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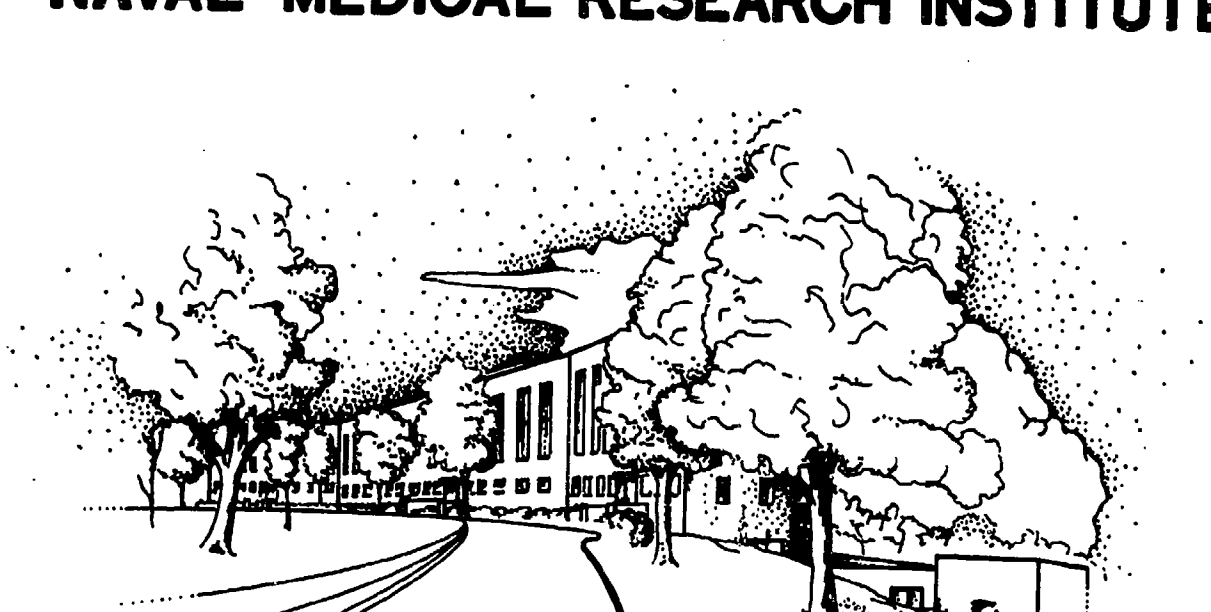

ACOUSTIC-OPTICAL DETECTION OF DECOMPRESSION SICKNESS IN HAMSTERS

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13. ABSTRACT

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ACOUSTIC-OPTICAL DETECTION OF DECOMPRESSION SICKNESS IN HAMSTERS

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ABSTRACT

Severe decompression sickness (defined herein by visible intravascular bubbles greater than 0.3 mm diameter) was produced by explosive decompression in twenty-three of forty-one anesthetized hamsters. Analysis of the post-decompression respiration pattern and ultrasonic transmission (through one thigh) led to two observations: 1) animals without apnea never demonstrated macroscopic bubbles, and 2) animals which gasped always demonstrated macroscopic bubbles. Ultrasonic attenuation was considered evidence of bubbles and preceded apnea by [0.18 (time ultrasound attenuation occurred) - 130] seconds. The ultrasound attenuation preceded gasping by [0.21 (time ultrasound attenuation occurred) + 30] seconds. It is postulated that the respiratory signs of early decompression sickness syndromes arose from bubbles of central (splanchnic) origin and that the later syndromes arose from both central and peripheral bubbles. The respiratory signs were considered to be a consequence of pulmonary aero-embolism. Pilot studies with 2-(4-phenyl-1-piperazinylmethyl) cyclohexanone HCl (PPCH), an antikinin/antihistamine that protects thin mice from decompression sickness, showed that it decreased mortality in unanesthetized hamsters but increased mortality in animals anesthetized with chloralose-urethane. Pilot studies with diphenhydramine in anesthetized hamsters showed no change in mortality rates; in contrast, ultrasonic evidence of bubbles lagged even further behind the onset of the respiratory signs. The expected ameliorative effect of the antihistamine drugs were not observed.

KEY WORDS

decompression sickness, ultrasound, aero-embolism, acoustic-optical imaging, hamster, antihistamine, drug, bubbles, mortality

INTRODUCTION

Accurate diagnosis of the early stages of decompression sickness has been difficult because of the variable manifestations of the disorder (2). Although the etiology of decompression sickness is not understood, bubbles are more frequently implicated as the initial insult than are any other agents (3). The goal of this project was to investigate an ultrasonic technique for bubble detection in small animals.

It is reasonable to assume that detectable quantities of bubbles develop in decompression sickness before the onset of tachypnea (8) or apnea and gasping (10). The initial size and growth rate of these bubbles is not known. By the time macroscopic ($\geq$ 0.3 mm diameter) intravascular bubbles have appeared, however, the prognosis is grave (4). The initial step in this experiment was therefore directed toward understanding the relationship between macroscopic bubbles and respiratory signs. The next goal was to detect the bubbles before the onset of the signs. Ultrasonic methods were particularly attractive because a large ultrasonic impedance mismatch occurs at liquid-gas interfaces. The techniques which have been developed utilize: acoustic-optical images (5), the doppler shift (13), echo scans (9), or through-transmission (7). Initially, the acoustic-optical imaging system seemed most promising; unfortunately, its practical resolution was only 0.7 mm (5). More quantitative information for populations of bubbles <0.7 mm could be obtained by modification of the acoustic-optical imaging system into a through-transmission detector.

The purpose of the experiments was to 1) correlate the signs of respiratory distress with the presence of the macroscopic bubbles which characterize severe decompression sickness in anesthetized hamsters, 2) correlate ultrasonic through-transmission in the rear limb of the hamster with their respiratory patterns, and 3) determine the effect of two drugs on decompression sickness as measured by mortality, respiratory signs, and ultrasonic through-transmission. An antihistamine, diphenhydramine HCl; and an antihistamine/antikinin, 2-(4-phenyl-1-piperazinylmethyl) cyclohexanone HCl* (PPCH), were tested (6). PPCH was chosen because of the profound protection reported against decompression sickness in thin siblings of hereditary obese mice (6). Diphenhydramine was chosen to help identify the role of histamine in the protection offered by PPCH.

PROCEDURE

Control Study. Forty-five fasted male golden hamsters weighing between 84 and 133 grams were anesthetized by the intra-peritoneal route with 0.8 to 1.0 cc/100 grams body weight (b.w.) of chloralose-urethane anesthetic (4). The left rear limb of each animal was cleaned with a dipilatory lotion (Nair); the hamster was entubated with a polyethylene tube (O.D. = 0.05 to 0.06 inches) and mounted on a lucite pallet (Fig. 1). The pallet held the limb of the animal firmly in contact with the interaction cell (Fig. 2) within a 1.0 cu. ft. TRW pressure chamber (Figs. 3 and 4). The ultrasonic transmission through the limb was monitored for 70 minutes. Recordings were made at 2.4

* The PPCH was kindly supplied by C. Chryssanthou of the Department of Pathology, Mount Sinai School of Medicine, City University of New York, New York.

second intervals (while sound transmission from a reference transducer was superimposed on the tracing at 30 second intervals). The 10 minute control period at 0 PSIG was followed by a 30 minute air dive at 170 to 190 PSIG, explosive decompression to 0 PSIG ($\leq$ 10 seconds) and a 30 minute post-decompression period. The respirations were continuously monitored with a thermistor probe in the endotrachael tube. Pallet temperature was generally maintained between 40 and 41°C. Following the post-decompression period, each animal was examined for macroscopic bubbles in the external limb veins. If no bubbles were found, laparotomy was performed and visceral veins were examined.

X-ray examination of the contralateral lower limb was occasionally performed to verify the presence of intravascular gas. A GE Maxmar-100 portable X-ray machine was used (55 KV, 7 MA, 1 sec and a focal-to-film distance of 15 inches).

Drug Studies and Mortality in Severe Decompression Sickness. Two pilot studies designed to assess the effect of drug treatment on mortality were done on animals subjected to a modified procedure. In the first study forty-five fasted male golden hamsters weighing between 78 and 119 grams were divided into 3 groups of 15 animals each. The controls were anesthetized with 0.8 cc/100 grams b.w. chloralose-urethane (all anesthetics were administered intra-peritoneally). The diphenhydramine group was anesthetized with a combination of 1.5 mg/100 grams b.w. diphenhydramine and 0.5 cc/100 grams b.w. chloralose-urethane; the PPCH group was anesthetized with a combination of 10 mg/100 grams b.w. PPCH and 0.5 cc/100 grams b.w. chloralose-urethane. Since the TRW pressure chamber could accomodate 15 animals per run, 3 identical dives

were performed using 5 animals at a time from each group. The chamber was continuously flushed with air to prevent carbon dioxide build up. The dive profile was 190 PSIG for 30 minutes followed by explosive decompression (190 to 0 PSIG in $\leq$ 10 seconds). The time following decompression required for palpable heart beat to cease was recorded.

The second study compared sedative doses of PPCH and chloralose-urethane. Eighty-five male golden hamsters weighing between 80 and 144 grams were divided into three groups. Group 1 (42 animals) was given 10 mg/100 grams b.w. PPCH I.P.; Group 2 (12 animals) was given 0.5 cc/100 grams b.w. chloralose-urethane I.P.; and Group 3 (31 animals) was given nothing. Equal numbers of animals from Group 1 and Groups 2 or 3 were together subjected to a 150 PSIG air dive which was terminated by explosive decompression. The schedule was reduced from 190 to 150 PSIG because lack of anesthesia rendered the animals more susceptible to decompression sickness. The mortality rate was recorded at the end of 15 and 30 minutes.

Drug Effects on Ultrasonic Transmission and Respiratory Signs. Thirteen hamsters were anesthetized with a combination of 1.5 mg/100 grams b.w. diphenhydramine and 0.5 to 0.7 cc/100 grams b.w. chloralose-urethane and tested as described in the Control Study.

Six animals were anesthetized with a combination of 10 mg/100 grams PPCH and 0.3 to 0.5 cc/100 grams chloralose-urethane and tested as described in the Control Study.

RESULTS

Respiratory Signs and the Presence of Bubbles in Anesthetized Controls. Apnea and gasping were the observed signs of respiratory distress. Gasping was characterized by an abrupt decrease (to 50 per cent or less) in respiratory frequency, a 2-fold increase in the respiratory amplitude, and a prolonged expiration phase. Approximately 70 per cent of the animals with respiratory distress demonstrated a sequence of 1) apnea for 80 to 270 seconds, 2) gasping (chokes) for 20 to 230 seconds, and 3) terminal apnea. Tachypnea (defined as $\geq$ 20% rise in respiration rate during post decompression period) occurred in 17% of all animals. It was not more frequent in the animals that showed intravascular macroscopic bubbles at autopsy. Comparison of the respiratory signs and autopsy findings are given in Table 1.

Gasping always indicated that macroscopic bubbles would be present at autopsy. This occurred in 19 control animals and 11 animals treated with either PPCH or diphenhydramine. Similarly, the absence of apnea indicated that no bubbles would be found at autopsy. This occurred in 16 of the control animals and 3 of the treated animals.

Respiratory Signs and Ultrasonic Through-Transmission in Anesthetized Controls. The respiratory signs and relative transmission were plotted as a function of time for each animal (Fig. 5). The ultrasonic transmission was stable throughout the control and dive periods; it was not stable during the decompression period. The onset of ultrasonic diagnosis was defined to be a 10 per cent attenuation of the ultrasonic transmission. The onset of apnea, onset of gasping, duration of

gasping, and fall time (time required for relative transmission of ultrasound to be attenuated from 10 to 90 per cent) were compared with the onset of the ultrasonic diagnosis. Only the onset of apnea and the onset of gasping showed significant correlation with the ultrasonic diagnosis time ($r = 0.78$ and $r = 0.82$ respectively). The fall time range was very wide, from 45 to 900 seconds. The data from the control group were plotted and recalculated after excluding 2 data points outside the $\pm$ 2 standard error boundaries (Fig. 6). Apnea always preceded gasping by approximately 160 seconds. There was a linear relationship between the attenuation of the ultrasound and the onset of respiratory signs. The ultrasonic attenuation preceded the appearance of any apnea episode in twelve per cent of the control animals. In thirty-one per cent of the hamsters the attenuation preceded the major apnea period. Ultrasound lead over apnea = [0.18 (time ultrasound attenuation occurred) - 130 seconds]. Ultrasound attenuation nearly always preceded the appearance of gasping, however; ultrasound lead over gasping = [0.21 (time ultrasound attenuation occurred) + 30] seconds (Fig. 6).

Drug Effects on Mortality in Decompression Sickness, Study 1. The mortality rate (80%) associated with explosive decompression in anesthetized animals treated with diphenhydramine was not significantly different from the control group (87%, Fig. 7). But, there was 100 per cent mortality in the anesthetized animals treated with PPCH and death occurred sooner than in the control hamsters (Fig. 7)

Study 2. The mortality rate 30 minutes after explosive decompression

in hamsters sedated with PPCH was 10% less than those sedated with chloralose and 20% less than hamsters not sedated at all (Table 2). The hamsters sedated with chloralose were less active than those sedated with PPCH.

Drug Effects on Ultrasonic Transmission and Respiratory Signs in the Anesthetized Hamster. Six of the thirteen animals treated with diphenhydramine developed decompression sickness. Only three of the six animals treated with PPCH developed the apnea, gasping sequence - the remaining three died quietly.

There was no correlation between the time of ultrasonic attenuation and the onset of gasping or apnea in the diphenhydramine-treated animals. Apnea in these animals preceded ultrasound attenuation by an average of 140 seconds. Gasping preceded the ultrasound attenuation by an average of 70 seconds.

The affects of PPCH were less profound - the ultrasound lead over gasping = [1.6 (time ultrasound attenuation occurred) + 10] seconds and the ultrasound lead over apnea = [0.05 (time ultrasound attenuation occurred) - 30] seconds.

DISCUSSION

The observations that 1) animals without apnea never demonstrated macroscopic intravascular bubbles, 2) animals with apnea usually demonstrated these bubbles, and 3) animals with gasping always demonstrated such bubbles, suggested that pulmonary aero-embolism was directly responsible for the signs. The tachypnea reported by Leverett

(8) in dogs with decompression sickness was observed to occur with equal frequency in both symptomatic and asymptomatic hamsters. Niden and Aviado (10) showed that injection of 5 grams of 125 μm glass beads into the right ventricle of dogs evoked immediate apnea followed by hyperpnea. The apnea was abolished by vagotomy and the hyperpnea was diminished by either thoracic sympathectomy or destruction of the carotid and aortic chemoreflex zones. Sasaki and Wagner (12) showed that capillary beds in unanesthetized rats acted as nearly perfect filters for 50 μm microspheres. The temporary nature, also, of the initial apnea helped identify the mechanism to be pulmonary in origin. (Gas embolism in the medullary respiratory center should have induced permanent apnea). The apnea that occurred in 2 of 18 animals without macroscopic bubbles (Table 1) was considered to be a result of bubbles too small to be seen. The effects of thermal, anesthetic, and positional stresses were not assessed. The observations led to the following conclusions: 1) apnea was the first sign of decompression sickness, 2) gasping was a late, critical and pathognomonic sign of decompression sickness, and 3) tachypnea was not a useful sign.

The expected sequence of events in a hamster with decompression sickness were: bubble nucleation in supersaturated tissues, embolization to the lungs, and respiratory signs upon sufficient embolization. Ultrasonic evidence of bubbles occurred at the same time or followed onset of respiratory distress in hamsters that developed problems in the first 10 minutes but preceded respiratory distress in those that developed signs in the last 20 minutes. This suggested that the principal insult was of

central origin in early-occuring syndromes and of peripheral origin in late-occuring states. Ultrasonic detection of the bubbles before signs appeared required that the transducer be located over the major source of bubbles. The transducer typically sampled a 6 per cent volume of the hamster thigh (0.2 cc of 3.5 cc). The proportion of muscle, fat, or blood vessels in that small volume was unknown. This is the most reasonable explanation for the wide range of lead and lag times in the ultrasonic diagnosis of decompression sickness and illustrated the necessity of determining whether or not there are specific anatomic regions that are particularly advantageous for detecting bubbles. The conclusions were: 1) ultrasonic through-transmission was a reliable method for detecting moderate or severe decompression sickness, 2) a more sensitive ultrasonic method was necessary for diagnosis of mild decompression sickness, and 3) susceptible sites of bubble formation needed identification (9,11).

Initially, it seemed logical that the hamsters demonstrating signs earliest after decompression would also suffer from more vigorous bubble nucleation. The lack of correlation between the onset and rate of ultrasonic attenuation (fall time) contradicted that hypothesis.

Chryssanthou demonstrated that PPCH completely protected thin mice from the decompression sickness associated with exogenous smooth muscle activating factor. He postulated that blockade of naturally occuring smooth muscle stimulants was responsible for the disorder (6).

The protection against death was not nearly as profound in hamsters (Table 2). The reason that PPCH and chloralose-urethane anesthetic together increased the mortality in hamsters with severe decompression sickness (Fig. 7) was not explained since either agent alone reduced mortality (Table 2). Sedation alone did not appear responsible either, since the PPCH group was more active after decompression than the chloralose group.

Diphenhydramine did not alter the mortality rate (Fig. 7) but the data did suggest that the respiratory signs occurred sooner after ultrasonic diagnosis in treated than in untreated animals. The small number of animals precluded proof of the altered response. Possibly, the antihistamine action allowed the splanchnic source of bubbles to empty more quickly without altering the total insult, therefore the respiratory distress would appear more quickly without altering the mortality. Another site of antihistamine action of diphenhydramine might be the smooth muscle in the lung. The adverse effect of diphenhydramine could not be explained on that basis, however.

GENERAL CONCLUSION

A workable but simplistic experimental model of decompression sickness that agrees with the observations of these experiments is a one-compartment model in which explosive decompression will induce bubble nucleation. The onset, rate and duration of degassing are all unpredictable, however.

The next series of experiments should be directed toward developing a bubble detector 10 to 100 times more sensitive than the acoustic-optical system and mechanically capable of monitoring unanesthetized animals.

Table 1. Comparison of Respiratory Signs and Autopsy Findings in Anesthetized Hamsters with Decompression Sickness.

	Macroscopic Bubbles			No Bubbles		
Group	Control	Diphen-hydramine	PPCH	Control	Diphen-hydramine	PPCH
number of animals	23(100%)	10(100%)	6(100%)	18(100%)	3(100%)	0
Signs*						
none	0(0%)	0(0%)	1(16%)	16(89%)	3(100%)	-
tachypnea	4(17%)	-	-	3(17%)	-	-
apnea, not terminal	21(92%)	9(90%)	4(67%)	2(11%)	0	-
gasping	19(83%)	8(80%)	3(50%)	0	0	-
terminal apnea only	1(4%)	1(10%)	1(16%)	-	-	-
gasping without preceding apnea	1(4%)	0	0	0	0	-

*Signs were not mutually exclusive unless otherwise specified. Dives were not of uniform depth; therefore the proportion of animals demonstrating bubbles varied from group to group.

Table 2. Mortality in 85 Unanesthetized Hamsters Subjected to Decompression Following 150 PSIG Air for 30 Minutes.

	42 with PPCH	12 with Chloralose	31 with no drug
after 15 minutes	16 dead (38%)	6 dead (50%)	24 dead (77%)
after 30 minutes	20 dead (48%)	8 dead (67%)	24 dead (77%)

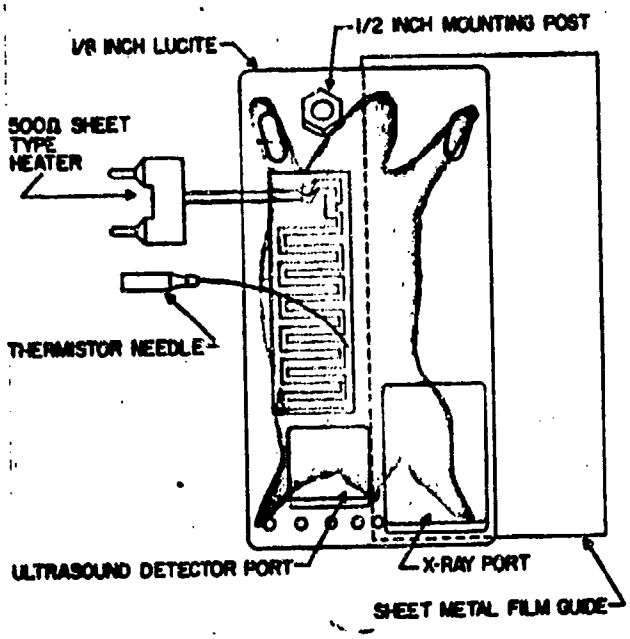

Figure 1. Heated Pallet for Hamster

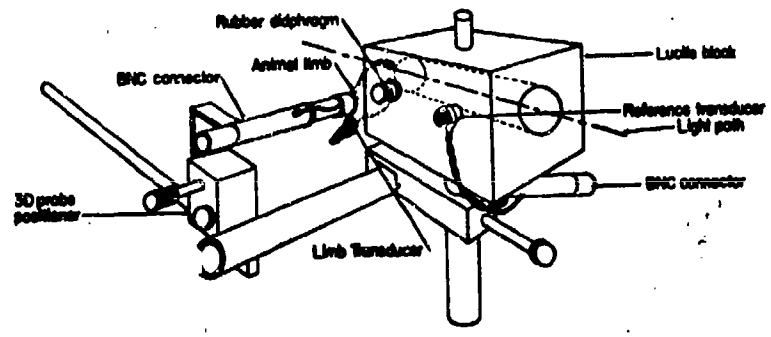

Figure 2. Acoustic-Optical Interaction Cell

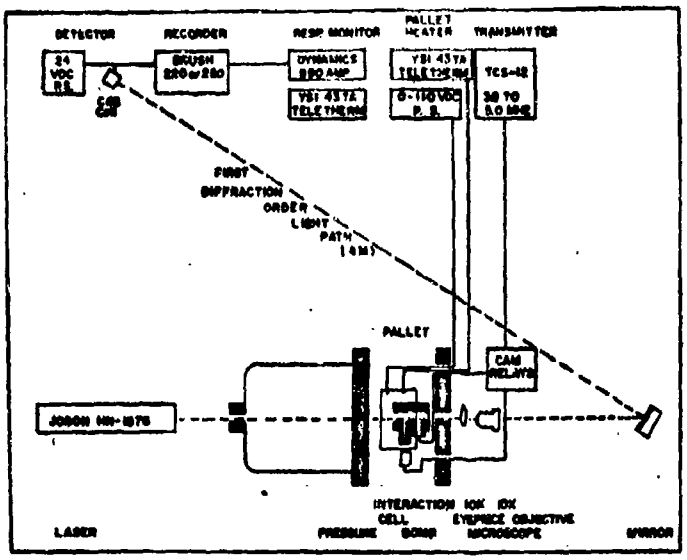

Figure 3. Acoustic-Optical Decompression Sickness Detector Equipment Arrangement

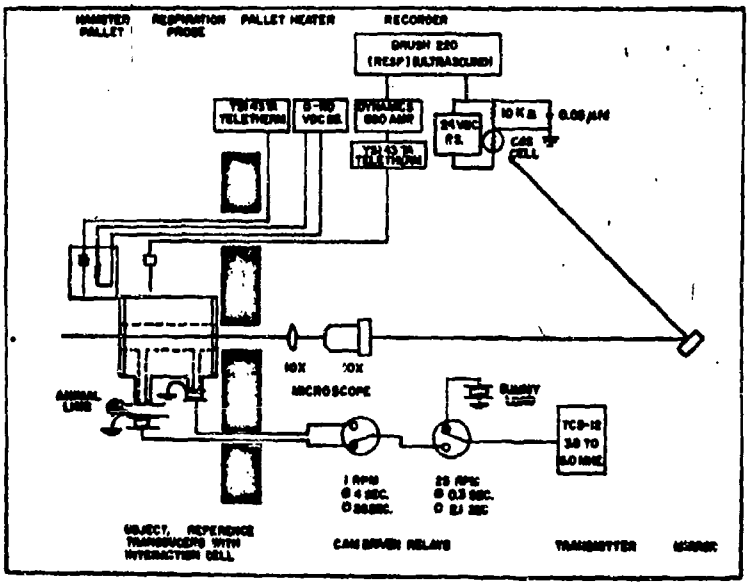

Figure 4. Acoustic-Optical Decompression Sickness Detector Electronic Block Diagram

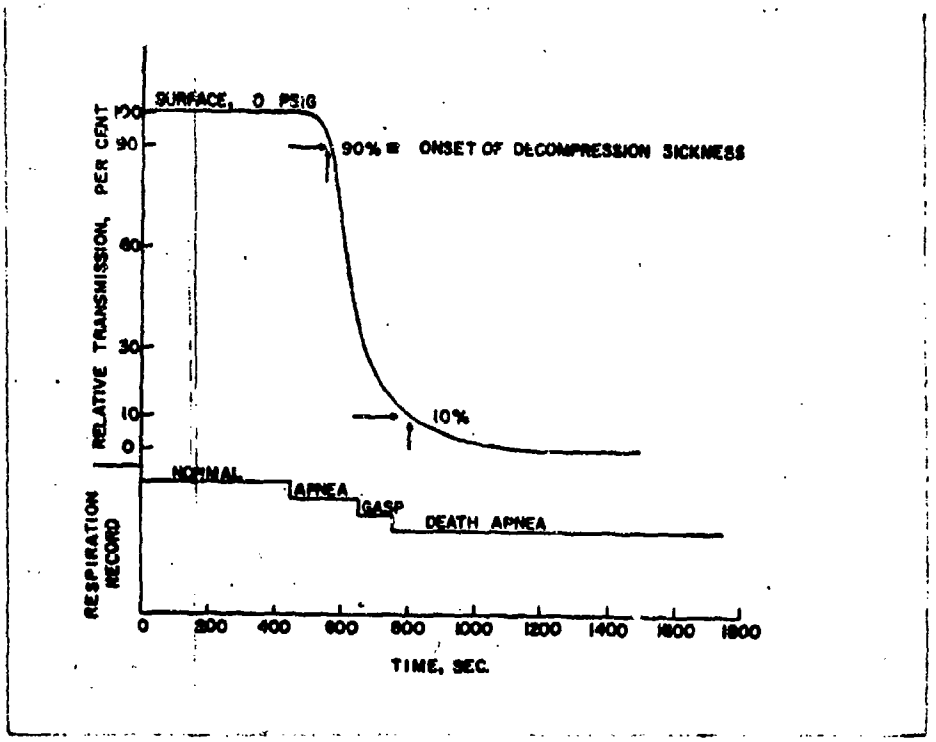

Figure 5. Record of Respiratory Signs and Ultrasonic Transmission in an Anesthetized Hamster with Decompression Sickness

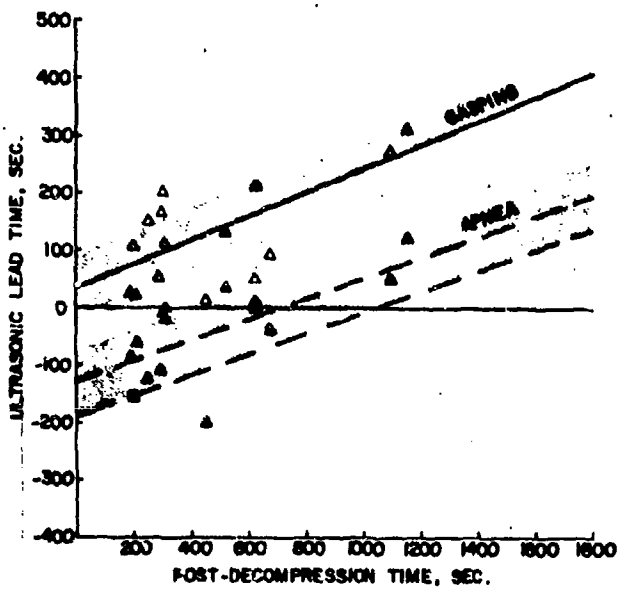

Figure 6. Length of Time by Which the Ultrasonic Detection of Bubbles Preceded the Onset of Apnea and Gasping in 16 Anesthetized Control Hamsters

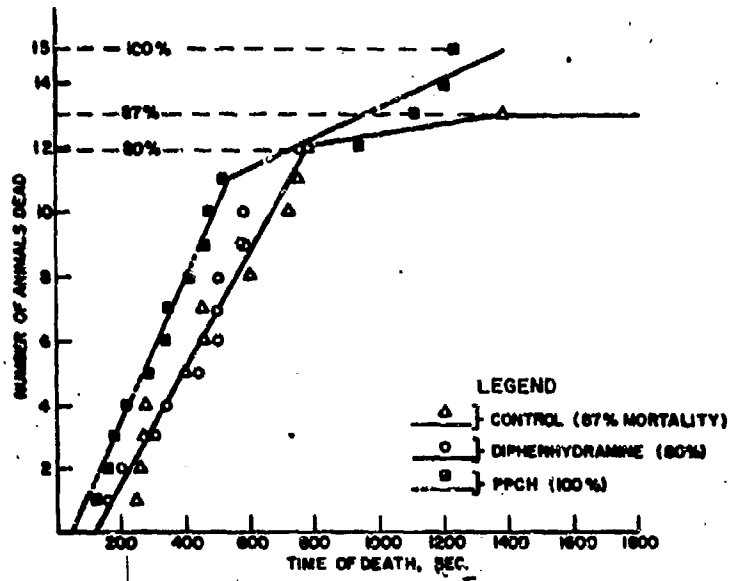

Figure 7. Onset of Gasping as a Function of Time of Ultrasonic Diagnosis in Anesthetized Hamsters with Decompression Sickness

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