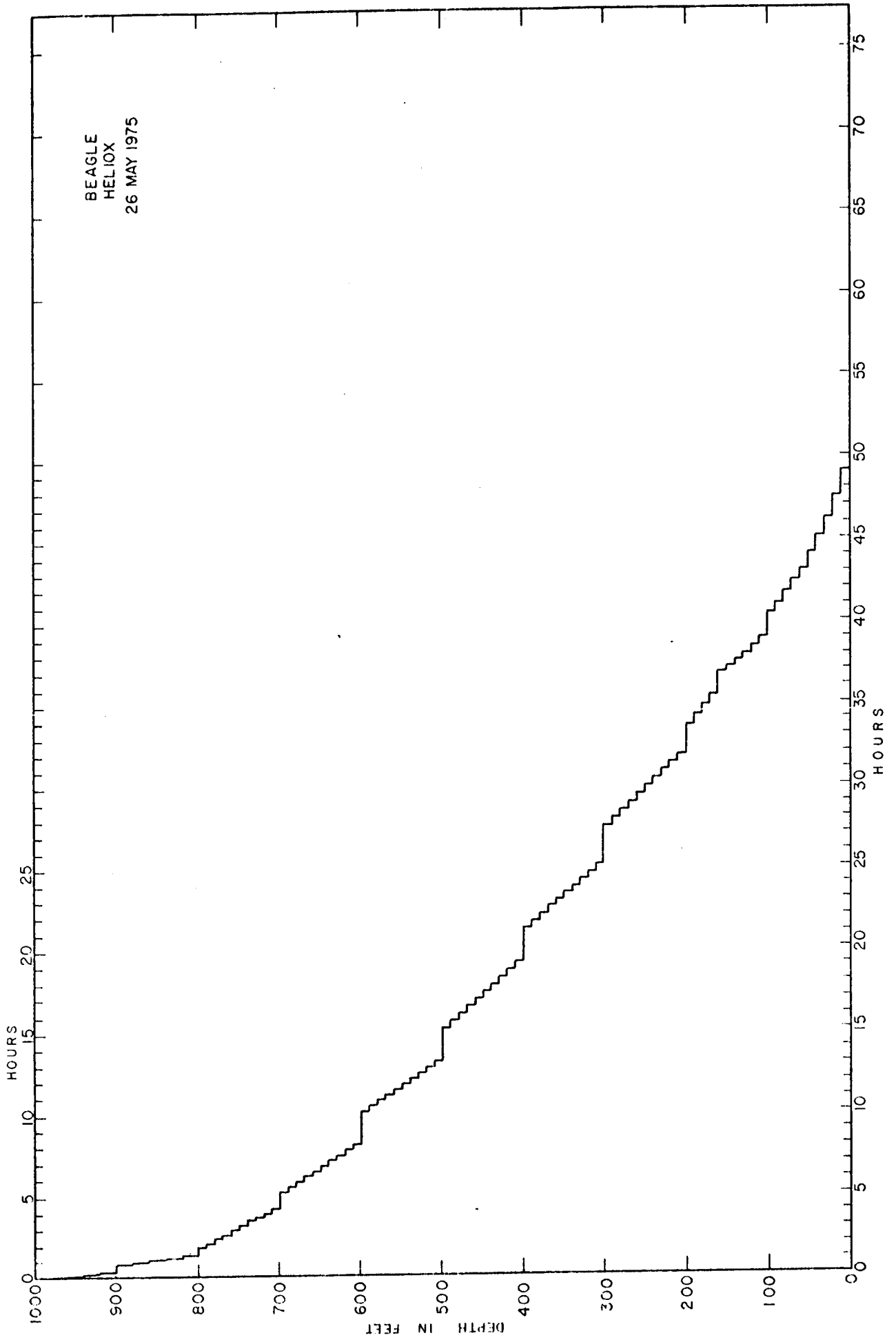


Figure 3.

ter was coupled with a temperature sensor and encapsulated in a mixture of beeswax and paraffin. The complete unit including battery was approximately 6 cm long and 3 cm in diameter and was molded in the hands to remove sharp edges and rough surfaces. It was implanted into the peritoneal cavity one week prior to the dive by a small mid-line abdominal incision. This type of unit operates continuously for about one month before batteries lose power. One such unit remained in an animal for 19 months during which time she has 2 litters. It caused no inflammatory reaction and still was serviceable when the battery was replaced.

Post-hydrox breeding experiments were carried out on selected dogs, both male and female. No medication was given either to enhance or inhibit fertility.

B. Protocol: Mouse

This work was ancillary to the primary program. It involved a study to determine the possible effect which hyperbaric hydrogen may have on various types of tumors. However, since exposure techniques are applicable to other hydrox diving the work on tumor-bearing mice will be briefly considered. It should be realized that all of the mice involved in this work had malignant tumors and that 100% mortality was virtually certain. The tumors employed were:

1. Squamous cell carcinoma
2. Ehrlich's ascites tumor
3. Leukemia

Tumors were mapped and animals were earmarked in accordance with standard procedures. They were placed in small chambers containing food, water, bedding, CO₂ scrubber. Heat was applied in accordance with the techniques found in Appendix 1.

Since these small chambers could not be flushed with water to remove urine they were brought to the surface every 3 to 4 days. Decompression from 300 FSW was carried out in three hours, while decompression from 1000 FSW was carried out in 6 hours. This was considered to be conservative decompression for mice but was done in deference to the debilitated condition of some of the animals. Except for these modifications the dive protocol was essentially the same as that described for dogs.

Tissue biopsies and necropsies were performed on some of the animals although in most instances studies consisted mainly of survival time.

C. Protocol: Rat

Studies carried out on rats were related to trying to determine the cause of death of two dogs which unexpectedly died while on a hydrox dive.

The first group of these rats was exposed to the identical profile used on the dogs which failed to survive. In the second dive, the animals were exposed to elevated levels of oxygen (500 mm Hg) and CO₂ (0.5%) in an effort to create the same symptoms seen in the fatal dog dive.

D. Protocol: Man

It should be emphasized that hydrogen studies with humans were supported by the Texas A&M University Sea Grant Program. They were completely independent of the Office of Naval Research-supported program. In this program, 15-minute and 30-minute bottom-time tables suitable for man were developed and tested.

Human subjects were drawn from the director and other laboratory personnel. All were divers with experience ranging from four years

to nearly 35 years. They ranged from 23 years to 58 years of age. All were in excellent physical condition and were involved in a regular swimming exercise program.

In our laboratory the development of man tables was carried out as follows:

A hydrox/heliox decompression table for a pig was generated, using parameters already developed and programmed. This program and this technique of using the pig as the first model has been operationally proven during the development of 420 FSW tables for a commercial diving company (21).

For each table the pig was subjected to its decompression profile to confirm its safety for that model. When it was determined to be safe for the pig, a "pig-to-man" extrapolation was carried out by computer and a man table was produced (Fig. 5). Previous experience showed that if the pig does not develop decompression sickness, the extrapolation will be approximately correct for man.

This table with a 15-minute bottom time was dived by an experienced diver using heliox. It then was slightly modified due to the appearance of mild bends and the same diver carried out a successful dive using heliox, followed by a successful dive using hydrox.

The table next was extended to 30-minute bottom time and was dived on hydrox by one of us (W.P.F.). The appearance of mild bends resulted in still another table (Table 2). It was dived by the same diver without difficulty, following which three other divers made dives on it. Since one of these divers developed a mild case of the bends, the table again was modified (Table 3). We believe this

Texas A&M University
Hyperbaric Laboratory
Man Hydrox Decompression Table 1

<u>Depth</u>	<u>Time</u>	300' -17 min	B.T.
300'	15 min B.T.		
270	1		
260	1	<u>Depth</u>	<u>Time</u>
250	1	90	6
240	1	80	7
230	3	70	10
220	3	60	11
210	4	50	13
200	6(Shift to 3% heliox-4 min) (Shift to 10% heliox-2 min)	40	15
190	3	30	18
180	5	20	22
170	4	10	<u>27</u>
160	7(Flush Chamber)		258 min total
150	8(With Air)		
140	9		
130	11		
120	15		
110	16		
100	8(Shift to Air) (Breathing)		

TABLE 2

TAMU Hydrox Decompression Table V

(MAN)

30 minutes - 300 feet

<u>Depth</u>	<u>Time</u>	<u>Depth</u>	<u>Time</u>
300'	30 min. (a)	60'	12 - O ₂
280	1	50	7 - O ₂
260	1		5 - air
250	2		4 - O ₂
240	2	40	15 - O ₂
230	2		5 - air
220	4		1 - O ₂
210	4	30	18 - O ₂
200	3 (b)		5 - air
190	2		6 - O ₂
180	6	20	13 - O ₂
170	6		5 - air
160	5		17 - O ₂
150	9	10	2 - O ₂
140	10		5 - air
130	12		20 - O ₂
120	14		5 - air
110	18		10 - O ₂
100	9 (c)	Surface	
90	10	Total	320 min.
80	8 (d)		
70	10		

including ascent
time (one minute
travel time between
stops)

(a) 3% hydrox

(b) shift to 3% heliox followed in 1 minute by shift to 10% heliox

(c) shift to 50% heliox

(d) shift to 50% nitrox

TABLE 3

TAMU Hydrox Decompression Table VI

(MAN)

30 Minutes - 300 Feet

<u>Depth</u>	<u>Time</u>	<u>Depth</u>	<u>Time</u>
300'	30 min. (a)	60'	12 - O ₂
280	1	50	7 - O ₂
260	1		5 - air
250	2		20 - O ₂
240	2		
230	2	40	5 - air
220	4		20 - O ₂
210	4		5 - air
200	3 (b)		20 - O ₂
190	2		5 - air
180	6	30	20 - O ₂
170	6		5 - air
160	5		15 - O ₂
150	9		5 - O ₂
140	10	20	5 - air
130	12		20 - O ₂
120	14		5 - air
110	18		5 - air
100	9 (c)		20 - O ₂
90	10	10	5 - air
80	8 (d)		20 - O ₂
70	10		5 - air
		Surface	<u>5 - air</u>
		TOTAL	345 min

(a) 3% hydrox

(b) shift to 3% heliox followed in 1 minute by shift to 10% heliox

(c) shift to 50% heliox

(d) shift to 50% nitrox

table now is safe to use for our further studies on hydrogen wash-out of various tissues, and will permit our technicians to dive with the dogs to a depth of 300 FSW for a bottom time of 30 minutes if this is needed.

Table 4.
Accumulated Hydrox Exposures

<u>Subject</u>	<u>Duration of Hydrox Exposure Hours:Min</u>	<u>Total Duration of Dives</u>
Dog	1140:18	1382:42
Rat-Mouse	5313:00	5378:15
Man	<u>4:37</u>	<u>41:27</u>
	6457:25	6802:24

These figures do not include animal dives carried out with heliox simulating a hydrox dive.

Table 5.

History of Hydrox Saturation Dives

Dog

<u>Name Animal</u>	<u>Depth</u>	<u>Total Dive Time Hrs:Min</u>	<u>Total Time on Hydrox Hrs:Min</u>	<u>Results</u>
Pioneer	300 FSW	34:15	23:30	Uneventful
Hydrox	1000 FSW	104:40	93:10	Bends. Hematuria once post dive. Heavy ammonia in chamber.
Zithy	1000 FSW	65:23	47:10	Bends - treated - cellulitis present from pre-dive in- fection
Cat	1000 FSW	85:10	68:00	Uneventful
Tweedle	1000 FSW	19:18	16:18	Died at depth due to hydrocarbon con- tamination of gas
Bandit	1000 FSW	19:18	16:18	
Cleo	1000 FSW	93:17	76:48	Uneventful
Lurch	1000 FSW	63:14	52:49	Contaminated gas. aborted dive - CNS treated OK.
Beagle	1000 FSW	98:25	83:08	Subcutaneous emphysema only. No other apparent complications.
Pattycake	1000 FSW	81:30	68:52	Uneventful
Ginger	1000 FSW	122:17	97:17	Missed decom stop, Possible slight bend self-treated - No other compoli- cations.
Five aborted dive due to failure of EEG transmitter.				
Sidekick	1000 FSW	117:05	97:10	Cerebral Hypoxia (?) and convulsions at 1000 FSW. Problem corrected and dog completely well and alert on reaching surface.

Table 5 (continued)

History of Hydrox Saturation Dives

Dog

<u>Name Animal</u>	<u>Depth</u>	<u>Total Dive Time Hrs:Min</u>	<u>Total Time on Hydrox Hrs:Min</u>	<u>Results</u>
Asher	1000 FSW	118:10	97:25	Uneventful
Patton	1000 FSW	118:02	96:48	Uneventful
Snap	1000 FSW	117:43	97:36	Uneventful
Sidekick	1000 FSW	120:08	107:59	Uneventful

Table 6.

History of Heliox Saturation Dives

Dog

<u>Name Animal</u>	<u>Depth</u>	<u>Time Dive Time Hrs:Min</u>	<u>Total Time on Helium Hrs:Min</u>	<u>Results</u>
Hydrox	1000 FSW	76:10	62:00	Bends but treated OK. Hematuria once post-dive.
Sally	1000 FSW	73:41	58:10	CNS-treated post- dive. Recovery OK.
Snow	1000 FSW	73:41	58:10	Muscular weakness Recovery OK.
Benjamin	1000 FSW	51:15	47:48	Uneventful
Lady	1000 FSW	51:15	47:48	Uneventful
Rover	1000 FSW	17:43	17:43	Fatal at depth due to contaminated gas. Died within 1 min of each other.
Red	1000 FSW	17:43	17:43	
Beagle	1000 FSW	93:04	54:57	Mild Bends, treated OK.
Sidekick	1000 FSW	93:04	55:10	Uneventful
Spot	1000 FSW	92:26	54:41	Uneventful
Patton	1000 FSW	92:45	54:52	Uneventful
Tasha	1000 FSW	<u>93:50</u>	<u>55:16</u>	Uneventful
		826:37	584:18	

Results and Discussion

This laboratory now has accumulated over 6450 hours of actual hydrox exposure to man and animals (Table 4). A total of 16 dogs have been involved in hydrox dives (Table 5) while 12 dogs have been involved in heliox dives (Table 6). Most of these dives with dogs were saturation dives to a depth of 1000 FSW. The results of these efforts now will be presented and discussed:

The earlier findings of Michaud, et al (16, 24) are puzzling. These workers have reported the appearance of brain abscesses, abnormal EEG changes, EKG abnormalities and sometimes death within less than six hours after the beginning of hydrox exposure at 7 ata pressure.

We have so far failed to observe these symptoms in our animals or in ourselves. As has been reported elsewhere, of the dogs which have been exposed to hydrox, we have lost only two during or after hydrox exposure (Table 5). Our studies of these two deaths subsequently revealed that they were due to the presence of toxic hydrocarbons in the breathing gas. There were no indications that hydrogen contributed to these deaths. Except for these two deaths, the only medical problems in the hydrox dogs have been related to bends during decompression or to what may have been cerebral hypoxia while at depth. It would appear that our animals received a sufficient hydrogen exposure to produce these symptoms since they were exposed to this gas continuously for as long as 97 hours. Brain biopsies for electron microscopic studies were carried out on two animals, and four brains were removed and sectioned.

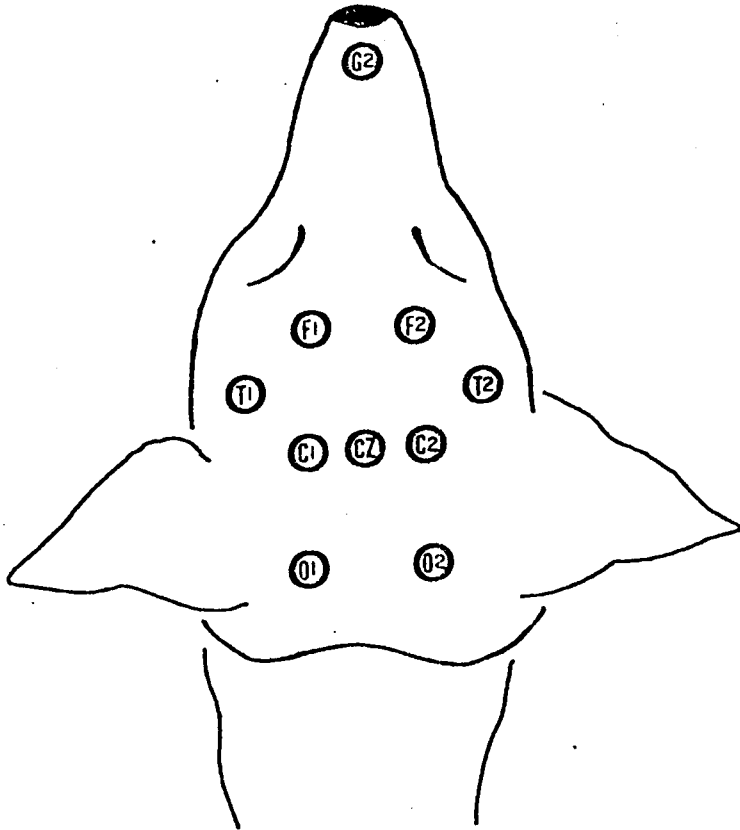
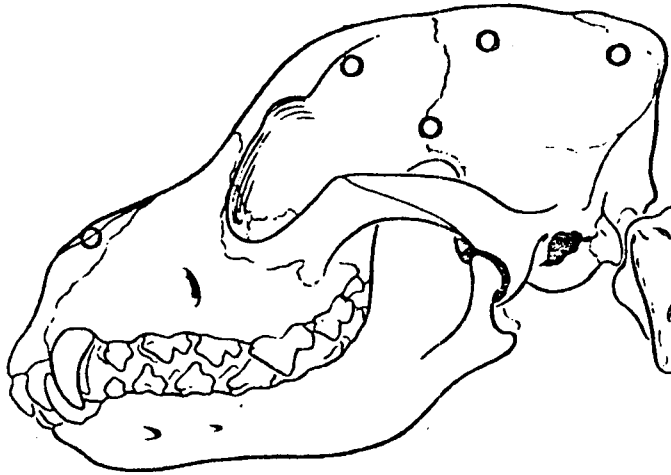
No lesions or other gross abnormalities were noted which resembled the earlier findings of Michaud.

BRAIN:

Only four animals have developed alarming neurological symptoms while at a simulated depth of 1000 FSW on hydrox. Two diving together and one diving alone apparently were subjected to gas contaminated with volatile hydrocarbons. These three animals demonstrated what appeared to be a CNS abnormality but only after remaining at 1000 FSW for from three to twelve hours. The two diving together died quickly after the first symptoms appeared. The third survived. The symptoms first appeared as general apprehensiveness and tachypnea followed by gradual loss of equilibrium and convulsions. In two instances this was followed quickly by a spastic-type paralysis of the forelimbs and the inability to stand erect or hold its head level. In one instance this progressed to an extensor-type muscle rigidity, opisthotonus, and the inability to stand or walk.

It is, perhaps, instructive to consider the 4th instance in some detail since it apparently was not related to contaminated gas. Further, the question of central nervous system abnormalities has been raised by other workers. This animal had been instrumented with an implanted EEG transmitter. Bipolar EEG leads were implanted in the skull in positions F-1, O-1, and C-Z with C-Z the common electrode as suggested by Klemm (26) (Figure 5). It, thus, was possible to obtain continuous EEG's by radio telemetry in the unrestrained animal. Using the pre-dive EEG as control (Figure 6), these two channels showed no apparent abnormal brain electrical activity for the first 22 hours of exposure to hydrox, 12 hours of which were at a depth of 1000 FSW (Figure 7).

FIGURE 5



Proposed electrode location and identification scheme. (From W. R. Klemm, "Attempts to Standardize Veterinary Electroencephalographic Techniques," *American Journal of Veterinary Research*, 29:1895-1900, 1968a).

Thirty-four hours after the beginning of hydrox exposure the dog developed opisthotonus, tachypnea, apprehensiveness followed by convulsions. During and immediately after the convulsions, it was, of course, impossible to record useful brain electrical activity due to the presence of overriding electrical muscle activity.

Since the onset of convulsions apparently developed over an approximately one-hour period and did not coincide with change of the gas source, pressure, or CO_2 levels, it was felt at the time that it possibly could be due to hypoxia, even though the oxygen level was 0.7% (0.6% equals a PO_2 of 160 mm Hg at 1000 FSW). For that reason when the convulsions appeared the oxygen level was quickly raised to 1.5% ($\text{PO}_2 = 400$ mm Hg) and decompression was begun. Upon reaching 890 FSW, the animal showed complete recovery from all postural symptoms although the EEG continued to show the loss of beta waves. As a result of this improvements, the animal was returned to 1000 feet for another 27 hours. This recompression created no adverse effects either behaviorally or in the EEG. During the subsequent sojourn at 1000 FSW, the EEG showed a gradual but continuous return toward normal. It did, however, reveal a loss of the superimposed beta band on the delta and theta waves. This activity is somewhat similar to that observed in humans suffering from HPNS⁽¹³⁾.

During the following decompression, this animal continued to show improvement in its EEG pattern until reaching a depth of 610 FSW. At that time, the inspired oxygen levels again were reduced due to operator error. Although the dog did not convulse, it

appeared to hold its head in an abnormally high position as though "star gazing". At that time, it again began to present a frankly abnormal EEG, containing overwhelming alpha waves. During and immediately after this episode muscle artifacts masked the EEG so that no valid recordings could be made. This episode quickly resolved itself as the oxygen levels were raised and within a few hours, the EEG appeared to be similar to those recorded before the dive. The last recording made before arrival at the surface could hardly be distinguished from the pre-dive recording.

The cause of these convulsions has not been confirmed. There is some circumstantial evidence to support the original theory that they were related to cerebral hypoxia even though the inspired oxygen levels were normoxic. A summary of the evidence is as follows:

a). The chamber (and thus, inspired) oxygen levels were accurately measured. A Beckman F-3 paramagnetic oxygen analyzer in series with a Beckman Model 777 were employed to provide on-line monitoring of chamber oxygen. These units were calibrated at least once each 24 hours against standard reference gases containing the same inert background gas and either zero or 3% oxygen. A variation at any time of more than 0.03% between these analyzers initiated immediate recalibration. We, therefore, are confident that the inspired oxygen levels were accurate to within this percentage at the time of the convulsions.

b). Chamber carbon dioxide levels were not elevated. They were constantly measured on-line with a Beckman infrared CO₂ analyzer. This unit was calibrated each 24 hours with standard reference gases containing zero CO₂ or 0.03% CO₂. Any apparent in-

stability in this unit initiated immediate recalibration. At the time of the convulsions, CO₂ levels were 0.001%.

c. The source of oxygen and hydrogen gases had been changed after the earlier problems with contaminated gas. Further, all lines, scrubbers, and mixing units had been thoroughly purged and at least 4 other dives had been carried out since the contamination problem occurred. There subsequently was found no evidence of contaminants in the gas used in this particular dive other than the presence of water vapor.

d. Perhaps the strongest evidence that these convulsions were hypoxia related was the fact that even though the animal had been subjected to hydrogen continuously for 22 hours, 12 of which were spent at a depth of 1000 FSW, the EEG's remained essentially normal and convulsions did not appear until the inspired oxygen levels had dropped to 0.7% (166.5 mm Hg). When convulsions appeared ascent was made to 900 FSW and oxygen was introduced into the chamber. By the time oxygen levels were raised to 1.5% (400 mm Hg) convulsions had ceased and the EEG lost some of its abnormal characteristics. In fact, the animal showed such marked EEG and behavioral improvement that it was returned to 1000 FSW for an additional 27 hours of bottom time.

Finally, after raising the oxygen levels, the improvement in EEG continued until 46 hours later during decompression. Upon reaching 610 FSW the inspired oxygen levels again were allowed to decrease to 133 mm Hg again due to operator error and the animal again presented both EEG and behavioral symptoms of impending convulsions "star gazing" with head elevated and a fixed stare. These symptoms disappeared quickly when oxygen levels again were raised to 380 mm Hg.

We are unable to identify any variable besides the oxygen levels which seemed to be temporarily related to the convulsions described in this dive.

This suggested that it was important to carry out an EEG study under varying degrees of tissue hypoxia at 1000 FSW, both with heliox and hydrox in an effort to quantify the level at which hypoxia in the presence of both hydrox and heliox begins to produce abnormal EEG's.

Preliminary studies to examine this problem were carried out in 5 anesthetized dogs. The use of the anesthetized dog for EEG clinical evaluation was suggested by Klemm (26) who showed that the EEG of the anesthetized dog was highly reproduceable and was quite free from many uncontrollable external factors which plague the EEG of the awake dog. These records still are undergoing analysis and interpretation. Recordings from the anesthetized dog results in a quite different EEG than that found in the awake animal. It seems probable from our early results that HPNS may be suppressed in the anesthetized dog. Further, while EEG's from anesthetized dogs reveal chronic cerebral abnormalities resulting from disease, we are presently uncertain if many of the subtle, reversible changes can be seen in the anesthetized animal.

We also believe it is important to measure cerebral tissue O_2 . We now have this capability and propose to measure it at 1000 FSW on hydrox.

High Pressure Nervous Syndrome

As indicated earlier, Brauer suggested that the HPNS did not appear during hydrox exposure until the subject reached a simulated depth of 1200 FSW (10). Brauer, Bennett (13) and others noted that with helium EEG changes were observed below about 600 FSW.

No clear evidence of HPNS was seen during any heliox dives. The few instances in which animals appeared to shiver while at 1000 FSW were attributed to being cold. This shivering could be controlled by raising chamber temperature.

No behavioral symptoms appeared which could be attributed to HPNS in any hydrox dives. However, in the convulsing dive described above (Figures 8, 9, 10, and 11), immediately after convulsions ceased and for a short time thereafter, the EEG pattern had elements which resembled HPNS similar to that described by Bennett.

It is not surprising that behavioral symptoms of HPNS were not observed in our dives because of the slow rate of compression. In early dives compression to 1000 FSW occupied 6 hours. In all of the dives in which the EEG was monitored, compression took 7.5 hours. We have conducted no studies of the effect of varying rates of compression on the EEG in the dog. This work, therefore, does not provide insight as to the resistance or susceptibility of a diver to HPNS while breathing hydrox. Recognizing that HPNS can be explained on the basis of a membrane effect, we cannot escape the question as to whether HPNS could contain an element attributable to cerebral hypoxia.

We know of no definitive studies in which cerebral tissue O_2 or CO_2 has been directly measured during the presence of HPNS. We have been struck by the fact that the EEG of the animal just described did not show a HPNS-type pattern during compression or for the first 34 hours of hydrox exposure. It was not until after the animal apparently developed cerebral hypoxia and con-

vulsed that the HPNS-type EEG appeared. This pattern disappeared after several hours of elevated inspired oxygen.

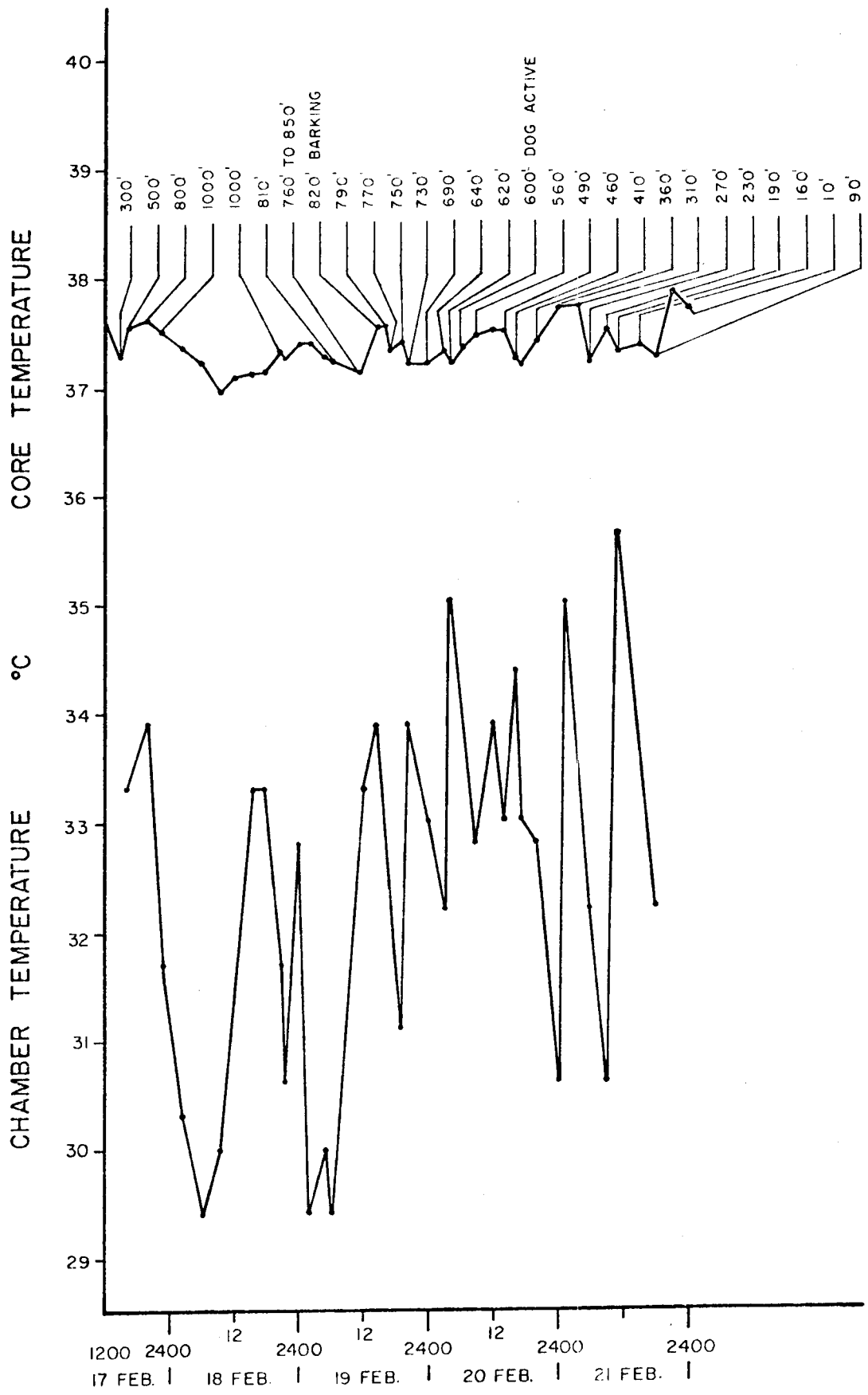
It must be recognized that the loss of the beta wave is not specific for HPNS. On the other hand, the relationship of cerebral oxygenation and beta waves at these greater depths needs to be examined under the controlled conditions in a dog instrumented for EEG and cerebral tissue oxygenation.

TEMPERATURE:

The importance of body heat loss in the hydrox environment cannot be ignored. Webb (22) has shown that at a depth of 800 FSW using heliox, the heat loss through respiration alone may be greater than can be replaced by metabolic activity even during heavy exercise. Since the specific heat of hydrogen is nearly twice that of helium, it was anticipated that this would create a problem when diving with hydrox. This can be overcome in the human diver by raising the temperature of the inspired breathing gas in the helmet or mask. In the case of the animal diver, when the chamber is flooded with hydrox, the convective heat losses through the skin are added to the major heat losses through respiration. This may become severe if the chamber temperature is held to that usually found in the laboratory.

This is demonstrated in Figure 17 which shows the relationship between the ambient temperature of a chamber compressed with hydrox, and the core temperature of the dog exposed to that environment.

It will be noticed that below about 600 FSW the trend in the core temperature of the subject generally followed the trend of the ambient temperature, dropping to 36.9°C when the chamber tem-



COMPARISON BETWEEN BODY CORE TEMPERATURE AND CHAMBER AMBIENT TEMPERATURE. THE NUMBERS ABOVE THE CORE TEMPERATURE REPRESENT CHAMBER DEPTH IN FSW.

Figure 17.

perature was lowered to 29.4°C. Above 600 FSW the core temperature appeared to be rather independent of the fluctuations of the chamber temperature, but appeared more often to roughly follow a circadian rhythm.

It appeared by visual observation that below 600 FSW the dog was more uncomfortable when its core temperature dropped than when the same drop occurred at a more shallow depth.

Although we have not yet correlated EEG with core temperature at depth, as indicated above it may be that the EEG abnormalities and subsequent death of the rabbits exposed to hydrox at 30 ata as reported by Michaud and his associates (16, 24) may actually have been due to loss of body heat rather than to any direct effect of hydrox.

Since all of our man dives have been in a chamber compressed with nitrogen, employing delivery of hydrox by masks, our subjects do not suffer from heat loss through the skin. Indeed, since the chamber contains 97% nitrogen the diver sometimes has been rather warm. The diver occasionally may remark as to the feeling of coolness of the inspired hydrox, but even though one of us (W.P.F.) has breathed hydrox continuously for two hours at a simulated depth of 200 FSW and for a total of 92 minutes at 300 FSW there was no indication of body heat loss. Inspired hydrox usually was at a temperature of approximately 22°C.

In animal and human dives using both heliox and hydrox, we are struck by the close similarity of feeling between the two. We who have dived on both heliox and hydrox are unable to sense any difference between the two gases. The voice differences subjectively appear to be indistinguishable; the breathing resistance subjectively

appears the same (although differences can be measured ⁽¹⁹⁾) and the temperature effects appear to be almost identical. When animals are placed in a chamber and taken to depth, the ambient temperature must be raised to about the same value with both hydrox and heliox to keep the body temperature at normal levels and the animal comfortable. Although we have employed temperature transmitters implanted in the abdomen of dogs, it appears that the chamber temperature can be maintained adequately by visually observing the comfort of the animal. If it shivers or lies next to the heated wall the temperature is elevated. If it avoids the wall and pants, the temperature is lowered.

It appears that the viable temperature range of the animal narrows when it is exposed to ambient hyperbaric hydrox or heliox. This, perhaps, should not come as a surprise since the lightly clothed swimmer or diver can detect, and be bothered by, water temperature changes of $\frac{1}{2}^{\circ}\text{C}$. The high specific heat of hydrox and heliox, together with the greatly increased density of these gases when compressed, increase body heat loss manyfold over that of nitrogen.

This apparent narrowing of the temperature tolerance has another consequence of interest as the heating system is designed. It appears that maintaining the chamber environment at a temperature of 34°C to 35°C at a depth of 1000 FSW will not accurately assure the comfort of the animal. The animal does, however, make fine temperature adjustments by changing its position in reference to the heated wall of the chamber. Instances have been noted when although the ambient temperature remained unchanged, animals purposefully changed their distance from the heated wall. In one instance the external heating source on a mouse chamber was moved

from its position at the side wall to the bottom so that the bottom and both sides were heated alike. Even though the ambient temperature was not altered, all animals died within 24 hours, apparently from overheating.

Comparison of Hydrox and Heliox Decompression

The present work makes it possible to study the difference between hydrox and heliox decompression.

The difference first was noted by Edel and Fife in their early work on dives to 1000 FSW with dogs. In this first study it was found that the same dog using the same decompression profile from a 1000-foot saturation dive developed decompression sickness at a depth of 425 FSW on hydrox while he did not develop a similar grade of bends with heliox until reaching 10 FSW. On the other hand, these observations were not viewed as a rigorous comparison because at the time of the first 5½-day hydrox dive no effort had been made to remove urine. As a result, bacterial action accelerated by the 35°C temperature had produced an extremely high ammonia level in the chamber. It is possible that these levels could have been detrimental to gas exchange and, perhaps, even caused pulmonary damage although there was no gross evidence of such damage. For this reason this observation was considered inconclusive.

It is, perhaps, useful to study what appear to be reproducible borderline decompressions from 1000-foot saturation dives. Such a profile is shown in Figure 18. This 66½-hour profile produced subcutaneous emphysema in only one dog while the remaining animals

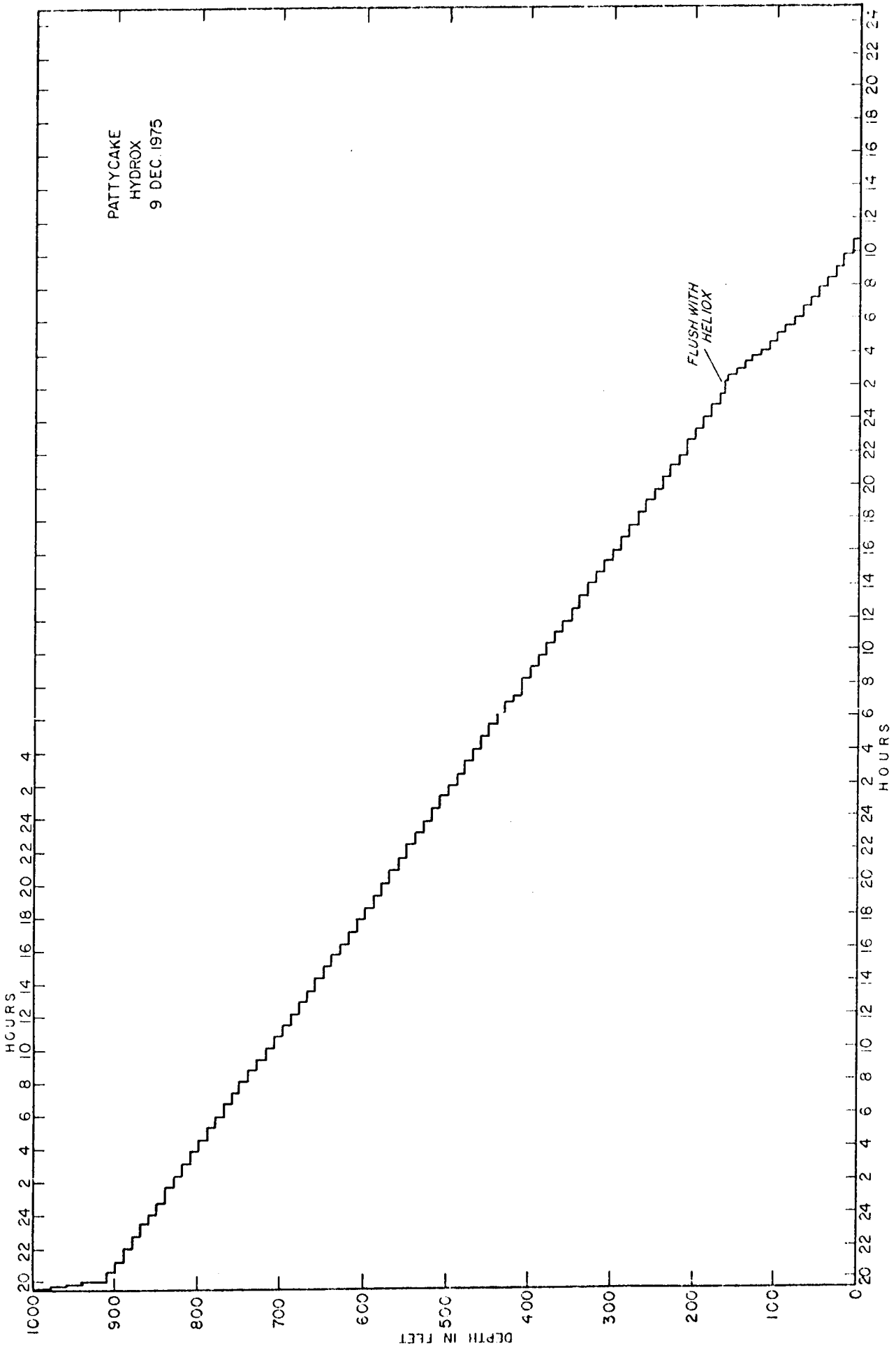


Figure 18

Showed no decompression sickness symptoms. However, any alteration which increased the rate of initial ascent by shortening the time deeper than 800 FSW resulted in bends. This may be seen in Figure 2, in which staged ascent with shortened stops was made from 1000 FSW to 800 FSW; and Figure 19, in which a direct ascent was made from 1000 FSW to 900 FSW without stops. In both of these instances the initial ascent apparently precipitated decompression sickness which appeared at 700 FSW and 750 FSW respectively. Both were CNS-type bends but were successfully treated.

It also was possible to develop a borderline decompression table for a 1000 FSW saturation dive using heliox in which the concentrations of helium were identical to those with hydrox. Such a profile may be seen in Fig. 1. This table required 49 hours as compared with 66½ hours for hydrox. It may be seen from Figure 20 that slightly increasing the rate of ascent by reducing the time at deeper stops resulted in decompression sickness. These symptoms appeared at a depth of 500 FSW and resulted in a temporary partial paralysis of one leg. It was resolved by treatment.

The question which must be asked is whether the increased tendency toward decompression sickness with hydrox is due to an increased rate of diffusion, increased solubility in fat tissue, increased solubility in aqueous tissue, or perhaps to several of the above factors. A review of the solubility properties of helium and hydrogen may be seen in the following table:

Table 4.

	Sol. in* H ₂ O	Sol. in* Olive Oil
N ₂	0.0141	0.076
He	0.0095	0.017
H ₂	0.0190	0.057

*Values determined at 38°C.

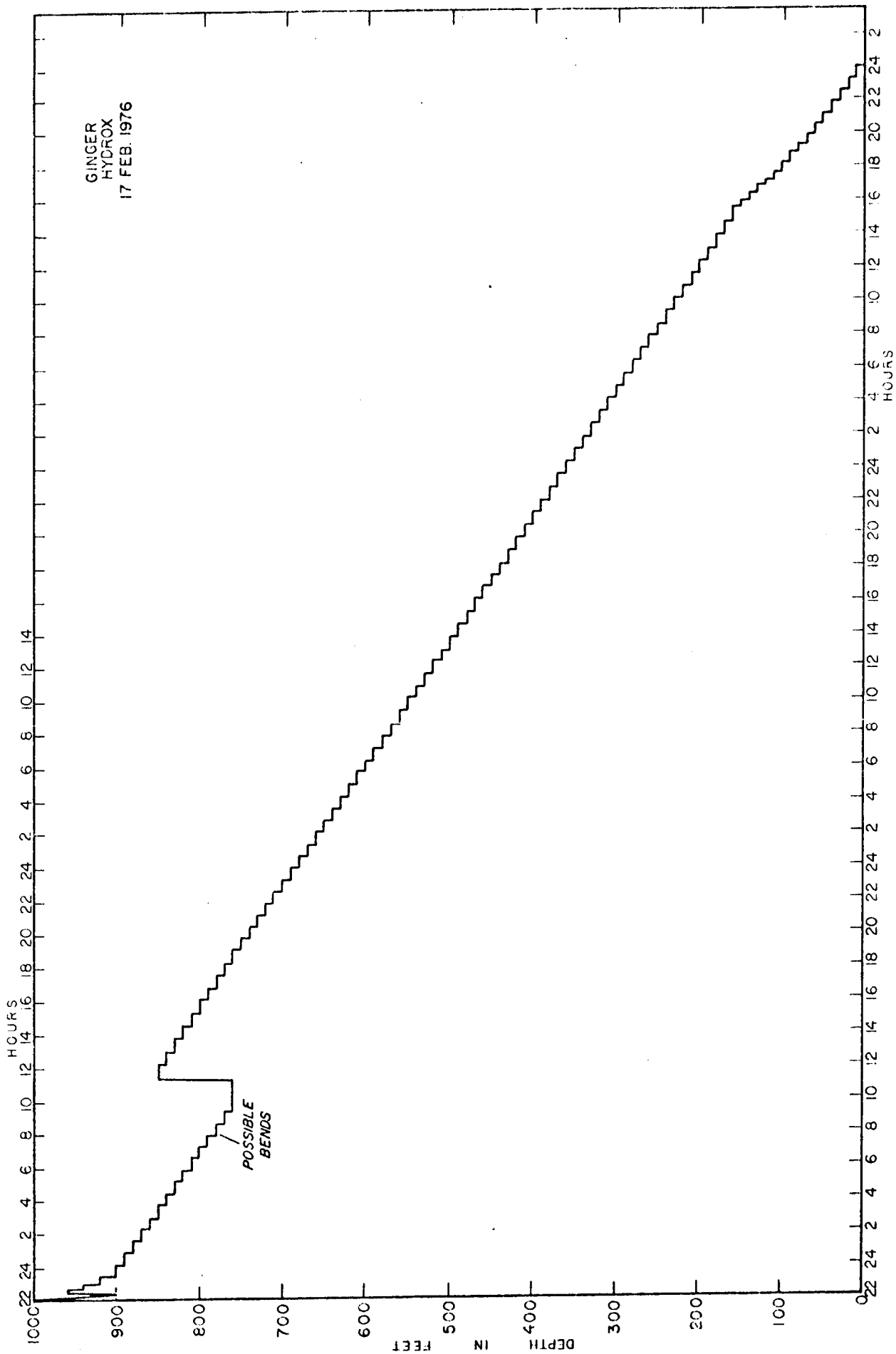


Figure 19.

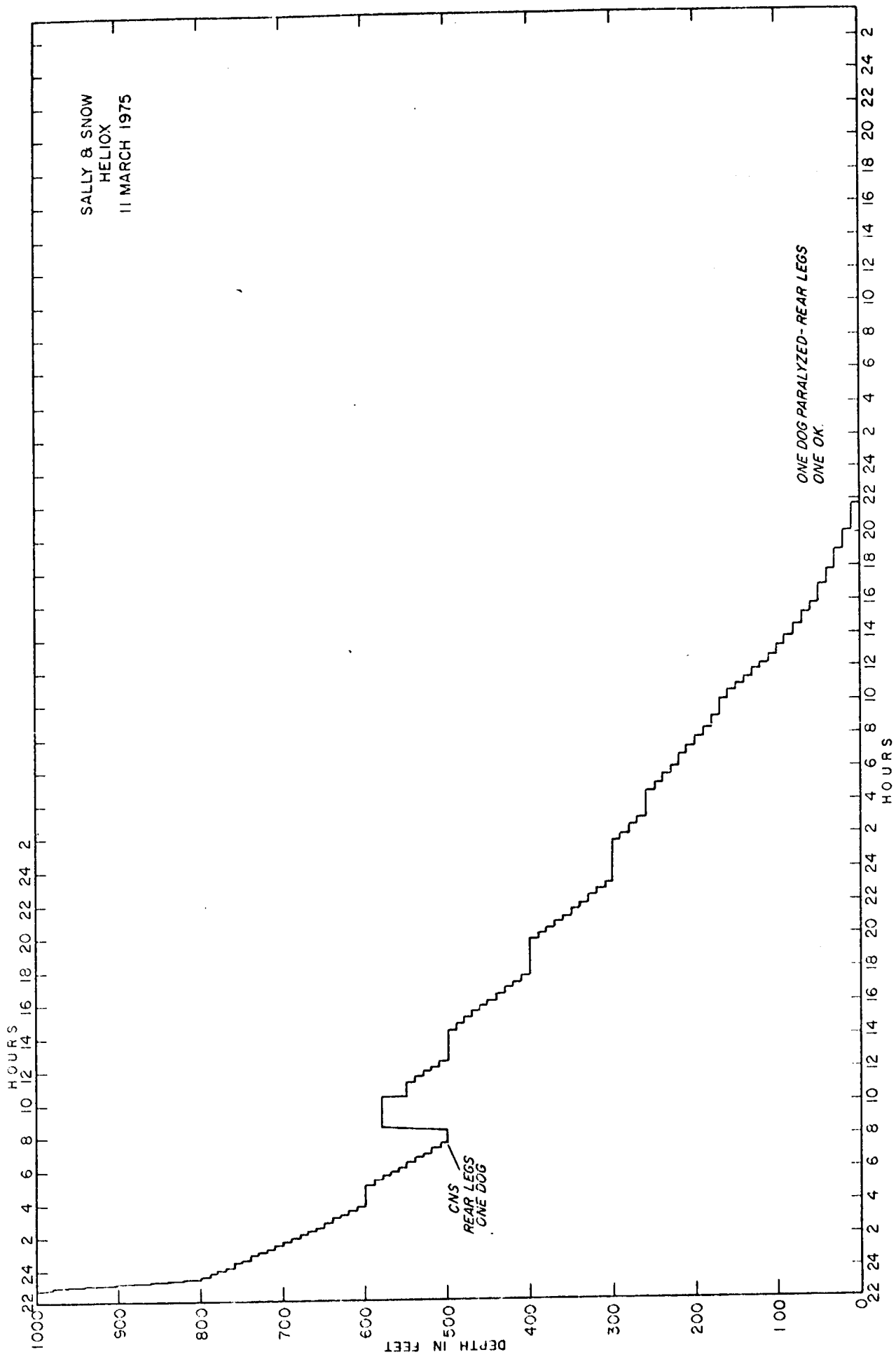


Figure 20.

H₂ is approximately 3.4 times more soluble in fat than is helium, while it is 2 times more soluble in the aqueous fraction.

The possibility that gas movement is diffusion limited must be considered. The diffusion of a gas is inversely proportional to the square root of its molecular weight. The following table shows this relationship for nitrogen, helium and hydrogen:

Table 5.

gas	mol wt	$\frac{1}{\sqrt{M}}$
N ₂	28.0134	0.188937
He	8.0052	0.35348
H ₂	2.0159	0.70423

It can readily be seen from this table that H₂ should diffuse about 2 times faster than does helium. During decompression this increased rate of diffusion should be an asset in favor of H₂ if it remains in solution. However, this property may be an additional hazard if supersaturation is exceeded since there is more likelihood of the coalescing of gas molecules. Further, once a bubble is formed the increased rate of gas diffusion would result in more rapid bubble growth in the case of hydrogen. As will be seen in Table 5, because of the increased solubility of H₂ in both fat and water, more H₂ molecules are present than after an identical exposure to helium. This would tend to further aggravate the growth of bubbles after exposure to hydrogen.

It may be instructive to consider the additive effects of solubility and diffusion. Although it is recognized that this relationship is a complex one, it may be simplified by the following table:

Table 6A.

<u>gas</u>	<u>Sol in olive oil</u>	<u>$\frac{1}{\sqrt{\text{mol wt}}}$</u>	<u>Bends Factor $\frac{1}{\text{sol} \times \sqrt{m}}$</u>
N ₂	0.076	0.1889	0.0144
He	0.017	0.3535	0.006
H ₂	0.057	0.7042	0.040

Table 6B.

<u>gas</u>	<u>Sol in H₂O</u>	<u>$\frac{1}{\sqrt{\text{mol wt}}}$</u>	<u>Bends Factor $\frac{1}{\text{Sol} \times \sqrt{m}}$</u>
N ₂	0.0141	0.1889	0.0027
He	0.0095	0.3535	0.0034
H ₂	0.0190	0.7042	0.0134

It would seem from a study of the bends factor that for an identical exposure and profile, the subject may be expected to develop decompression sickness approximately 7 times more readily on H₂ than on He if fat is the critical tissue; or approximately 4 times more readily if aqueous fraction is critical. It is clear that the borderline bends tables presented here do not permit a critical conclusion to be drawn concerning the importance of aqueous and lipid compartments in bends susceptibility with hydrox. However, for hydrogen, lipid tissue has a "Bends Factor" of 7 over that for helium. The same factor for aqueous compartments is approximately 4. The fact that it only was necessary to extend decompression time by about 22% for hydrox suggests that some other factors must have a major influence on bends susceptibility with hydrox. The observations point to the need to study the relationship between hydrogen and nitrogen since their solubilities in aqueous and lipid fractions are reversed. An examination of the borderline

decompression tables for both H₂ and He (Figures 1-4) shows that following saturation at 1000 FSW, the H₂ decompression tables required a 22% extension in decompression time over those for He.

The Effects of Hydrox on Fertility:

No evidence has been found that exposure to hydrox has any effect on fertility. Normal litters were born to seven dogs which had been subjected to 1000-foot hydrox dives. Two of these dogs were approximately one-month pregnant at the time of their dives. Three of these litters were sired by dogs which also had been subjected to 1000-foot hydrox dives.

Further, one litter of mice was born during a 300-foot hydrox dive. All but one of the young survived and subsequently reached maturity.

Although no sperm counts of other fertility tests were carried out, we were unable to see anything unusual in the cycle of any of the dogs, or any unusual differences in the size or sex ratio of the litters. The ratio of males to females appeared to be about the same for dogs which had not been subjected to hydrox.

Ultrastructure of the Lung:

Pre- and post-dive lung biopsies were carried out on a total of 25 dogs and were studied by electronmicroscopy. Standard techniques were used to prepare and view tissues. This included buffered glutaraldehyde fixation, osmium tetroxide staining, methylemethacrylate imbedding and viewing by a transmission electronmicroscope.

A critical evaluation of this work showed little remarkable. In some animals there appeared to be a slight increase in the amount of collagen but in all instances it was felt that the conditions which precipitated this were not related either to hydrox or heliox for two reasons: Firstly, it appeared to be present in most pre-dive specimens. Secondly, it was felt that there was insufficient time for collagen to develop during a dive even if an appropriate insult occurred. As a further indication that such an insult did not occur, attention is directed to the animals which had a second series of lung biopsies related to subsequent dives. There was no evidence that these animals suffered any long-term or late-developing ultrastructural changes which could be attributed to a previous dive.

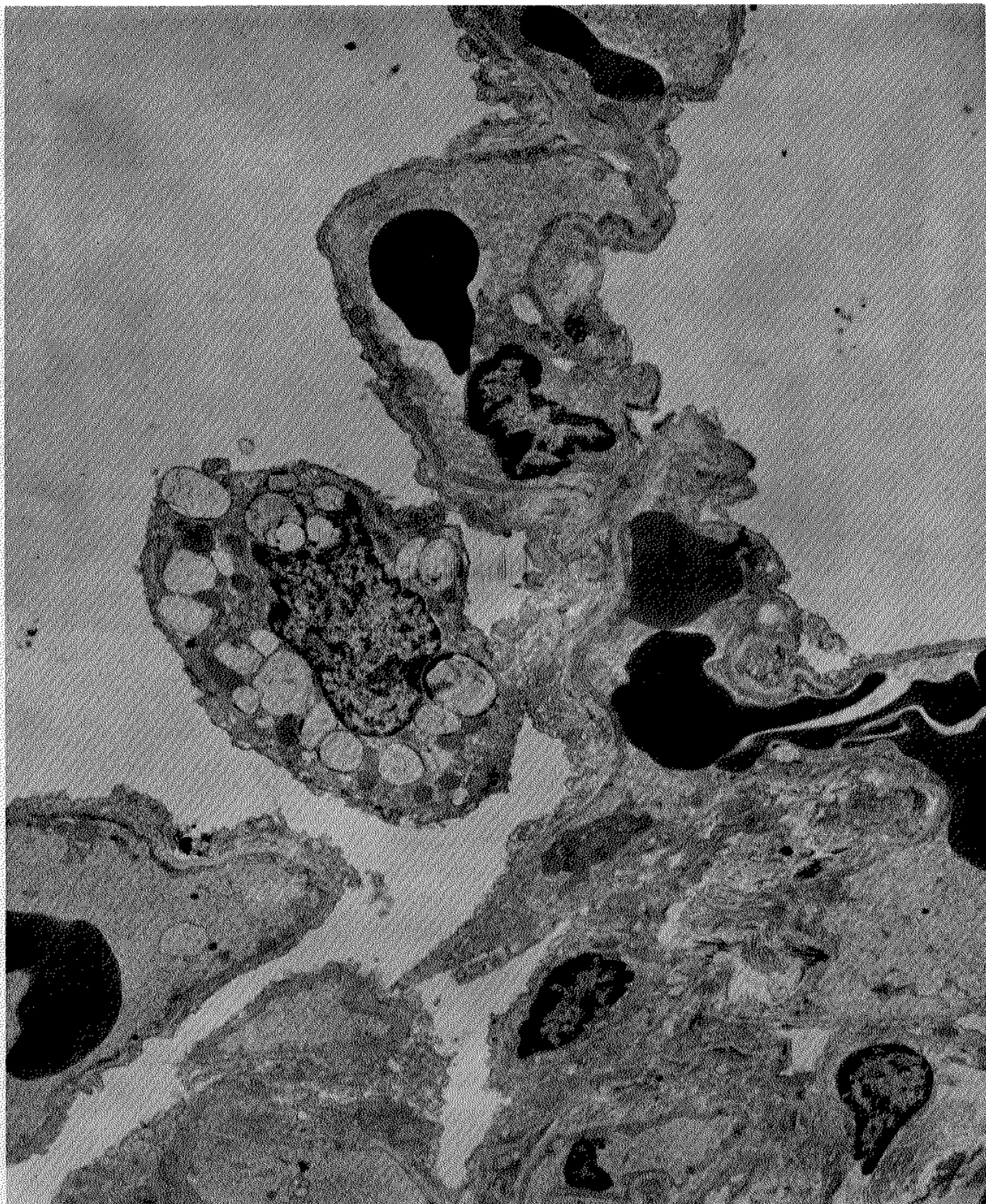
Special attention was paid to the alveolar lining. The Type B cells appeared to be completely normal, containing a normal number of osmophilic lamellar bodies, mitochondria and other structures. Further, the pulmonary capillary endothelium appeared normal in all respects. Occasionally, erythrocytes were observed in the alveolar spaces; however, these appeared to have been displaced during tissue mincing and subsequent processing. There was

no evidence that these cells were the result of hemorrhage or an inflammatory process.

Basement membranes and pulmonary interstitium were closely examined. There were some areas in which the back-to-back relationship of the epithelial and endothelial basement membranes could be distinguished. However, there was no evidence of basement membrane separation, degeneration, thickening or edema as a result of either hydrox or heliox exposure.

The general conclusion was that there is no evidence that exposure to hydrox to a depth of 1000 FSW for periods of up to 97 hours results in any temporary or permanent pulmonary damage or in any way interferes mechanically with gas exchange. Figures 20-22 present typical pre-and post-dive electromicrographs for both heliox and hydrox exposures.

It is recognized that this limited number of micrographs cannot present a representative sample of the lung or represent a basis for critical evaluation. More than 300 other micrographs have been studied as a part of this project.



Dog Lung - Pre Dive

(21000x)



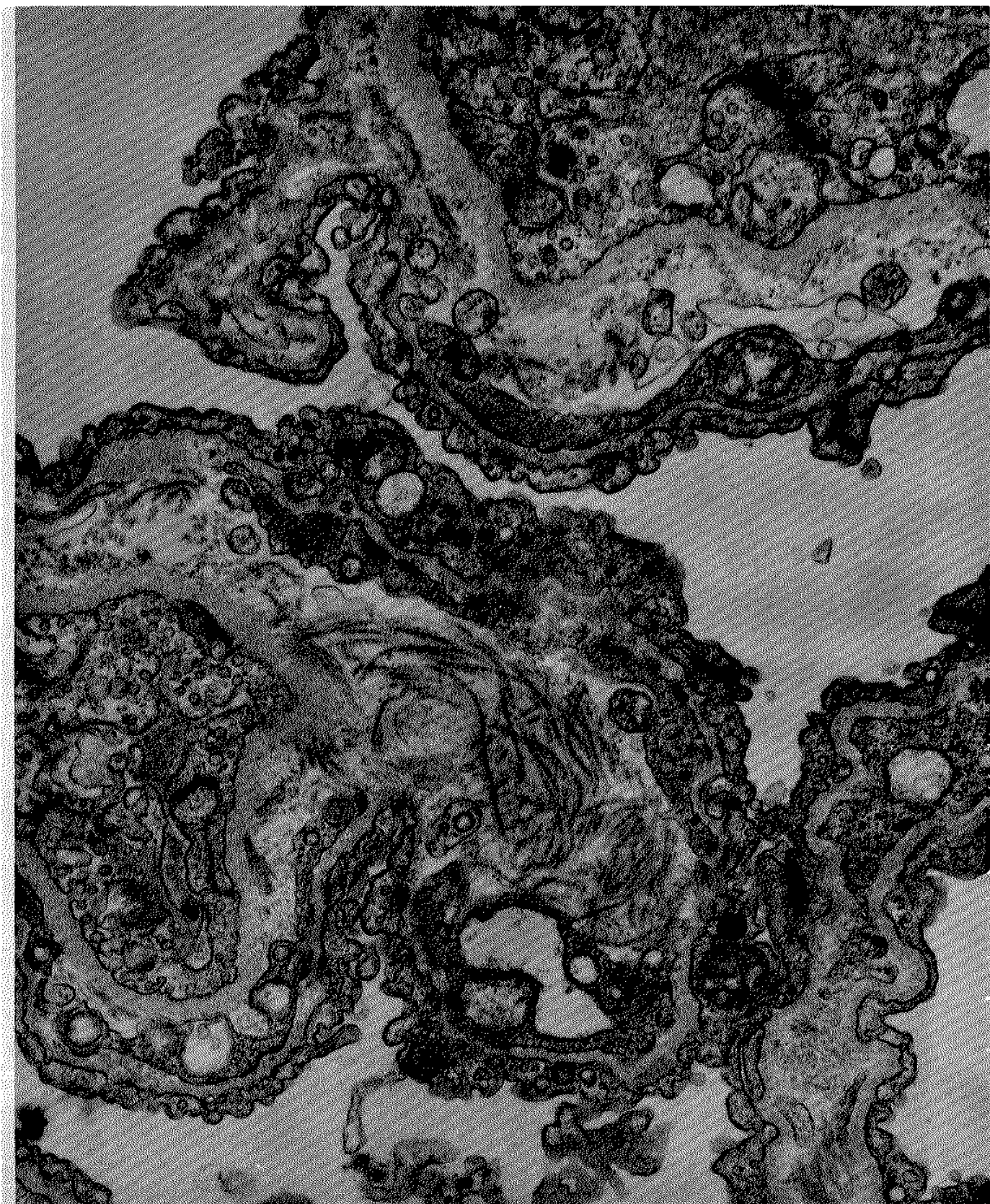
Dog Lung - Post Dive Hydrox

(33000x)



Dog Lung - Post Dive Hydrox

(35000x)



Dog Lung - Post Dive Heliox

(58000x)

Hematology:

Extensive blood studies were conducted using the animal as its own control.

Blood samples were drawn from a foreleg vein. The following determinations were made:

1. Serum alkaline phosphatase (S.A.P)
2. Total serum calcium (T.S.C.)
3. Creatine phosphokinase (C.P.K.)
4. Lactate dehydrogenase (L.D.H.)
5. SGOT
6. SGPT
7. Cholesterol
8. Total protein
9. Urea nitrogen
10. Glucose
11. Hematocrit
12. RBC
13. WBC
14. Serum phosphorus

Serum Alkaline Phosphatase (S.A.P), Cholesterol and Serum Protein (S.P.) were slightly increased while Total Serum Calcium (T.S.C.) was decreased in the post-dive samples from both groups of dogs. Creatine Phosphokinase (C.P.K.) and Lactate Dehydrogenase (L.D.H.) were elevated in the post-dive samples from dogs on 97% Helium: 3% Oxygen and decreased in dogs on 97% Hydrogen: 3% Oxygen. These changes were not statistically significant and no consistent trend was detected in the other parameters measured (Table 7).

Increased S.A.P. and decreased T.S.C. raise the possibility of a change in calcium homeostasis in dogs inhaling these gas mixtures. This possibility requires further investigation because of possible effects on neuromuscular irritability and homeostatis. The increased cholesterol seen in both groups of dogs may be due to endogenous lipid mobilization but this is by no means clear. Elucidation of the underlying mechanism would necessitate evaluation of fat metabolism and endocrine balance. The increases in C.P.K. and L.D.H. in dogs inhaling heliox may reflect increased muscular activity or subclinical intravascular hemolysis. Increases in C.P.K. and L.D.H. were not seen in dogs on heliox. This mixture may be safer. However, further study is necessary to evaluate this hypothesis.

It is gratifying to see that exposure to hydrox does not appear to cause hematological changes which suggest that hydrox is more harmful to the body than is heliox. Indeed, there appears to be no clinically significant difference between the post-hydrox and post-heliox values compared to their own pre-dive values except for the decrease in the post-dive CPK value for hydrox exposure, and for the quite significant rise in post-dive cholesterol values for both gases.

Of special interest is the significant rise in post-dive serum cholesterol levels. This has been reported previously by other workers and seems to be a consistent finding. It would seem to be especially important to determine the source of this cholesterol. If it comes from mobilization of cholesterol deposits it is possible that this could be of benefit to the body, particularly

if it resulted in reduction of such deposits in the cardiovascular system. We hasten to note, however, that there is no indication at present that this is the case. On the other hand, if this rise in circulating cholesterol is the result of new synthesis it may reflect an undesirable condition. It could represent an unwanted source of cholesterol and result in further cholesterol deposits. It possibly could reflect the loss of precursors of vitamin D or the steroid hormones.

A summary of blood values may be seen in Tables 7 for those blood enzymes and components of interest.

The Question of Hydrox Inertness in the Body:

The large number of successful hydrox dives to a simulated depth of 1000 FSW, and the survival of these animals for up to 7 years in apparently normal health suggest that dissolved molecular hydrogen even at a partial pressure of 23400 mmHg (31.1 ATA) does not result in identifiable irreversible physiological damage. On the other hand, there are several indications that molecular hydrogen may not be completely inert in the body, particularly at elevated partial pressures. Earlier work by Dole⁽²⁸⁾ indicated that at high partial pressures (500 TORR) molecular hydrogen scavenged alkyl radicals in polyethylene. Further studies by Dole, Wilson, and Fife⁽²⁹⁾ indicated that hyperbaric molecular hydrogen at an ambient partial pressure of 8 ATA may have altered the course of ultraviolet-induced squamous cell carcinoma in the hairless mouse. Recently, Fife⁽²⁸⁾ has carried out unpublished preliminary studies suggesting that hyperbaric molecular hydrogen at an ambient partial pressure of 8 ATA apparently suppressed the early development of

precancerous skin lesions which appeared within 5 days in control mice painted with methylcholanthrene and croton oil.

The possible biological effect of molecular hydrogen related to the development, prevention or treatment of neoplasms will not be elaborated upon except to suggest that molecular hydrogen may scavenge highly active alkyl and hydrox free radicals or superoxides, some of which are a by product of normal cellular metabolism, without disturbing lower energy metabolic activities. However, this would not explain the possibility that the partial pressure of inspired oxygen can decrease to a lower level in the presence of helium than in the presence of hydrogen without developing adverse neurological symptoms. If molecular hydrogen enters in some way into normal metabolic pathways, it may decrease the efficiency of oxygen metabolism, producing the same effect as reducing the partial pressure of cellular oxygen. We found no instance in heliox diving to 1000 FSW in which lowering the partial pressure of oxygen to 166 mmHg (0.22 ATA) resulted in abnormal EEG's or convulsions.

TABLE 7

	Pre Dive		Post He-O ₂		Post H ₂ -O ₂	
	<u>MEAN</u>	<u>S.D.</u>	<u>MEAN</u>	<u>S.D.</u>	<u>MEAN</u>	<u>S.D.</u>
Alk.Phos. I.U.	25.36	21.64	28.00	23.08	30.00	8.21
SGOT I.U.	33.82	19.42	36.80	9.50	21.75	7.14
SGPT I.U.	39.30	25.99	28.60	2.70	35.67	25.54
CPK I.U.	267.00	195.17	376.50	85.58	92.67	59.05
LDH I.U.	319.36	288.58	427.20	234.89	324.10	477.59
Cholesterol MG/DL	175.67	43.08	207.20	21.38	210.50	58.26
Total Prot. GM/DL	6.97	1.38	7.60	1.23	7.52	1.80
UREA N MG/DL	13.92	4.54	12.14	2.17	13.63	3.77
Creatinine MG/DL	0.93	0.32	0.98	0.29	0.98	0.38
Ca MG/DL	10.53	0.72	9.98	1.41	9.31	1.18
P MG/DL	5.45	1.07	4.68	0.51	5.43	1.45
Glucose MG/DL	82.73	14.14	102.40	16.82	55.50	31.30
HCT	43.57	7.36	43.75	6.18	39.80	4.00

SPECIAL TECHNICAL PROBLEMS AND THEIR SOLUTIONS

HYPERBARIC CHAMBER; SPECIAL PROBLEMS AND THEIR SOLUTIONS:

Many hyperbaric chambers now in operation could be made suitable for hydrox diving without major modifications. Any chamber which is safe for use with oxygen should be even safer for use with 3% O₂ in hydrogen, provided care is exercised to avoid leaks. In fact, we believe that flooding a chamber with oxygen-enriched gas is far more hazardous than flooding the chamber with hydrogen. This is perhaps best illustrated by the work of Doerr and Schreiner (23) who showed that even compressed air under pressure could sustain a vigorous fire. On the other hand, as already mentioned, a gas mixture containing 3% oxygen and 97% hydrogen could not be ignited even with an open spark.

Great care must be taken to assure that hydrogen gas will not leak out of the chamber into the laboratory. While such leaks may occur at any penetration, door or port, our experience has shown that leaks are more likely to occur at electrical penetrations and on large diameter hose or pipe fittings. Potting techniques described in Appendix 5 have been successfully used in our laboratory for several years with little trouble. Most of our penetrations are tested at a pressure of 1800 psi with helium and are completely leak proof. Some of these have been in constant use for over three years without failure.

If hydrogen is to be used in the chamber it is essential that the chamber and all ancillary components be tested with helium to at least 10% over the proposed working pressure before the dive begins. Many leaks not apparent with nitrogen will become major problems with helium or hydrogen at the same pressure. Rarely will

a leak appear with hydrogen if it did not appear during a check with helium.

All fittings, valves, regulators, doors and ports are checked for leaks every 100 feet during compression and at least once during each subsequent 4 hours. In addition, an electronic hydrogen warning system is in constant operation in the chamber area.

An additional precaution has been taken by installing a closeoff valve at every chamber penetration except for the electrical penetration. In this way hoses, gauges, etc. can be isolated and replaced in event of failure without aborting a dive. Although electrical penetrations do not have close-off valves installed, the penetration is constructed in such a way as to permit wires to be cut and the penetration capped in event a leak occurs.

CARBON DIOXIDE REMOVAL

Any chamber dive which is to last more than a few minutes must have provisions for CO₂ removal. This may be accomplished either by an internal or external scrubber. One unit in common use in the diving industry is the Lindberg-Hammar Model M-9.* This unit employs a sealed explosion-proof 24-volt AC/DC motor with magnetic coupling to the fan. It also contains a basket in which any of the usual granular CO₂ absorbers may be used. This unit may be placed inside the chamber and be controlled with a variable auto transformer outside the chamber. Another satisfactory motor is manufactured by Rotron.**

We also have built an external scrubber, using the same type of Lindberg-Hammar motor and basket. This was accomplished by constructing a four foot long container from high pressure oil-field pipe.

* Lindberg-Hammar Inc. Fort Worth, Texas

** Rotron Inc. New York, New York

The motor was permanently mounted in the bottom section while the scrubber basket may be inserted or removed from the top. One inch diameter high pressure hoses connected the unit to the chamber.

CO₂ scrubbers for small animal chambers are easily made in the laboratory. They usually consist of a can 5-6 inches in diameter with a screen at each end. The fan is driven by an induction type motor built for continuous operation. The condenser for the motor is removed to the outside of the chamber. In this way there are no commutator brushes inside the chamber and no external pressure is placed on the condenser which may be oil filled. These scrubbers have operated successfully in our laboratory for over 5,000 hours of hydrox exposure.

It is desirable to use slow-blow fuses in the motor circuit. These fuses should be rated slightly below the locked rotor current flow of that particular motor.

Consideration should be given to the CO₂ absorbent used. We chose Soda-Sorb since its CO₂ absorbing capacity is greater than some other types. Initially we obtained this from either a medical or scientific supply distributor. However, after a long series of problems it was discovered that there may be a great variation in CO₂ absorbing capacity between batches of this absorber. Further, it apparently is possible for scrubber material to meet medical grade specifications and still not have enough CO₂ absorbing capacity to satisfy our own needs. As a result, arrangements were made with a manufacturing company to produce a supply of fresh Soda-Sorb having a CO₂ capacity at least 4 times better than the usual medical grade. In some instances 10 pounds of absorber would adequately remove CO₂ produced by a 60 pound dog for

up to 3 days. These problems would suggest that larger users of CO₂ absorbers may find it wise to deal directly with a factory producing absorbent material. Soda-Sorb especially manufactured for use in diving now is available.*

Heat Loss:

As discussed elsewhere, it is extremely important to maintain ambient chamber temperature in the range of 33°C to 35°C when hydrox is the diving gas. Further, since the temperature tolerance of the subject seems to be narrowed in this environment it is highly desirable to allow the subject to make his own fine adjustments in his temperature exposure. This can be accomplished by heating the chamber from one side wall only, using an external heat source. In one of our larger chambers (30 cu ft) a 220 v thermostatically controlled electrical space heater is placed 6 inches from the outside wall of the chamber. In small chambers an infrared bulb or a 110 v heating strip, thermostatically controlled may be employed.

Ammonia Control:

It is essential the urine be removed from the chamber as quickly as possible since the high temperature of the chamber greatly accelerates breakdown of urine into ammonia. It is not clear how serious the ammonia build-up is on the subject. On an early dive of 5½ days duration no effort was made to remove urine. As a result, ammonia levels became high enough to bother the technician when the dog was removed from the chamber at the termination of the dive. The dog showed no gross evidence of pulmonary damage, and no subsequent respiratory problems appeared. On the other hand, intuitively it would seem that ammonia build-up

*Soda-Sorb H.P.

Dewey-Almy Chemical Div., Atlanta, Ga.

should be avoided. Studies on the effects of ammonia on the body under pressure have been much too brief to draw valid conclusions since in the past considerable precautions have been taken to avoid ammonia build-up in the chamber. In the larger chambers this removal is accomplished by flushing water over the chamber subfloor. In the smaller mouse and rat chambers absorbent flooring is used which is changed periodically by bringing the chamber to ground level. These small animals can be brought to the surface and returned to depth in 3-6 hours, depending upon the depth of exposure.

SAFETY CONSIDERATIONS

Combustibility of Hydrox:

Of primary concern is the question related to the combustibility of hydrogen-oxygen gas mixtures. The first concern over this matter appears to have been raised by Case and Haldane (2) who apparently consulted Professor A.G.C. Egerton, Secretary of the Royal Society, before they undertook their study of hydrox in 1941. Professor Egerton assured them that a mixture containing 4% oxygen and 96% hydrogen was entirely safe from explosion, as was a mixture containing 68.7% hydrogen and 31.1% air. More recent and carefully documented work by Dorr and Schriener (23) place the safety limit at 5.2% oxygen and 94.8% hydrogen. There has been word that some Russian work suggests the safety limit is somewhat lower than that identified by Dorr and Schreiner but this report cannot yet be confirmed.

In our laboratory we have considered the work by Dorr and Schreiner to be reliable. However, we, like most other workers have arbitrarily limited oxygen levels to about 3%. We have an absolute maximum permissible oxygen level of 3.5% to allow for slight variations in commercially produced hydrox. However, our own mixing system makes it possible to hold oxygen levels to $3\% \pm 0.05\%$.

The 3% oxygen limitation poses no serious problem to the life of the diver who is deeper than 200 FSW since it represents a normal oxygen level at that depth (7 ata). As the diver goes deeper he may wish to lower his inspired oxygen to less than 3%. The rationale behind this is further discussed in Appendix 1 and 2.

Perhaps the best indication of the safety with which hydrox can be used is to consider a safety record. We have carried out in excess

of 6,061 hours of animal and human exposure to hydrogen-oxygen mixtures in chambers over the past 10 years without accident. These range from simulated dives to depths of 200 feet of sea water (FSW) to 1000 FSW. Total times of individual dives range from 5 hours to over 5 days in duration. It should be noted that in all but about 20 hours of hydrox diving we have elected to flood the chamber with the hydrox mixture. So far, in the human dives the hydrox has been delivered by mask with overboard dumping of exhaled gas. On the human dives the chambers have contained 160 mm pO₂ at all depths with the balance nitrogen.

Hydrogen Embrittlement:

The matter of hydrogen embrittlement has become of increasing concern as interest in hydrogen for fuel and other uses has increased. Perhaps the greatest concern to divers came with reports by Michaud (24) and his associates who noted that door failure occurred in one of their animal chambers, apparently due to hydrogen embrittlement.

A considerable amount of work has been carried out to study this problem. There is no doubt that such embrittlement does, indeed, occur, and that it varies with the type of metal and alloy employed.

On the other hand, many of these embrittlement studies were conducted using extremely pure grades of hydrogen. This becomes of special importance in view of the work by Kesterson (25), who showed that in some alloys, the embrittlement was reduced in direct proportion to the increased partial pressure of oxygen in the hydrogen gas. If these data are replotted and extrapolated to bring the oxygen levels within the range used in diving, it can

be seen that within the range of the data points experimentally derived the reduction in embrittlement appears to be significant if oxygen is present in the hydrogen. For example, in one type of stainless steel the data show that when hydrogen contains 150 ppm (.015% of oxygen) there is only a 7.5% reduction in strength. If these data points are graphed it can be seen that the reduction in strength is a semi-logarithmic function of the oxygen content. Thus, by extrapolation, when oxygen levels reach 500 ppm (.05%) it would appear that the strength would be reduced by only 0.1% from its original strength. Since the metal tested also had been irradiated it is possible that non-irradiated metal may suffer even less loss of strength.

It is clear that different alloys respond differently to hydrogen embrittlement. Indeed, the above cited metal (CW301 stainless) showed much greater loss of strength than the other 3 alloys tested at that time.

It should be realized that the hydrogen in diving chambers always contains a far greater amount of oxygen than 500 ppm. In fact, at a depth of 4,000 FSW the oxygen levels probably would be about 0.4% (380 mmHg).

While this observation does not provide a totally satisfactory answer to the concern over hydrogen embrittlement it would suggest that even with the reduction of strength from embrittlement a chamber still may be well within the design tolerance of the device if high quality steel has been used.

Flash-back Arrestor:

Since the hydrox chamber will be venting hydrox almost constantly this gas must be exhausted in a safe manner. It is not

enough simply to vent the exhaust to the outside of the building. While 3% hydrox is not in itself explosive, at low flow rates air will move retrograde through the exhaust line, bringing oxygen to the exhaust valve of the chamber. This would create an explosive mixture in the exhaust line. This problem easily can be overcome by directing the exhaust line to the bottom of a steel drum filled with water. Exhaust gas then must bubble up through the water, thus effectively isolating the hydrox line both from the back flow of air and from any flame which might develop above the drum. The pipe leading into the water drum must, of course, be metal for at least the last 10 feet of its length.

Gas analysis exhaust tubing may be handled in two ways. One is to place an aquarium air stone on its end and place it 4" below the surface of a water-filled metal container. The purpose of the air stone is to eliminate varying back pressure which is created by large bubbles. This may affect the analyzers. The other method would be to place a mine safety screen over its end. This would prevent the flash-back of a flame but would not prevent retrograde diffusion of air when the flow rate is slow.

APPENDIX 1

Protocol for Hydrox Diving with Animals:

The following detailed procedure is used for hydrox chamber diving with animals:

1. Complete the pre-dive check list
2. Close chamber door and begin compression to 200 FSW with pure helium or nitrogen.

Comments: At the start of compression the chamber contains 21% oxygen at a partial pressure of 160 mm Hg. Upon reaching 200 FSW (7 atmospheres absolute (ata)) the chamber still contains approximately 160 mm Hg of O₂, reduced by the amount metabolized. However, this oxygen now represents only 3% of the total gas. As previously indicated, since a gas mixture containing less than 5% oxygen and 95% hydrogen cannot be ignited by a spark, hydrogen now may safely be introduced into the chamber. Further, since at 7 ata a gas mixture containing 3% oxygen still represents 160 mm Hg partial pressure of oxygen, the subject is exposed to normal life-sustaining oxygen levels.

3. Check all fittings and ports for leaks.

Comments: If the chamber is never allowed to contain oxygen levels above 5%, there is no danger of explosion or fire inside the chamber. However, leaks may be serious since such leaks will allow hydrogen to be mixed with the 21% oxygen of the air. Protection against chamber, cylinder or regulator leaks, therefore, is essential. Thus, leak checks are made before introduction of hydrogen, and after reaching each additional 100 feet of depth. If leaks are discovered further compression is prohibited until

they can be stopped.

4. Flush chamber with 3% hydrox (3% oxygen in 97% hydrogen).

Comments: This flush is designed to eliminate the helium and nitrogen gases which are present upon reaching 200 FSW. The amount of gas required to carry out this gas exchange depends upon the size of the chamber, the amount of residual nitrogen or helium which can be tolerated, and the efficiency of gas mixing in the chamber. Practice has shown that for a 30 cu ft chamber, approximately 1400 cu ft of hydrox is required to reduce the other inert gases to 0.5%. This figure would, of course, vary with individual facilities. It should be noted that since the chamber contained 3% oxygen before flushing began, and that the flushing gas also contained 3% oxygen, the diver would see no change in oxygenation.

5. Compress to the desired depth with pure hydrogen. Pause each 100 FSW for leak checks.

Comment: It is important to keep the chamber oxygen levels within a normal physiological range. If no additional oxygen is added, the partial pressure of oxygen remains at about 160 mm Hg. Since it is gradually reduced by metabolic requirements of the diver, if compression is quite slow it may be necessary to add more oxygen. This must be done in the manner described in paragraph 6 below. The rate of compression must be carefully controlled as the dive goes deeper due to the possibility that the diver will develop the high pressure nervous syndrome (HPNS). For dogs, a pause of 30 minutes each 100 feet for leak checks appears to allow a dive to 1000 FSW on hydrox without the presence of HPNS.

6. Oxygen replacement. This is accomplished by introducing a hydrox mixture containing 3% oxygen.

Comment: It is essential to avoid introducing an explosive oxygen mixture into the chamber. Use of already mixed hydrox will allow the input of 3% oxygen with the balance being hydrogen. Since dives deeper than 200 FSW will call for less than a 3% oxygen level in the chamber, adequate oxygen levels can be maintained by adding the 3% mixture. It usually is possible to establish a steady bleed-in of hydrox to just balance oxygen utilization while the dive is in progress.

If the oxygen level must be raised during compression it may be accomplished by carrying out a part of the compression with hydrox in place of pure hydrogen as described in paragraph 5 above. When the oxygen concentration again reaches the desired level, compression is continued with pure hydrogen.

Decompression:

1. Begin ascent by opening the exhaust valve. Bleed in hydrox as required to raise the oxygen level.

Comment: It is necessary not only to follow carefully the planned decompression table, but to maintain adequate oxygenation as well. If the depth is below 200 FSW, the oxygen levels will have been held at less than 3%. (For example, at 1000 FSW the oxygen level in the chamber should be between 0.6% and 1.5%.) By the time the diver ascends to 200 FSW the oxygen level must be returned to 3%. This is accomplished by flushing in 3% hydrox. As the diver approaches 200 FSW, the quantity of hydrox utilized in the flushing becomes increasingly greater.

2. Upon reaching 200 FSW, begin flushing with 3% oxygen-97% helium (heliox).

Comment: The flushing with 3% heliox will assure that at no time hydrogen will be present with more than 3% oxygen. This flushing at 200 FSW also assures adequate tissue oxygenation since as indicated above, 3% oxygen provides 160 mm Hg partial pressure at 7 ata.

Flushing is continued until the hydrogen level is reduced to below 3%, at which time it no longer is an explosive mixture with air.

3. Introduce the desired oxygen or nitrogen-oxygen (nitrox) mixture required by the decompression profile.

Comment: If it is desired to decompress on heliox, it is only necessary to bleed-in sufficient oxygen to raise the chamber oxygen content to the desired levels as decompression proceeds. If compressed air or other nitrox mixture is desired, it is necessary to flush with that mixture to eliminate helium. Consideration should be given to possible oxygen toxicity if an oxygen-enriched environment is contemplated.

After elimination of the hydrox mixture from the chamber, decompression may proceed in accordance with usual chamber decompression practices.

APPENDIX 2

Protocol for Hydrox Chamber Diving with Humans:Compression:

1. Proceed through both the 24-hour pre-dive checklist and the "Day of Dive" checklist.

2. Diver enters and door is secured. Compression begins to 100 FSW using pure nitrogen.

Comment: The diver may breathe chamber air during this descent, or may breathe compressed air by mask, depending on the table used. This compression begins to reduce the percentage of oxygen within the chamber without reducing its partial pressure. Although there is an increased percentage of nitrogen in the inspired gas, it is not sufficient to create unacceptable nitrogen narcosis. The extra nitrogen uptake must be considered during subsequent decompression.

3. Diver begins to breathe 10% oxygen-90% helium by mask, and compression toward 200 FSW is resumed using pure nitrogen. Exhaled gas is exhausted overboard into a flashback arrestor.

Comment: Although 10% oxygen is slightly hyperoxic to the diver, it does not create a toxicity problem during the relatively short time it is used. Further, this mixture is standard in mixed gas diving. Compression of the chamber further reduced the percentage of ambient oxygen so that upon reaching 200 FSW (7 ata), the chamber atmosphere is only 3% oxygen. This will not create an explosive mixture in event hydrogen gas subsequently leaks into the chamber. At the same time, in event of mask failure the diver can breathe the chamber atmosphere without danger to life. Of course, the chamber cannot be flushed with air to remove a build-up of hydrogen. Further, flushing with pure nitrogen would remove excess hydrogen, but

would lower oxygen levels. The chamber atmosphere then no longer would support life in case of mask failure. H_2 leaks into the chamber should be avoided.

4. Shift breathing mixture to the 3% heliox manifold for two minutes.

Comment: This is done instantly by closing the 10% heliox line and opening the 3% heliox line between breaths. Since the intake line is cleared of the previous gas in 3-4 breaths, the 1-minute breathing period provides an adequate safety factor to purge the intake line and regulator. Since the exhaled gas expands upon leaving the chamber, the exhaust line to the flashback arrestor completely is cleared in two breaths. This assures that the exhaust line also will not contain more than 3% oxygen by the end of the 2-minute period. This 2-minute period also allows time for the chamber attendants to shift gas lines to the hydrox manifold.

5. Shift breathing mixture to the 3% hydrox manifold.

Comment: The diver now is breathing a 3% hydrox mixture. Overboard dump of exhaled air assures that there will not be a buildup of hydrogen in the chamber.

6. Compress to desired bottom depth with pure nitrogen.

Comment: The diver may remain on the 3% hydrox breathing mixture for an indefinite period down to a depth of 475 FSW. Based on Lamberstsen's work, he could go much deeper for shorter periods of time without being in danger of serious oxygen toxicity. Indeed, our work suggests that he may be able to remain for a week or more at a depth of 625 FSW while breathing 3% hydrox. If there is concern over the effects of oxygen at greater depths, the breathing mixture may be shifted to one containing less oxygen.

Decompression:

1. Ascend to 200 FSW by reducing chamber ambient pressure in accordance with the decompression schedule.

Comment: The diver remains on the mask during this phase. His breathing mixture should be shifted back to 3% hydrox if it had been changed to a lower oxygen percentage during the dive. If the chamber had been compressed with pure nitrogen to a depth significantly deeper than 200 FSW, the atmosphere will contain less than 3% oxygen. As a safety precaution 3% oxygen in nitrogen should be flushed in to bring oxygen levels to about 3%. In this way it will be assured that the diver will have adequate oxygen in event of mask failure. Chamber oxygen levels should not be raised above 3% as long as the diver is breathing hydrogen.

2. Shift breathing gas to 3% heliox for 2 minutes.

Comment: This will assure that hydrox is flushed out of the entire breathing system. The question of isobaric bends may be raised since there is an almost instantaneous shift from hydrogen to helium. This problem has not been observed in our work.

3. Check chamber gas to be sure it contains no more than 3% hydrogen.

4. Shift breathing gas to 10% heliox.

Comment: This will flush out the 3% heliox and make it possible to bring the diver to within 33 FSW of the surface without danger of hypoxia.

5. Decompress in accordance with standard techniques.

Comment: Examples of both animal and human decompression tables developed in this laboratory are presented. They are discussed in more detail elsewhere.

APPENDIX 3

Lung Biopsies:

Lung biopsies on dogs may be carried out in a manner almost identical to that used on humans. The technique has been outlined elsewhere but is briefly described as follows:

The dog is tranquilized with Acepromazine administered inter-muscularly. The usual dosage is 1 mg per kg of body weight. The right chest area is shaved and cleansed as for other surgery and a location is selected, exercising caution to avoid the heart. This may be done by bringing the elbow of the foreleg back to mid-chest. The angle of the elbow is the approximate location of the heart. A dependent lobe will be entered by staying at least 4 cm caudal to this angle. An area over the center of a rib is selected as the point of penetration and is infiltrated with Xylocane or other local anesthetic agent, following which a 2 or 3 mm incision is made perpendicularly through the skin directly over the rib with a stylet-type blade. The incision should penetrate to the lateral surface of the rib. A sterile Vim True Cut biopsy needle then is inserted perpendicularly through the incision to the surface of the rib. Upon contacting the rib the needle is slid cephalad while still held perpendicular to the skin. When the tip of the needle can be felt to slide over the anterior border of the rib it is forced directly inward, penetrating the parietal pleura. Care must be exercised to keep the needle as close as possible to the rib border to avoid penetration of the intercostal vessels. When the tip of the needle has penetrated the parietal pleura it will rest on the visceral pleura covering the lungs.

This can be confirmed by the tendency of the back of the needle to move in a cephalad or caudal direction in synchronization with respiration. At the moment of greatest exhalation the coreing needle is quickly advanced into the lung tissue, rapidly followed by the outer sleeve which cuts the biopsy plug. The needle then is quickly withdrawn. Since the point of penetration through the skin slides caudally a few millimeters it closes and seals the needle pathway. This tends to reduce the possibility of pneumothorax. The animal recovers in a few minutes and resumes normal activity.

Lung biopsy tissues then may be transferred to glutaraldehyde fixative and prepared in a standard manner for electron microscopic study.

Appendix 4

Protocol for Liver Biopsy:

This biopsy may be done with a Menghini needle or with a Vim True Cut needle. Our technique employs a modified Menghini needle of our own design.

1. The animal is tranquilized by acepromazine as described above.
2. The chosen area, usually subcostal, is shaved, sterilized and infiltrated with Xylocaine to the depth of the peritoneum.
3. An incision through the skin to the peritoneum is made with a stylet.
4. The Menghini needle is attached to a 6cc syringe containing 3cc of sterile saline. The needle then is advanced through the incision so that the tip rests on the surface of the liver capsule.
5. Approximately 1cc of saline is ejected to clear the needle. It then is pressed against the capsule.
6. The plunger of the syringe is withdrawn a few cm to produce a vacuum and seal the liver to the needle tip. While holding the vacuum, the needle is quickly advanced to the desired depth and without pause quickly and completely withdrawn. Upon leaving the body, the vacuum draws the biopsy tissue into the saline solution in the syringe. The needle then can be removed and the tissue expressed into the fixative.

Appendix 5

Potting Techniques:

The potting of electrical penetrations for the CO₂ scrubber becomes critical since the development of even a small leak in such a fitting often results in the requirements to abort the dive. A number of epoxy-type electrical potting materials were tried without success. The potting material finally settled on was Scotchcast #5. This material will withstand the stress of repeated compression even in a hydrogen environment.

The potting process is as follows:

1. Insulation is removed from the multistranded wires for a distance of 1-2 inches at the point where they will pass through the fitting. The strands are soldered together to make a single solid bundle. The area then is coated with the potting compound to provide a good bonding.
2. The inside of the fitting is cleaned on the inside with solvent and scraped to the bare fresh metal with a circular file. It then is coated with the potting compound.
3. The epoxy-coated wires are passed through the fitting and a plug of clay or gum rubber is molded around one end to provide a seal.
4. Additional potting solution is poured in the open end, and the entire unit is placed in an oven at a temperature of 140°F. Care is exercised to assure that the wires are not touching each other or the fitting wall. The fitting is allowed to cure for 24 hours undisturbed. It then may be removed, cooled, and tested.

Penetrations ranging from ¼" to 1" may be prepared in this way.

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