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18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Hyperbaric Physiology; Respiratory Physiology; Cardiopulmonary Physiology; Neurophysiology; Decompression; Decompression Sickness; Bends; Oxygen Toxicity; Hydrostatic Pressure Effects; High Pressure Neurological Syndrome; Inert Gas Narcosis; Body Heat Loss; Psychomotor Performance;

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The Program featured state-of-the-art reviews by eminent authorities, followed by shorter research papers selected by the Symposium Governing Board from submitted mini-papers. In response to the Call for Papers, more than 100 contributions were received, of which 46 were selected for oral presentations in symposia and 37 were programmed as poster presentations. Symposia included the following topics:

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Oxygen Toxicity
Oxygen Sufficiency and Utilization within the Cell
Metabolism and Thermal Physiology
Molecular and Cellular Effects of Hydrostatic Pressure
High Pressure Nervous Syndrome
Cardio-Respiratory Responses to Exercise
Inert Gas Exchange and Decompression
Health Hazards

Attendance for the Symposium was gratifying, with a total of 298 registrants representing 25 countries. The majority (65%) were from countries other than the U.S.A.

SEVENTH SYMPOSIUM ON UNDERWATER PHYSIOLOGY

Secretariat and Meeting Management: Federation of American Societies for Experimental Biology
9650 Rockville Pike, Bethesda, Maryland 20014, U.S.A. TELEPHONE: 301 – 630-7010

(11) 10 Jul 80

July 5-10, 1980
Athens, Greece

(12) 98

PLEASE REFER REPLY TO:

(9) FINAL REPORT. 1 Aug 78 - 31 Jul 80.

(6) 7th Symposium on Underwater Physiology (U)
July 5-10, 1980
Athens, Greece.

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Cardio-Respiratory Responses to Exercise
Inert Gas Exchange and Decompression
Health Hazards

There appeared to be a broad consensus that the return to presentations of intensive current status reviews produced some unusually fine papers, and that the 7th Symposium was a professionally rewarding experience.

A copy of the Program, Abstracts and Mini Papers booklet is enclosed which will serve as the final technical report for the symposium.

(14) Arthur J. Bachrach

for

N00014-78-G-0211

(16) PR04101 (17) PR04101-2

Arthur J. Bachrach, Ph.D.
Symposium Chairman

7TH SYMPOSIUM ON UNDERWATER PHYSIOLOGY

UNDERSEA MEDICAL SOCIETY ANNUAL SCIENTIFIC MEETING
EUROPEAN UNDERSEA BIOMEDICAL SOCIETY ANNUAL MEETING

A Satellite of the XXVIII International Congress of Physiological Sciences

July 5-10, 1980
Athens Hilton
Athens, Greece

PROGRAM, ABSTRACTS, AND MINI-PAPERS

7th SYMPOSIUM ON UNDERWATER PHYSIOLOGY
GOVERNING BOARD

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Naval Medical Research Institute, Bethesda, MD

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**In Memorium*

Sponsors of the 7th Symposium:

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Address for both the Symposium Secretariat and Undersea Medical Society:
9650 Rockville Pike, Bethesda, Maryland, 20014, U.S.A.

PROGRAM, ABSTRACTS AND MINI-PAPERS

THE UNDERSEA MEDICAL SOCIETY ANNUAL SCIENTIFIC MEETING

THE /TH SYMPOSIUM ON UNDERWATER PHYSIOLOGY

THE EUROPEAN UNDERSEA BIOMEDICAL SOCIETY ANNUAL MEETING

JULY 5 - 10, 1980
The Athens Hilton Hotel
Athens, Greece

TABLE OF CONTENTS

GENERAL INFORMATION

REGISTRATION AND INFORMATION

Athenian Lobby, Athens Hilton

Hours:

Saturday, 5 July ..... 1200 - 1800
Sunday, 6 July ..... 0800 - 1700
Monday, and Tuesday, 7-8 July ..... 0830 - 1700
Wednesday, 9 July ..... 0830 - 1300
Thursday, 10 July ..... 0830 - 1700

For information of any kind, consult the Symposium Registration/Information Desk.

Notices about Symposium events will be posted on bulletin boards near the Information Desk.

SECRETARIAT

Symposium and UMS staff will be available at the Information Desk in the Athenian Lobby throughout the hours shown above.

MESSAGES

Those who wish to leave messages for registrants during the above hours should ask the hotel operator (Athens Hilton telephone number: 720-201) for the 7th Symposium Information Desk, Athenian Lobby. Messages will be posted on the bulletin board adjacent to the Information Desk.

BANQUET AND LUNCHEON TICKETS

Available at the Registration/Information Desk, Athenian Lobby.

AN EVENING IN PIRAEUS

The Symposium Banquet will be held at the National Yacht Club of Greece in Piraeus on 8 July. The Club is on a promontory overlooking the Aegean, in Turkillmann (Bay of Turks), and Athens (ten miles away) can be seen from the deck where cocktails will be served. The Acropolis, lighted in the summer, adds to the spectacular view. Dinner (Fish, Veal Jardinier, Greek Salad, and unlimited service of Achaia Clauss Rose Wine) and entertainment follow.

Tickets are 1260 Drachmas per person and must be purchased by 1500 Hours on Sunday, 6 July.

The price includes cocktails, dinner, wine, entertainment, and transportation to and from the Hilton, with a tour through the ancient harbor en route

UMS LUNCHEON

The Undersea Medical Society Annual Business Meeting, presentation of awards and the Suzanne Kronheim Memorial Lecture, will take place during a luncheon on 9 July, at the Athens Hilton Hotel.

Tickets are 500 Drachmas per person and must be purchased by 1200 Hours on Monday, 7 July.

VISITOR INFORMATION

Information on Athens attractions, museums and tours is available at the Symposium Registration/Information Desk, Athenian Lobby.

CURRENCY EXCHANGE

Exchange of foreign currencies may be made at the Ionian and Popular Bank of Greece, located off the main lobby of the Athens Hilton Hotel.

AIRLINE RESERVATIONS

Several of the major airlines have offices in the Athens Hilton. **DO NOT FORGET TO RECONFIRM YOUR RETURN FLIGHT.**

HOTEL DINING AND LOUNGE FACILITIES

The Athens Hilton facilities include the Trattoria, an Italian specialties restaurant; the Taverna Ta Nissia, a tavern following the Greek style; a Roof Top Supper Club overlooking the Acropolis; the Pan Piano Bar; and the Byzantine Coffee Shop which is open 24 hours daily. **The Coffee Shop is extremely busy and, accordingly, the service can be rather slow so allow sufficient time in your schedule if you intend to breakfast in the hotel.**

SYMPOSIUM PROCEEDINGS

The PROCEEDINGS of the 7th Symposium will be published shortly after the meeting. If you wish to be included on the mailing list to recieve order forms for the PROCEEDINGS when available, please leave your name and address at the Registration/Information Desk.

CONTINUING MEDICAL EDUCATION CREDITS

The program of the 7th Symposium, including the Undersea Medical Society and European Undersea Medical Society sessions, has been certified for one CME hour credit for each hour of scientific sessions attended. Certification forms are available at the Symposium Registration/Information Desk, Athenian Lobby.

WEEK AT A GLANCE

	Saturday, 5 July	Sunday, 6 July	Monday, 7 July	Tuesday, 8 July	Wednesday, 9 July	Thursday, 10 July
MORNING		0800-1700 *Regis. & Info.* **UMS** 0815-1000 *Sess. 1*-Decompression 0830-1200 *Sess. 2*-Posters 1030-1200 *Sess. 3*-Hydrostatic Pressure	0830-1700 *Regis. & Info.* **7TH SYMPOSIUM** 0830-1200 *Sess. 7*-Oxygen Toxicity 0900-1200 *Sess. 8*-Posters-Psychom. Perf. & HPNS *Sess. 9*-Posters-Cardio-Resp. Effects	0830-1700 *Regis. & Info.* 0915-1220 *Sess. 15*-Molec. & Cell. Effects of Hydrost. Press.	0830-1300 *Regis. & Info.* 0900-1200 *Sess. 19*-Cardio-Resp. Responses to Exercise	0830-1700 *Regis. & Info.* 0900-1200 *Sess. 20*-Inert Gas Exchange & Decompression
AFTERNOON	1200-1800 *Symposium Regis. & Info.* If you have not purchased tickets for "An Evening in Piraeus" or the UMS Lunch, do so today.	1500-1645 *Sess. 4*-Oxygen I 1500-1900 *Sess. 5*-Posters 1715-1900 *Sess. 6*-Oxygen II	1200-Duke Film 1500-1650 *Sess. 10*-Oxygen Suff. & Utiliz. Within Cell 1500-1900 *Sess. 12*-Posters-Molec. & Cell. Effect of Hydros. Pressure *Sess. 13*-Posters-Inert Gas Exchange & Decompression *Sess. 14*-Posters-Health Hazards 1720-1830 *Sess. 11*-Metab. & Thermal Phys.	1500-1900 *Sess. 16*-HPNS 1500-1900 *Sess. 17*-Posters-Metab. & Thermal Phys. *Sess. 18*-Posters-Oxygen Toxicity	1215-1500 *UMS Annual Bus. Meeting & Lunch* *Afternoon & evening free for individual plans.*	**EUBS** 1500-1830 *Sess. 21*-Health Hazards 1830-1930 *EUBS Annual General Meeting*
EVENING		2030-*7th Symp. Opening Reception*		1930-*An Evening in Piraeus*		

PROGRAM

SATURDAY, 5 JULY

REGISTRATION AND INFORMATION - Athenian Foyer
1200 to 1800 Hours

SUNDAY, 6 JULY

REGISTRATION AND INFORMATION - Athenian Foyer
0800 to 1700 Hours

UNDERSEA MEDICAL SOCIETY ANNUAL SCIENTIFIC MEETING

WELCOME AND OPENING REMARKS

0815 - Terpsichore Ballroom

JEFFERSON C. DAVIS, President, Undersea Medical Society

SESSION 1

DECOMPRESSION — Terpsichore Ballroom
Co-Chairmen: **H. V. HEMPLEMAN** and **B. G. D'AOUST**

0830 Evaluation of different decompression schedules by agarose gel bubble. **Y. MANO, M. SHIBAYAMA** and **H. MAEDA**

0845 The development and testing of high altitude diving tables using extrapolated U.S. Navy critical tissue pressure criteria. **R. L. BELL, A. C. THOMPSON** and **R. E. BORGWARDT**

0900 Non-Haldanian decompression schedules. **T. D. KUNKLE, E. L. BECKMAN** and **D. E. YOUNT**

0915 The perfusion/diffusion dilemma: resolution and clarification by isobaric gas switching. **B. G. D'AOUST, C. YOUNG, R. WHITE,** and **R. DUNFORD**

0930 Pitfalls in the diagnosis of dysbaric osteonecrosis and the significance of suspected lesions. **J. K. DAVIDSON, W. P. TROWBRIDGE** and **D. N. WALDER**

0945 Scuba diving in pregnancy. **J. H. G. RANKIN, E. N. LANPHIER, M. K. STOCK** and **D. F. ANDERSON**

SESSION 2

POSTER PRESENTATIONS — Nectar/Ambrosia Room

0830-1200 (Coffee with the authors 1000-1030.)

Board #

1 Treatment of cardiovascular dysfunction resulting from cerebral air embolism. **D. E. EVANS, A. I. KOBRINE, E. T. FLYNN** and **M. E. BRADLEY**

2 Neurophysiological and biochemical studies in He-N_2-O_2 atmosphere at 11 ATA. **I. STOILOVA, V. KOLEV, I. DOSSEVA, L. VENKOV, Ts. TENCHEVA, A. DISHELOV** and **A. VARBANOVA**

3 Visceral malformations, resorptions, and birthweight among fetal rats exposed to air at increased atmospheric pressure. **M. E. BOLTON** and **A. L. ALAMO**

4 Brainstem evoked potential changes associated with variations in middle-ear pressure. **B. M. CLOPTON** and **J. M. MILLER**

5 Analysis of medical reasons for withdrawing medical certification of fitness in commercial divers in the U.K. **W. A. CROSBIE**

6 Modeling, measurements, and moments of inert gas exchange. **P. K. WEATHERSBY** and **L. D. HOMER**

7 The effects of cold stress on venous gas bubble production in man following a no-decompression dive. **R. DUNFORD** and **J. HAYWARD**

8 Size distribution of intravascular bubbles induced by decompression. **B. D. BUTLER, B. A. HILLS** and **T. E. SUTTON**

9 Thermal effects of recompressed bubbles. **R. G. BUCKLES, M. E. COX** and **J. B. ECKENHOFF**

10 Results of validation testing of flying-after-diving schedules. **B. E. BASSETT**

11 An analysis of the effects that hyperbaric oxygen has upon pressure reduction tolerances in rats and humans. **D. E. YOUNT** and **D. A. LALLY**

12 Physicochemical properties of the nonionic surfactants surrounding gas cavitation nuclei (microbubbles). **J. S. D'ARRIGO**

SESSION 3

HYDROSTATIC PRESSURE — Terpsichore Ballroom
Co-Chairmen: **J. C. ROSTAIN** and **P. B. BENNETT**

1030 Acute injection of phenytoin and long latency evoked potentials in guinea pigs under high pressure helium. **P. G. KAUFMANN, J. C. FARMER, JR.** and **F. G. HEMPEL**

1045 Evaluated microvibration on cat under the compression effect to 51 ATA (He-N_2-O_2). **K. SEKI, H. NAKAYAMA** and **M. MATSUDA**

1100 H.P.N.S. in human during 38 hours compression to 450m with N_2 injections. **J. C. ROSTAIN, B. GARDETTE, M. C. GARDETTE-CHAUFFOUR** and **R. NAQUET**

1115 Diazepam under hyperbaric conditions in rats. **L. GRAN, R. COGGIN** and **P. B. BENNETT**

1130 Changes in red cell membrane enzymes in man during simulated dives of up to 55 bar in helium-oxygen. **J. A. PACIOREK** and **R. F. CARLYLE**

1145 The effect of hydrostatic pressure on enzymes involved in the oxygen metabolism. **E. MORILD** and **J. E. ØLMHEIM**

SESSION 4

OXYGEN I — Terpsichore Ballroom
Co-Chairmen: **Y. G. ZORBAS** and **M. D. FAIMAN**

1500 The effect of hyperbaric oxygen inhalation upon the ultrastructure of the lung alveoli. **T. K. AKERS**

1515 Alterations in oxidative metabolism during recovery from pulmonary oxygen toxicity. **W. D. CURRIE, P. C. PRATT** and **A. P. SANDERS**

1530 On the influence of exogenous and endogenous substrate accumulation on drug induces variations in glutamic acid decarboxylase activity prior to oxygen high pressure exposure. **B. E. SEGERBO**

1545 Oxygen convulsions in mice. Influence of nitrogen admixture. **N. BARTELSON, O. CRIBORN** and **A. MUREN**

1600 Hop-induced cerebral vasoconstriction, its contribution to CNS-toxicity kinetics. **B. BLEIBERG, A. LANIR** and **D. KEREM**

1615 Tolerance of mice to pulmonary oxygen toxicity. **A. LANIR, D. KEREM** and **D. GERSHON**

1630 CNS and pulmonary oxygen toxicity during intermittent exposure to hyperbaric oxygen and air. **D. KEREM, C. BITTERMAN** and **B. BLEIBERG**

SESSION 5

POSTER PRESENTATIONS — Nectar/Ambrosia Room

1500-1900 (Coffee with the authors 1645-1715)

Board #

1 Stress and mental performance under water. **P. G. A. M. JORNA**

2 Noninvasive continuous monitoring of diver pulmonary performance. **M. J. ACKERMAN**

3 Hydrostatic pressure: Its effects on cellular membrane ion transport. **W. R. GALEY, P. S. VAN NICE** and **C. V. BEATO**

4 The effects of prone immersion on lung function. **I. DASKALOVIC, A. HASHIMOTO, E. H. LANPHIER** and **W. G. REDDAN**

5 Thoracic shape, lung volume and diaphragmatic contraction during immersion. **V-D. MINH** and **G. F. DOLAN**

6 Blood metabolites in resting and exercising rats at various partial pressures of nitrogen and oxygen. **R. de G. HANSON, R. M. GRAY, P. SMYTHE** and **K. G. M. M. ALBERTI**

8 Emergency thermal protection for saturation diving. **G. H. EGSTROM** and **A. DICHARO**

9 Heat stress during dives in warm water. **I. HOLMER** and **G. KIHLSTROM**

10 Effect of body temperature and composition on recovery from hypothermia. **J. B. MORRISON, J. S. HAYWARD** and **M. L. CONN**

11 An electromyographic study of shiver in immersed human subjects. **P. A. IAIZZO, R. W. PETRY** and **R. S. POZOS**

12 An analysis of emergency heating requirements for personnel transfer capsules. **E. H. WISSLER**

SESSION 6

OXYGEN II — Terpsichore Ballroom
Co-Chairmen: **E. KINDWALL** and **D. ELLIOTT**

1715 Induction of cytochrome P-450 by hypoxia and hyperoxia in vivo and in vitro. **H. A. ROWE, S. F. GOTTLIEB** and **I. S. LONGMUIR**

1730 Hydrogen oxygen exposure of rabbits at 30 ATA with multiday survival. **H. E. ÖRNHAGEN, C. E. G. LUNDGREN** and **A. MUREN**

1745 Effect of normobaric and hyperbaric oxygen on cyanide intoxication. **T. TAKANO, Y. MIYAZAKI, I. NASHIMOTO** and **K. KOBAYASHI**

1800 Hyperbaric oxygenation: Tissue oxygen characteristics in chronic, soft tissue wounds. **P. J. SHEFFIELD**

1815 Adrenergic and cardiopulmonary responses to exercise with air and helium-oxygen at 1 ATA. **E. T. FLYNN, D. E. EVANS, K. M. GREENE, D. C. LeGRYS** and **R. P. LAYTON**

1830 Differential performance behavior after a 40-hour compression to 450 MSW. **C. LEMAIRE**

1845 Influence of exercise on ventilatory capacity at depth. **A. PASCHE** and **C. LUNDGREN**

7TH SYMPOSIUM OPENING RECEPTION

2030 Hours - Pool Area

HOSTED BY THE GREEK GOVERNMENT

MONDAY, 7 JULY

REGISTRATION AND INFORMATION - Athenian Foyer

0830 to 1700 Hours

7TH SYMPOSIUM ON UNDERWATER PHYSIOLOGY

WELCOMING REMARKS

0830 Hours - Terpsichore Ballroom

A. J. BACHRACH, *Symposium Chairman*

C. J. LAMBERTSEN, *University of Pennsylvania Medical Center*

S. G. ALIVISATOS, *University of Athens*

S. MARKETOS, *Secretary General, Ministry of Social Services*

SESSION 7

OXYGEN TOXICITY — Terpsichore Ballroom
Chairman: **H. SALTZMAN**; *Co-Chairman:* **M. W. RADOMSKI**
Rapporteur: **A. B. FISHER**

0900 Review: Current concepts of oxygen toxicity. **J. CLARK**

0930 Mechanism(s) of central oxygen toxicity: A re-evaluation. **M. D. FAIMAN, R. J. NOLAN, D. E. DODD, J. M. WAECHTER, R. C. DIRKS, K. HAYA** and **J. A. ZEMPEL**

0950 The central role of ammonia in OHP induced convulsions. **E. W. BANISTER** and **A. K. SINGH**

1010 **Coffee and Poster Presentations**

1040 Changes in cell volume following hyperbaric exposure: A manifestation of oxygen toxicity. **J. POOLEY** and **D. N. WALDER**

1100 Lung ATP turnover during oxidant stress. **A. B. FISHER**

1120 Protection from pulmonary oxygen toxicity by treatment with low doses of bacterial endotoxin. **L. FRANK, M-J. CHIANG** and **D. MASSARO**

1140 Evolution of pulmonary diffusing capacity after deep saturation dive with high O_2 level during decompression. **R. H. HYACINTHE** and **B. BROUSSOLLE**

SPECIAL FILM
1200 Hours - Terpsichore Ballroom
The Duke 650 Meter Dive. **P. B. BENNETT**

POSTER PRESENTATIONS — Nectar/Ambrosia Room
0900-1200

SESSION 8

PSYCHOMOTOR PERFORMANCE AND HIGH PRESSURE NERVOUS SYNDROME

Board #

2 A theory of inert gas narcosis. **B. FOWLER**

3 Assessment of the high pressure neurological syndrome (HPNS): A new method of measuring tremor in an animal model. **J. A. BAKER, M. J. HALSEY, B. WARDLEY-SMITH** and **R. T. WLOCH**

4 Genetics of variability in susceptibility to HPNS Type I seizures in mice. **R. D. McCALL** and **D. FRIERSON, JR.**

5 Criteria analysis of selection for deep diving (EEG and performance). **J. C. ROSTAIN, C. LEMAIRE, M. C. GARDETTE-CHAUFFOUR, S. DOUCET** and **R. NAQUET**

6 Modification of electrophysiological sleep under the hyperbaric environment (31ATA, He-N_2-O_2, 34 days, 3 divers). **K. SEKI, H. NAKAYAMA** and **M. MATSUDA**

SESSION 9

CARDIO-RESPIRATORY EFFECTS

Board #

7 Inertance as a factor in uneven ventilation in diving. **J. R. CLARKE, M. A. FISHER** and **M. J. JAEGER**

8 The arrhythmogenic potency of hydrostatic pressure on cardiac conduction. **T. J. DOUBT** and **P. M. HOGAN**

9 The effect of alcohol on the cardiovascular adjustments of the dive reflex in man. **L. E. WITTMERS, JR., L. FAIRBANKS, S. BURGSTAHLER** and **R. S. POZOS**

10 Pulmonary function in divers. **M. CIMSIT** and **V. FLOOK**

11 Regulation and frequency of heart rate during open-sea saturation diving. **S. M. GOSOVIĆ** and **A. I. RADOVIĆ**

12 Influence of the inspiratory effort and swallowing on the cardiovascular response to simulated diving and breath-holding. **T. F. HUANG** and **C. T. PENG**

13 Ventilation, pattern of breathing and activity of respiratory muscles in awake cats during oxygen-helium simulated dives. **G. IMBERT, Y. JAMMES, N. NARAKI, J. C. DUFLOT, M. HUGON** and **C. GRIMAUD**

14 Physiological responses to immersion at 31 ATA (Seadragon IV). **M. MATSUDA, S. K. HONG, H. NAKAYAMA, H. ARITA, Y. C. LIN, J. CLAYBAUGH, C. LUNDGREN** and **R. M. SMITH**

15 The effect of water temperature on vital capacity during head-out immersion. **D. I. KURSS, C. E. G. LUNDGREN** and **A. J. PASCHE**

SESSION 10

OXYGEN SUFFICIENCY AND UTILIZATION WITHIN THE CELL — Terpsichore Ballroom
Chairman: **A. KOVACH**; *Co-Chairman:* **J. C. DAVIS**
Rapporteur: **L. A. KIESOW**

1500 Review: Current concepts of oxygen sufficiency and utilization within the cell. **F. F. JOBSIS**

1530 Use of aortic body and carotid body chemoreceptors as internal probes to monitor tissue oxygenation. **S. LAHIRI**

1550 Heterogeneity of capillary distribution and capillary circulation in mammalian skeletal muscles. **E. M. RENKIN, S. D. GRAY, L. R. DODD** and **B. D. LIA**

1610 Retinal oximetry with hypercapnia and hyperbaric oxygen. **F. G. HEMPEL, S. R. BURNS** and **H. A. SALTZMAN**

1630 A mechanism for the beneficial effect of hyperbaric oxygen on staphylococcal osteomyelitis. **J. T. MADER** and **G. L. BROWN**

1650 **Coffee and Poster Presentations**

SESSION 11

METABOLISM AND THERMAL PHYSIOLOGY — Terpsichore Ballroom
Chairman: **K. BONDI**; *Co-Chairman:* **M. MATSUDA**
Rapporteur: **G. EGSTROM**

1720 Review: Current concepts of metabolism and thermal physiology. **P. WEBB**

1750 An analysis of heat stress under hyperbaric conditions. **K. R. BONDI**

1810 Contribution of metabolic and respiratory heat to core temperature gain after cold water immersion. **M. L. CONN, P. A. HAYES** and **J. B. MORRISON**

1830 The metabolic and thermal status of divers during simulated dives to 55 bar. **M. P. GARRARD, P. A. HAYES, R. F. CARLYLE** and **M. J. STOCK**

SESSION 12

MOLECULAR AND CELLULAR EFFECTS OF HYDROSTATIC PRESSURE

Board #

1 A study of the specific action of "per se" hydrostatic pressure on fish considered as a physiological model. **L. BARTHELEMY, A. BELAUD** and **A. SALIOU**

2 Osmotic fragility of erythrocytes: Effects of hydrostatic pressure and pentanol. **A. C. HALL** and **A. G. MACDONALD**

3 A mathematical analysis of high pressure and anaesthetic effects. **M. J. HALSEY, A. F. MOTT, C. C. SPICER** and **B. WARDLEY-SMITH**

4 Contrasting actions of hydrostatic pressure and helium pressure on growth of *saccharomyces cerevisiae*. **S. R. THOM** and **R. E. MARQUIS**

5 Effects of different normoxic hyperbaric exposures on glucose, lactate and glycogene brain concentrations. **T. OBRENOVITCH** and **F. BRUE**

6 Toxic effects of oxygen on the functions of pulmonary cytochrome P-450. **G. H. GURTNER, A. SYBERT, A. KNOBLAUCH, N. BRENNEN, M. PEAKE and J. T. SYLVESTER**

SESSION 13

INERT GAS EXCHANGE AND DECOMPRESSION

Board #

7 Study on definition of maximum permissible gas flow in lungs during decompression. **J. PARC** and **J. LE CHUITON**

8 Evaluation of decompression tables by a model describing bubble dynamics in tissue. **S. MEISEL, Y. TALMON** and **D. KEREM**

9 Computer simulation of diffusive gas mixing in the lung at 10 ATA. **H. D. VAN LIEW**

10 Some recent experiments on bubble formation in supersaturated gelatin. **D. E. YOUNT, C. M. YEUNG** and **T. D. KUNKLE**

SESSION 14

HEALTH HAZARDS

Board #

11 Microbiological studies on acute otitis externa in saturation divers. **S. R. ALCOCK**

12 An epidemiological study of fatal diving accidents in two commercial diving populations. **M. E. BRADLEY**

13 Drug therapy of decompression sickness. **B. BROUSSOLLE**

14 Decompression sickness in commercial diving population. **M. R. CROSS** and **L. A. BOOTH**

15 An evaluation of cardiopulmonary resuscitation techniques for use in a diving bell. **R. MYERS** and **M. E. BRADLEY**

SESSION 15

MOLECULAR AND CELLULAR EFFECTS OF HYDROSTATIC PRESSURE — Terpsichore Ballroom

Chairman: **L. BARTHELEMY**; *Co-Chairman:* **M. J. HALSEY**
Rapporteur: **A. G. MACDONALD**

0915 Review: Current concepts of molecular and cellular effects of hydrostatic pressure. **A. G. MACDONALD**

0945 Effects of hyperbaric conditions on the multiplication of Echo 11 Herpes Simplex Virus (Type 1 and Type 2) in tissue culture. **C. CHASTEL, L. BARTHELEMY, A. BELAUD** and **A. MICHAUD**

1005 Effect of hydrostatic pressure on active transport, metabolism and the Donnan equilibrium in human erythrocytes. **J. M. GOLDINGER, B. S. KANG, R. A. MORIN, C. V. PAGANELLI** and **S. K. HONG**

1030 **Coffee**

1100 Effects of high hydrostatic pressures on Na^+ transports across isolated gill epithelium of sea water acclimated eels *Anguilla anguilla*. **A. J. R. PEQUEUX**

1120 A quantitative description of pressure-induced alterations in ionic channels of the squid giant axon. **B. B. SHRIVASTAV, J. L. PARMENTIER** and **P. B. BENNETT**

1140 Transient versus steady state effects of high hydrostatic pressure. **K. T. WANN, A. G. MACDONALD, A. A. HARPER** and **M. L. J. ASHFORD**

1200 The effects of high pressures of inert gases on cholinergic receptor binding and function. **J. F. SAUTER, L. BRASWELL, P. WANKOWICZ** and **K. W. MILLER**

SESSION 16

HIGH PRESSURE NERVOUS SYNDROME

Chairman: **R. NAQUET**; *Co-Chairman:* **J. VOROSMARTI**
Rapporteur: **D. MILLAR**

1500 Review: Current concepts of high pressure nervous syndrome. **J. HALLENBECK**

1530 The effects of general anaesthetics on post-synaptic responses. **H. J. LITTLE** and **W. D. M. PATON**

1550 Pharmacological investigation of the high pressure neurological syndrome: Brain monoamine concentrations. **S. DANIELS, A. R. GREEN, D. D. KOBLIN, R. G. LISTER, H. J. LITTLE, W. D. M. PATON** and **E. B. SMITH**

1610 Prevention of HPNS: The possible use of structuralisomers of anaesthetics. **B. WARDLEY-SMITH** and **M. J. HALSEY**

1630 Rapid compression with trimix (He-N_2-O_2). **P. B. BENNETT, R. COGGIN, J. ROBY** and **J. N. MILLER**

1650 **Coffee and Poster Presentations**

1720 The effect of high pressure on cooperative lipid-protein interactions. **H.-J. GALLA** and **J. R. TRUDELL**

1740 Currents in a voltage-clamped vertebrate neuron at hyperbaric pressure. **J. J. KENDIG**

1800 Differential effects of pressure on the mammalian central nervous system. **P. G. KAUFMANN, P. B. BENNETT** and **J. C. FARMER, JR.**

1820 Somatic evoked potentials in monkey during saturation dives ($He-O_2$ and $He-N_2-O_2$). **M. HUGON, K. SEKI, L. FAGNI** and **J. C. ROSTAIN**

1840 Differentiation of the two components of the convulsion stage of the HPNS in vertebrates. **R. W. BRAUER, R. W. BEAVER, H. W. GILLEN, W. M. MANSFIELD, JR.** and **R. D. McCALL**

POSTER PRESENTATIONS — Nectar/Ambrosia Room

1500-1900

SESSION 17

METABOLISM AND THERMAL PHYSIOLOGY

Board #

7 Energy and body fluid balance during a 14-day dry saturation dive at 31 ATA (Seadragon IV). **H. NAKAYAMA, S. K. HONG, J. CLAYBAUGH, N. MATSUI, Y. S. PARK, Y. OHTA, K. SHIRAKI** and **M. MATSUDA**

8 A computer model designed to make rapid predictions of diver temperature changes. **S. WILCOCK** and **V. FLOOK**

SESSION 18

OXYGEN TOXICITY

Board #

9 Comparative effects of various protective agents upon acute cerebral hyperbaric oxygen toxicity in mice: Particular interest of some benzodiazepines. **F. BRUE, P. JOANNY, A. CHAUMONT, J. CORRIOL** and **B. BROUSSOLLE**

10 Effect of excessive oxygen upon the capability of the lungs to filter gas emboli. **B. D. BUTLER** and **B. A. HILLS**

12 SEM observations of oxygen toxicity in guinea pigs exposed to continuous 100%, 85%, or 75% oxygen at 1 ATM. **A. E. McKEE** and **M. E. BRADLEY**

13 The influence of inert gas concentration on pulmonary oxygen toxicity. **M. R. POWELL** and **H. D. FUST**

14 Brain GABA and cGMP as indices of metabolic lesions in CNS during acute oxygen toxicity. **M. W. RADOMSKI** and **W. J. WATSON**

15 Pulmonary prostaglandin metabolism during normobaric hyperoxia. **C. L. SCHATTE** and **M. M. MATHIAS**

AN EVENING IN PIRAEUS

1930 Hours

Buses pick up registrants at the Athens Hilton, arriving at the National Yacht Club in Piraeus at 2000 for cocktails, dinner and entertainment. **See General Information section for ticket information.**

Buses depart National Yacht Club at 2300 Hours for return to the Hilton.

SESSION 19

CARDIO-RESPIRATORY RESPONSES TO EXERCISE — Terpsichore Ballroom

Chairman: **C. E. LUNDGREN**; *Co-Chairman:* **B. BROUSSOLLE**
Rapporteur: **A. A. BOVE**

0900 Review: Current concepts of cardio-respiratory responses to exercise. **L. FAGRAEUS**

0930 Exercise metabolism in humans on acute exposure to a 5.8 bar normoxic oxyhelium environment. **R. de G. HANSON, R. M. GRAY, M. M. WINSBOROUGH, R. S. McKENZIE AND K. G. M. M. ALBERTI**

0950 Comparison of metabolic responses and growth hormone release during submaximal exercise in man breathing heliox or air at normal barometric pressure. **J. RAYNAUD, P. VARENE** and **J. DURAND**

1010 **Break**

1040 Effects of exercise and hyperbaric air on ventilation and central inspiratory activity. **C. M. HESSER** and **F. LIND**

1100 Inspiratory dyspnea during exercise at 47 ATA. **J. SALZANO, E. M. CAMPORESI, B. STOLP, H. SALTZMAN, W. BELL** and **D. SHELTON**

1120 Carbon dioxide retention with underwater work in the open ocean. **J. DWYER, J. W. MACDONALD, B. W. STOLP** and **A. A. PILMANIS**

1140 Cardiopulmonary functions and maximal aerobic power during a 14-day saturation dive at 31 ATA (Seadragon IV). **Y. OHTA, H. ARITA, H. NAKAYAMA, S. TAMAYA, C. LUNDGREN, Y. C. LIN, R. M. SMITH, R. MORIN, L. E. FARHI** and **M. MATSUDA**

UNDERSEA MEDICAL SOCIETY ANNUAL BUSINESS MEETING AND AWARDS LUNCHEON

1215 to 1500 Hours - Hesperides Room

The Suzanne Kronheim Memorial Lecture, presentation of awards, and business meeting. **See General Information section for ticket information.**

SUZANNE KRONHEIM MEMORIAL LECTURE
Mental activity related to the blood flow and metabolism of the brain. **D. H. INGVAR,** University Hospital, Lund, Sweden

PRESENTATION OF AWARDS
The Albert R. Behnke Award, The Stover-Link Award, and The Oceaneering International Award

REMARKS BY THE INCOMING PRESIDENT, PAUL WEBB

FOLLOWING THE LUNCHEON, AFTERNOON AND EVENING FREE FOR INDIVIDUAL PLANS.

SESSION 20

INERT GAS EXCHANGE AND DECOMPRESSION — Terpsichore Ballroom
Chairman: **H. V. HEMPLEMAN**; *Co-Chairman and Rapporteur:* **K. D. REIMANN**

0900 Review: Current concepts of inert gas exchange and decompression. **P. WEATHERSBY**

0930 Species independent maximum no-bubble decompression from saturation dive. **Y. C. LIN**

0950 Determination of safe tissue tension values during the surface interval in surface decompression schedules for helium-oxygen dives. **P. O. EDEL**

1010 **Break**

1040 Assessment of decompression profiles and divers by doppler ultrasonic monitoring. **R. Y. NISHI, K. E. KISMAN, B. C. EATOCK** and **G. MASUREL**

1100 Monitoring bubble formation with an integrating pulse-echo ultrasonic method. **S. DANIELS, J. M. DAVIES, W. D. M. PATON** and **E. B. SMITH**

1120 Migration of lung surfactant to pulmonary air emboli. **B. A. HILLS** and **B. D. BUTLER**

1140 Prevention of decompression sickness by combined cyproheptadine-amphetamine treatment. **C. CHRYSSANTHOU, L. RODRIGUEZ** and **P. BRANDEN**

EUROPEAN UNDERSEA BIOMEDICAL SOCIETY

SESSION 21

HEALTH HAZARDS — Terpsichore Ballroom
Chairman: **A. A. BOVE**; *Co-Chairman:* **C. CHRYSSANTHOU**
Rapporteur: **D. H. ELLIOTT**

1500 Review: Current concepts of aural barotrauma. **J. C. FARMER, JR.**

1530 Mechanisms of aural barotrauma. **J. MILLER, A. AXELSSON, D. McPHERSON** and **W. POTTER**

1550 Water-borne microbial pathogens and diving environments. **O. P. DAILY, S. W. JOSEPH, J. D. GILLMORE, R. J. SEIDLER, D. A. ALLEN** and **R. R. COLWELL**

1610 Management of health hazards associated with the salvage of toxic chemicals using a saturation diving technique. **A. MARRONI, J. GETHING** and **D. ZANNINI**

1630 **Break**

1700 Review: Current concepts in bone necrosis research. **D. N. WALDER**

1730 Abnormal bone and cartilage collagen metabolism in experimentally induced dysbaric osteonecrosis. **D. B. PARSONS, M. E. BRADLEY**

1750 A detailed histological and radiological controlled study of selected bones from divers. **C. R. WEATHERLY, W. M. PARK, M. HADDAWAY** and **I. CALDER**

1810 The efficacy of spinal anesthesia at high pressure. **H. F. NICODEMUS, H. McELROY** and **R. J EVY**

EUROPEAN UNDERSEA BIOMEDICAL SOCIETY
ANNUAL GENERAL MEETING

1830 to 1930 Hours - Terpsichore Ballroom

D. H. ELLIOTT, President, EUBS

-END-

ABSTRACTS

UNDERSEA MEDICAL SOCIETY

EVALUATION OF DIFFERENT DECOMPRESSION SCHEDULES BY AGAROSE GEL BUBBLE. Y. Hano, M. Shibayama* and H. Maeda*. Dept. of Public Health, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo, 113, Japan.

Decompression schedules after dive are actually different in countries like as United States, England, France and Japan, and it is too difficult to appraise them because of the difficulty to know the relation between the schedule and the bends incidence.

As one of the methods, bubble formation work by an agarose gel has been researched in a dry chamber controlled the temperature to evaluate different decompression schedules like as U.S.N. Manual table and Japanese Standard table.

Agarose gel bubbles are only physically formed by pressure changes and it is obvious that decompression sickness is due to the bubble formation plus our physiological body reaction after bubble formation, our physical conditions and so forth.

But it should be remarkable that the bubble formation must be most important as a first sympt_m occurrence factor. And a bubble is formed according to the physical decompression ratio. So, it can be estimated which kind of decompression schedule is better to keep the lower number of bubbles and the lowest bubble number after diving would be introduced the safer decompression schedule.

The decompression schedules for this research were nine tables for both divers and compressed air workers. The total decompression time is quite different in each schedule even though the depth and the bottom time are same. Agarose gel devided to from 12 to 16 cells was pressurized to the predetermined pressure and time, decompressed according to the each schedule, counted the bubble number in each 0.27 ml of the cells and those schedules were evaluated by the bubble number.

THE DEVELOPMENT AND TESTING OF HIGH ALTITUDE DIVING TABLES USING EXTRAPOLATED U. S. NAVY CRITICAL TISSUE PRESSURE CRITERIA. R. J. Bell, A. C. Thompson* and R. E. Borgwardt*. Departments of Chemical Engineering and Physiological Sciences, University of California, Davis, California, 95616.

The critical tissue pressure curves obtained by the U. S. Navy were extrapolated to obtain predicted critical tissue pressures for altitude exposures. Using these extrapolations, no-decompression limits were predicted. A new set of repetitive groups which extend the U. S. Navy groups to reduced subsmpheric pressures were defined and repetitive diving tables were calculated. In addition, the RNI resulting from equilibration at low altitude followed by slow ascent to altitude was accounted for in "arrival tables." The no-decompression schedules calculated in this study either were not listed or were calculated as decompression schedules in the Galfetti, Bont (Buhlman) or Cross Tables. These tables were tested using 15 subjects and a total of 168 chamber and water exposures at Lake Tahoe, California (elevation 6,200 feet). Circulating platelet levels, four plasma clotting factors and three clotting times were monitored for evidence of disseminated intravascular coagulation (DIC) and pre-cordial doppler bubble detectors were used to listen for bubbles. There was no clear objective or subjective evidence that any subject encountered decompression sickness using the proposed tables.

NON-HALDANIAN DECOMPRESSION SCHEDULES. T.R. Kunkle*, K.L. Beckman and D.R. Yount. Department of Physics and Astronomy and Department of Physiology, University of Hawaii, Honolulu, Hawaii 96822.

The recent development of an explicit physical model for bubble nucleation in supersaturated fluids has permitted the computation of decompression schedules based entirely on established physical principles. The procedure differs from most current schemes in that it employed a pressure-difference principle instead of a pressure-reduction ratio and attempts to predict bubble-free and not just asymptomatic profiles. In computing these schedules the established practice of characterizing the body by a number of tissue time constants is retained, but the conventional M-value calculation of the pressure reduction limits is replaced by a computational algorithm which traces the evolution of cavitation nuclei through the pressure history. The resulting computer program can handle five component breathing gas mixtures along with gas interchanges, and explicitly treats the metabolism of oxygen, including the effects of the "oxygen window" and the possibility of oxygen bends. In all of the new tables the first stop is much deeper than that stipulated in the corresponding US Navy or RNPL schedules. The total decompression time is, however, similar to that of the US Navy tables. Representative schedules are shown and compared with existing tables and with field experience such as Nippon Salvage Company's field experience in salvaging the Liner Cariblia. The new schedules are in this manner shown to be reasonable. Because they are believed to be virtually bubble-free decompressions, the use of such schedules should not result in chronic conditions such as aseptic bone necrosis.

THE PERFUSION/DIFFUSION DILEMMA: RESOLUTION AND CLARIFICATION BY ISOBARIC GAS SWITCHING. A. A. D'Aoust, C. Young*, R. White*, R. Dunford. Virginia Mason Research Center, Seattle, Washington, U.S.A.

The interdependent problem of inert gas exchange on the one hand and bubble formation and growth on the other have confounded experimental approaches to their clarification, particularly when decompression is used as the supersaturating technique. We have previously demonstrated the asymmetry of gas elimination (J. Appl. Physiol., 41:MM, 1976) following decompression as compared with saturation, indicating that decompression is a cardiovascular stress. For those reasons, we have begun isobaric studies (Science, 197:889, 1977) at pressures where the supersaturation induced by the unequal equilibration rate of inert gases having unequal diffusion rates and/or tissue-blood partition ratios allows estimation of both supersaturation and the molar time constants of the body without the confounding effects of decompression. The diffusion coefficients and tissue to-blood (fat/water) partition coefficients of inert gases predict unique supersaturation or subsaturation pressures in animals and man according to the order of switching and thus such experiments can demonstrate major perfusion vs. diffusion-dependent time constants of the body. Using this technique with doppler bubble detection, we have now crudely "titrated" both the minimum time of equilibration in awake goats and the minimum critical depths by *transient* isobaric gas switching of nitrogen, helium, neon, and argon. Results indicate that whereas switching from saturation on nitrogen (5.1 ATA O_2) to either helium or neon causes bubbles, switching from neon to helium causes almost none *in spite of the more rapid diffusion of helium than neon*. Thus, although the importance of diffusion differences cannot be entirely ruled out, the above results indicate strong support for the classical perfusion dependent model first set forth by Krogh in 1919. Supported by NIH grant HL 22406, HL 22414, HL 20012, and ONR Contract #N00014-76-C-0749 to Virginia Mason Research Center.

PITFALLS IN THE DIAGNOSIS OF DYSBARIC OSTEONECROSIS AND THE SIGNIFICANCE OF SUSPECTED LESIONS. J.K. Davidson, W.D. Dysart-Gore, D.N. Calder. MRC Decompression Sickness Central Registry, Newcastle upon Tyne, UK.

The identification of well developed lesions of dysbaric osteonecrosis should be straightforward. By subjecting divers and compressed air workers to regular radiographic examination an attempt is made to detect the condition as early as possible to try to identify the causal incident and also, perhaps, enable protective measures to be established promptly. Early diagnosis is based on changes in the trabecular structure of the bone usually associated with a change in density. The difference between these early pathological depletion and bizarreries and normal variance, in both trabecular and cortical bone, which make up the syndrome of radiological diagnosis, will be discussed. Sometimes an abnormality has to be reported as a 'suspected' lesion. Using the data in the Newcastle Decompression Sickness Central Registry we have looked at the occurrence of 'suspected' juxta-articular (JA) and head, neck and shaft (HNS) lesions, which eventually become 'definite'. The results are as follows:

Lesion Type and Site	Time taken for definite Divers	CA workers	If the only factor influencing the diagnosis of 'suspected' lesions was the radiologists uncertainty of the presence of a definite lesion, we might expect
JA shoulder & hip	30% (17/40)	28% (35/124)	about 50% of such decisions
HNS shoulder & hip	14% (2/14)	10% (3/29)	
HNS femur & tibia	13% (3/23)	39% (68/176)	

care in treating a joint eventually to be confirmed as definite. That this does not happen may indicate that early bone changes are repairing successfully in some cases. (This research is supported by an MRC grant.)

SCUBA DIVING IN PREGNANCY. J.H.G. Rankin, J.N. Langhier, M.S. Stuck* and D.L. Anderson*. Departments of Physiology and Gynecology-Obstetrics and Biotron, University of Wisconsin, Madison, WI 53706.

The effect of simulated standard, no-decompression dives to 100 ft. and 60 ft. of seawater was tested in 12 near-term sheep carrying 16 fetuses. Six surgically prepared fetuses were dived to 100 ft. Five died within 20 min. of ascent and the 6th suffered severe cardiac arrhythmia and hypotension. At autopsy all fetuses were observed to have massive bubbling in the arterial system and the heart. Five fetuses were dived to 100 ft. without surgery. Two were alive 3 hours later and no bubbles were present at autopsy and 3 animals were born alive at term. *The difference between* the response of the fetuses subjected to surgery and that of the fetuses with no surgery was significant $P<.001$. With the 60 ft. dives, 3 fetuses were subjected to surgery and all suffered massive bubbling. Two fetuses were dived to 60 ft. without surgery, 1 was alive after 6 hours and the other was born alive at term. With the 60 ft. dives the differences between fetuses with surgery was significantly different from that of the fetuses without surgery $P<.03$. We conclude that surgery and monitoring result in the formation of post-dive gas bubbles which would not otherwise appear. In the immediate post-dive period there were no significant changes in fetal blood pressure, fetal placental or renal blood flow but the maternal blood pressure was elevated by 5% and the maternal placental blood flow was depressed by 18%. Fetuses which have not been subjected to surgery and monitoring do not appear to suffer any damage from standard, no-decompression dives of 100 ft. and 60 ft.
Supported in part by the University of Wisconsin Sea Grant College Program, National Oceanic and Atmospheric Administration, U.S. Department of Commerce and by the State of Wisconsin and NIH grant 9011185.

TREATMENT OF CARDIOVASCULAR DYSFUNCTION RESULTING FROM CEREBRAL AIR EMBOLISM. D.E. Evans, A.I. Kobylinc, E.P. Flynn, and M.E. Bradley. Naval Medical Research Institute, Bethesda, Maryland 20014.

In previous investigations of possible mechanisms of sudden death after dysbaric air embolism, we found in animals that air infused into the cerebral circulation alone caused acute hypertension and severe cardiac arrhythmias. These acute cardiovascular events were accompanied by a sharp increase in intracranial pressure and a 100-200 fold increase in circulating catecholamines. The present series of experiments were designed to test possible therapeutic approaches to the treatment of cardiac arrhythmias and other deleterious effects of cerebral air embolism. Intravenous lidocaine was the first agent to be tested because of its widespread use as an antiarrhythmic agent. Lidocaine (5 mg/kg i.v.) was administered 5 minutes before air was infused into the vertebral artery of anesthetized, ventilated cats. Lidocaine was found to eliminate the severe cardiac arrhythmias after cerebral air embolism and to reduce significantly the acute hypertensive response. Also significantly reduced were the rise in intracranial pressure and the increase in plasma catecholamines after cerebral air embolism. Larger doses of lidocaine were found to be even more effective in attenuating the cardiovascular effects of cerebral air embolism. These preliminary findings suggest that lidocaine may be a useful therapeutic agent in treating the severe cardiac arrhythmias and acute hypertension resulting from cerebral air embolism.

NEUROPHYSIOLOGICAL AND BIOCHEMICAL STUDIES IN He-N_2-O_2 ATMOSPHERE AT 11 ATA. I. Stoilova*, V. Kolev*, I. Doseva*, I. Venkov*, Tm. Tencheva*, A. Dishkelov* and A. Varbanova*. *Central Laboratory for Brain Research, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria.

A joint Soviet-Bulgarian experiment "HELIUX-100" was carried out in the USSR in 1978. Three aquanauts were examined under conditions of 14-day stay in pressure chamber, 7 days spent at 11 Ata, using He-N_2-O_2 atmosphere in different ratios. The main aim of the experiment was to study the changes taking place in the human organism during and after continuous exposure to high pressure conditions in He-N_2-O_2 medium.

In the course of the experiment recordings were made of the EEG, both spontaneous and in functional tests, of the evoked potentials (EP) after light stimulation, as well as polyphysiographic sleep recordings. Lipid metabolism - total lipids, phospholipids, cholesterol and fatty acids - as well as the acid-base equilibrium, were studied parallel with the electrophysiological data. The electrophysiological analysis show that the experimental conditions had different effects on the different subjects due to the individual adaptation *possibilities and they were a factor influencing* EP *generation*. The longer latencies of the EP components observed in the course of the experiment should be assumed to be one of the indicators of the general physiological stress under hyperbaric conditions. A readjustment of the metabolic processes requires considerable energy expenditure which is compensated by a general intensification of lipid metabolism

VISCERAL MALFORMATIONS, RESORPTIONS, AND BIRTHWEIGHT AMONG FETAL RATS EXPOSED TO AIR AT INCREASED ATMOSPHERIC PRESSURE. M.E. Hulton and A.L. Alone*. University of Florida, Gainesville, Florida, U.S.A.

Maternal exposure to air at greater than 1 atmosphere absolute pressure (ATA) has been associated with amniotic and fetal intravascular bubbles in several animal species. However, previous teratogenic investigations have involved animals subjected to less than 1 ATA air, and did not reveal increased frequency of fetal malformation or death. The purpose of this research was to determine if pregnant rats subjected to maximum "bends-free" exposure to air at 6 ATA would have an increased frequency of fetal death, resorptions, low birthweight or malformations. Ninety pregnant rats were assigned to one of three exposure schedules during organogenesis: days 9-11, 12-14, or 15-17; and randomized between one treatment and two control groups. The treatment group was subjected to 6 ATA for 70 minutes, with compression and decompression at a rate of 60 fsw/min. Control groups were exposed to either 1 ATA of air within the hyperbaric chamber, or 1 ATA of air outside the chamber. For 10 minutes following decompression, chamber treated animals were placed in a slow, motor-driven rotating cage, and assigned a "bends score" based on gait disturbances (Aerosp. Med., 1972, 43: 1240-1244). On day 20 of gestation, laparotomy was performed, and corpora lutea, implantations, and resorptions were counted. Fetuses were fixed, sectioned, and examined for visceral malformations. Minor visceral anomalies were present in 16.2 of all fetuses; however, there were no significant differences between groups. Similarly, there were no significant differences when number of resorptions, number of dead fetuses, mean fetal weights, and malformations in treatment and control groups were compared by analysis of variance. Finally, there was no significant relationship between "bends score" and any of the above variables. These results indicate that exposing rats to air at increased atmospheric pressure does not affect fetal health or survival.

BRAINSTEM EVOKED POTENTIAL CHANGES ASSOCIATED WITH VARIATIONS IN MIDDLE-EAR PRESSURE. Ben M. Clopton* and Jozef M. Miller, Department of Otolaryngology RL-30, School of Medicine, University of Washington, Seattle, Washington, 98195.

The brainstem evoked response (BSER) to clicks presented to an ear in which middle-ear pressure was varied in guinea pigs served as an indicator of pressure effects. The magnitude of wave V was observed over a 60 dB range of click intensity as middle-ear pressure was varied from -300 to +300 mm H_2O in 50 mm steps. The magnitude of the BSER was approximately linear with dB stimulus magnitude providing estimates of equivalent changes in stimulus magnitude. All negative middle-ear pressures produced attenuation of the BSER magnitude, the greatest effect being at -100mm H_2O. Increasingly positive pressures produced increasingly greater reductions in response magnitude, but of lesser effects than negative pressures. These results agreed in magnitude and form with those seen using peripheral measures of middle-ear pressure effects, thus supporting the BSER as a convenient alternative correlate of middle-ear pressure effects.

ANALYSIS OF MEDICAL REASONS FOR WITHDRAWING MEDICAL CERTIFICATION OF FITNESS IN COMMERCIAL DIVERS IN THE U.K.
W.A. CROSBIE, King's College Hospital Medical School, London, and R.N.M.C., Great Yarmouth, U.K.

Diving regulations in the U.K. demands that a diver be medically examined every 12 months to assess his fitness to work underwater. A body of "Approved doctors" administer the system in the U.K. but problems arise when a diver is found to have developed some abnormal medical condition. He is then usually referred to a specialised unit for further investigation. Over the past 1-2 years, 17 such men have been referred for investigation of respiratory abnormalities and it is the object of this paper to describe their findings and subsequent progress.

The age range was 20-48 years and commercial diving experience 1-20 years. 10 were found fit and returned to unlimited diving while 7 were advised to stop diving. In the first group 6 had evidence of early airflow obstruction (1 subsequently developed breathing difficulties under water), 3 had evidence of lung barotrauma and both fully recovered, 1 had temporary toxic inhalation damage and 2 had abnormal lung shadows on x-ray, later consider innocuous. In the second group, 3 were found to have significant asthma, 2 emphysema and 1 suspected narcotic abuse. On review the major problem was the assessment of degree of airway disease

MODELING, MEASUREMENTS, AND MOMENTS OF INERT GAS EXCHANGE. P.K. Weathersby* and L.D. Homer* (SPONS: J. Yamamoto) Naval Medical Research Institute, Bethesda, Maryland 20014

Measurements of xenon gas exchange over 7 h in anesthetized dogs were fitted to a number of mechanistic models of capillary gas exchange. Both simple blood perfusion and simple tissue diffusion models failed to adequately fit the data. Models that combined blood perfusion with either radial or axial diffusion in the capillary fit the data, but only with implausible values of physiologic variables. Thus, it was concluded that some form of capillary heterogeneity must be formally included in reasonable models of tissue gas exchange. The models used could be summarized by the moments (e.g. mean and standard deviation) of the distribution of gas molecule transit times in the capillary. These moments provided a very useful framework for model comparison and development because models that fitted data well gave similar estimates of moments, regardless of which physical mechanisms of gas transport were assumed. Poorly fitting models gave different values of moments. Numerical values and properties of the moments established constraints for any generically useful model.

THE EFFECTS OF COLD STRESS ON VENOUS GAS BUBBLE PRODUCTION IN MAN Follow ING A 30 DECOMPRESSION DIVE. Richard Bonford and John Hayward*. Virginia Mason Research Center, Seattle, Washington, U.S.A., and University of Victoria, Victoria, British Columbia, Canada.

The effect of cold stress on venous gas bubble production was studied using doppler ultrasonic monitoring. Ten subjects participated in four exposure regimes carried out at 78 feet on an underwater platform for 18 minutes of light exercise in 10°C water off Victoria, B.C. Two cold exposures (C) using light neoprene wet suits and two warm exposures (W) using dry insulated suits were each followed by rewarming in a heated bath (H) or by endogenous heat production while insulated in a sleeping bag (I). The four regimes for each individual (WH, WI, CH, CI) were designed to affect changes in peripheral circulation. Pre-exposure measurements included mean skin fold, anthropometry, and predicted work capacity at 170 heart beats per minute (PWC170).

Results showed that for the cold exposure compared to warm exposure 1) air consumption increased 39%, 2) rectal temperature dropped 0.8°C at the end of the dive, 3) mean skin temperature dropped an average of 11°C, and 4) cooling rate correlated significantly with both mean skin fold and endomorphy ($p<.001$). A three-fold increase in bubble counts ($p<.07$%) was observed following the warm exposures compared to the cold exposures. The effects of rewarming regimes on bubble production after cold exposure was not conclusive. The WH combination showed a faster decline in bubble production than the WI method and was significantly different ($p<.05$) at 140 minutes post dive. The results suggest that cold stress affects peripheral circulation to inhibit inert gas uptake in the periphery

This project was supported by a grant from the Max and Victoria Dreyfus Foundation, Inc., and by the National Research Council of Canada, Grant #A6077.

SIZE DISTRIBUTION OF INTRAVASCULAR BUBBLES INDUCED BY DECOMPRESSION. R.D. Butler, J.A. Hills and F.L. Sutton,* Marine Biomedical Institute and Dept. of Physiol. & Biophy., Univ. of Texas Medical Branch, Galveston, Texas 77550.

Although in most cases, bubbles found in the venous system during decompression are trapped in the lungs, it is still most desirable to know their size distribution in attempting to predict their effects. Dogs (18-24 kg) were anaesthetized and compressed to various depths ranging from 120 to 220 fsw for exposures lasting to 3 hours. Prior to compression cannulae were placed into the sinus venarum cavarum for sampling venous blood containing the decompression-induced bubbles. The cannula was connected to a high-pressure blood-sampling valve which passed through the chamber wall. Size distributions of the bubbles were determined from 50 ml aliquots drawn from the venous cannula for periods up to 2 hours post decompression. A coulter-counter was used for bubble size measurement. Bubble sizes ranged from 19-179 μm for the lower end of the scale while larger bubbles, hundreds of microns in diameter, were measured after various intervals post-decompression. Quantities of smaller bubbles usually appeared immediately post-decompression while larger bubbles tended to appear later. The research reported here has been supported under the Office of Naval Research with funds provided by the Naval Medical Research and Development Command.

THERMAL EFFECTS OF RECOMPRESSED BUBBLES. R.G. Buckles, M.E. Cox* and J.H. Eckenhoff*, Department für Anaesthesie der Universität Basel, Kantonsspital, 4031 Basel, Switzerland; Department of Physics, University of Michigan-Flint; and Alza Corporation, Palo Alto, California.

Experimental studies were carried out to evaluate bubble behavior during and after recompression as is used to treat divers who suffer decompression sickness. It is traditionally believed that bubbles rapidly lose their heat of compression and are thus isothermal. Theoretical considerations suggest that there may be incomplete thermal equilibration at the current rates of recompression. Inert gas bubbles of He and N2 were suspended in saline or human plasma and their size monitored during recompression from 1 ata to 3.5 or 6.0 ata at rates of 1/3 or 1 atm/min. The fluids were pre-equilibrated with either N2 or He at 1.0 ata and 37°C. Bubble dimensions were holographically recorded at frequent intervals during and following the recompression until bubbles were fully dissolved (Cox, M.E., Buckles, R.G., Whitlow, D.: Cineholomicroscopy of small animal microcirculation. Appl. Optics 10:128-131, 1971). The bubble dissolution behavior at high compression rates to the maximum depth (analogous to a U.S.N. Table 8.3 or 8.4 treatment) exhibited anomalous behavior consistent with extensive heating of the bubble due to imperfect heat loss during compression. Calculations show that mean bubble temperatures in excess of 45°C occurred; subsequent bubble behavior suggests that thermal denaturation of plasma proteins occurs during this treatment procedure. Recompressed bubbles in water and under conditions of slower rates and lower pressures do not exhibit these effects.

RESULTS OF VALIDATION TESTING OF FLYING-AFTER-DIVING SCHEDULES. H. J. Bruggeti, Lt Col, USAF, BSC, USAF School of Aerospace Medicine, Crew Protection Branch, Brooks AFB TX 78235.

Hyperbaric exposures conducted at sea level and followed by immediate ascent to elevations greater than sea level, and hyperbaric exposures conducted at elevations greater than sea level cannot be conducted using decompression procedures designed and tested for use at sea level only. Exposure schedules for compressed air dives to depths from 10.75 fsw to 130 fsw were calculated using limiting tissue nitrogen values (B_0 values) adjusted to an altitude of 10,000 feet above sea level. The B_0 values were derived by using the same surfacing ratios (Tissue P_{N_2}/Barometric Pressure) used in the U. S. Navy Standard Air Decompression Tables. Twenty different volunteer military divers were exposed to each of six calculated exposure profiles: 130/7; 100/10; 80/14; 60/20; 40/34; and 10.75/1440. The hyperbaric exposure was followed by a 5-minute ascent to 10,000 feet in an altitude chamber, 4 hours at 10,000 feet, a 1.5-minute ascent to 14,000 feet, 1 hour at 14,000 feet and a 4-minute descent to sea level. Precordial Ultrasonic Doppler monitoring for venous gas emboli (vge) was conducted during the altitude exposures. A total of 98 subjects participated in 110 exposures which resulted in 12 (10.9%) terminated exposures. Five (4.6%) cases of pain only bends occurred while 7 (6.4%) additional exposures produced vge scores which resulted in early termination of the exposures. In addition, of the 98 completed exposures, vge were detected to a lesser degree in 14 exposures (14.3%). These results indicate: (1) the calculated schedules do not prevent bubble formation; (2) some previously published schedules and procedures are highly suspect; and (3) the U. S. Navy Standard Air Decompression Table B_0 values for surfacing at sea level may not be sufficiently conservative.

AN ANALYSIS OF THE EFFECTS THAT HYPERBARIC OXYGEN HAS UPON DECOMPRESSION TOLERANCES IN RATS AND HUMANS. D. E. Gerth and L. A. Lally*, Department of Physics and Astronomy and Department of Physiology, University of Hawaii, Honolulu, Hawaii 96822.

Oxygen is widely used at elevated partial pressures to facilitate decompression, yet the optimum dosage and the magnitude of the beneficial effects are poorly known. Mainly this is because oxygen enhancements, expressed as increases in the allowed pressure reductions, are small and easily masked by variations in the tolerances of individual subjects. Furthermore, oxygen can produce both beneficial and detrimental results, and the range from a therapeutic to a toxic level is narrow. Berghage and McCracken have recently carried out two massive investigations involving 1100 rats and 60 experimental conditions. These authors suggest that the conventional concept of an "equivalent air depth" is no longer tenable and that oxygen should not be disregarded in calculating the total tissue gas tension. We find instead that the observations of Berghage and McCracken are, in fact, compatible with a more detailed model in which the tensions of oxygen and carbon dioxide dissolved in tissue are estimated from their respective blood dissolution curves and added to the tensions of the other dissolved gases that are present.

PHYSICOCHEMICAL PROPERTIES OF THE NONIONIC SURFACTANTS SOLUBILIZING GAS CAVITATION NUCLEI (MICROBUBBLES). J.W. D'Arrigo* (UPON: D.A. Lally), Physiology Dept., U. of Hawaii Sch. of Medicine, Honolulu, Hawaii 96822 and CAVITATION-CONTROL Technology, Kailua, Hawaii 96734, U.S.A.

Surface-active substances in aqueous liquids, usually detected as trace organic contaminants, have long been known to affect both the cavitation threshold and stability of bubbles formed in these liquids. Such data and other findings have recently led to the proposal that pre-existing gas cavitation nuclei normally present in aqueous liquids (e.g., body fluids) are surrounded and stabilized by "skins" of surface-active compounds. Subsequent experimental work, using a newly developed agarose gel method, has shown that the surfactants which stabilize these gas nuclei are nonionic. To gain additional chemical data about these nonionic surfactants, the present study examined the relative effectiveness of 42 different electrolytes (each at 0.4 M) in decreasing the degree of bubble production in agarose gels in response to a fixed pressure schedule. Five different commercial preparations of ultrapure agarose were compared for each of the 42 different electrolytes in order to better identify reproducible and significant trends in the chemical data on cavitation. The precise anion and cation sequences obtained, which contain many similarities with published data in the physicochemical literature for salting out of identified nonionic surfactants, carry clear implications as to the structural characteristics of the nonionic surfactants stabilizing gas cavitation nuclei. For example, the pronounced and very similar anion sequences, combined with other data, suggest that the polar portions of these nonionic surfactants represent either weak bases or more probably amide groups. This view is in accordance with the relative position of Sr^{++} in the cation sequences, indicating either strong or moderate salting out in each case, and hence rendering it quite unlikely that ether linkages contribute to the hydrophilicity of the nonionic surfactants stabilizing gas nuclei.

ACUTE INTERACTION OF PHENYTOIN AND LONG LATENCY EVOKED POTENTIALS IN GERBILS PRESSURIZED IN HIGH PRESSURE HELIUM. F.G. Kaufmann, J.C. Farmer, Jr., and I. G. Sieggel. E.G. Hall Environmental Laboratory, Duke University Medical Center, Durham, N.C. 27710.

We have previously shown (Undersea Biomed. Res., 1979 Supp., p. 51) that in spite of its effectiveness in altering the course of electroshock seizures in experimental animals, diphenylhydantoin does not alter either the convulsion threshold pressure of guinea pigs exposed to high pressure helium or the large increases in amplitude of short latency (14 msec) cortical potentials evoked by electrical stimulation of the optic nerve. We now describe the effects of high pressure helium and phenytoin on long latency (120-200 msec) evoked responses. Under barbiturate anesthesia, ten guinea pigs were prepared with chronically indwelling electrodes in the optic nerve, lateral geniculate nucleus of the thalamus, and visual cortex. After several days of recovery, a cannula was implanted in the femoral vein under halothane anesthesia. After 1-2 hrs a control series of responses to electrical stimulation of the optic nerve were recorded. Diphenylhydantoin (15-60 mg/kg) was then administered intravenously either at surface or at 50 bars of pressure. At surface, phenytoin injection tended to shorten the duration of the long negative wave at the cortex beginning 120 msec after stimulation. At pressure the duration tended to be lengthened. The greatest effects were seen in the augmentation, by pressure, of afterdischarges beginning at about 175 msec after stimulation. Our findings are consistent with the view that pressure and phenytoin interact to exacerbate symptoms of HPNS. Supported by the Office of Naval Research Contract N00014-75-C-0551 with funds provided by the Naval Medical Research and Development Command, and by NIH grant NS12696.

H.P.N.S. in human during 38 hours compression to 500m with N_2 injections, J.C. ROSTAIN[1], B. GARDETTE[2], M.C. GARDETTE-CHAUFFOUR[1], R. NAQUET[1], 1. CNRS-GIH PHYSIOLOGIE HYPERBARE Fac Med.Nord Bd. Pt Dranguet 13326 MARSEILLE Cedex 3 - 2. COMEX-CFB Avenue de la Seude 13274 MARSEILLE Cedex 2 - 3. CNRS-LPN 91190 GIF-SUR-YVETTE, FRANCE.

In March-April 1979 at COMEX 8 French navy and Comex divers have reached a $He-N_2-O_2$ dive to 650MSW, the compression procedure (profil, steps and Nitrogen injections) is selected from : 1° analysis of Human deep $He-O_2$ dives to 500MSW and beyond. (PHYSALIE V-VI ; SAGITTAIRE II-IV); 2° Results of the $He-N_2-O_2$ saturation dives in monkey Papio papio to 1000MSW and beyond. The compression duration was 38 hours. The compression rates followed an exponential function of the depth. They were different every 100MSW (v_1, v_2, v_3 ... v_7 : 14MSW/min). The compression included four 150min stops every 100MSW from 100MSW. The nitrogen injections (1.5 ATA; were made before each stop to have 4,82 (2,7 ATA) at 650MSW. The method used (pt III, N_2 injections) limited some clinical symptoms of HPNS (tremor, dysmetria, hyperkinesia) ; It did not stop the appearance of EEG modification (increase in theta activities, decrease in alpha and beta activities). These modifications increased between 180MSW and 300MSW and the maximal values were found several hours after the stop at 650MSW. The intensity was different according to the subjects ; It was function of the individual susceptibility to hyperbaric conditions (compression, pressure, breathing mixture) and was not the same for every symptoms. These modifications decreased from the 24th hour of the stay and the physiological conditions of all the divers appeared better than in the $He-O_2$ dives.

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CHANGES IN RED CELL MEMBRANE ENZYMES IN MAN DURING SIMULATED DIVES OF UP TO 55 BAR IN HELIUM-OXYGEN. D.A. Purchase*, Biochemistry Department, Kings College, London WC2 and R.F. Carlyle*, Physiological Laboratory, AMTE, Fort Road, Alverstoke, Gosport, Hampshire, England (PRON R.N. Hemplemann)

The red cell isoenzymes of carbonic anhydrase, CA I and CA II, assayed by rocket immunoelectrophoresis, were found to decrease during saturation dives to 31, 42 and 55 bar with incomplete recovery during decompression. Red cell ghost preparations (Duman, Seco and Marikovsky, 1956) showed that approximately 90% of the apparently lost CA I was reloralised on the red cell membrane. Concomitant decreases in progesterone and CA I during decompression with only partial recovery of both hormone and enzyme during decompression are similar to those found in pregnancy. Following morphological changes in the red cell membrane (Carlyle, Nichols, Purchase, Rowles and Spencer, 1979) there may be trapping of molecules such as CA I, superoxide dismutase and loss of other molecules such as 2,3 DPG. The significance of these molecular alterations remains speculative but several interesting implications arise. First, changes in cell membrane structure may influence deformability and ability of the red cell to traverse capillary beds as well as gas exchange in those situations. Second, if changes in membrane binding of macromolecules occurs in other cell types then it may be involved in the origin of pathological changes such as HPNS. Lastly, there is the theoretical implication that membranes separating gas phases during decompression may not be morphologically or behaviourally the same as during the compression phase of a dive.

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INSTANTANEOUS MICROVIBRATION ON CAT UNDER THE COMPRESSION EFFECT TO 51ATA (He-N_2-O_2). K.Seki, H.Nakayama and M.Matsuda. Japan Marine Science and Technology Center (JAMSTEC), Natsushima-cho, Yokosuka 237, Japan.

This study is hyperbaric simulation on microvibration of 2 cats. Maximum depth was 51ATA(500msw). Experimental schedule was as follows; pre-dive(1ATA,air), 2ATA(He-N_2-O_2), 51ATA(He-N_2-O_2), decompression and post-dive(1ATA,air) -- 1days respectively, total: 10days.

This results shows the change of microvibration. Minor tremor sensor was fixed on the cranium of 2 cats(♂, body weight: 5.0kg, 4.8kg) and lead line extended to the outside of chamber via connector fixed on the heads of cats. EEG activity was recorded by bipolar(Fp-C). The change of amplitude and frequency of microvibration was estimated on the basis of the amplitude of EEG on slow wave sleep stage(frequency was 5 and 7 Hz).

The amplitude of microvibration progressed to increase during the compression, then the amplitude was 2-5 times as much as the control values at 1ATA(air) and 2ATA(He-N_2-O_2). Remarkable increase of amplitude manifested itself after the depth reached 51ATA. During 51ATA period, the amplitude of microvibration gradually decreased with the passage of time, and returned to the control level. However, all during the experiment, the frequency of microvibration did not show change, was about 11 Hz.

Data are discussed in relation; mixgas, compression rate, partial pressure of He per se, stage of pre-determined depth and duration.

DIAZEPAM UNDER HYPERBARIC CONDITIONS IN RATS. L. Grau*, R. Coggin* and P.B. Bennett. E.G. Hall Laboratory, Department of Anesthesiology, Duke University Medical Center, Durham, N.C. 27710.

The anesthetic effect of diazepam, expressed as a loss of the righting response and the symptoms of HPNS were studied in 28 rats at 90 ATA. Eighteen rats were given 7.5 mg/kg body weight diazepam S.C. and compared with 18 untreated rats in He/O_2 (p_{O_2} = 1 ATA). The loss of righting response caused by diazepam was found to be reversed as a linear function of decompression. At 60 ATA and beyond the righting response had returned in all animals. The pressure reversal cannot be explained as a gradual wearing off of the S.C. administered dose: an undived control group (18 rats) also given 7.5 mg/kg had a maintained loss of the righting reflex (in 70% of the animals) for 2.5 hrs. Symptoms of HPNS were graded 0 - no symptoms, 1 - single jerks, 2 - tremor, 3 - convulsions. Single jerks and tremor began to appear at 50 ATA. At 90 ATA all untreated animals had some degree of HPNS. Convulsions were observed in 67% of the animals without diazepam but did not occur in the diazepam treated group. To evaluate a dose response relationship 24 additional animals were given doses ranging from 0 to 15 mg/kg and compressed in the same manner as the previous groups. These animals showed that the amount of diazepam required to prevent convulsions (2.4 mg/kg) is less than that producing a loss of righting response (4.1 mg/kg). Diazepam can be regarded as a potent anticonvulsive drug under hyperbaric conditions. The severity of HPNS is markedly reduced. For hyperbaric emergency situations, when medical treatment has to be given, no contraindications for the use of diazepam are foreseen from these investigations.

THE EFFECT OF HYDROSTATIC PRESSURE ON ENZYMES INVOLVED IN THE OXYGEN METABOLISM. Eddie Østlid and Jan E. Ødegaard*, Norwegian Underwater Institute, N-5034 Ytrebygd, Bergen, Norway.

About 80 pct of the oxygen consumed by respiring organisms enters into the respiration chain and is metabolized in the ordinary way. The rest enters into other pathways where its product metabolites may be the cause of oxygen toxicity. The fate of these oxygen molecules at high hydrostatic pressure is mostly known.

One particular interesting pathway is the following reaction

$$O_2 \xrightarrow{e^-} O_2\cdot^- \xrightarrow{2H^+} H_2O_2 \xrightarrow{e^-,\ H^+} \cdot OH \xrightarrow{e^-,\ H^+} H_2O$$

Both $O_2\cdot^-$, H_2O_2 and $\cdot OH$ are known to seriously damage living tissue at high partial pressure of oxygen, and also at low total hydrostatic pressure. The question raised in this work is how high pressure per se influence the enzyme system involved in this reduction process. The activity of the O_2 producing enzyme xanthine oxidase and those of the scavenger enzymes for H_2O_2 and $O_2\cdot^-$, catalase and superoxide dismutase, have been investigated to 1500 bar. All enzymes have their activities reduced by pressure, and in particular the dismutase enzyme

THE EFFECT OF HYPERBARIC OXYGEN INHALATION UPON THE ULTRASTRUCTURE OF THE LUNG ALVEOLI. L.K. Akers. Department of Physiology, University of North Dakota School of Medicine, Grand Forks, North Dakota 58202.

The contribution of sympathoadrenomedullary pathways to the pulmonary pathogenesis associated with convulsive reactions of oxygen have been studied extensively. However, the contribution of these pathways to the development of pulmonary oxygen poisoning at sub-convulsive oxygen levels has not been well investigated. In this study, the role of the sympathetic nervous system in the development of pulmonary oxygen toxicity at sub-convulsive oxygen tensions was evaluated by examining the changes in the ultrastructure of the alveoli in the lungs. Three groups, each containing 56 adult male guinea pigs, were used in this study. These groups were *delineated* by the type of premedication used: no drug (control) and dibenzyline. Three experimental conditions were used. Animals from each group were subject to one ATA ambient air (PO_2 160 mm Hg) and 1 ATA or 20 ATA (PO_2 with 500 mm Hg PO_2). Six-day exposures of guinea pigs at 1 ATA or 20 ATA (PO_2 with 500 mm Hg PO_2), produced death in 1% percent of the animals and pulmonary congestion and edema in the rest. Scanning electron micrographs showed characteristic alveolar wall thickening, proliferation of alveolar-type II cells, and macrophage involvement occurring in alveoli of untreated guinea pigs. The total lung protein cholesterol and lung wet weight/dry weight were significantly elevated over the control animals. There was an increase in vacuolization of alveolar type I pneumocytes and endothelial cells with increased duration of O_2 exposure. There was also an increase of laminar figures in type II pneumocytes upon longer exposure to O_2. Catecholamine blockade or depletion seem to produce opposite vacuolization.

ALTERATIONS IN OXIDATIVE METABOLISM DURING RECOVERY FROM PULMONARY OXYGEN TOXICITY. W. D. Currie, P. C. Pratt*, A. P. Sanders. Duke University Medical Center, Durham, North Carolina, 27710.

Exposure of rats to 1.5 and 2.0 atmospheres absolute (ATA) for 0 to 24 and 12 hr, respectively, decreased ATP levels and the activity of enzymes involved in energy metabolism in lung tissue. Alterations in oxidative metabolism and in cell morphology following O_2 exposures and recovery periods were compared. The QO_2 values obtained with α-ketoglutarate as substrate were decreased to a greater degree than with succinate immediately following the O_2 exposures (413 - 681), however, following two days of recovery under room air conditions all QO_2 values had returned to control levels or higher. The lung ATP levels were reduced (<15%) after the exposures, but returned to normal after only one day of recovery. ATPase and cathepsin activities were unchanged or decreased after the O_2 exposures and following recovery periods. Morphological studies conducted simultaneously with the biochemical studies showed damage to the lungs following exposures to oxygen that markedly *improved* following recovery periods of 1-2 days. Despite the apparent severe damage to many endothelial cells, it is notable that interstitial edema was inconspicuous. The lamellar bodies and mitochondria of type II cells were damaged by the O_2 exposures; however, improvement was observed following recovery periods of 1-2 days. The rapid recovery of normal appearance of the mitochondria following return to room air conditions has not been reported, however it is entirely consistent with the rapid recovery of metabolic activity. The importance of oxidative metabolism in repair of injured lung tissue is reflected in the rapid, simultaneous increase in mitochondrial function and morphological improvement during recovery periods.

ON THE INFLUENCE OF EXOGENOUS AND ENDOGENOUS SUBSTRATE ACCUMULATION ON DRUG INDUCED VARIATIONS OF GLUTAMIC ACID DECARBOXYLASE ACTIVITY PRIOR TO OXYGEN HIGH PRESSURE EXPOSURE.

<u>B.Kr. Regnström</u>. Dept. of Neurobiology, University of Gothenburg, Sweden

Oxygen high pressure (OHP) exerts its effects on cellular metabolism by inactivation of enzymes involved in the early stages of hydrogen transfer and in the citric acid cycle. By interfering with enzyme activity and substrate supply prior to OHP-exposure the seizure mechanism has been studied. Unsymmetrical dimethylhydrazine (UDMH), a convulsant agent, inhibits glutamic acid decarboxylase (GAD) probably by tying up its cofactor upon reducing the formation of succinate through the GABA-shunt pathway. Pyridoxine (PYR) is a B_6 vitamine and a cofactor for GAD. The results show that UDMH has influence on seizure triggering, probably by controlling GAD-activity. The length of the induction period and the severity of convulsions were correlated to the dose of UDMH given. It was found to be possible to give UDMH in so small a dose to provoke convulsions in air 1 ATA but yet sufficient for significant decrease in GAD activity and OHP-resistance. The resistance to OHP was also found to be related to the access to substrate. Additional succinate given by parenteral administration during acute severe UDMH-poisoning proved to be protective against OHP-toxicity but the effect decreased successively. Injection of PYR 5 min after the first UDMH-seizure period at 4 ATA resulted in a dramatic and immediate increase in resistance to OHP (6 ATA) in both succinate treated and non-succinate treated rats, indicating that GAD inhibition by UDMH meant endogenous accumulation of substrate sufficient for enzyme saturation without exogenous supply of succinate at the time of GAD reactivation by PYR.

OXYGEN CONVULSIONS IN MICE, INFLUENCE OF NITROGEN ADMIXTURE

<u>N. Hertelsen*, O. Criborn* and A. Muren</u>

Defence Materiel Administration, Naval Materiel Department and National Defence Research Institute, Stockholm

The oxygen convulsion treshold is relatively well established, especially in small animals. There is, however, a wide variation between individuals and groups. Attempts have been made to exclude some of the factors responsible for these variations. Apart from the standardization of the experimental procedure and the use of a homogenous strain of mice, the influence of age, body weight and body temperature has been studied. Diurnal cycle effects have also been taken into consideration. During evaluation of the influence of these factors on the oxygen treshold, attention was focused on the importance of the purity of the gas, primarily with regard to the contamination of oxygen with nitrogen.

A total of just over one hundred CBA mice, all of which were males at an age of 70-80 days were exposed to 5 ATA of oxygen, five at a time, in a transparent pressure chamber. Pure oxygen as well as different nitrox mixtures were used. The time from arrival at pressure to the appearance of obvious tonic convulsions was recorded individually. With pure O_2 (98-99%) the mean time was 320 seconds. With the admixture of 28%*N_2 the time was reduced to 240 sec and with 45% N_2 170 sec. The differences between these groups are significant. *With 4 as well as 6 ATA oxygen the effect of N_2-admixture gave rise to corresponding results.

HOP-INDUCED CEREBRAL VASOCONSTRICTION, ITS CONTRIBUTION TO CNS-TOXICITY KINETICS. <u>D. Elsiberg*, A. Lavie* and D. Keren</u> (UPON: K. Pirnamin). Dept. of Physiology, Faculty of Medicine, Technion, Naval Medical Institute, P.O.B. 8040, and Israel Oceanographic & Limnological Research, P.O.B. 8030, Haifa, Israel.

The contribution of cerebral vaso-activity during HOP breathing to the form of the pressure vs. time curve for the CNS-toxicity threshold was studied in awake rats. Animals bore chronically implanted electrodes through which a continuous record of the electrocorticogram was obtained, and its first electrical discharge was used as an end-point for toxicity. A control group was compared to an experimental group in which cerebral vasoconstriction was prevented by the addition of 30 mm Hg CO_2 to the inspired oxygen at all pressures. Part of the animals were tested at one pressure only and for some, individual curves were constructed. The pressure/time curves were approximated to rectangular hyperbolae with both time and PO_2 asymptotes. The combined curve of the experimental group showed a leftward and downward shift over most of the pressure range. At pressures higher than 6 ATA, latencies progressively increased, abolishing the time asymptote. We conclude that: 1. Cerebral vasoconstriction does not contribute to the basic shape of the pressure/time curve, which is probably dependent on cellular kinetics. 2. By lowering mean tissue PO_2, the vasoconstriction shifts the curve to a higher pressure asymptote and longer latencies in the 3-6 ATA range. 3. Beyond 6 ATA, a dominant narcotic effect of CO_2 may delay FEB appearance.

TOLERANCE OF MICE TO PULMONARY OXYGEN TOXICITY. <u>A. Lavie*, D. Keren and D. Gelman*</u> (UPON: Y. Helamedi). Naval Medical Institute, P.O.B. 8040, Israel Oceanographic & Limnological Research, P.O.B. 8030, and Dept. of Biochemistry, Technion, Haifa, Israel.

Young (3-4 months) and adult (21 months) mice (C57-B1) were exposed to 0.6, 0.8, 0.85 and 1.0 ATA of O_2. Survival time and changes in lung, liver and blood antioxidant enzyme activity (superoxide dismutase, catalase and some indoleamine 2,3 dioxygenase measurements) in response to hyperoxia were determined. No significant changes in enzyme activities were observed in the liver and blood during long-term (3-8 days) hyperbaric oxygen exposures. During the initial 30 hours of the 95-hour mean survival time at 1 ATA O_2, lung superoxide dismutase activity increased by 15% and then fell progressively to 75% control level before death. Prolonged exposure of mice to either 0.6 or 0.8 ATA of O_2 did not induce antioxidant enzyme systems in the lung, nor did it improve their resistance to further exposure to 1.0 ATA of O_2. The results clearly show that young and adult mice are incapable of overcoming the high oxygen environment challenge. Moreover, antioxidant enzyme induction and the associated partial protection from pulmonary toxicity are not the general rule in mammalian lung exposed to subtoxic oxygen levels. Preliminary water proton magnetic relaxation studies in the injured lungs of the mice revealed that NMR can be used as a quantitative tool for studying the development of cell damage by hyperoxia.

CNS AND PULMONARY OXYGEN TOXICITY DURING INTERMITTENT EXPOSURE TO HYPERBARIC OXYGEN AND AIR. D. Keren, C. Ritterman* and B. Bleiberg*. Israel Oceanographic & Limnological Research, P.O.B. 8030, Naval Medical Institute, P.O.B. 8040, and Dept. of Physiology, Faculty of Medicine, Technion, Haifa, Israel.

Unanesthetised rats, chronically implanted with cortical electrodes, were individually exposed at 5 and 6 ATA to alternating oxygen and air. CNS toxicity end-point was the first electrical discharge (FED) in the electrocorticogram, and pulmonary toxicity was judged by dispnea and p.m. histopathology. The main results and conclusions are: 1. Consecutive HOP exposures at 6 ATA up to FED, seperated by 10 min periods of air-breathing, had essentially unaltered latencies (mean latency 9.35 min). 2. A profile of alternating 7 min periods of oxygen and air breathing at 6 and 5 ATA markedly increased CNS-toxic-free total exposure time and cumulative oxygen time but did not prevent FEDS, which were observed during both oxygen and air breathing periods. 3. Of the latter, 63% occurred immediately after switching to air and as such could have been due to a reduction in the narcotic potency of the breathing mixture. 4. CNS-toxic-free exposures of 90 min and over permitted the development of pulmonary toxicity which could limit such profiles. 5. Existing predictive indices for oxygen toxicity appearance do not fit these results and should be modified accordingly.

POSTER PRESENTATIONS

SESSION V

STRESS AND MENTAL PERFORMANCE UNDER WATER. P.G.A.M. Jorna* (SPONI A. Schouten), Institute for Perception TNO, P.O. Box 23, Soesterberg, The Netherlands.

A submerged diver will find himself in an environment for which he is not naturally adapted. A diver is not only physically loaded but also experiences mental load. In this study divers were tested to evaluate to which extent it was possible to process a mental task in the underwater situation. The task consisted of auditory presented letters with intervals of 3 sec. The diver had to detect certain target letters as instructed before the dive. In addition the number of targets had to be counted selectively. Two levels of difficulty were used (2-4 targets). Test divers were made by divers in training at three phases in the training course. Performance increased with progress in training. Divers detected more target letters and counted them more often correctly. This effect was most prominent for the difficult tasks. Dry control tests indicated no improvement due to task learning. Reaction times, measured for distorted targets, showed no improvement. It is assumed that an experienced diver will be more adapted to the stressful underwater environment and therefore better capable to execute a task. This notion is supported by test dives made by experienced divers who showed no significant improvement in underwater performance. Dry performances did not discriminate inexperienced from experienced divers. Inexperienced divers reached the performance level of their more experienced colleagues at the end of their training, although reaction times were still faster for experienced divers.

Finally, heart rate and respiration were recorded continuously during the dive. Spectral analyses on the R-R intervals were used to inspect the 0.1 Hz component of heart-rate variability. This component seems to be sensitive for mental effort.

NONINVASIVE CONTINUOUS MONITORING OF DIVER PULMONARY PERFORMANCE. G.L. Ackerman. Naval Medical Research Institute, Bethesda, Maryland 20014.

A noninvasive, real-time device for pulmonary monitoring, usable in the diving environment, is described. The device consists of posture-on electromagnets and electromagnetic sensors attached to the diver and a microprocessor control/readout unit located on the surface. Breath-by-breath analysis consisting of tidal volume, minute volume, and breathing rate is available as well as a partitioning of tidal volume into chest and abdominal components. Results of the analysis may be displayed digitally or on an analog recorder and may also be stored for archival purposes. A portable spirometer is used for easy calibration of the device, even with a man in the water, and the calibration is stable over long periods of time. The data we obtained using this device with the subject in the water or in the dry agreed with data obtained from a plethysmograph under the same conditions to within 98% accuracy.

HYDROSTATIC PRESSURE: ITS EFFECTS ON CELLULAR MEMBRANE ION TRANSPORT. W. R. Galey, Ph.D., P. S. Van Nice and C. V. Beaty, (SPONI B. C. Wood) Dept. of Physiology, University of New Mexico School of Medicine, Albuquerque, New Mexico, 87131, USA.

Ion movement across the cellular membrane of nerve cells is responsible for the conduction of nerve impulses. Since perturbation of nerve function by anesthetic agents is antagonized by hydrostatic pressure (pressure reversal of anesthesia) and hydrostatic pressure itself can induce abnormalities in nerve activity (High Pressure Nervous Syndrome), we have studied the effects of hyperbaric pressure on the movement of ions across cell membranes. Our studies have used the red cell membrane as a model for investigating the effects of pressure on membrane active and passive fluxes of $^{22}Na^+$ and $^{42}K^+$. It was observed that under conditions where 69ATA of pressure were exerted on a red cell suspension by the non-narcotic inert gas helium active transport of both Na^+ and K^+ was inhibited by 20%. Furthermore, it was seen that the active influx of K^+ into red cells decreased linearly between 15 and 117ATA of helium exerted pressure. The effect of the pressure appears to be associated with active transport since the influx of $^{42}K^+$ in cells poisoned with 10^{-4} M Ouabain was not affected by hydrostatic pressure. The effect was also antagonized by adding the narcotic elemental gases Ar or N_2 to the helium gas. The activity of the Na, K ATPase enzyme system of red cell ghosts was not inhibited by hydrostatic pressure suggesting that the effect of the hydrostatic pressure was not on the enzyme activity per se but on its ability to transport ions across the membrane. The results of these studies are very similar to those seen in mechanically induced hydrostatic pressures. Supported in part by ONR Contract N00014-76-C-0015 and NIP Grant RR 07129-04.

THE EFFECTS OF PRONE IMMERSION ON LUNG FUNCTION. I. Daskalovic, A. Hashimoto*, W.B. Laughlin and W.G. Reddan*. Department of Preventive Medicine, University of Wisconsin, Madison, WI 53706.

Studies of upright (or "head-out") immersion (UI) have shown a number of unfavorable respiratory effects. Prone immersion (PI) is more common in swimming and scuba diving but has largely been neglected. We determined pulmonary effects of PI in 7 healthy subjects and in 5 with chronic obstructive pulmonary disease (COPD). Lung volumes, flows, and gas exchange were measured using standard clinical procedures adapted for immersion. Closing volume (CV) was determined by the single-breath N_2 technique. In going from upright posture on land (UL) to supine on land (SL) and to UI, ERV decreased markedly while VC, TLC, and FEV_1/FVC showed smaller decreases. In PI, all of these variables returned toward UL values. RV was unchanged. CV (%VC) increased progressively from UL to SL to UI in healthy subjects but was unchanged in COPD. In PI, however, CV fell below UI values in both groups and even below UL values in some COPD subjects. The volume ERV-CV, normally positive, became negative in UI and returned toward UL (+) values in PI. The shift was most notable in the COPD group, which included negative values even on land. DLCOgn showed a small increase with PI in healthy subjects but no change in COPD. We confirmed potentially deleterious changes in UI and found that these were accentuated in COPD. In contrast, the effects of PI were largely neutral and appear in some instances to be beneficial. (Supported by the University of Wisconsin Sea Grant Institute.)

THORACIC SHAPE, LUNG VOLUME AND DIAPHRAGMATIC CONTRACTION DURING IMMERSION. Yu-Pinh Shih and G.L. Dahan*. The VA Medical Centers of Long Beach, CA, and St. Louis, MO; and University of California, Irvine, U.S.A.

Diaphragmatic contraction and configuration were studied in the dog during head-up immersion to a mid-neck level, using bilateral electrical phrenic stimulation over a large range of lung volume (V). In six animals, the strength of diaphragmatic contraction was measured as the change in alveolar pressure within the occluded respiratory system (Pmus). In five other animals, diaphragmatic configuration (during relaxation and diaphragmatic contraction) was documented in air and in water. It was found that: a) Pmus by constant-stimulus diaphragmatic contractions decreased with lung inflation, both in water and in air; b) immersion attenuated the effect of inflation on Pmus; c) at low-V, immersion was associated with parallel changes in Pmus and diaphragma lc length (DL), the latter was measured from a lateral costal diaphragmatic insertion to the other, along the diaphragmatic contour on anteroposterior chest radiographies; d) the distortability of the diaphragm was not parallel to that of the whole respiratory system, resulting in an effect of immersion on Pmus most pronounced at higher lung volumes; and f) DL appeared to be a prevailing factor controlling diaphragmatic contraction, whereas diaphragmatic curvature seemed to be of lesser importance.

BLOOD METABOLITES IN RESTING AND EXERCISING RATS AT VARIOUS PARTIAL PRESSURES OF NITROGEN AND OXYGEN. R. de G. Hanson, R.M. Gray*, P. Smythe* and K.G.M.M. Alberti*. Physiological Laboratory (AMRE), Tort Road, Alverstoke, Gosport, Hampshire, and Southampton University, Hampshire, UK.

This series of experiments was designed to see if some or all of the changes found in earlier experiments in hyperbaric air (Hanson et al, 1977) could be due to the increase in pN_2 or pressure per se and to see if a degree of hypoxia could show effects opposite to those in hyperbaric air. Four atmospheres were chosen: A (pO_2 0.14 bar pN_2 0.86 bar), B (pO_2 0.21 bar pN_2 0.79 bar), C (pO_2 0.21 bar pN_2 3.79 bar), D (pO_2 0.82 bar pN_2 3.18 bar). 48-hour starved rats (225-265 g) were exposed to these atmospheres in batches of 6. Half of each batch remained resting while the other were forced to swim. After 30 minutes they were decapitated and the first drops of blood collected in chilled perchloric acid for analyses of glucose, lactate, pyruvate, ketone bodies alanine and glycerol; the remaining blood was collected for assay of insulin and non-esterified fatty acids (NEFA). Results showed the expected differences between exercising and resting rats in all environments. There were some differences between the group of resting animals, those in D (hyperoxic) showing a lower blood glucose ($P < 0.05$) compared to A and B (normobaric) and an increase in NEFA ($P < 0.05$) compared to B. Both hyperbaric environments (C and D) had higher ketone levels than the normobaric ones. The exercising animals in C and D showed a lower glucose level than in A and B unaccompanied by a difference in insulin. The animals in D had lower lactate and pyruvate levels ($P < 0.05$ and $P < 0.01$) than the other environments and higher NEFA and 3-Hydroxybutyrate ($P < 0.05$). The series confirmed earlier findings and produced others which were new probably due to improvements in technique.

Reference: Hanson R de G, Gray R M, Smythe P and Alberti K G M M (1977). Med Aeronautique et Spatiale, Med Subaquatique et Hyperbare 17, p255-260.

Emergency Thermal Protection for Saturation Diving. Glen H. Egstrom and Anthony DiChiaro*, Commercial Diving Center, Wilmington, California and Kinergetics, Inc., Tarzana, California

Loss of power and heat during saturation dives has resulted in casualties in circumstances where breathing gas supplies and CO_2 elimination capability were adequate for an extended period of life support. The loss of environmental control has quickly shifted ambient conditions to 0-2°C, relative humidity 100%, in an HeO_2 environment. Death in a short time is the not unexpected end result.

A study conducted in the Commercial Diving Center's saturation facility involved a survival device developed at Kinergetics, Inc. A 24 year old, 178 cm, 75 Kg, male commercial diver and safety diver were saturated at 3 ATA on an 82% He, 13% O_2 gas mix. Overall heat loss was tamated to be kept below 100 watts per hour. During the initial 24 hour exposure, the chamber temperature was kept between 0-3°C with a relative humidity of 83-100%. Comparative data was recorded each 30 minutes for 27½ hours. Monitored diver parameter ranges included: heart rate (42-109), rectal temperature (36.3 - 37.1°C), and skin temperature (36.6 - 36.9°C). Subjective evaluations of comfort indicated "too warm" except during sleep periods when he was "comfortable".

RESULTS:

1. A thermal protective device maintained diver comfort during a 24 hour exposure in a HeO_2 environment at 0-3°C ambient temperature. The diver's initial rectal temperature of 36.9°C and the hour 24 rectal temperature of 37°C indicated stable heat balance.
2. Reduced metabolic activity during rest and sleep did not result in hypothermic discomfort or abberations of EKG.

HEAT STRESS DURING DIVES IN WARM WATER. I. Holmér* and G. Kihlström* (SPONI A. Auren). Dept. of Occupational Health, National Board of Occupational Safety and Health, S-171 84 Solna, Sweden and the State Power Board, S-162 87 Vällingby, Sweden.

Divers are exposed to warm, or even hot water in fuel basins in nuclear power plants. In order to investigate the thermal strain associated with dives in warm water, two divers performed light work on a bicycle ergometer alternating with periods of rest. The experiments were performed in a tank filled with water, controlled at 34, 38 and 42°C, respectively, and exposure time was 60 min. The divers were underwear and a rubber diving suit. The thermal strain increased with increasing temperature of the water. In water at 42°C body and mean skin temperature were higher than 39.0°C, subjects felt the conditions intolerable and exposure was interrupted after 30-45 min. An ice vest, worn under the suit, reduced the thermal strain, resulting in less increase in body and mean skin temperature and, consequently a lower rate of body heat storage. The heat stress during dives in warm water necessitates limitations of the duration of the dive with respect to activity and temperature of the water. The cooling power of an ice vest makes possible to double the exposure time in water at temperatures 39-43°C.

EFFECT OF BODY TEMPERATURE AND COMPOSITION ON RECOVERY FROM HYPOTHERMIA. J.B. Morrison, J.S. Hayward* and M.L. Copin*. Dept. of Kinesiology, Simon Fraser University, Burnaby, V5A 1S6 B.C. and Dept. of Biology, University of Victoria, Victoria, V8W 2Y2, B.C., Canada.

Inhalation warming has been promoted as a process which can be easily administered in remote environments. Its effectiveness has been challenged, however, and experimental studies appear to be contradictory. Comparison of various studies may be confounded by differences of physiological conditions and body composition. After cooling in 11.8°C sea water, 14 subjects having varied core temperatures were rewarmed by inhalation of saturated air at 44°C. Multiple linear regression analyses were computed for best possible subsets relating rectal and tympanic rewarming rates (r = .41) to physiological and anthropometric measures. It was found that although there was a good correlation (0.61 < R < .71 between 3 (1=R,T) and the corresponding mean metabolic or ventilatory rates, rewarming rates (1) could be more closely predicted by a combination of initial core and skin temperatures (r=0.75, 0.78; 1=R,T). The best predictive equations of rewarming rate (°C/hr) were

$\dot{R}$ = 29.08 1.044 T_{RQ} 17.56h/wt r = .5.9

$\dot{T}$ = 100.8 1.023 T_{TQ} 0.947 T_{TQ} 18.06h/wt r = 0.88

where T_{RQ}, T_{TQ} are initial rectal and tympanic temperatures, (h/w) is the height/weight ratio (cm/kg), and r is the adjusted multiple correlation. Results indicate that the rate of rewarming from hypothermia is strongly influenced by initial core and skin temperatures and by body composition. Comparisons of rewarming data obtained in different investigations and with other treatment methods are likely to be misleading unless experimental protocols and subject groups are carefully matched.

AN ELECTROMYOGRAPHIC STUDY OF SHIVER IN UNDRESSED HUMAN SUBJECTS. P.A. Iaizzo*, R.W. Petry*, and R.R. Pozos* (SPONI R.J. Bentley). Department of Physiology, School of Medicine, University of Minnesota, Duluth, Duluth, Minnesota 55812.

Although shivering is an intense muscular activity, relatively little attention has been paid to an analysis of the frequency and amplitude of electromyograms (EMG's) from the involved musculature. Therefore, this study was undertaken to quantitate such parameters and also to determine the muscle or group of muscles which first demonstrate electrical activity in response to immersion into cold water (15 19°C). Bipolar surface electrodes were placed on the following muscles: masseter, trapezius, pectoralis major, rectus abdominus, external oblique, latissimus dorsi, quadriceps, soleus, biceps, triceps, deltoids, and gluteus maximus. The EMG's were recorded on a Hewlett Packard FM tape recorder for later frequency and amplitude analysis on a PDP 12 digital computer linked to a CDC cabet 721. The records were taken before, during, and after immersion. The core temperature was monitored using both rectal and tympanic measurements. In addition, peripheral temperatures were recorded from selected locations using Bailey surface thermocouples. Initial results indicate that the predominant frequencies of oscillation appear in several bands between 5-12 Hz. Cross correlation analysis indicates that the muscles were not firing in phase. Further, in this study the observed shiver was due to a drop in peripheral temperature without a significant drop in the core temperature. In several subjects exploration increased the amplitude of shiver. These findings may provide additional information concerning spinal and supra spinal control of shiver.

AN ANALYSIS OF EMERGENCY HEATING REQUIREMENTS FOR PERSONNEL TRANSFER CAPSULES. F. M. Wiegler, University of Texas at Austin, Austin, Texas 78712.

Recent fatal accidents in which personnel transfer capsules have been dropped in the North Sea suggest that emergency heating systems should be available on PTC's which are used in cold waters. Otherwise, accidental loss of power from the surface support vessel rapidly subjects divers to severe cold stress. Several possible solutions for this problem have been proposed. One is to provide passive insulation in the form of blankets and "sleeping bags" which help the diver conserve metabolic heat. Another is to use various chemical heat sources which supply hot water either to heat the capsule or to heat individual divers. In this paper the various alternatives are analyzed using a comprehensive mathematical model of the human thermal system together with technical information obtained from published papers and reports. The following factors were considered in the analysis: (1) water temperature, (2) depth, (3) gas composition, (4) type of garment worn - wet suit or dry suit, (5) form of supplemental heating, and (6) time of exposure. Case studies employing a computer model were used to evaluate the importance of each factor. Results obtained to date indicate that active heating is required when the environmental gas is helium and individual heating requires significantly less energy than space heating. Specific energy requirements are presented for representative systems. The research reported in this paper was supported under the Office of Naval Research contract N000014-76-C-0951 with funds provided by the Naval Medical Research and Development Command.

INDUCTION OF CYTOCHROME P-450 BY HYPOXIA AND HYPEROXIA. H. A. Rose*, S. L. Gottlieb and L. S. Langmuir, Department of Biochemistry, N. C. State University, Raleigh, NC 27650, and Biological Sciences Division, Purdue University, Fort Wayne, IN 46805, U.S.A.

Both hypoxia and hyperoxia produce physiological effects. Some of these effects may be described as acclimative in that they tend to minimize the deleterious consequences of oxygen deficit or excess. Cytochrome P-450 has been shown to play a role in facilitating the transport of oxygen in liver and some other tissues [Longmuir (1970) Symp. 6th Int. Conf. Microcirculation, pp. 3-7] and across the placenta [Gartner & Burns (1974) *Proc. Natl. Acad. Sci. USA* 71:368] and the lung [Burns & Gartner (1975) *Drug Metabolism and Disposition* 3:84]. An increase in the amount of this pigment might be expected to increase the efficiency of oxygen transport in hypoxia. By virtue of its relatively high affinity for oxygen, cytochrome P-450 might also have a protective effect in hyperoxia [Wittenberg, J. B., *Oxygen and Physiological Function* (1977) p. 238] Longmuir, *Fed. Proc.* (1977) p. 244]. For these reasons, we studied the effect of hypoxia and hyperoxia on the levels of mouse liver cytochrome P-450. The animals were exposed to various ambient P_{O_2} values for varying periods prior to sacrifice. Exposure to hypoxia resulted in a doubling of the level of this pigment in half an hour, after a two and-one-half hour lag. Exposure to hyperbaric oxygen produced an even greater effect in one hour, but without a lag phase. To test if the mechanism of this response is localized in hepatocytes, we studied the effect of various ambient P_{O_2} values on the level of cytochrome P-450 in isolated hepatocytes. Exposure to anoxia resulted in reduction, there was a slight reduction in normoxia and significant increases in hypoxia and hyperoxia. These increases were abolished by inhibitors of transcription and translation, but heme synthesis inhibition had no effect. Thus the whole mechanism of induction is present in the isolated cell and involves protein synthesis.

HYDROGEN OXYGEN EXPOSURE OF RABBITS AT 30 ATA WITH MULTIDAY SURVIVAL. H.T. Bennagen, C.E.G. Lundgren and A. Myren, Laboratory of Aviation and Naval Physiology, University of Lund and National Defence Research Institute, Sweden.

After the termination of the first series of Hydrox dives by Zetterstrom in 1945, experimentation in this field was not resumed until the late sixties. The results from Hydrox exposures of different species including man are to a great extent encouraging, but there are also reports on toxic effects of Hydrox. According to a French group the survival of rabbits breathing Hydrox at 30 ata is less than one hour. Since these reports are seriously influencing the expected applicability of Hydrox as a diving gas, it was decided to try to reproduce the experiments and look further into these problems.

Three rabbits were compressed at a time, each placed in a separate compartment in a 400 litre pressure chamber. Electrocardiogram and subcutaneous temperature were recorded continuously. Compression was first made with air to 1.2 ata and pure nitrogen was then added to a pressure of 8 ata. At this pressure the chamber atmosphere was changed to 3% O_2 in H_2 (Hydrox). Further compression to 30 ata was made with Hydrox and pure H_2. Bottom times were 24 or 48 h. During exposure the PO_2 was kept at 0.2 - 0.5 ata, the PCO_2 at 0.005 - 0.01 ata and the chamber temperature at 30 - 34° C.

Seven rabbits have been exposed. Of these, four have been exposed 2 or 3 times with some weeks in between. So far all the animals have survived these exposures without evidence of toxic or other ill effects.

Supported by National Swedish Board for Technical Development.

EFFECT OF NORMOBARIC AND HYPERBARIC OXYGEN ON CYANIDE INTOXICATION. Takehito Takano, Yoshifumi Miyazaki*, Ichiro Nashimoto and Koh Kobayashi*, Dept. of Hygiene, School of Medicine, Tokyo Medical and Dental University, Tokyo, Japan; Dept. of Hygiene, Saitama Medical School, Saitama, Japan.

In order to evaluate the effect of normobaric and hyperbaric oxygen on cyanide poisoning, the intracellular oxidation-reduction state was observed in sixteen New Zealand White rabbits by detecting the fluorescence of reduced pyridine nucleotide which represented intracellular redox state and indirectly indicated the function of the respiratory chain. Animals were anaesthetized with urethane (1 g/kg, s.c.) and pentobarbital (10 mg/kg, i.v.), and immobilized with pancuronium bromide. The trachea and femoral artery and vein were cannulated for ventilation, measurement of arterial blood pressure and administration of 1000 ppm KCN solution. The animals were maintained on a Harvard respirator at the rate of 450 ml/kg/min. The left kidney was carefully exposed on the back above the retroperitoneum cavity and then the optical fiber for fluorometric measurement was set on it. Alteration of tissue oxygen tension was estimated using platinum polarographic electrodes (0.2 mm in diameter), and electrocardiograms were monitored. The data obtained in this study indicated that oxygen had an anticyanide activity, and administration of hyperbaric oxygen appeared to enhanced the cyanide detoxification. Some interesting implications were discussed from toxicological points of view on the results obtained.

HYPERBARIC OXYGENATION: TISSUE OXYGEN CHARACTERISTICS IN CHRONIC, SOFT TISSUE WOUNDS. P.J. Sheffield, Hyperbaric Medicine Division, Brooks AFB, Texas 78235, U.S.A.

The healing wound represents a dynamic mixture of cellular metabolism, local blood flow and gradients of normoxia/hypoxia. There are a number of disease entities in which these parameters become deranged and result in a chronic, nonhealing wound. Only through optimum wound capillary blood flow and tissue oxygenation is the wound able to heal. One mechanism by which HBO apparently aids the healing of ischemic and hypoxic soft-tissue wounds is to raise the wound oxygen sufficiently to support tissue metabolism. Our clinical investigation is a study of the changes in wound oxygen tension during normobaric and hyperbaric oxygen administration.

Wound oxygen tension was measured in chronic nonhealing, soft-tissue wounds with a polarographic oxygen electrode. Measurements were taken prior to HBO and at weekly intervals during the course of treatment. Measurements were recorded for each patient at 1 ATA and 2.4 ATA pressure. Three long-term (8-24 weeks) and ten short-term (2-8 weeks) patients were evaluated with tissue oxygen measurements (four patients were also evaluated with concomitant radioisotope scan metabolic studies along with the tissue oxygen measurements).

These initial studies indicate that the use of tissue oxygen measurements, particularly when combined with metabolic studies such as thallium 201 radioisotope scanning technique, promise to be valuable adjuncts in the medical decision-making process when dealing with difficult, non-healing soft tissue wounds.

ADRENERGIC AND CARDIOPULMONARY RESPONSES to EXERCISE WITH AIR AND HELIUM-OXYGEN AT 1 ATA. J. A. Flynn*, D. J. Evans, K. B. Greene, D. C. Lecroy*, and R. P. Layton. Naval Medical Research Institute, Bethesda, Maryland 20014.

Ten male subjects performed continuous bicycle exercise at approximately 50% of aerobic capacity for 30 min in the H-P Laboratory. Oxygen consumption, heart rate, cardiac pre-ejection period, ventricular ejection time, and cardiac output determined by thoracic impedance were measured at 5-min intervals and found to be the same whether the subject breathed air or an 80% helium-20% oxygen mixture. In contrast, pulmonary ventilation ($\dot{V}_E$) and respiratory frequency were increased 4.5% and 9.1%, and tidal volume decreased 6.0% on helium ($P < .05$). Plasma epinephrine increased linearly from 40 pg/ml to 110 pg/ml over the 30-min exercise interval. The response was identical with both air and helium. Plasma norepinephrine also increased with exercise, but the relative change was smaller in magnitude and considerably more variable. No clear difference between air and helium was apparent. These findings suggest that helium-oxygen breathing at 1 ATA does not alter the adrenergic or cardiovascular response to exercise significantly. Small changes in pulmonary ventilation can be detected, however.

INFLUENCE OF EXERCISE ON VENTILATORY CAPACITY AT DEPTH. A. Piscitelli and C. Lundgren, Hyperbaric Res. Lab., Dept. of Physiol., SUNY, Buffalo, NY 14214.

Exercise enhances ventilatory capacity at 1.0 atm as measured by maximal voluntary ventilation and expiratory flow. The present study investigates the same phenomenon in submerged subjects at depth. Five subjects performed maximal voluntary ventilation (MVV) and forced expirations during rest, exercise (50, 125 and 200w) and CO_2-air inhalation while being submerged at pressures of 1.45, 2.8 and 4.6 atm. Spontaneous ventilation during maximal exercise was measured separately. Independent of pressure, MVV increased by about 15% at the heavier workloads and expiratory flow at 40% of vital capacity increased by about 40%, the latter increase disappearing within 2 min after exercise. At 4.6 atm the exercise caused an 8 mm Hg increase in end-tidal CO_2 tension. Carbon dioxide inhalation increasing the end-tidal CO_2 by up to about 20 mm Hg during rest had no effect on MVV and a slight to moderate effect on flow, increasing it by a maximum of 20% at 4.6 atm. As at 1.0 atm, MVV at depth increases with breathing frequency. However, in general, the breathing frequency used by our subjects decreased during exercise. It was concluded that the enhancing effect of exercise on MVV and expiratory flow at depth presumably was mainly due to modified autonomic nervous activity reducing pulmonary flow resistance, that CO_2 accumulation played a negligible role, that passive distension of airways played no role, and that the exercise enhancement of MVV occurred in spite of a possible retarding influence on MVV by the breathing frequencies during exercise. Submersion (i.e. water inertia) did not affect MVV or maximal exercise ventilation. A considerable individual variation in the relation between spontaneous ventilation during maximal exercise and MVV (0.61 - 0.93) could be ascribed mainly to variations in MVV. (Funded by ONR and NAMRL (ONR Contract No. N00014-78-C-0705). *Fellow under the auspices of Norwegian Underwater Institute).

DIFFERENTIAL PERFORMANCE BEHAVIOR AFTER A 40-HOUR COMPRESSION TO 450 MSW. Christian LEMAIRE. Hyperbaric Research Center - COMEX - 13270 - Marseille Cedex 2 - France.

The effects of a 40-hour compression to 450 msw and a 48-hour consecutive sojourn were studied on 8 subjects from a performance point of view. The tests in use were 2 sensorimotor tests (manual dexterity MD and visual choice reaction time VCRT) and 2 intellectual tests (number ordination NO and double figure crossing DFC). The tests were performed always on morning, twice during the pre-dive at 10 msw (oxy-helium ; P_1O_2 = 0.4 bar ; P_1N_2 = 0.8 b ; 46 hours) and twice at 450 msw (oxy-helium ; P_1O_2 = 0.4 b ; P_1N_2 = 2.7 b). The results show an increase in performance between the two series at 10 msw (3 and 4 % for the sensorimotor tests, 7 and 10 % for the mental ones). At arrival at 450 msw, compared to the last series of the pre-dive, a mean decrement is present for all the tests, with values as : 10 % for MD, 6 % for VCRT, 11 % for NO and 2 % for DFC. As noticed during a previous dive (JANUS IV ; 8 subjects ; P_1N_2 = 1.6 b , compression to 400 msw in 24 hours ; LEMAIRE and CHARPY, Rev. Med. Aero. Spat. Med. Sub. Hyp. 16, 1977), recovery is evident 24 hours after the end of the compression, but in a differential mode, total for VCRT, moderate for mental test and negligeable for MD. The common conclusion (7 dives at 400 and 450 msw, with 16 subjects) is that, 24 hours after the end of the compression, vigilance/attention is no more impaired, that mental ability is longer to recover and that manual dexterity doesn't improve during sojourn. These results can differentiate the effects of compression versus pressure. This knowledge constitutes a reliable basis to consider for the consequences on operational capacity of the diver in this range of depth. (Research supported by a DRET grant 79/131).

ADDITIONAL ABSTRACT (NOT PROGRAMED)

HYPERTHERMIC EFFECTS ON OPERATOR PERFORMANCE IN THE ONE-ATMOSPHERE DIVING SYSTEM, JIM. M. D. Curley, A. J. Bachrach, and R. C. Longsworthy. Naval Medical Research Institute, Bethesda, MD. 20014, USA.

This study assessed operator performance of the one atmosphere diving system (JIM) while maneuvering the JIM system in mild (60°F) and warm (90°F) water. The operators were 4 U.S. Navy divers and 1 NOAA diver, all healthy males between 25 and 38 years old, and experienced in the operation of JIM. Testing was conducted at the U.S. Navy Experimental Diving Unit's indoor pool. Each operator completed a minimum of 4 dives under each of the 2 water temperatures. On each dive 3 walks of 50' and 3 sets of step maneuvers were conducted. Task completion times, HR, respiration rates, core and cabin temperatures were recorded. Walk completion times at 30°F ($\bar{x}$ = 68.4 sec) were significantly faster ($p < .01$) than walk times at 20°F ($\bar{x}$ = 71.1 sec); however, heart rates were higher at 20°F during walks ($\bar{x}$ = 135.3 bpm at 30°F ($\bar{x}$ = 127.9, $p < .001$). On the step maneuver, completion times at 30°F were significantly faster ($\bar{x}$ = 60.5 sec, $p < .001$) and heart rates higher ($\bar{x}$ = 151.5, $p < .05$) than completion times ($\bar{x}$ = 99.5 sec) and heart rates ($\bar{x}$ = 145.8) at 20°F. Respiration rates did not vary significantly as a function of task or water temperature. After 40 minutes of working the suit in 90°F water, core temperatures reached as high as 38.5°F, cabin temperatures averaged 92.6°F with high humidity. This data and post dive reports by the operators suggest that the duration of JIM dive operations in warm water may be limited by hyperthermic stress.

MINI-PAPERS

7TH SYMPOSIUM ON UNDERWATER PHYSIOLOGY

In the interest of space, references have been eliminated from the following mini-papers; however, all papers will be printed in full, including references, in the Symposium PROCEEDINGS.

MECHANISM(S) OF CENTRAL OXYGEN TOXICITY: A RE-EVALUATION. H. P. Fatman, R. J. Bolan, D. F. Dodd, John H. Waechter, Richard C. Dirks, K. Kaya and I. A. Zempel. Dept. of Pharmacology and Toxicology, University of Kansas, Lawrence, KS 66045.

Many theories have been proposed to explain the mechanism(s) by which oxygen at high pressure produces convulsions. These include: the oxidation of key enzyme sulfhydryl groups, alterations in the GSH/GSSG redox ratio, lipid peroxide formation, oxidation of pyridine nucleotides and subsequent inhibition of energy metabolism, a decrease in intracellular high energy phosphates, formation of superoxide anion and hydroxy radicals, and formation and accumulation of H_2O_2 in brain cells leading to increased oxidant stress. The maintenance of normal brain γ-aminobutyric acid (GABA), an inhibitory neurotransmitter, also has been suggested to play an important role in oxygen-induced convulsions. In view of the many theories proposed throughout the last 100 years, systematic in-depth studies in the intact animal were undertaken to reexamine the many proposed mechanism(s) of oxygen convulsions.

Mice were exposed to various pressures of 100% oxygen in a modified hyperbaric chamber so constructed that the animals could be sacrificed without the need for chamber decompression, thus eliminating potential decompression effects. Mice were exposed to the oxygen pressures under study for various periods of time, with these exposures reflecting a pre-convulsive period. Mice also were sacrificed at various stages of central oxygen toxicity, such as hyperactivity and seizure onset. These several exposure regimens were chosen in an attempt to correlate any observed biochemical changes with the onset of symptoms of central oxygen toxicity.

After exposure of the mice to the high oxygen pressure for the appropriate time period, the animals were sacrificed, the hyperbaric chamber decompressed, the mice removed, and the cerebral cortex excised. The various biochemical substrates to be studied were then determined.

In mice exposed to 6 atm of 100% oxygen, no changes in cortical ATP, oxidation of non-protein sulfhydryls, reduced glutathione, superoxide dismutase, and lipid peroxide formation were found. Cerebral $NADP^+$ was increased and NADPH decreased, with these changes occurring as soon as the mice were exposed to the 6 atm pressure. No change in cortical NAD was found, although NADH decreased at various oxygen exposure times and stages of central oxygen toxicity investigated. Similar changes in $NADP^+$ and NADPH were found in mice exposed to 1, 4.5 and 6 atm of oxygen, while similar changes in NAD^+ and NADH were found at 4.5 and 6 atm. The CT_{50}'s for 1, 4.5 atm and 6 atm of 100% oxygen were 16 hrs, 102 and 16 min respectively. Therefore, the perturbation of cerebral levels of pyridine nucleotides cannot be correlated with seizure onset.

Cerebral GABA and glutamate decreased as soon as mice were exposed to 6 atm of oxygen. Glutamic acid decarboxylase (GAD) also decreased, but longer exposure times were necessary. Cerebral glutamine increased at the various exposure periods. No correlation between decreased cortical GABA and increased susceptibility to oxygen convulsions was observed. Furthermore, increasing brain GABA by inhibiting GABA transaminase (GABA-T) did not prevent oxygen convulsions. GABA uptake into synaptosomes of cerebral cortex was markedly inhibited by oxygen, but this inhibition could not be correlated with oxygen-induced seizures.

In conclusion, these results from detailed in vivo studies do not support the various theories previously proposed to explain the mechanism(s) of oxygen convulsions. Misconceptions as a result of speculation, and a lack of in-depth studies have propagated erroneous theories in explaining the cause of oxygen convulsions. A new hypothesis is needed. Furthermore, several of the biochemical parameters studied ($NADP^+$/NADPH, NAD^+/NADH, GSH/GSSG, lipid peroxidation, SOD) are not altered in brain but have been reported by others to be altered in lung of animals exposed to non-convulsive oxygen pressures for prolonged periods. It appears that different mechanism(s) may be operative by which oxygen causes central and pulmonary toxicity. (Supported in part by NIH grants HS-07797 and GM-22357, and by ONR Contract Number N00014-75-C-0160.)

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THE CENTRAL ROLE OF AMMONIA IN CNS TOXICITY. R. W. Ramster and A.K. Singh. Kinesiology, Simon Fraser University, Burnaby, B.C., CANADA V5A 1S6.

Ammonia is formed extensively in many tissue during the course of normal metabolism and its rate of formation is considerably increased during abnormal states.

Ammonia is released to blood from muscle in particularly large amounts during exercise (Parnas & Mozolowski, 1927) and by both tetanus and convulsions (Schwartz, Lawrence & Roberts, 1958). In muscle ammonia formation is accompanied by a decrease in the level of total adenine monona leotides (Marrichi & Mommaerts, 1959). Ammonia production from both nerve tissue and brain slices by electrical stimulation is well documented (Weil-Malherbe, 1967; Viba, 1957).

Lowenstein (1967) has observed that the amount of ammonia formed by brain slices during electrical stimulation greatly exceeds the amount that could be formed by deamination of adenine nucleotides alone, and that an additional probable source is deamination of amino acids via α-ketoglutarate or glutamate.

Iverson and Simonsen (1970) have reported the normal rate of synthesis of norepinephrine stores in rat brain stem and cerebellum to be 0.100 μg·g^{-1}·hr^{-1} which is elevated to 0.152 μg·g^{-1}·hr^{-1} after electroconvulsive shock treatment.

Schildkraut, et al, (1966) have also observed an increased turnover of brain catecholamines mediated by lithium ions and oxygen exposure. They proposed an enhanced intraneuronal discharge and deamination of catecholamines to account for this observation. This implication of brain catecholamines as a definite source of brain ammonia during periods of intense oxidative activity is important.

The experiments reported here have been to investigate:

1) the time course of change in the concentration of GABA, Ammonia, glutamate, glutamine, adrenaline and norepinephrine in oxygen toxicity,
2) catecholamines as a potential source of ammonia brain, exposures to high oxygen pressure (HBO),

MATERIALS AND METHODS

Animal Groups

a) Time course of brain and blood metabolites during hyperoxia. Groups of rats (n=5) were allocated to control and oxygen exposure up to the production of convulsive activity. Blood and brain samples were taken for analysis of γ amino butyric acid (brain only) ammonia, adrenaline and noradrenaline glutamate and glutamine.

b) Catecholamines as a potential ammonia source during oxygen exposure. Groups of rats (n=5) were exposed to control and high pressure oxygen after drug treatment with 6-hydroxy dopamine, hexamethonium, α-methyl-p-tyrosine or adrenalectomy respectively to alter the concentration of catecholamines in the blood or brain. Ammonia, glutamate, glutamine, γamino butyric acid (brain only), adrenaline and noradrenaline concentrations were measured in blood and brain tissues of both control and oxygen convulsed animals. Oxygen exposure of the animals and the preparation of brain and blood tissue for analysis was carried out as described previously (Bannister et al, 1976).

Biochemical Analysis:

Catecholamines. Blood samples were centrifuged with S-adenosyl-L-(methyl-^{3}H) methionine and COMT for 1 h as described by Passon and Peuler (1973). After incubation, the metanephrines were separated by TLC, extracted by toluene, and the radioactivity was determined in each fraction (Passon and Peuler, 1973).

Brain Catecholamines. Brain samples were homogenized with cold 0.2N perchloric acid (1:4, v/w) and centrifuged. The pH of the supernatant was adjusted to 7.5 and 0.1 mL was used for estimating A and NA as described by Passon and Peuler (1973).

Blood and Brain Ammonia and Amino Acids:

Blood. Serum was separated from the blood by centrifugation after allowing clotting and an equal amount of citrate buffer was added and the solution was kept at room temperature for 30 minutes. Protein was precipitated with 80% ethanol and free amino acids extracted twice. Alcohol was removed from the final extract by evaporation on a water-bath at 50°C and amino acids in 0.05-0.1 ml of the residue were analyzed by the procedure of Benson, Gordon & Patterson (1967).

Brain. After exsanguination, the brain was quickly removed, weighed and kept cold. It was homogenized in 5 ml phosphate buffer (pH 7.5). The homogenate was centrifuged for 15 minutes (2,000 g) and the supernatant removed. It was deproteinized and amino acids extracted twice with 80% ethanol. Alcohol was removed from the final extract by evaporating on a water-bath at 50°C. Amino acids in 0.05 -0.1 ml of the final residue were analyzed as previously described for blood.

RESULTS

Table 1 shows the time course of change in concentration of brain tissue concentrations of GABA, ammonia, glutamate, glutamine, noradrenaline and adrenaline during high pressure oxygen exposure. It is apparent in normal animals that there is relatively little change in the major fraction of brain catecholamines (only adrenaline changes significantly). However, a significant increase occurs in brain ammonia and GABA is significantly depleted. We have previously observed (Bannister & Singh, 1979) that noradrenaline, adrenaline and ammonia concentrations all increase significantly in the blood until convulsions occur during hyperoxia.

Table 2 shows the effect of various procedures which interfere with catecholamine concentration in the brain and blood.

The effect of 6-OH dopamine is to produce a chemical sympathectomy by replacing NA in the vesicles of the nerve endings. Adrenalectomy effectively removes the circulating catecholamines from the adrenal medulla. Hexamethonium acts on the acetylcholine receptor site at the pre/post synapse to interfere with catecholamine release in the post ganglionic fibre. α-methyl-p-tyrosine inhibits tyrosine hydroxylase an essential enzyme in the synthesis of catecholamine in the brain.

Adrenalectomy and hexamethonium both reduce circulating catecholamines in rats and despite a large variability brain NA and A seemed to accumulate more in these animals than in groups treated with other drugs. The point of convulsion in adrenalectomized and hexamethonium treated animals was considerably delayed although the final concentration of all the metabolites studied did not vary significantly, in these groups, from the others. 6-OH dopamine reduced, significantly, the catecholamine concentration of the brain in the pre-oxygen exposure condition concomitantly brain ammonia was significantly elevated and GABA significantly depleted. Convulsive latency during oxygen exposure under these conditions was considerably abbreviated. α-methyl-p-tyrosine caused a significant depletion in brain catecholamines in the control state prior to oxygen exposure and 6OH treatment produced a further significant depletion but convulsion latency remained unaltered from that of the undrugged control animal. The general effect of producing a depletion of catecholamines in the brain or blood by enhancing their release, and hence catabolism, rather than by preventing their release (i.e. adrenalectomy or hexamethonium treatment) is to increase brain and blood ammonia, decrease brain GABA, increase glutamine asparagine and decrease glutamate.

DISCUSSION

The catecholamines have long been implicated in toxicity resulting from oxygen at high pressures (Bean 1961). It may be the facility with which, catecholamines and, more generally, ATP and some amino acids, become deaminated forming toxic ammonia that finally determines the convulsive state. Glutamic acid seems to lie at the centre of a wealth of events leading to the induction of convulsions. Quastel (1974) has designated glutamate, glutamine and GABA as forming a glutamate system one of whose function is to exert a buffer action for ammonia converting the dicarboxylic amino acid glutamate to its amide glutamine. It is the preferential use of glutamate in this action rather than in its role as a precursor for GABA which may precipitate convulsive activity when ammonia production is excessive. GABA is a CNS depressant and a putative inhibitory neuro transmitter in the peripheral nervous system. There is evidence (Berl et al, 1965a & Berl et al, 1965b) for the buffering of induced ammonia directly by CO_2 fixation system which would spare glutamic acid in its buffering capacity within the glutamate system. Whether the demands made on an CO_2 fixation system for the direct buffering of ammonia are sufficient when ammonia production becomes excessive remains uncertain. Certainly, direct buffering of brain ammonia would spare the conversion of α-keto-glutarate by transamination and preserve the integrity of the Krebs cycle to support ATP production. Collaborative evidence for CO_2 fixation and information on the

adequacy of the pathway when ammonia production increases has recently been presented by Wayne et al. (1978). During hypercapnia these authors observed that brain glutamine and GABA increased and glutamine and aspartic acids decreased. Hypercapnia also stimulated ammonia formation but brain ammonia did not increase in the first hour of hypercapnia since CO_2 fixation and amidation sufficed to buffer it. Glutamate concentrations naturally would first have to increase to initiate the buffer action and early in the hypercapnic period one might assume that an enhanced GABA formation would also occur as was indeed observed. When ammonia production became too great to be buffered by a balancing CO_2 fixation then glutamic and aspartic acid concentrations declined. Thus limitations in the capacity of the CO_2 fixing systems provide an explanation for the above observations.

Figure 1 illustrates the multi demands placed upon glutamate concentrations during hyperoxia; as a potential deviator of α-keto-glutarate from the Krebs cycle, as a component of the γglutamyl cycle (Meister, 1973) producing glutathione for amino acid transport and free radical scavenging, as precursor in the formation of GABA a neuronal depressant and emphasizes the complex hierarchy of events leading to convulsive action within which ammonia and glutamate occupy central roles.

In the experiments described here where ever experimental manipulation of the animals has been able to attenuate the production of ammonia from oxidative deamination of brain and circulating catecholamines convulsive activity has been delayed. Figure 1 depicts the possible inter relationship of the events described above and attempts to rationalize the phenomenon of convulsive activity in hyperoxia.

References will appear in PROCEEDINGS, Table 1, 2 and Figure 1 follow.

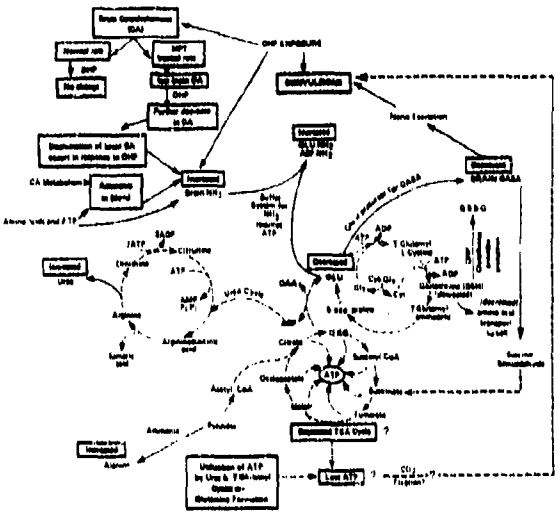

Fig. . Contributing effects of Catecholamine deamination, and ammonia to convulsive activity in hyperoxic states.

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TABLE 1. BRAIN GABA (μmole/gt), AMMONIA (μg/gt), GLUTAMATE (μmole/gt), GLUTAMINE (μmole/gt) AND NORADRENALINE (ng/gt) IN NORMAL RATS EXPOSED TO 6ATA FOR DIFFERENT TIME INTERVALS.

	Control	10 min	15 min	20 min	25 min	30 min	Convulsions
GABA	1.15 ±12	1.46 ±.15	1.04 ±.16	0.93 ±.05	0.89 ±.05	0.59* ±.06	0.75* 10
NH_3	5.25 ±.72	5.42 ±.51	5.72 ±.50	9.09* ±.80	10.20* ±.8*	14.18* ±.54	17.23 ±.1
Glu	9.42 ±.64	8.80 ±.60	5.70* ±.00	4.56* ±.51	1.56* 0.6	5.16* 0.62	5.18* .49
Gln NH_3	0.07 ±.0	1.74* ±.25	2.02* ±.21	2.83* ±.20	2.56* ±.15	2.91* ±.52	2.88* ±.5
BS	128.9 5.6	90.1* 10.4	85.0* 10.5	98.1* 5.6	105.9* 12.2	140.6 4.0	121.6 10.1
A	7.2 0.4	0.12* .01	0.56* .03	1.64* .001	1.75 .14	1.87 .58	1.95* .1

*p < .05 when compared with control.

Table 2. Time to Convulsion (min) and Brain GABA (μmole/gt), Ammonia (μg/gt) Glutamate (μmole/gt) noradrenaline (ng/gt) and Adrenaline (ng/gt) levels in normal, 6-Diazo, Aspartate, Monomethionine, α-Methyl-P Tyrosine treated and Adrenalectomized Rats BEFORE AND AFTER CONVULSIONS (±SE Mean) (N=5)

	NORMAL		6-DIAZO		IN VAN DUNKEN		α-Methyl-p-Tyrosine		STARVED		ADRENALECTOMIZED	
	C	CON	C	CON	C	CON	C	CON	C	CON	C	CON
GABA	1.15 ±.11	0.75* .02	.87 .13	.20* .15	1.35 .26	.50* .07	1.20 .10	.29* .10	1.20 .19	.66* .05	1.20 .26	.69* .12
NH_3	5.25 .72	17.8* .90	8.24* .62	19.0* .90	5.5 1.3	18.56* 1.11	4.74 .75	17.35* 2.50	6.81 .52	17.1* 2.5	5.6* .4	16.0* 2.7
Glu	9.42 ±.64	5.18* .44	6.70 .87	4.14* .87	8.04 .89	4.09* .94	8.48 1.77	4.58 2.5	8.37 .83	4.10* .90	7.20 .80	4.40* .60
Gln NH_3 ASP. NH_3	.07 .17	2.88* .73	.20 .22	3.22* .55	.90 .19	2.72* .25	0.66* .11	2.18 .70	.93 .08	3.52 .65	1.38 .30	3.02* .65
NA	120.9 5.6	124.6 29.3	64.80 6.4	91.4 18.6	141.4 31.9	172.6 60.2	98.8* 11.4	56.2* 14.5	139.4 12.0	124.0 9.2	140.8 15.0	121.5 8.8
A	1.22 .10	1.35 .12	0.51* .07	0.41* .10	2.54 .57	1.46 .46	1.28 .45	1.21 .16	1.92 .25	1.15 .10	2.05 .25	2.54 .12
CONVUL SION LATENCY		43.0 3.8		20.6* 6.1		48.0* 9.0		43.0* 8.0		26.4* 5.0		102.8* 5.0

* P < 0.05 when compared with normal control

+ P < 0.05 when compared with corresponding drugged control

CHANGES IN CELL VOLUME FOLLOWING HYPERBARIC EXPOSURE: A MANIFESTATION OF OXYGEN TOXICITY. G. Bentley and D.N. Walder. University of Newcastle upon Tyne, NE1 4LP, UK.

A decrease in blood flow has been observed in rabbit femoral bone marrow during simulated air dives by Bentley and Walder in 1978. It was postulated that an increase in marrow fat cell volume might occur during hyperbaric exposure, and that this, by increasing the resistance to intramedullary blood flow, could account for the observation. To examine this hypothesis experiments have been performed to determine the cell volume distribution in a fat cell suspension and to investigate the effect of exposure of the cells to air and other gas mixtures at pressures above atmospheric.

Simultaneously, a study of the morphological appearance of fat cells, following hyperbaric exposure, has been carried out.

For the purposes of comparison the work was extended to include an investigation of red cell volume.

The suspensions of isolated fat cells were prepared from the epididymal fat pads of adult white rats using a technique described by Smith in 1971. In this procedure, the complete fat pad was removed from one epididymis of a rat and 4 blocks of tissue weighing 300-400 mgm. were excised. This tissue was incubated in Krebs-Ringer bicarbonate buffer containing collagenase. After incubation, the liberated fat cells were separated by centrifugation. This preparation provided both a control and a test suspension.

The control suspension was maintained at 37°C at atmospheric pressure. The test suspension was placed in a thermostatically controlled compression chamber and maintained at 37°C during exposure to compressed air at 3-0 A.T.A. for periods of up to 3 h. At the end of this time an assessment of the volume of the fat cells in the suspensions was made by means of a Coulter counter and channelizer which displayed the result as a volume distribution curve.

By superimposing the volume distribution curve obtained from the test suspension on that of the control, any change in the volume distribution of the fat cell suspension occurring as a result of exposure to compressed air could be detected and the direction of the change determined.

Microscopic examination of both stained and unstained preparations of the fat cell suspensions after exposure to compressed air was carried out by direct microscopy, dark ground and phase contrast techniques.

From the recordings obtained, illustrated in Fig. 1, it can be seen that the volume distribution curve of the cell suspension exposed to compressed air lies to the right of the control suspension. This was found to be the case for cell suspensions exposed to compressed air at pressures ranging from 3-8 A.T.A. for periods of time of 2-3 h. No evidence of gas inclusion in the cell of the test suspensions was seen following the exposure to compressed air when using any of the microscopic techniques. These results indicate that an increase in fat cell volume occurs in vitro as a result of exposure to compressed air.

To elucidate the mechanism of the observed increase in fat cell volume, the separate effects of increased HCO_3^-, PN_2 and pressure was investigated. Because, as was stated by Robinson in 1970, it is probable that colloid osmotic pressure is an important factor in the pathological swelling of cells, the effect of increasing the colloid osmotic pressure of the suspending medium on fat cell volume was investigated. The effect of hyperbaric exposure on red cell volume, in vitro, was also determined.

Using the technique described above, the effect of exposing fat cell suspensions to the following gas environments was determined.

a. Trimix Normal HCO_3^- and PN_2 with He to 6 A.T.A.

b. Oxygen mixture - Normal PO_2 with N_2 to 6 A.T.A.

c. Oxygen 100% at 1 A.T.A.

Subsequently, the effect of increasing the colloid osmotic pressure of the suspending medium was investigated by repeating the experiments with fat cells suspended in Krebs-Ringer bicarbonate buffer containing bovine albumin at concentrations of 2% or 4% w/v.

Finally human venous blood samples, 2.0 ml volume, were placed in heparinised plastic containers, maintained at 37°C and exposed to compressed air at pressures ranging from 3 A.T.A. - 8 A.T.A. for periods from 2-3 h duration. At the end of this time the volume distribution curve of the Red cells was determined, using a technique similar to that described above, and compared with that of a control sample from the same donor kept at atmospheric pressure. The effect on the observed cell volume changes of introducing Lithium ions into the venous blood sample prior to exposure to compressed air was also investigated.

The results of these experiments may be summarised as follows:

1. The volume distribution curves of fat cells exposed to 100% oxygen at 1 A.T.A. were moved to the right when compared with those of control suspensions exposed to air at 1 A.T.A. (Fig. 2).

2. The volume distribution curves of fat cells exposed to high partial pressures of Helium or Nitrogen but with normal PO_2 were unchanged from those of control suspensions exposed to air at 1 A.T.A.

3. When the cells were suspended in a medium of Krebs-Ringer bicarbonate buffer containing albumin 4% w/v, the volume distribution curves of fat cells exposed to compressed air at pressures ranging from 3-8 A.T.A. and also fat cells exposed to 100% oxygen at 1 A.T.A. were to the left of those of control suspensions. No volume change occurred in cells exposed to high partial pressures of helium or nitrogen when suspended in this medium.

4. The volume distribution curves of Red cells exposed to compressed air at pressures in excess of 5.0 A.T.A. were to the right of those of control suspensions. This volume change was found to be prevented or reversed by the presence of Lithium ions in the blood samples.

From these results it is concluded that:

1. Fat cells in suspension increased in volume on exposure to 100% oxygen at 1 A.T.A. This increase in volume was similar to that seen following exposure to compressed air at 4-6 A.T.A.

2. Hyperbaric exposure to gas mixtures of helium or nitrogen containing oxygen at a normal partial pressure had no effect on the volume of fat cells.

3. A decrease in fat cell volume was seen following exposure to both compressed air and 100% oxygen when Albumin 4% w/v had been added to their suspending medium prior to exposure. But, no change in volume of fat cells occurred in a medium containing albumin was produced by hyperbaric exposure to gas mixtures containing nitrogen or helium.

4. Red cells in vitro increase in volume were exposed to compressed air at pressures in excess of 5.0 A.T.A. This volume increase is modified by the presence of Lithium ions in the suspending medium.

Changes in volume of fat cells in vitro have been demonstrated following exposure to both compressed air and 100% oxygen.

The use of the Coulter Counter and Channelizer for measuring the volume distribution of particulate material is widely documented.

Inaccuracies in assessing fat cell volume because of wide size distribution (15-100μ dia. in rat) and individual and tissue differences have been avoided by using a cell suspension prepared from one epididymal fat pad of a given rat to provide both test and control samples.

The increase in fat cell volume following exposure to compressed air appears to result from the high partial pressure of oxygen. Hyperbaric exposures in which the PO_2 remained normal resulted in no change in cell volume, excluding pressure per se as a causal factor.

Decompression were not performed according to tables as it was felt that the dynamics of gas equilibration in these in vitro preparations would not resemble that of perfused tissue. However, as an increase in fat cell volume was demonstrated to occur following exposure to 100% oxygen, requiring no decompression, it is concluded that the increased cell volume observed following exposure to compressed air occurs during the exposure, while at pressure, and results from an increased partial pressure of oxygen.

As the maintainance of a constant volume is a basic function of mammalian cells, this increase in fat cell volume is considered to be a manifestation of oxygen toxicity.

It is now accepted that the mechanism by which cells achieve a constant volume is by the active extrusion of sodium ions to maintain an osmotic gradient across the cell membrane, exactly balancing the colloid osmotic pressure of the intracellular ions. The increase in fat cell volume resulting from exposure to high PO_2 can therefore be explained by postulating a toxic action of oxygen acting at the level of the sodium pump and the reversal of the volume change by the presence of extracellular albumin is then understood.

Controllable evidence has been accumulated implicating the sodium pumping system as a target for oxygen toxicity. The increase in Red cell volume following exposure to compressed air would appear to have a similar basis particularly when considering the observed 'protective' action of Lithium ions in the context of the proposed mechanism of the action of Lithium in C.N.S. toxicity.

In summary, increase in the volume of fat cells exposed to increased partial pressures of oxygen has been demonstrated to occur in vitro. The occurrence of this swelling in the fat cells of bone marrow, which are contained in an inextensible cavity, would account for the decreased blood flow through bone marrow previously demonstrated to occur during exposure to compressed air.

This work is supported by the British Medical Research Council.

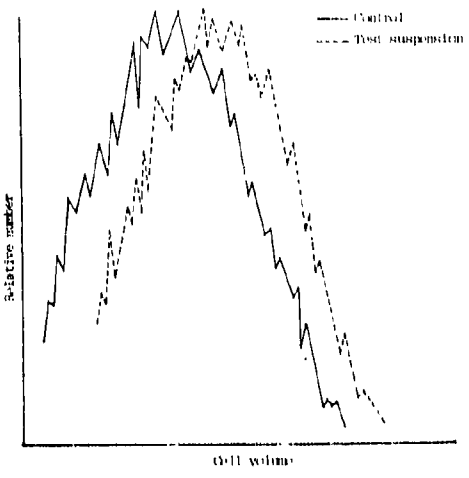

Fig. 1.

Volume distribution curve of a fat cell suspension following exposure to compressed air at 6 A.T.A. compared with control suspension maintained at 1 A.T.A.

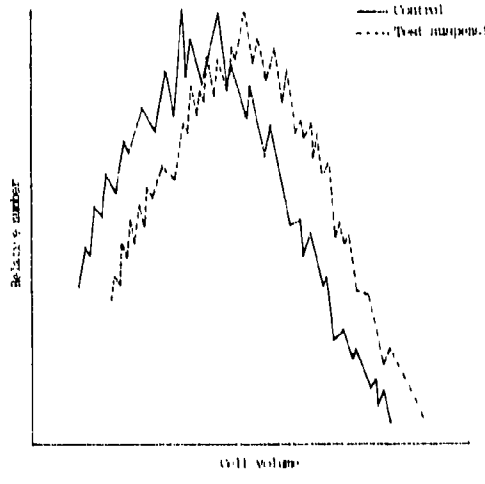

Fig. 2

Volume distribution curve of a fat cell suspension following exposure to 100% oxygen compared with control suspension exposed to atmospheric air.

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LUNG ATP TURNOVER DURING OXIDANT STRESS. A.B. Fisher, Dept. of Physiology, Univ. of Pennsylvania Sch. of Medicine, Philadelphia, PA. 19104

Alteration of energy balance has been postulated as a mechanism for the early manifestations of oxygen toxicity, but this hypothesis has not been tested in the intact lung. In this study, we evaluated the effect of oxidants (hyperbaric oxygen and paraquat) on ATP turnover and tissue energy state using the isolated perfused rat lung model.

Rat lungs were continuously ventilated and perfused with hemoglobin-free artificial medium maintained at 37° and pH 7.4. Rates of production of lactate, pyruvate, and $^{14}CO_2$ were calculated from analysis of samples of perfusate and expired gas during two hours of perfusion. Parallel experiments for $^{14}CO_2$ production were carried out with [U-^{14}C]- or 6-^{14}C]-glucose; $^{14}CO_2$ production was partitioned into that derived from mitochondrial and pentose shunt pathways. ATP turnover was calculated by assuming net generation of 1 mole ATP per mole of lactate or pyruvate produced plus 1/1 mol ATP per mole of mitochondrial CO_2 produced (glycolytic ATP production) and 6 mol ATP per mol mitochondrial CO_2 (mitochondrial ATP production). Adenine nucleotides (ATP, ADP) were measured by enzymatic methods on extracts of lungs that were rapidly frozen at the temperature of liquid nitrogen at the end of one hr of perfusion. Studies were carried out under control conditions (ventilation with air or O_2 at 1 atm) and during ventilation with 95% O_2 or perfusion with 0.2 mM dinitrophenol in order to establish the range of pulmonary response to maximal inhibition or uncoupling

of mitochondrial metabolism. For experimental studies, lungs were perfused with 1.5 mM paraquat or ventilated with O_2 in a hyperbaric chamber pressurized with oxygen at 5 ata. In another series, rats were exposed to 4 ata O_2 for 1 hr and then evaluated for lung energy status under control perfusion conditions.

Control lungs (air ventilation) had a calculated rate of ATP synthesis of 358 umol/hr/g. dry wt. ATP production was 85% by mitochondrial pathways and 15% via glycolysis (Table 1). Tissue adenine nucleotides showed a normally high ATP/ADP (Table 2). During ventilation with 95% CO, there was marked decrease in mitochondrial activity and total ATP synthesis decreased by 54% despite increased glycolytic flux; tissue ATP content and ATP/ADP also decreased markedly. During perfusion with DNP, the "equivalent rate of ATP synthesis" almost tripled while tissue ATP and ATP/ADP decreased. These results provide models for interpretation of effects of oxidants on lung metabolism. Results for lung ATP turnover (Table 1) and adenine nucleotide content (Table 2) were similar to control during ventilation with O_2 at 1 ata. However, during perfusion of lungs in the hyperbaric chamber with O_2 at 5 ata, there was increased glycolytic and mitochondrial ATP production accompanied by a decrease in lung ATP content and ATP/ADP. During the 2nd hour of perfusion in the hyperbaric chamber there was a further increase in ATP turnover. During perfusion with paraquat, changes in lung energy balance was similar to those observed with hyperbaric O_2. Lungs from rats pre-exposed to O_2 at 4 ata and then perfused had normal lung ATP and ATP/ADP levels (Table 2).

These data indicate that the early effects of oxidants (paraquat and hyperbaric oxygen) upon lung metabolism are increased energy requirements that are met by increases in both glycolytic and mitochondrial ATP generation but resulting in depressed lung ATP content. ATP generation under these conditions appears to be responsive to metabolic control mechanisms. Contrary to previous suggestions, exposure to hyperbaric oxygen initially stimulates rather than depresses mitochondrial metabolism, but these effects appear to be rapidly reversible.

TABLE 1

ATP SYNTHESIS OR ITS EQUIVALENT BY ISOLATED RAT LUNGS DURING PERFUSION WITH INHIBITORS OR OXIDANTS

Condition		"ATP synthesis"[a], umol/hr/g dry wt glycolytic[b]	mitochondrial[c]	total	% of control
Control (0.2 ata O_2)	(3)	52	306	358	
CO, 0.95 ata	(4)	124	42	166	46?
DNP, 0.8 mM	(3)	91	966	1057	2952
O_2, 0.95 ata	(7)	94	294	388	97?
PQ, 1.5 mM	(1)	66	432	498	139*
HBO, 5 ata, 1st hr	(1)	101	480	581	1631
HBO, 5 ata, 2nd hr	(1)	157	606	761	2132

Results are mean values for number of experiments indicated in parentheses. Lungs were perfused for 2 hrs with Krebs bicarbonate buffer (pH 7.4) containing 5.5 mM glucose and 3% fatty acid-poor bovine serum albumin. CO = carbon monoxide; DNP = dinitrophenol; PMS = phenazine methosulfate; PQ = paraquat; HBO = hyperbaric oxygen.

[a]Calculated from rate of production of lactate & pyruvate + $1/1\ CO_2$ from mitochondrial oxidation of glucose.

[b]Calculated as 6 X rate of mitochondrial oxidation of glucose to CO_2.

TABLE 2

ADENINE NUCLEOTIDE CONTENT OF ISOLATED RAT LUNGS AFTER 1 HR OF PERFUSION WITH INHIBITORS OR OXIDANTS

Condition	n	ATP umol/g dry wt.	% of Control	ATP/ADP ratio	% of Control
Control (0.2 ata O_2)	8	10.5 ± 0.1		7.9 ± 0.2	
Control for HBO only	5	8.7 ± 0.6		6.2 ± 0.5	
CO, 0.95 ata	4	6.9 ± 0.2	67	2.7 ± 0.7	34
DNP, 0.8 mM	1	9.1 ± 0.2	89	5.0 ± 0.1	64
O_2, 0.95 ata	17	10.5 ± 0.2	100	7.9 ± 0.1	100
PQ, 1.5 mM	4	8.1 ± 0.2	78	5.1 ± 0.1	65
HBO, 5 ata [a]	5	7.2 ± 0.4	81	2.5 ± 0.2	31
HBO pre-exposure [b]	4	9.8 ± 0.6	97	8.0 ± 0.7	101

Results are mean ± SE for n experiments. Perfusion conditions and abbreviations as in Table 1.

[a]Lungs perfused in hyperbaric chamber.

[b]Rats exposed to O_2 at 4 ata for 1 hr, lungs were subsequently perfused under control conditions for 60 mins.

PROTECTION FROM PULMONARY OXYGEN TOXICITY BY TREATMENT WITH LOW DOSES OF BACTERIAL ENDOTOXIN. I. Frank, M.J. Chiang and D. Hussain, The Pulmonary Toxicology Laboratory, V.A. Hospital and the Calvin and Flavia Oak Asthma Research Center, Pulmonary Division, University of Miami School of Medicine, Miami, Florida, U.S.A.

Exposure of adult rats to 95-100% O_2 at one atm. results in severe lung damage and substantial mortality within 72 hrs. It was recently discovered that purified bacterial lipopolysaccharides (endotoxins) from a variety of gram-negative organisms given to rats immediately before and during exposure to >95% O_2 gives a marked degree of protection against O_2-induced lung damage. (J. Clin. Invest. 61:269, 1978)(Survival rate at 72 hrs. = 265/275 (97%) for endotoxin-treated vs. 66/201 (33%) for untreated rats). Since these initial studies we have been concerned with several major questions: 1) will endotoxin given after the onset of exposure to >95% O_2 at one atm. provide protection; 2) will endotoxin provide protection against the more chronic effects of oxygen toxicity; and, 3) what is the mechanism by which endotoxin confers protection?

We have now found that administration of a single dose of endotoxin to rats at zero time (just prior to the start of >95% O_2 exposure) or at 12 or 24 hours after the start of hyperoxic exposure results in nearly 100% survival at the end of 72 hours (Figure 1). A single dose of endotoxin administered after 36 hours of hyperoxia resulted in a 75% survival rate. All these treatment groups had statistically significant increases in survival compared to the 33% survival rate of the rats simultaneously exposed to O_2 but not given endotoxin (p<0.05). Endotoxin given after 48 hours in hyperoxia did not increase survival (35% survival rate)(Figure 1).

In addition to significantly increased survival per se, animals treated with endotoxin have demonstrated a marked reduction in the usual pathological manifestations of O_2 toxicity including pulmonary edema, pleural fluid accumulation (and lung hemorrhage):

Treatment	Survival (%)	Pleural fluid (ml)	Lung wt/Body wt
Air-control	10/10 (100)	.15 ± .06	.507 ± .101
O_2+saline	4/15 (27)*	9.79 ±1.70*	.769 ± .045*
O_2+endotoxin[a]	14/15 (94)	.58 ± .21	.597 ± .077

* p<0.05 compared to other groups.
[a] 500 ng/kg dose just prior to O_2 exposure (72 hrs; >95% O_2).

The degree of protection against experimental O_2 toxicity resulting from endotoxin treatment has been supported by repeated histological studies at both the light microscopic and electron microscopic levels. Whereas lung sections from untreated O_2-exposed rats characteristically demonstrate diffuse perivascular, peribronchiolar, interstitial and intra-alveolar edema & areas of alveolar hemorrhage, the endotoxin-treated O_2-exposed animals show minimal evidence of such O_2-induced alterations except for some focal lung areas with alveolar septal thickening due to edema and/or hypercellularity. In electromicrographs, the endotoxin-treated animal lungs demonstrate a preservation of the pulmonary capillary endothelium, which is disrupted very early during hyperoxic exposure in the untreated animal - initiating the cascade of increased vascular permeability, progressive pulmonary edema (& hemorrhage), and compromise of respiratory function.

It has been shown that the improved tolerance to hyperoxia conferred by endotoxin is associated with an increase in the anti oxidant enzyme superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GP)(J. Appl.Physiol. 47:577, 1979).(A large number of recent studies from many laboratories have established the important role these inherent antioxidant defense systems play in providing potential protection from oxidant lung damage due to hyper-oxidant stresses). We therefore compared the time course of appearance of these lung enzyme activity changes to the development of pulmonary edema in our treated and untreated O_2-exposed animals. We reasoned that if these enzymes were important in protecting the lung against the development of severe pulmonary edema, increased levels should be detected in endotoxin treated O_2-exposed rats before the usual time of development of severe lung edema in O_2-exposed rats not given endotoxin. We found that, indeed, these enzymes (SOD, CAT, GP) increased in activity in the lungs of O_2-exposed endotoxin-treated rats by 36 hours of exposure, 12 hrs. before the onset of detectable increases in lung water in the rats exposed to hyperoxia but not given endotoxin. The enzyme levels continued to increase while lung water remained constant in the treated animals during the rest of the O_2-exposure period. Untreated animals showed no such increases in lung anti oxidant enzyme activity, and progressive edema formation occurred.

We did two additional types of experiments to further explore the role of these enzymes in the protection conferred by endotoxin against the lethality and lung damage produced by >95% O_2 at one atm. First, we treated rats with diethyldithiocarbamate (DDC) which is known to inhibit SOD activity. We found that DDC treatment blocked the rise in SOD in endotoxin-treated rats exposed to hyperoxia and also completely nullified the protective action of endotoxin. Second, we treated mice with endotoxin and found that endotoxin treatment in mice exposed to >95% O_2 at one atm. did not result in any increase in pulmonary antioxidant enzyme activity (SOD, CAT, or GP) and had no protective effect against pulmonary O_2 damage or against the lethal effect of hyperoxia.

We have recently tried some longer-term (7-day) O_2 exposure experiments to try to determine if the protective effect of endotoxin treatment against the acute manifestations of O_2 toxicity would be maintained over a longer period of hyperoxic challenge and if treatment would also affect some degree of protection from the more chronic changes seen in the lungs of animals that do manage to survive prolonged >95% O_2 exposure.

RESULTS OF 7 DAY EXPOSURE TO >95% O_2

Treatment	Survival (%)
Air control	14/14 (100)
O2+saline	5/30 (14)*
O_2+endotoxin (as) grouped	40/42 (95)
endotoxin 1 dose (500 ng.kg)	27/28 (96)
endotoxin 2 doses	5/5 (100)
endotoxin 1 dose	12/11 (92)

* p<0.05 compared to all other treatment groups.

After the surviving animals from these experiments were maintained in room air for a 6-week recovery period, special stains for fibrotic lung changes revealed a much reduced deposition of collagen and reticular fibers in the O_2-exposed endotoxin-treated rats compared to the increased fibrosis demonstrable in the untreated O_2-exposed survivors. Analysis for lung hydroxyproline content gave supportive biochemical evidence for a reduction in chronic lung changes (fibrosis) in the endotoxin-treated animals.

We have further explored the biochemical basis by which endotoxin confers tolerance to hyperoxia by measuring its effect on lung DNA, RNA and the ratio of RNA to DNA. In rats breathing room air endotoxin results in an increase within 24 hrs in total lung DNA and RNA without any change in the RNA/DNA ratio; these findings persist for at least 72 hrs. In rats exposed to >95% O_2 at one atm, but not given endotoxin, there is a smaller rise in total lung DNA and RNA but no change in the RNA/DNA ratio except at 72 hrs. of exposure time in the few rats who survive without endotoxin treatment. In contrast, in O_2-exposed rats given endotoxin, a significant rise in the ratio of RNA to DNA occurs by 48 hrs. of O_2 exposure. This suggests an "activation" of the lung to increased cell division plus biosynthetic activity.

We conclude that 1) endotoxin confers protection against acute O_2 toxicity even when given in a single dose (500 µg/kg or 1/50th LD_{50} dose) as late as 36 hours after the onset of O_2 exposure; 2) the antioxidant enzymes of the lung - SOD, CAT, and GP - play an important role in the protective effect produced by endotoxin; and, 3) endotoxin treatment may protect against the delayed (fibrotic) changes which follow acute pulmonary O_2 damage. We suggest that endotoxin acts as a mitogen in the lung (increased DNA) and that it "activates" lung cells to respond to metabolic perturbation as evidenced by a rise in the ratio of RNA to DNA in the endotoxin-treated O_2-exposed animals (compared to the treated, but unperturbed rats breathing room air). We think it may be this "activation" which facilitates a rapid increase in synthesis of antioxidant enzymes in response to hyperoxic free radical stress in the endotoxin-treated O_2-challenged animal.

Studies to further define the mechanism for the marked protective action of endotoxin against pulmonary O_2 toxicity may hopefully lead to the development of still other agents with similar protective actions but perhaps less toxic potential than endotoxin itself, agents that may be of some future clinical use in helping to circumvent the lung injury associated with prolonged treatment with life-giving O_2.

Acknowledgement. The initial studies with endotoxin were performed in cooperation with Dr. Robert J. Roberts, Depts. of Pharmacology and Pediatrics, University of Iowa School of Medicine, to whom the authors express their appreciation and gratitude.

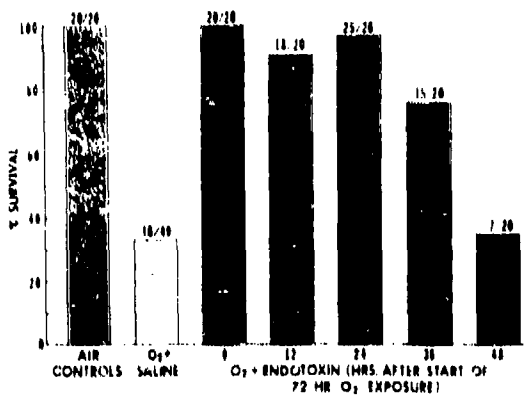

Figure 1. Effect of delayed endotoxin treatment on survival of adult rats exposed to hyperoxia (95-98% O_2, 72 hrs.). Animals were treated with a single 500 µg/kg dose of endotoxin, i.p., either at 0 time (just before being placed in hyperoxia) or at 12, 24, 36 or 48 hours after the onset of O_2 exposure. O_2-control group received equivolume phosphate-buffered saline (PBS) and air-controls received either endotoxin or equivolume PBS at 0 time. Survival rates for O_2-control group and endotoxin groups 0, 12, 24, 36 hours are all significantly greater than O_2-control group survival rate, p<0.05.

EVOLUTION OF PULMONARY DIFFUSING CAPACITY AFTER DEEP SATURATION DIVE WITH HIGH O_2 LEVEL DURING DECOMPRESSION. R.H. Hyacinthe and R. Broussolle.CEBIO B.P 610 83800 Toulon Naval,France.

The beneficial effects of breathing oxygen during a decompression have long been recognized.However it's very little known about the optimal oxygen level for a lung exposure to a high pressure.The calculation of UPTD and the decrement in forced vital capacity are not satisfactory when the PI_{O2} is low and when oxygen is combined with other gases. For long deep saturation dives with heliox it's recommend not to exceed 4 days with P_{O2} of 0,6 ATA in helium.

We studied the evolution of carbon monoxide lung diffusing capacity (DL_{CO}) after two saturation dives. The first one was a 47 ATA Heliox saturation dive with incursion at 501 meters in open sea. The profile of P_{O2} during the 8 days of decompression was a series of decrements function of pressure from 0,8 to 0,4 ATA. The 6 divers breathe suroxygenated mixtures at the end of decompression. The second dive was a 46 ATA trimix saturation simulated dive.The profile of P_{O2} during the 9 days of decompression was an exponential decrement function of pressure from 0,7 to 0,5 ATA. 4 of 8 divers breathe suroxygenated mixtures by cycle of 25/5mn. 1 to 4 times a day during 2 days 48 hr after the start of decompression and the last two days of decompression. We measure DL_{CO} by BATES steady state method on subject at rest and breathing a mixture of 500 PPM of CO in air.

Compared to control measurements, DL_{CO} decreased in all but one subject during the post O_2 measurements obtained 0,5-16 hr after termination of the dive, the range was +9 to -20,9% with a mean decrease for all subjects of 13,4% ($P<0,01$). At the time of follow up measurements determined 5-9 days after termination of the dive, DL_{CO} measured on 17 subjects was below the control values in all but one subject. Compared to control values the mean decrease was 17,9 % with a range between + 10 to - 42 % ($P<0,01$). Two weeks after the termination of the dive DL_{CO} measured on 4 subjects was below the control values in all but one subject. Compared to control values the mean decrease was 10,4% with a range between +1 to -26% .

Six weeks after the termination of the dive DL_{CO} measured on 5 subjects was returning toward normal in all but one subject with a mean increase for all subjects of 1,4% and a range between - 12,7 to + 21,7 %.

The abnormal changes in DL_{CO} two weeks after the termination of the dive indicate changes in pulmonary function which are slowly reversible. For the determination of optimal oxygen level for a long exposure to high pressure it's necessary to consider the exposure pressure,the exposure time and the physiological sensitivity of the divers (cf. bends and pulmonary O_2 toxicity.

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A THEORY OF INERT GAS NARCOSIS. Barry Fowler. York University, 4700 Keele St, Downsview, Ont., Canada.

One approach to the analysis of the behavioral effects of inert gas narcosis is to postulate a disruption of one or more of the various information processing mechanisms which control performance. If a pattern of effects can be established, it is hoped that performance on complex tasks can be predicted. There are a number of studies using either hyperbaric air or N_2O (nitrous oxide) which can be interpreted in terms of this model and which form a coherent pattern.

Narcosis affects the kinesthetic system (Chapman, et al, 1972)but not vision (Biersner, 1972) or audition (Fowler, et al, 1980). Narcosis increased reaction time by a constant amount, irrespective of the number of choices in a card sorting task, (Summerfield, 1964) and irrespective of the size of the stimuli in a visual recognition task (Banks, et al, 1979). On the other hand, in a task where a response was required to previously learned sets of digit pairs, a proportionate increase in reaction time was found as a function of set size (Whitaker and Findley, 1977). Following the reasoning of Sternberg (1969), the lack of an interaction in the card sorting and visual recognition tasks and its presence in the digit response task implicates a narcotic effect on some aspect of memory processing but not stimulus-response or visual processing. Memory and learning deficits have been reported by a number of workers. This evidence has been summarized by Fowler, et al, (1980), who argued that these effects reflect a LTM (long-term memory) input deficit and that STM (short-term memory) is unaffected.

The purpose of this paper is two-fold. First, to report two experiments designed to examine the effects of narcosis on a neglected but important information processing mechanism, attention. Second, to propose a model of narcotic effects on the basis of the current evidence.

In the first experiment twelve subjects were required to remember a list of words presented to one ear alone or with a distracting list in the other ear when breathing either $75\%N_2O$ or air. A recognition paradigm was used to test recall and the results are illustrated in Fig.1. A paradoxical effect is apparent. The distracting list has relatively less effect on recall when breathing the narcotic mixture than with air. There are two likely interpretations for these results. The first is that narcosis leads to a fixation of attention on the to-be-remembered words so that the distracting words have less effect. The second is that the distracting words interrupt memory and narcosis blocks this interruption in some manner. These hypotheses were tested in a second experiment which followed a similar paradigm to the first but included the following conditions: 1) detection of target words in the list during presentation 2) recall of the list after presentation 3) recognition of words after presentation. The results from this experiment suggest that the second explanation rather than the first is the correct one.

The present evidence suggests that, at least up to moderate dose levels, narcosis has a remarkably specific effect on certain mechanisms, namely the kinesthetic memory system and memory, and that other mechanisms remain unaffected. A model which takes these facts into account and which can explain a wide range of narcotic effects involves three assumptions. First, narcosis causes a slowing in the rate of access of a stimulus to LTM. Accessing LTM is a critical mechanism in the information processing model (Schneider and Shiffrin, 1977; Shiffrin and Schneider, 1977), and it can be argued that disruption of this mechanism will lead to an increase in reaction time and slowing on such complex tasks as mental arithmetic. The second assumption is that a consequence of this disruption is a failure of memory trace consolidation. The third assumption is that task errors produced by narcosis are due to a shift in the speed-accuracy criterion (Kantowitz, 1974) rather than the intermittent failure of some processing mechanism.

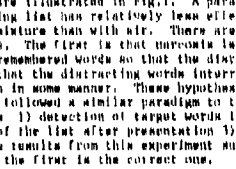

Fig. 1

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ASSESSMENT OF THE HIGH PRESSURE NEUROLOGICAL SYNDROME (HPNS): A NEW METHOD OF MEASURING TREMOR IN AN ANIMAL MODEL. J.A. Baker[1], M.J. Rainey[2], Bridget Yardley-Smith[2] and R.T. Winch[1]. [1]Division of Anaesthesia and [2]Underwater Physiology, Clinical Research Centre, Harrow, Middlesex, England.

One of the signs of HPNS seen in both man and mammals is the onset of tremor. The tremor threshold pressure varies between species (for example, in man it is about 90 ATA, in rats about 60 ATA) but it is a reproducible end-point (Brauer et al, 1976), and a useful parameter for the assessment of the severity of HPNS. The main advantage in using tremor to monitor the progress of HPNS is that it can be measured by a non-invasive technique. This is of particular importance when using an animal model since, if the animal is reasonably free of apparatus, a more realistic situation is likely to be obtained. This paper is concerned with the method we have developed to measure tremor in the rat; detailed results of the pharmacological experiments using this new technique will be presented elsewhere.

In man, there are several satisfactory methods for measuring tremor, both simple and complex, but they all require a degree of motivation. Previous workers have used three main approaches for monitoring tremor in animals: 1) behavioural observation of the animal, 2) electrical recording from implanted electrodes and 3) non-invasive techniques.

Behavioural observation of the animal is essential in any experiment. However, reliable assessment of tremor onset pressure by this method requires one individual making all observations; also it is difficult to assess any small changes in tremor by observation alone.

Invasive techniques in animals such as e.m.g. (de Luca, 1979) can give a reliable estimate of tremor but the necessity for a moderately restrained animal, and the discomfort of implanted electrodes, make it more suitable for use in anaesthetized animals.

Two non-invasive methods have been previously used in animals: 1)magnetic induction, 2) mechanical transducers. The magnetic induction device consisted of a magnet taped to the leg of a small animal which was then placed in a cage over a coil. Movement of the limb would cause the magnetic lines of force to intersect the coil, thereby generating an electro-motive force (Dill, Dorman and Hickey, 1968). This system has been used for tremor measurement in the guinea pig (Ackerman and Gruenau, 1978). However, unless the orientation of the magnet is fixed with respect to the coil, i.e. the animal is severely restrained, quantitative assessment becomes difficult.

The mechanical transducer (Walker, Muskie and MacDonald, 1977) consisted of a small cage on steel springs suspended over a phonograph cartridge. This method was mainly useful for recording onset and duration of tremor, rather than frequency or amplitude.

We are developing a device which we hope will be more versatile. In our experiments we have used rats, but the principle of the system is suitable for any size of animal.

Abdominal respiration detector

Before finalising our present design for tremor measurement, we tried several different methods. The first involved a modified abdominal respiration monitor (Wright, 1977). It consists of a simple pressure transducer: a small plastic cylinder, closed at one end and with a rubber diaphragm on the other end. A flexible tube leads from the cylinder to a variable parallel plate capacitor, which responds to changes in pressure within the cylinder. The transducer was taped either directly onto an anaesthetized rat or beneath a small rat cage. This system gave an excellent signal, but it proved difficult to remove all the artifacts caused by the environmental pressure constantly changing.

Strain gauge

We have now developed a system incorporating a small silicon strain gauge (Entran Pixie Type 8120). It consists of four nylon pillars attached to a perspex base plate and a rectangular metal frame which is mounted on the four pillars. Three nylon straps, attached to the frame, support a small rat cage. The strain gauge itself (1.2 mm x 2 mm) is bonded to a strip of 2" gauge beryllium copper shoot, to reduce fragility, and the assembly is sewn underneath the central support strap.

Initial experiments with extruded aluminium mesh produced a cage which exhibited unacceptable mechanical resonance. The version currently in use consists of netting over a simple, loop shaped wire framework attached to a 4 mm thick rubber base (21 x 8 cm) with a fixed perspex panel at the front and a removable perspex panel at the rear. A V shaped hole is cut in the latter to permit protrusion of the rat's tail. Several turns of 'Elastoplast' prevent the tail being pulled through the hole, and this maintains the rat in a fairly constant position without causing fear or discomfort. With this arrangement we have found only a low level of resonance which can be electronically suppressed.

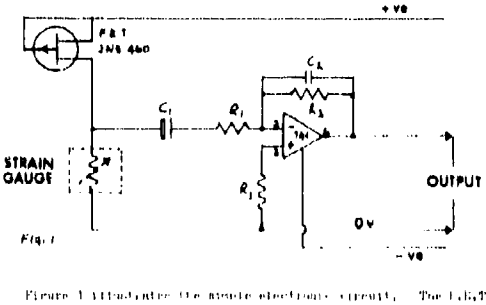

Fig.1

Figure 1 illustrates the strain gauge circuit. The F.E.T. provides a constant current through the strain gauge and the resultant potential difference is amplified by a factor of 10 (determined by R_2) by an operational amplifier (Type 741). Any movement causes the centre of gravity of the rat to shift sufficiently to affect the strain gauge and produce a change in resistance. The resulting change in potential difference is amplified and appears at the output.

Results

We have successfully used the above equipment to monitor tremor and associated rate levels in a pressure chamber (Washington[3], Baker and Rainey, 1977). In addition to recording tremor, the system responds to any pressure movements, respiration and the ballistocardiograph (B.C.G.). However, the B.C.G. is seen only at amplified rate, and it is necessary to filter out the noise introduced by vocal movements. We have found that a low frequency signal can be recorded reliably at pressures as low as 30 ATA. Image noise can be considerable. It is quite difficult to distinguish from the constantly changing frequency signal associated with voluntary movement of the rat, and to exclude artifacts of this nature.

PSYCHOMOTOR PERFORMANCE AND HIGH PRESSURE NERVOUS SYNDROME

The onset of tremor, as the pressure is increased, can be seen as short bursts of tremor, lasting for up to 500 ms occurring every few seconds. These initial spells of tremor establish the frequency of tremor for the individual rat, and we have found it to remain constant (usually between 11-14 Hz). As tremor gets worse, the episodes occur more often, and have a longer duration, until finally tremor is almost continuous.

Once tremor is well established, we have used this method to detect any improvement in HPNS by infusing drugs into the pre-cannulated tail vein of the rat. Improvement in tremor is seen initially as a reduction in amplitude, followed by abolition of the basic tremor frequency.

Summary

Tremor in small animals is an important parameter used in the study of HPNS, but its measurement is very difficult to quantitate. Problems include a) distinguishing it from gross movement (including small convulsions), postural changes, small movements such as "washing", respiration and b.c.g., and the natural resonance of the restraining cage; b) quantitative analysis in terms of both amplitude and frequency; c) the necessity for a non-invasive technique which requires no manual adjustment during the course of a high pressure experiment; d) limitations as to the bulk of the detector; e) independence of other environmental variables such as pressure, temperature and lighting conditions.

We report our findings with a simple strain gauge device specially developed for the purpose, which appears to overcome the majority of the above constraints and which we are now using in the pharmacological studies of HPNS.

References will appear in PROCEEDINGS.

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GENETICS OF VARIABILITY IN SUSCEPTIBILITY TO HPNS TYPE I SEIZURES IN MICE. R. D. McCall and E. Eriksson, Jr. Institute of Marine Biomedical Research, University of North Carolina, Wilmington, North Carolina, USA.

The constellation of phenomena associated with vertebrate CNS hyperexcitability under pressure, generally referred to as the High Pressure Nervous Syndrome (HPNS), has been described from a number of perspectives. To the description we now add an aspect of the problem of genetic variability in susceptibility to the clonic (Type I) seizure phase of the HPNS.

Our interest in the genetics of the Type I seizure stems from four well-documented facts: (1) the seizure is phylogenetically a wide-spread phenomenon; (2) in every vertebrate experimental population there is considerable variability in seizure threshold (and presumably in human also); (3) an individual adult animal's seizure threshold is stable and reproducible over a significant portion of its life span; and (4) the magnitude of the differences in mean seizure threshold among inbred mouse strains compared to variation within each strain is so large as to suggest genetic involvement in the differences. These data suggest a potentially significant contribution to the safety of divers working in the deep sea if one could understand and eventually exploit the biological basis for variability of HPNS susceptibility in human populations: personnel selection based on identification of divers at low risk of developing the life-threatening seizures might have the effect of increasing the safe working depths by 50% without requiring any other new technology.

Inbred mouse strains are the experimental animals of choice because of their manipulability, their amenability to genetic analysis, and their apparent suitability as a mammalian model of the phenomenon. To explore further the nature of the mean threshold strain differences mentioned in (4) above, it was necessary to know whether they reflected a simple, Mendelian inheritance mode or one more complex. The Type I convulsion is manifestly a complex behavioral event, and pedigree data from two inbred mouse strains served to support the hypothesis of complexity. The seemingly 'backcross generations' seizure thresholds were continuously distributed, meaning that the classification of each animal into one or another class could not be made with certainty. Until recently, such a situation was thought to require a "quantitative" or "polygenic" approach to analysis of the distributions. A corollary of that approach was that there could be only a remote possibility that the contribution of a single gene to the expression of the character in question could be identified, on the assumption that the character was determined by the actions of a large number of genes each with small, equal, and (usually) additive effect. However, if the strains are well separated in the character of interest, as the strains we selected are, it is now possible, with pedigree data, to estimate the likelihood of specific genetic models using maximum likelihood methods.

Our approach was to expose individuals of the C57BL/6J and DBA/2J inbred mouse strains, their F1 hybrids, and both backcrosses to compressed air in a helium atmosphere in the manner described in Brauer, et al. (1970). The compression was stepwise in equal 2 atm increments at a rate of 100 atm/hr. The Type I seizure threshold data for parental, F1, and backcross generations are presented in Figure 1.

The variance in the backcrosses compared to an estimate of the common variance in C57BL/6J, DBA/2J, and their F1 hybrid is larger ($F_{BC\ C57BL/6J}$, $p < 0.05$; $F_{BC\ DBA/2J}$, $p < 0.1$) confirming the presence of a genetic contribution to the variation. Maximum likelihood methods were applied to these data to help determine which of eleven selected genetic models best described the observed distributions. The number of models tested do not, of course, exhaust the possibilities but are adequate, we believe, to differentiate the cases involving a simple inheritance pattern from the complex "multifactorial" patterns.

If we write $N(\cdot,\cdot)$ for a normal distribution, with mean $\cdot$ and variance $\cdot$, then in each model we assumed that the C57BL/6J distribution was $N(\cdot,\cdot)$, the DBA/2J distribution was $N(\cdot,\cdot)$, and the F1 distribution was $N(\cdot,\cdot)$. The theoretical backcross distributions varied from model to model, but in each case were assumed to be mixtures of normal distributions. For all the models $\cdot$, $\cdot$, $\cdot$, and $\cdot$ were regarded as unknown parameters to be estimated. First we generated maximum likelihood estimates of the unknown parameters in each model using a computer program which included a sub-program developed for this purpose by Kaplan and Elston (1972), and then obtained the natural logarithms of the models' likelihoods maximized with respect to the various parameters.

Brief descriptions of the models are necessary before one can offer interpretations of the results presented in Table 1. The names assigned to the models are the same as those used by Elston and Stewart (1971) and Stewart and Elston (1973). Models A-10 and A-11 assume a large number of equal and additive unlinked loci acting in concert to produce the backcross distributions which, for DBA/2J is $N(\alpha_1 + \alpha_2)/2, \sigma^2_e)$, where $\sigma^2_e = \sigma^2 + C\,(\alpha_1 - \alpha_2)^2$. Here, C can equal zero (in A-10) or be positive (as in A-11). The backcross distribution for C57BL/6J is similar with substitution of the appropriate subscripts. Model A-2 specifies two additive unlinked loci of equal effect, so that the backcross to C57BL/6J is distributed as $\frac{1}{4}N\,(\mu_{11}, \sigma^2) + \frac{1}{2}N\,(\mu_{12}, \sigma^2) + \frac{1}{4}N\,(\mu_{22}, \sigma^2)$, and the distribution of the backcross to DBA/2J is similar. The model designated A-1 has a theoretical backcross to DBA/2J distribution of $\frac{1}{2}N\,(\mu_{12}, \sigma^2) + \frac{1}{2}N\,(\mu_{22}, \sigma^2)$.

The B- models specify two linked loci with the backcross to C57BL/6J having as its distribution $\frac{1}{2}(1-\lambda)\,N\,(\mu_{11}, \sigma^2) + \frac{1}{2}\lambda\,N\,(\mu_{12}, \sigma^2) + \frac{1}{2}\lambda\,N\,(\mu_{21}, \sigma^2) + \frac{1}{2}(1-\lambda)\,N\,(\mu_{22}, \sigma^2)$ where λ is the recombination fraction or the expected proportion of new genetic combinations unlike the parental combinations of two linked loci produced by crossing over ($0 \leq \lambda \leq 0.5$) and μ_{11} and μ_{22} are the means of the recombinant genotypes. The backcross to DBA/2J is similarly distributed. Model B-A0 had an additivity restriction built into the model, namely that $\mu_{11} + \mu_{21} = \mu_{12} + \mu_{22}$ and $\mu_{12} + \mu_{21} = \mu_{11} + \mu_{22}$. A symmetry restriction was placed on model B-0B, so that $(\mu_{12} - \mu_{21})/(\mu_{11} - \mu_{22}) = (\mu_{12} - \mu_{22})/(\mu_{11} - \mu_{21})$. Neither restriction was in effect for model B-00.

In the C- models, we assume that the experimental distributions result from the expression of one genetic locus of major effect and a large number of interacting loci each with small, equal, and additive effect. The backcross to C57BL/6J is distributed as $\frac{1}{2}N\,(\mu_{12}, \sigma^2_e) + \frac{1}{2}N\,(\mu_{22}, \sigma^2_e)$, and σ^2_e has the same meaning as before. Likewise, C is equal either to zero (as in C-00 and C-A0) or is an unknown positive constant (in C-0C and C-AC) to be estimated along with the means μ_{12} and μ_{22}. Similarly, the backcross DBA/2J is distributed as $\frac{1}{2}N\,(\mu_{21}, \sigma^2_e) + \frac{1}{2}N\,(\mu_{11}, \sigma^2_e)$. In C-A0 and C-AC both the additivity and symmetry restrictions were imposed.

Listed in Table 1 are the $\log_e$ likelihoods for each of the models maximized with respect to the parameters. In the following, we base our interpretations upon the approximate criteria for significance of Stewart and Elston (1971) in which a $\log_e$ likelihood difference between two models of less than 1.0 is considered not significant, between 1.0 and 2.0 is "suggestive but not conclusive", and greater than 2.0 is "probably significant".

On this basis considering their associated $\log_e$ likelihoods, we exclude as candidates for the "preferred" model all except the major locus models C-0C and C-00 and possibly B-00 (two linked loci). We tend to exclude B-00 as well since all non-zero arbitrary initial estimates of the additional parameter λ quickly converged, by iteration, to 0.5, the value of λ at which linkage cannot be distinguished from the case in which the loci are located on different chromosomes. This result implies that the relatively high likelihood of B-00 may be due to unequal effects of the genes and/or some mode of genic interaction other than additivity, because in the latter case B-00 would be equivalent to model A-2 (2 equal, unlinked, additive loci) which is associated with a much lower likelihood. In general, imposition of the additivity restriction resulted in lowering the likelihoods (C-A0, C-AC, and B-A0). Models C-0C and C-00 have the highest likelihoods but, as they differ by only 0.04 $\log_e$ units, are probably indistinguishable.

It should be pointed out that our approach provides no more than a first order approximation to the actual situation and that further breeding tests are required before a "preferred" model can be confirmed. However, the discriminative power of the method is apparent from consideration of the likelihood ratio between the most likely and least likely models which equals $e^{6.8}$, i.e., the C-0C model is about 120 times more likely to be an adequate "explanation" of the data than model A-10.

The major finding of this study is that a single major locus "accounts for" 64% of the difference in mean Type I seizure thresholds between the parental strains both in model C-0C (from $\sigma^2/\frac{1}{2}$ where σ^2 is as before) and C-00 (from $(\mu_{12} - \mu_{22}) + (\mu_{21} - \mu_{11})/(\mu_{12} - \mu_{22})$). This suggests the possibility of (1) identifying critically the major locus involved in terms of its physiological and biochemical actions, and (2) identifying the position of the locus in the mouse genome which may in turn open the way to exploring the effects of the locus upon others involved in Type I seizure etiology and of identifying the loci so affected. Attempts to do both are currently underway. Preliminary results of a test of the model accord well with the major locus hypothesis.

TABLE 1

$\log_e$ LIKELIHOOD OF ELEVEN GENETIC MODELS OF HPNS TYPE I SEIZURE SUSCEPTIBILITY MAXIMIZED WITH RESPECT TO MODEL PARAMETERS

MODEL	DESCRIPTION	$\log_e$ LIKELIHOOD	COMMENTS*
C-0C	One major locus	444.820	$\sigma^2_e = 0.0111$
C-00	One major locus	445.460	
B-00	Two linked loci	445.860	$\lambda = 0.5$
B-0B	Two linked loci	446.706	$\lambda = 0.5$
C-A0	One major locus	446.810	
C-AC	One major locus	447.148	$\sigma^2_e = 0.0$
A-10	Many unlinked loci	447.961	$\sigma^2_e = 0.05119$
B-A0	Two linked loci	448.120	$\lambda = 0.44210$
A-2	Two unlinked loci	448.136	
A-1	Simple Loci	449.525	
A-10	Many unlinked loci	454.596	

*The symbol $\cdot$ indicates "constrained to".

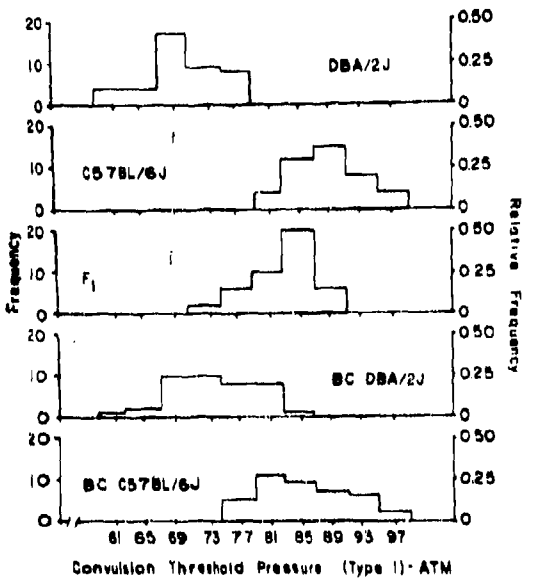

Figure 1 - Frequency distributions of HPNS Type I seizure threshold in DBA/2J, C57BL/6J, F_1 hybrids, backcross to DBA/2J and backcross to C57BL/6J.

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CRITERIA ANALYSIS OF SELECTION FOR DEEP DIVING (EEG AND PERFORMANCE) - J.C. Rostain (1), C. Lemaire, (2) M.C. Gardette-Chauffour, (1) S. Bouquet, (3) H. Naquet (4)

Introduction.

The various dives to saturation by man in a helium-oxygen atmosphere have shown that an inter-individual sensitivity to the HPNS existed (Brauer et al 1969 ; Bennett and Towse 1971 ; Rostain and Naquet 1974, 1978). It would be very important to be able to determine which divers are the most sensitive to hyperbaric conditions during dives equal to or superior to 300 meters and most likely to induce a HPNS.

It has been noticed that it was possible to provoke HPNS symptoms in certain divers during "military" or "excursion" dives (rapid compression in 10-15 mins. to 180 meters ; stay at the bottom not exceeding 105 mins. to avoid saturation). The symptoms occur , generally with a latency of from 30-60 mins. after arrival at the bottom.

It seemed interesting to find out if the divers who showed certain HPNS symptoms at 180 meters in tests of EEG or performance were the same subjects in whom one finds the most marked disturbances during dives to greater depths.

Methods of diver selection.

Twenty-four professional divers (18 COMEX commercial divers and 6 French Navy divers) were put through a series of tests at the surface and at 180 meters. These tests included :

- EEG tests at rest and during intellectual work,
- psychometric tests made up of two sensory-motor tests (manual dexterity and visual choice-reaction time) and two intellectual tests (number ordination and symbol recognition).

The tests were carried out during reference series at the surface, in normoxia, then at the surface with a heliox hypoxic mixture (0,12 bar) and during dives to 180 m. (compression 15 min ; stay . 105 min. ; $He-O_2$ mixture with 6% oxygen).

The subjects were classified according to the evolution of the EEG activities between the surface and 180 meters, the results gathered and processed as previously described (Rostain and Naquet 1974-1978). Three groups were distinguished

Group 0 (6 subjects) - EEG not or slightly modified (less than 20% increase in theta activity).

Group 1 (15 subjects) - EEG significantly modified (between 20% and 100% increase in theta activity).

Group 2 (3 subjects) EEG very modified (theta activity increase beyond 100%)

PSYCHOMOTOR PERFORMANCE AND HIGH PRESSURE NERVOUS SYNDROME

Eight subjects were selected to make the dive to 450 meters, three from groups 0 and 1, and two from group 2. Three of the eight, one from each group, were pressurized to 180 meters with a $He-N_2-O_2$ mixture (N_2 1,9 bar). The eight divers were pressurized at the same time. The EEG tests, measured simultaneously for 8 and the psychomotor tests were carried out during confinement (duration 48 hours He - 0,8 bar ; N_2 - 0,8 bar, O_2 0,4 bar), during compression (duration 30 hours ; progressive introduction of N_2 in the $He-O_2$ mixture until reaching 2,2 bars of N_2 at 450 meters) and finally during the stay at 450 meters (PIO_2 - 400 meters ; T° = 32° ± 2 ; H_2O - 40 to 60%)

Results

1) EEG

a) At arrival at the bottom the EEG modifications found during this dive compared to those obtained during the trial dives to 180 m. give the following observations :

- The two subjects whose recording were the most modified at 180 meters (group 2) are those who showed the greatest increase in theta activity at 450 met. (600% and 1,000%)

- In the two subjects of group 0, the power spectra of the theta activity clearly increases and is located between 100 and 200% ; in the third, it does not vary.

- Two of the subjects of group 1 show only a relative increase in the power spectra of their theta activities (100% and 300%). The third presents very important variations similar to those of the most affected subject of group 2. It is interesting to point out that the power spectra of theta activities of the latter had increased almost 950% during the trial test to 180 meters with an $He-N_2-O_2$ mixture.

b) During the stay at 450 meters the EEG records improve in the same way in all the subjects so that at the end of the stay they remain classified as they were at the time of arrival at the bottom.

2) Psychometer performance.

- The data provided by psychomotor tests show that on the group level, average variations in performance between the surface and 180 m. and between the surface and 450 m. are a function of the depth.

- The same is not true on an individual level the great inter-individual variability at 180 meters is not found at 450 meters where the subjects have a more homogeneous behavior.

- If the subjects are classed not by the difference between two situations, but as a function of their absolute performance in each of the situations, the classification varies little from the surface to 450 meters.

Conclusions

On the basis of the psychomotor tests it is possible to predict that there will be a lessening in the performance of all the subjects. However, there exists such an inter-individual difference at the surface with or without hypoxia and at 180 meters that it is impossible to preclude the behavior of each subject at 450 meters where considerable constraints diminish this variability.

The EEG behavior of a subject at 450 meters can be predicted by a dive to 180 meters made with rapid compression especially if the same respiratory mixture is used. The subjects who present the greatest modifications at 180 meters with the $He-O_2$ mixture will also show the greatest modifications at 450 meters with the $He-N_2-O_2$ mixture. The subjects who present the least modifications at 180 meters with the $He-O_2$ mixture are those who will be most likely to have the least at 450 meters with the $He-N_2-O_2$ mixture, but this is not always true.

The test at 180 meters with the $He-O_2$ mixture is not sufficient ; it would be necessary to have a test at 180 meters with the same mixture as that used at 450 meters. We have seen that a subject could have little modification of his EEG at 180 meters with the $He-O_2$ mixture, and show substantial modifications at the same depth with the $He-N_2-O_2$ mixture, or at 450 meters with the same mixture but using a different mode of compression.

These results added to the already known data reveal once more that the subject reacts differently to pressure as can be seen in their EEG, their clinical symptoms or their performance.

Furthermore, in a given subject, the sensitivity of each of his symptoms may differ according to the mode of compression as well as the gas mixture used or the pressure itself. What remains to be defined in the symptom which would be the most useful in selecting not only the best divers to already - explored depths, especially those between 450 and 600 meters, but also those divers who could reach down to greater depths. It would be tempting to ignore the EEG symptoms if the performance remains good ; however, on the basis of data gathered from patients at great depths, an epileptic seizure can be anticipated and it is possible that the oncoming seizure would be detected only by the EEG signs and thereby prevented in time.

Acknowledgment

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References will appear in PROCEEDINGS.

PSYCHOMOTOR PERFORMANCE AND HIGH PRESSURE NERVOUS SYNDROME

MODIFICATION OF ELECTROPHYSIOLOGICAL SLEEP PATTERN IN THE HYPERBARIC ENVIRONMENT (1ATA, He-N_2-O_2, 14 days, 3 divers)

K.SEKI, H.NAKAYAMA and M.MATSUDA, JAPAN MARINE SCIENCE AND TECHNOLOGY CENTER (JAMSTEC), Laboratory of Human Physiology, 2-15 Natsushima-cho, Yokosuka-shi, 237-Japan.

Three divers (24, 31, 36 years old) forced to live under the He-N_2-O_2 hyperbaric environment, was carried out after 14 days in the hyperbaric chamber (Pre-dive 1ATA air 5 days, Compression: 1 day, 26ATA: 7 days, 31ATA: 7 days, Decompression: 12 days and Post-dive 1ATA air: 4 days). Respective partial pressure of environmental gas was as follows; PO_2=0.4atm, PN_2=0.78atm, PHe=the other. Throughout the sleep, to which EEG, EMG, EOG and ECG were polyraphically recorded everyday and sleep pattern was analyzed (RECHTSCHAFFEN & KALES, 1968). The result was as follows:

1. The ratio of REM time to total sleep time for 3 divers decreased at the first night after the compression from 1ATA to 26ATA (Fig.1), then the total sleep time showed nonsignificant change for control value.
2. The compression from 26ATA to 31ATA did not affect the sleep pattern.
3. At first night after the decompression, the ratio of awake time to total sleep time increased significantly in comparison with control value, 26ATA and 31ATA.
4. With the passing of the experimental days, the ratio of awake time and time of sleep stage (I+II) to total sleep time increased, then the ratio of time of sleep stage (III+IV) to total sleep time decreased (Fig.2).
5. As to the ratio of REM time to total sleep time, there was nonsignificant difference between 1ATA and 26ATA period, and then between 1ATA and 31ATA period the ratio increased significantly in the latter.
6. The sleep cycle fluctuated widely in 26ATA, 31ATA and decompression periods.

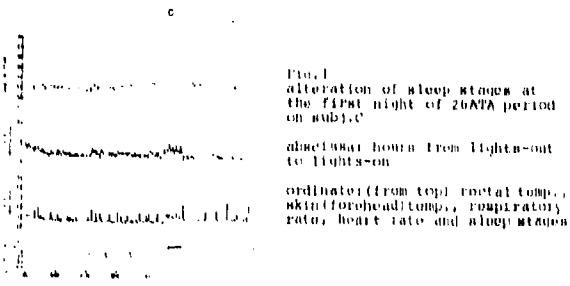

Fig.1
alteration of sleep stages at the first night of 26ATA period on sub).C

abscissa: hours from lights-out to lights-on

ordinate: (from top) rectal temp., skin(forehead)temp., respiratory rate, heart rate and sleep stages

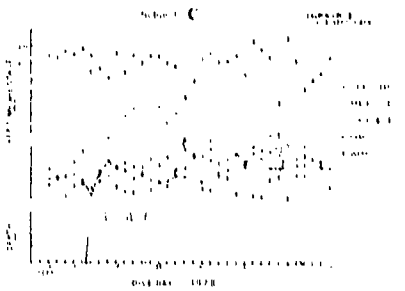

Fig.2
alteration of sleep stages during experimental period (every night) on sub).C

abscissa: days

ordinate: depth, sleep time and stages

CARDIO-RESPIRATORY EFFECTS

INERTANCE AS A FACTOR IN UNEVEN VENTILATION IN DIVING. J. V. Clarke, H. A. Fisher, and M. A. Jaeger. Naval Medical Research Institute, Bethesda, Maryland and Dept. of Physiology, School of Medicine, Univ. of Florida, Gainesville, Florida, U.S.A.

An increase in alveolar arterial P_{O_2} difference (A-a DO_2) was noted a decade ago in three subjects on a chamber dive to 11 ATA (1000 feet) (Overfield et al., 1969). One explanation for the increase, mentioned but not seriously considered at that time, was a redistribution of ventilation resulting from increased gas density. This possibility should now be reconsidered since Fredberg and Mead (1976) have noted that gas inertance at 1 ATA can influence the distribution of flows in parallel but unequal resistance airways.

Two factors contribute to the inertance of the respiratory system: gas density and airway geometry (Mead 1956). Narrowing of airways and increases in gas density increase inertance and thus elevate the respiratory pressures required to breathe. Although Fredberg and Mead (1976), using air at 1 ATA, reported inertance phenomena at frequencies which are not physiological (2 to 10 Hz), the work presented here demonstrates that at high pressure, gas inertance theoretically may result in profound inequalities of ventilation at normal respiratory frequencies and may well have participated in the widening of A-aDO_2 seen by Overfield et al. (1969).

METHODS

Although gas inertance can be measured readily (Mead 1956), the inertial effects of a dense gas cannot be experimentally isolated from the effects on resistance. A theoretical treatment is therefore required. The historical and successful two compartment model of the lung (Otis et al. 1956) has been used to our work, with the addition of inertance for its electrical equivalent, inductance) to a common upper airway and to each of the lower or branch airways. Normal inertance values at 1 ATA were selected from Mead's 1956 paper (0.011 cm H_2O l^{-1} sec^2), yielding at 1000 fsw (31 ATA) a value of about 0.1 cm H_2O l^{-1} sec^2, assuming no change in airway geometry and a 99% He 1% O_2 gas mixture. The ratio of upper airway to lower airway (bronchial) inertance was maintained at 2:1 as suggested by Mead (1956). From the branch and total impedance equations were obtained alveolar pressure, branch and total flows, and compartment volumes. Phase angles between branch flows and between mean alveolar pressure and total flow, as well as ratios of compartmental tidal volumes, were determined for various conditions of branch resistance, lung compliance, respiratory frequency, and inertance.

RESULTS

Although other factors (uneven ventilation at 1 ATA have been normal in the post-dynamic compliance, Otis et al. 1956; phase difference [PD] between alveolar pressure and flow (Dimarco et al. 1976; Rodarte et al. 1976), the ratio of compartmental tidal volumes (V_1 : V_2) was found to be the only consistent indicator of uneven ventilation whenever inertance was elevated (Fig. 1). As respiratory frequency was increased, both V_1 : V_2 (Fig. 2) and the difference in magnitude of branch flows rose to a peak at a frequency corresponding to the resonance frequency (minimum impedance) of the lower airway alveolar.

The resonance frequency could be lowered by increases in either inertance (I) or lung compliance (C). The inertance could in turn be elevated by an increase in gas density or by a reduction of airway cross sectional area. For example, a doubling of either C or I reduced the resonance frequency seen in Fig. 2 from 9% to 6% Hz. For the case where C and I in both compartments were 0.1 l cm H_2O^{-1} and 0.1 cm H_2O l^{-1} sec^2, respectively, at 60 breaths per minute (BPM) V_1:V_2 equalled 2.1 when airway resistance equalled 2.0 and 0.2 cm H_2O l^{-1} sec. To model a constriction of the low resistance airway, we doubled both the resistance and inertance on that side, after which the V_1:V_2 at 60 BPM rose from 2.1 to 6.4; the degree of unevenness almost doubled. At 10 BPM, V_1:V_2 increased by 12% from 1.2 to 1.4.

Elevations of resistance alone under conditions of unequal ventilation also led to increased unevenness as Otis et al. demonstrated in 1956. However, when resistance and inertance were increased in both compartments, as with an increase in gas density, unevenness increased even more. (In general, when gas density increases inertance increases much faster than does resistance.) However, with inertance, an associated increase in dynamic compliance (Mead 1956) causes each lung unit to receive more gas per tidal cycle for a given respiratory effort than if resistance alone had increased. Resistance decreases ventilation for a given pleural pressure swing, whereas inertance increases ventilation.

This change in ventilation mode, of course, affect ventilation perfusion ratios ($\dot{V}/\dot{Q}$) and their scatter. To test this, we added compartmental perfusion to the model; perfusion was matched to the unequal ventilation of each compartment at 1 ATA. When airway resistances were increased threefold and inertance increased tenfold while modeling the dense atmosphere of a deep dive, total ventilation at 60 BPM increased by 65%. The $\dot{V}/\dot{Q}$ ratio which had been originally 1.0 with no scatter, dropped to 0.86 ± 0.86 (mean ± standard deviation) when resistance alone increased. With both resistance and inertance elevated, the $\dot{V}/\dot{Q}$ distribution for this simple case was equal to 1.28 ± 0.96. The increase in disparity (standard deviation) between the compartmental $\dot{V}/\dot{Q}$ ratios is thus another undesirable effect of inertance.

DISCUSSION

The inertance effect described above depends upon the existence of some uneven ventilation at 1 ATA. The greater the unevenness at the surface, the greater will be the ventilation mismatching under pressure. Some unevenness appears unavoidable, however, and apparently contributes to the normal A-aDO_2 found in young, healthy subjects (Overfield et al. 1970). $\dot{V}/\dot{Q}$ distributions and A-aDO_2 are lower, related whenever anatomic shunt and shunted blood saturation (with consistent (Jones et al. 1976; Whipp and Wasserman 1969; Overfield et al. 1970).

Aging increases uneven gas distribution (Buchthal 1954; Chiang et al. 1971), as does smoking. Knudtzon et al. (1976) found that the Pb between mean alveolar pressure and flow at the mouth (a measure of unevenness at 1 ATA) correlates with smoking history. Of Overfield's (1969) subjects, the one exhibiting the greatest widening of A-aDO_2 as pressure increased was also the oldest subject (42 years). Interestingly, a study by Leotant (1966) revealed in seven Navy divers at 1 ATA a $\dot{V}/\dot{Q}$ distribution, percentage shunt, and wide

$A\text{-}aDO_2$ comparable to that found in older nondivers. Smoking histories, unfortunately, were not given.

Pendelluft, a phenomenon where one lung unit fills while another empties, increases physiological dead space, impairs gas exchange (West 1977), and exists whenever compartmental time constants are unequal. Inertance slightly increases Pendelluft at some respiratory frequencies, but reduces it to near zero at the lower airway resonance frequency. This effect may reduce, but certainly not obviate, the consequence of gross ventilation unevenness at that frequency.

Initially, some reports would appear to refute the present theoretical observations. In two studies using either a dense gas at 1 ATA (SF6, Gledhill et al. 1978) or air at 7 ATA (Saltzman et al, 1971), $A\text{-}aDO_2$ was seen to decrease. But, in both studies respiratory frequencies (7 to 25 BPM) were lower than those expected to produce a major inertance effect. Furthermore, there are undoubtedly density-dependent phenomena not included in the model that may aid in gas mixing (Wood et al, 1976). In spite of these reservations, one of Saltzman's (1971) three subjects had no change of $A\text{-}aDO_2$ at 7 ATA, in spite of a decreased respiratory frequency and increased total ventilation, compared to the 1 ATA control (a change that should have minimized $A\text{-}aDO_2$). At 1 ATA this subject had the highest $A\text{-}aDO_2$ (21 mm Hg) and therefore might have been the most susceptible to inertance effects if the $A\text{-}aDO_2$ had been related to a wide $\dot{V}/\dot{Q}$ distribution. The inertance effects presented in this paper may therefore help explain some of the variability seen in other works.

Unfortunately, the present model is limited by the number of respiratory variables that must be considered. Consequently, the degree or even the certainty of an increase in uneven ventilation under pressure cannot be defined. Nevertheless, it is axiomatic that the higher the respiratory frequency the more likely it is that inertance effects will be manifested. That those effects may include the redistribution of flows at currently obtainable pressures has not been previously appreciated. Certainly, any factor that potentially impairs respiration at high pressures, even though the impairment be small, is of concern to diving physiologists, and should be added to the list of factors that nibble away at a diver's respiratory reserve. This study further illustrates yet another example in which respiratory inadequacies may be amplified by pressure.

The ultimate concern of any study of uneven ventilation, $\dot{V}/\dot{Q}$ ratios, or of $A\text{-}aDO_2$, should be arterial oxygenation. Hypoxemia is not usually a concern in diving because of the high inspired P_{O_2}. Nevertheless, Spaur et al. (1977), while noting P_{aO_2}'s in between 120 and 190 mm Hg during rest and exercise at 50 ATA, did observe unexplained swings of both $A\text{-}aDO_2$ and P_{aO_2} as pressure varied. Because it is not known how rapidly P_{aO_2} can deteriorate under increased pressure, monitoring of P_{aO_2} and $A\text{-}aDO_2$ during exercise appears warranted on future deep saturation dives.

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References will appear in PROCEEDINGS.
Figures 1 and 2 follow.

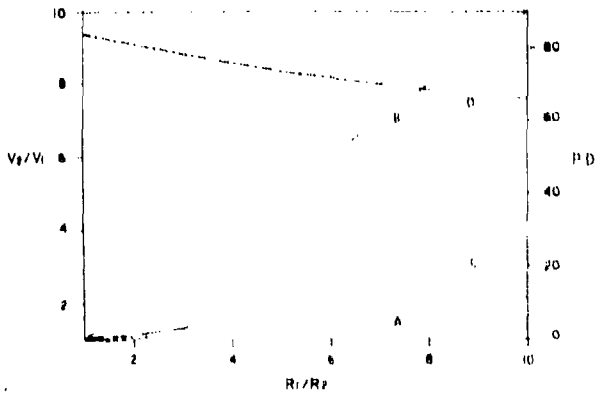

Fig. 1. Changes in compartmental tidal volume ratios (V_A/V_B curves A and B) and phase difference (PD) in degrees between mean alveolar pressure and total flow vs. R_I/R_E as the ratio of branch resistances (R_A/R_B) is changed from 0.2 to 10 sec. Curves A and B are without inertance, C and D with an inertance of 0.1 cm $H_2O\ l^{-1}\ sec^2$.

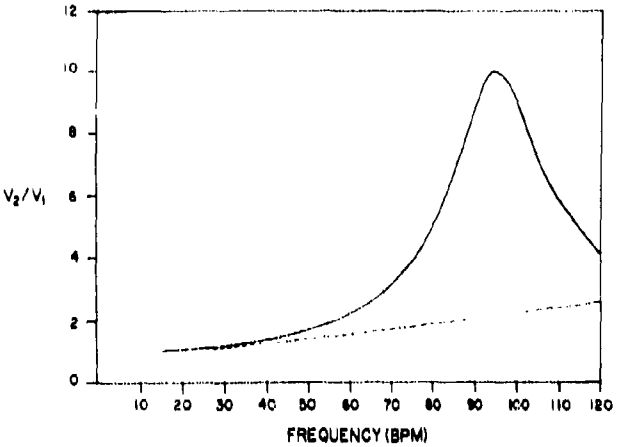

Fig. 2. Ventilation inequalities versus frequency for the case with inertance (solid line) and without inertance (broken line). Branch resistances equal to 2.0 and 0.2 cm $H_2O\ l^{-1}\ sec$, compartment compliances each equal to 0.1 cm $H_2O\ l^{-1}$, upper airway and branch inertances each equal to 0.1 cm $H_2O\ l^{-1}\ sec^2$. Total equivalent inertance = 0.15 cm $H_2O\ l^{-1}\ sec^2$.

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THE ARRHYTHMOGENIC INFLUENCY OF HYDROSTATIC PRESSURE ON CARDIAC CONDUCTION.
T. J. Doubt and P. M. Hogan.* Department of Physiology, State University of New York at Buffalo, Buffalo, New York, U.S.A.

Exposure of humans to hyperbaric environments often produces alterations in cardiac rate and rhythm (1, 2). Elucidation of the mechanisms responsible for these arrhythmias is complicated by the multiplicity of contributing factors; the most notable of these include hydrostatic pressure per se, narcogenic responses, breathing gas composition, and oxygen toxicity.

Evidence has accumulated indicating that hydrostatic pressure alone is capable of disturbing normal cardiac electrical behavior. Pure hydrostatic compression of intact animals (3) and isolated sinus node preparations (4) have demonstrated that this environmental parameter can produce bradycardia and/or cardiac arrhythmias. The latter study also illustrated that hydrostatic pressure was capable of producing a conduction arrhythmia, namely intermittent sino atrial exit block (see I of Table 1).

Subsequent investigations from our laboratory have been directed toward delineating the mechanisms underlying the pressure effects on the electrogenesis process in heart cells. During the course of this investigation various cardiac arrhythmias were encountered that are explicable on the basis of what is now known about the effects of hydrostatic pressure on normal membrane events during the cardiac cycle. The present report details the occurrence of arrhythmias in several types of cardiac tissue, the predisposing factors associated with the arrhythmias, and the probable cellular mechanisms responsible for the development of the aberrant rhythms.

In all experiments, the cardiac tissue were subjected to pure hydrostatic pressure by employing a special steel chamber fitted with a window for the horizontal mounting of the chamber, so that the tissue could be immersed in a continuously superfused physiological solution kept constant at 0.5 ATA, and the temperature maintained within 0.1°C of the selected set point. Further, the tissue was placed at a constant rate using bipolar pulses set at 0.5 Hz rhythm.

Our previous reports (5, 6) have documented the basic effects of hydrostatic pressure on cardiac conduction. Briefly, elevations in pressure up to 100 ATA initially depress conduction velocity. Part of the decrease in velocity can be attributed to reductions in membrane excitability. In addition, slowing of conduction velocity is associated with a reduction in the maximum upstroke velocity (dV_{max}/dt) of the action potential. At the full range of testing pressures, these changes in phase 0 can be anticipated, indicating depressed membrane responsiveness. The refractory period is increased at pressure, an effect due to both depressed membrane excitability and increased action potential duration (APD).

Table I presents the several arrhythmias encountered throughout the test. The arrhythmias are indexed in order of their appearance as pressure is increased. Sino atrial conduction occurred in a typical 4:3 pattern. Further, the arrhythmias were not precipitated by compression, and could be duplicated by decompression from 3 ATA. Rhythm strip recordings of this type could be presented as evidence of an arrhythmia on at least one occasion. The data is shown in Fig. 3, and depicted extracellularly appeared at 100 ATA. The figure shows the action potential traces recorded from a cardiac Purkinje cell at 1 and 100 ATA. The arrows point to the normal stimuli delivered at one pulse/second. The extrasystolic pattern could be eliminated by either an increase in stimulation frequency or by decompression. In view of our findings regarding the effects of pressure on cardiac electrical events, the most plausible explanation for this arrhythmia is recovery of the excitatory process during conditional block and slowed conduction in the Purkinje pathway.

It is evident from Table I that the arrhythmogenic potency of hydrostatic pressure is enhanced when combined with other stressors known to depress conduction. Cooling Purkinje tissue at 1.0 ATA resulted in the occurrence of aberrant conduction in 6 of 11 experiments (see IV in Table I). In two instances a 2:1 conduction block developed that was a function of the APD. At 1.0 ATA reducing the temperature by 2°C lengthened the action potential beyond the basic cycle length (CL). Thus, the recovery of excitability in the preparation was incomplete at the time the next pulse was delivered to the tissue (conditional), and blocked responses were elicited during application of alternate Purkinje pulses. Warming the tissue to 36°C restored the APD duration so that it could be paced 1:1 conduction.

In the other two examples of pressure temperature stress an oscillation occurred in the conduction time between the stimulating and recording sites. There was no appreciable variation in APD, suggesting that membrane excitability, not refractoriness *per se*, was alternately diminished at 27°C/150 ATA. Warming the tissues to 30°C abolished the arrhythmia.

Combinations of rate stress and pressure are also potentially arrhythmogenic. As noted in Table I, arrhythmias developed in 25% of the rabbit atria (II) and 50% of dog Purkinje preparations (V), always in conjunction with faster rates and higher pressures. Abnormal atrial conduction appeared as a 3:2 block at 100 ATA when the pacing rate was increased to 200 pulses·min^{-1}. Increasing the pressure demonstrated the additive nature of the rate/pressure stress. At 150 ATA the 3:2 block was evident at a slower rate of 150 pulses·min^{-1}. Increasing the rate to 200 pulses·min^{-1} increased the conduction deficit, resulting in a 2:1 block.

A 2:1 conduction block was also encountered in 2 Purkinje fiber preparations (see V in Table I) subjected to rapid stimulation at 150 ATA. In these fibers the APD of the conducted impulse was markedly longer than the stimulus cycle length (250 msec). Thus the next stimulus was delivered during the relative refractory period of the tissue, and therefore unable to evoke a propagated response. The resultant dropped beat enabled the tissue to recover sufficiently to respond to the subsequent stimulus, establishing the 2:1 conduction pattern.

Other rate and pressure related arrhythmias in Purkinje fibers (V of Table I) were identified by an oscillation in impulse conduction time. In these examples, every other stimulus pulse occurred during the terminal repolarization limb of the preceding Purkinje action potential. The resultant response was initiated from a depolarized level of membrane potential. As a result, this action potential had a reduced V_{max} and conducted more slowly than the preceding response. The APD of the "slow" response was shortened such that repolarization was complete prior to the occurrence of the next stimulus. The next response, originating from the fully polarized membrane, propagated more effectively. Thus, an oscillatory conduction pattern was thereby established.

The present findings offer insight into the arrhythmogenic potency of elevated hydrostatic pressure. High pressure reduces the safety margin for cardiac conduction by depressing excitability, decreasing membrane responsiveness, and prolonging the refractory period. These pressure-induced perturbations may be of sufficient degree, under certain circumstances, to lead to the development of overt arrhythmias.

Decreases in temperature or increases in frequency have an additive effect with pressure to further lower the safety margin for conduction. This fact is evident in the present report, where arrhythmias were encountered at 100-150 ATA when either the temperature was lowered to 27°C or when the stimulus rate exceeded 150 pulses·min^{-1}.

These results may have direct application to diving man. Typically, depth, work load, and hypothermia are three of the primary safety concerns during an open-water dive. Our *in vitro* experiments may simulate the conditions of a diver working in cold water. Our findings suggest that a diver under these conditions may have an increased probability of developing an aberrant cardiac rhythm. Obviously, the occurrence and severity of any arrhythmia will also be dependent on other factors (coronary circulation, humoral factors acting on the heart, etc.) contributing to the decrease in cardiac safety margin. Awareness of the risk factors could enhance the overall safety of manned exploration of the sea.

References will appear in PROCEEDINGS.
Figure 1, Table 1, follow.

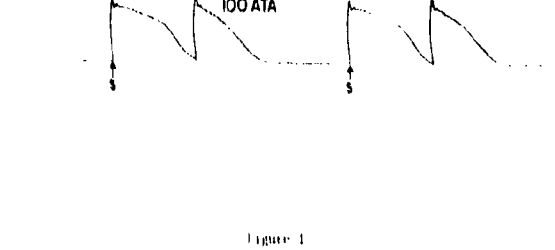

Figure 1

Action potential traces from a canine Purkinje cell at 1 and 100 ATA. Normal stimulus pulses, marked by the arrows, were at a rate of 60 min^{-1}. Coupled extrasystoles appeared upon compression to 100 ATA.

TABLE I

Arrhythmias encountered during hydrostatic compression

	Tissue	Pressure	Temp.	Rate	Arrhythmia	Reference
I	Sinus node	60-150 ATA	23-32°C	-	Bradycardia, Exit block	4
II	Atria (2 of 8)	100-150 ATA	30°C	150-200	3:2 block, 2:1 block	5
III	Purkinje (1)	100 ATA	37°C	60	Coupled extrasystoles	Present report
IV	Purkinje (4 of 11)	150 ATA	27°C	90	2:1 block, Oscillatory conduction	Present report
V	Purkinje (4 of 8)	150 ATA	37°C	240	2:1 block, Oscillatory conduction	Present report

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THE EFFECT OF ALCOHOL ON THE CARDIOVASCULAR ADJUSTMENTS OF THE DIVE REFLEX IN MAN. J. E. Whitney, Jr., J. Fairbanks, S. Rungsiyakul, and R. S. Rozos, Department of Physiology, School of Medicine, University of Minnesota, Duluth, Duluth, Minnesota 55812.

The cardiovascular changes observed when an animal submerges in water (decrease in heart rate and peripheral vasoconstriction) have been defined as the "dive reflex". This reflex is considered oxygen conserving and life preserving in many diving species. Although attenuated, this reflex is present in man. The studies presented here deal with the effect of alcohol consumption on the dive reflex in man.

The subjects for these experiments were healthy male and female volunteers, ranging in age from 20 to 40 years. Heart rate was continuously monitored by a single lead telemetry system and rate calculated over a 5 beat mean or for very slow rates by R-R interval length. Blood pressure (systolic-diastolic) was measured with a semi-automatic pneumatic cuff and stethoscope system. The dive reflex was elicited by submerging the subject's face (up to level of the ears) in cold water (6 ± 1°C). Exerted lung volumes were measured with a spirometer. Blood alcohol levels were estimated by sampling and analyzing end alveolar gas. All subjects were exposed to the experimental set at least once prior to the actual experiment in order to familiarize them with the equipment and experimental design. Prior to the experimental period (control) 4 alcohol consumption) all subjects fasted for 12 hours. The subject was seated comfortably with his head bent forward over a pan of water. The subject took a breath to either vital capacity or some intermediate lung volume (70% vital capacity), and either held that lung volume (as long as possible) in air or submerged in water. The subject then exhaled into a spirometer. Subjects drank the alcoholic beverage of their choice at a controllable rate until a blood alcohol level of 0.1 mg% was achieved. At that point the breath holding maneuvers at both lung volumes in air and water were repeated.

The experiments were designed to study the effects of alcohol on the dive reflex and in light of previous work that indicated that breath holding alone may cause considerable changes in heart rate and blood pressure and that alterations in these parameters may be affected by lung volume at the time of apnea. Heart rate response in air appears minimal whereas in water there is an initial increase in heart rate followed by a fall (representing a 40% decrease from control levels). At vital capacity the heart rate changes in air or water appear are not altered by alcohol ingestion. Water apnea results in an initial rise (15-25 mm) in blood pressure of approximately 25 sec followed by a slow continual rise throughout the period of apnea. Mean blood pressure changes in these experiments were similar in both the both (or control and after alcohol consumption) however, after alcohol consumption the mean blood pressures were approximately 10 mmHg lower than in the control studies. Air apnea is associated with a slow continuous rise in blood pressure.

Results obtained at the intermediate lung volumes differ in the following respects. The initial increase in heart rate seen in water immersion does not appear. Further blood pressure changes in the control experiments show a delayed rise in both air and water apnea. After alcohol ingestion there is no increase in blood pressure following water immersion.

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PULMONARY FUNCTION IN DIVERS. N. J. Cotes and V. J. Cook, Department of Physiology, University of Aberdeen, Aberdeen, AB9 1AS, Scotland.

Divers make up an increasingly important subgroup in the population seen by medical practitioners and there is now a need for evaluation of the "normal" values of important physiological parameters for the diving population. The ventilatory system more than any other is subject to continuous stress during diving and therefore might be expected to show changes related to the diving history of the individual. At medical examination the diver is judged to be fit according to the standards set for the non-diving population and there is growing evidence that these standards are appropriate. Crosby et al published in 1972 data showing that the average forced expiratory volume in one second (FEV_1) for divers is 1% above predicted and the forced vital capacity (FVC) is 10% above predicted when compared with the Kory (1961) prediction nomogram. These authors did not have information which would allow them to relate the changes in lung function to diving history.

227 commercial divers and 54 Royal Navy divers took part in this study. CIBIA forms and the MRC questionnaire on respiratory symptoms were used to obtain information about the diver's medical, diving and smoking histories. The weight, height and age of each diver was recorded. Measurements were made of:- vital capacity (VC), forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) using a Vitalograph and following a strict protocol. The Kamburoff (1973) nomogram was used to give predicted values for FEV_1 and FVC for each diver. This nomogram was chosen because it gives the closest agreement with results from more recent population studies and gives the highest predicted values, the Kory nomogram giving predicted values 8-10% below those currently accepted. Use of the Kamburoff nomogram would thus minimise the effect of diving.

The basic measurements were used to calculate:- FEV_1.FVC; actual to predicted FEV_1 ratio (FEV_1a.FEV_1p); actual to predicted FVC ratio (FVCa.FVCp); the ratio of actual to predicted FEV_1/FVC ($(FEV_1/FVC)a$. $(FEV_1/FVC)p$) and the difference between vital capacity and forced vital capacity (VC - FVC).

The average values for 280 divers gave a significantly high FEV_1, 4.2% higher than predicted; a significantly high FVC, 8.6% higher than predicted; a significantly low FEV_1:FVC, 4% below predicted; P less than 0.001 in all cases. The average FEV_1:FVC was 81.5% and the mean VC-FVC 82 ml.

Further statistical analysis did not demonstrate a significant difference between Royal Navy and commercial divers nor between smokers and non-smokers therefore the 280 were not divided for the more detailed analysis of results.

Linear regression statistics were used to evaluate the influence of age, the influence of the number of years diving and the influence of the maximum depth at which each diver had worked. Divers who had done saturation diving were compared with those who had not.

FEV_1:FVC decreased significantly with increasing age ($P < 0.001$), VC-FVC increased significantly with age ($P < 0.05$) and FVCa.FVCp increased significantly with age ($P < 0.001$). The number of years for which the subject had been a diver correlated significantly with a decrease in FEV_1:FVC and with an increase in VC-FVC. The effect of age and of number of years diving are not independent of each other and further statistical analysis is necessary to separate the two effects.

FVCa/FVCp and FEV_1a.FEV_1p both increase significantly with increased maximum depth to which the subject had dived ($P < 0.01$) and are changed significantly by saturation diving compared with non-saturation diving. In general a diver who has done saturation diving has dived deeper and therefore further analysis is necessary to separate the two effects.

In conclusion diving causes significant changes in FEV_1, FVC and FEV_1:FVC and these changes remain significant when compared with the values predicted from nomograms which allow for the effect of age. It is therefore suggested that the nomograms and prediction equations available from studies on a non-diving population are not appropriate for use in divers. A "divers nomogram" should be used which would automatically bring FEV_1/FVC values into line with those which medical practitioners use for non-diving populations.

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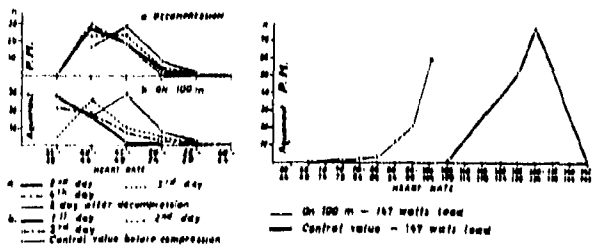
REGULATION AND FREQUENCY OF HEART RATE DURING OPEN-SEA SATURATION DIVING. S. M. Golavić and A. I. Radović, Naval Medical Institute Split and Institute of Aviation Medicine, Zemun, Yugoslavia.

The ECG and instantaneous heart rate of four aquanauts during a four-day open-sea saturation diving at 100 m with excursions down to 120 m were recorded on an eight-channel Beckman biomedical recorder. The histographic analysis of the instantaneous heart rate was obtained by a modified Parin-Baevski method.

During the first 24 hours at 100 m, the heart rate of three of the four aquanauts decreased by 21,14% in relation to the control value. This was accompanied by a marked shift of the instantaneous pulse rate's histographic curve to the left, indicating vagotonia. At this stage, the histographic-curve peaks ranged between 35-39 and 50-54 beats .whereas the control values were between 45-49 and 60-64. During the next three days at 100 m and decompression, the histographic curves were stabilized between the control curve and the histographic curve obtained for the first 24 hours at 100 m. The histographic curves ranged between 40-44 and 55-59 beats per minute and the average heart rate was 9,77% slower than under control conditions. During sleep, the heart rate of all four aquanauts was on the average 13,6% slower than during the day. At 100 m, exercise on an ergocycle under a 147-watt load produced tachycardia in all four aquanauts. However, the tachycardia was on the average 24,5% lower than tachycardia produced by the same physical effort under control conditions. Whereas histographic-curve peaks ranged between 130 and 134 beats per minute under control conditions, under hyperbaric conditions they were between 100 and 104 beats at 100 m and markedly shifting the histographic curve to the left.

The study confirms the findings according to which bradicardia is characteristic for the hyperbaric environment. This bradicardia is the most pronounced during the first 24 hours at 100 m (adaptation) and during sleep. The study also shows vagotonia as characteristic during saturation diving and decompression. However, even under these unnatural conditions the human organism reacts with a regular circard an rhythm - further slowing of the pulse rate during sleep and tachycardia during exercise.



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INFLUENCE OF THE INSPIRATORY EFFORT AND SWALLOWING ON THE CARDIOVASCULAR RESPONSE TO SIMULATED DIVING AND BREATH-HOLDING. T. F. Huang and C.T. Yang*. Dept. Physiol.,Coll.Med.,Nat'l Taiwan U., Taipei, Taiwan, Republic of China.

Characteristic cardiovascular responses to diving is well known. Gandevia et al.(1978) reported that reflex bradycardia during diving is reduced by inspiratory effort against a closed glottis or by swallowing. However their effect on reflex vasoconstriction remains to be clarified.

Twenty-one healthy young men volunteered as the subjects. The subject was seated leaning forward, and held his breath for 30 sec. After a rest for 3-5 min with normal respiration, he repeated breath-holding during which an inspiratory effort against closed airway was made for 5 sec or swallowing was performed. After taking another rest with quiet breathing, his face was immersed for 30 sec in a basin of water at room temperature to simulate diving. He repeated face immersion,during which an inspiratory effort or swallowing was performed. ECG and finger plethysmogram were monitored on a Grass model 7 polygraph.

Heart rate decreased from 77.7±3.9 bpm for control to 66.5±2.9 bpm during diving ($P < .01, n=20$), and from 78.8±2.8 bpm for control to 74.2±3.2 bpm during breath-holding ($P < .01, n=21$). Intervention with inspiratory effort or swallowing during diving or breath-holding attenuated reflex bradycardia,while it did not affect reflex vasoconstriction. After emersion, heart rate recovered and vasodilation occurred.

It was reported that inspiratory effort and swallowing can activate centrally the respiratory neurons and peripherally the intrapulmonary receptor resulting transient tachycardia. However we observed that vasoconstricting response to diving was not affected by these interventions. Our previous study showed that heart rate did not decrease, while vasoconstriction appeared during Valsalva maneuver. Either an increase or decrease in intrathoracic pressure may affect cardiovascular response to diving. Apparently bradycardic and vasoconstricting responses to diving and breath-holding seem to be independent in human subject, while vagotomy or selective destruction of the chemoreceptor abolished both bradycardic and vasoconstricting responses to diving (Huang,1976). It is well known that arrhythmia may develop during diving or breath-holding. However we noted that an intervention with inspiratory effort or swallowing during diving did not induce serious arrhythmia in healthy subjects.

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VENTILATION, PATTERN OF BREATHING AND ACTIVITY OF RESPIRATORY MUSCLES IN AWAKE CATS DURING OXYGEN-HELIUM SIMULATED DIVES. G. Imbert, Y. Lemaire, R. Naïtali, J.C. Bulbal, M. Bugon and J. Grimaud. C.E.R.S. de Physiologie Hyperbare, C.N.R.S Marseille, France.

Respiratory distress has been reported in animals during exposures at high pressures of oxygen-helium and related to the increase in the airways resistance due to dense gas breathing (St Denis et al., 1965; Clouteau, 1971). It has been shown in man that the enhancement of the mechanical work of breathing (i.e. of the activity of respiratory muscles) essentially results from an important limiting effect of the dynamic compression of airways, which counters the expiratory flow rate even during quiet breathing (Vaïsse et al., 1976). This paper deals with the ability of ventilatory muscles to sustain breathing in cats at pressures up to 90 atmospheres of oxygen-helium (900 msw).

METHODS.

Ten cats were used, weighing 2.1 to 3.5 kg and 10 to 18 months old. Myographic potentials were recorded through platinium bipolar electrodes soldered to a stainless, teflon-coated wire. The electrodes were inserted in the cupola of the diaphragm, the external obliquus (abdominal muscle) and in 2 cats, in the intercostal muscles (3rd and 10th spaces). Surgery was performed 10 to 15 days before diving. During the recovery period, the animals were trained to stay in a whole body volume-displacement plethysmographic box. The plethysmographic box was connected to a Knight's spirometer coupled with an angular displacement sensor (Penny and Giles Ltd, AP1 2/1). The response of this system was verified at depths up to 1000 msw, using a ventilatory pump able to work against high pressures. No change was found in the response between sea level and depth within the range of ventilatory frequencies of cats (20 to 90 min^{-1}). The plethysmograph (volume 15 l) containing the animal was fitted into an hyperbaric chamber (volume 165 l), connected to an external life support system which allowed the removal of carbon dioxyde and volatile pollutants. The

oxygen partial pressure (0.20 to 0.45 atm.), the relative humidity (30 to 50 p. cent) and the ambient temperature (30 to 34°C) were automatically monitored. Compression of the chamber was achieved by injecting pure helium. The compression rates were progressively decreased with the depth (100 to 15 msw.hr^{-1}). Steps at varied durations (from 2 to 20 hours) took place at 300, 600 and 900 msw for physiological measurements. The total duration of decompression from 900 msw to sea level was 46 hours, using an exponential curve corrected for the body weight of rats (Gardette et al.,1979). Nine animals survived decompression, thus allowing post-dive ventilatory and electromyographical controls.

RESU

Changes in ventilation and pattern of breathing.

In all rats, an important hyperventilation was observed during compression, associated to lengthening periods of motor excitement. However, high pressures increased minute volume of ventilation ($\dot{V}_E$), this effect being enhanced by high rates of compression. Between 600 and 900 msw, $\dot{V}_E$ could reach 2.7 l BTPS.min^{-1} (i.e. more than 5 times the control value at sea level). This was achieved by increasing the tidal volume (V_T) and the respiratory rate (f_R). During the steps, the animals continued to hyperventilate but breathed more regularly. $\dot{V}_E$ was analyzed in terms of four variables, independant with respect of the control of breathing : f_R, T_I/T_{TOT} (ratio between the duration of inspiration and the total cycle), V_T and V_T/T_I (mean inspiratory flow rate), $\dot{V}_E$ being equal either to the $f_R.V_T$ or the $(V_T/T_I).(T_I/T_{TOT})$ product (Milic-Emili and Grimaldi, 1976). Ventilatory datas from different animals were pooled together with respect to the dive profiles. Figure 1 collects the relative changes in ventilatory variables measured during steps in 4 rats compressed from 0 to 900 msw over a period of 16 hours, using an identical compression procedure. Twenty successive breaths were taken into account for each animal and for each conditions. The changes are expressed in percentages of the reference values obtained at sea level. Significant changes in each variable between steps were assessed in each animal by a Student's t test. From 300 msw, significant ($2p \leq 0.05$) increases in V_T and V_T/T_I occured. No significant change, neither in f_R nor in T_I/T_{TOT} could be observed up to 900 msw.

Changes in activities of respiratory muscles.

Inspiratory activities. As shown in (fig. 2), an important increase in the integrated E.M.G. of the cupolae of the diaphragm was observed. This increase in recruitment of motor units appeared from 600 msw onwards and was associated with the disappearance of the post-inspiratory discharge. The normal diaphragmatic pattern was recovered when animals were returned to sea level.

Expiratory activities. During compression also from 300 msw onwards, an expiratory abdominal activity was observed. This active expiration occured for all cycles and continued when the compression was stopped. It disappeared during decompression at about 200 msw. In one animal, transient expiratory activities were observed at very high pressures (800 msw) in internal intercostal muscles (10th space). This activity was associated with sudden bursts of activity in the diaphragm during the expiratory phase.

DISCUSSION

The changes in activity of respiratory muscles seemed essentially to result from an increase in the airways resistance due to dense gas breathing. A control experiment was performed at sea level. The cat were on oronasal mask connected to a two-ways valve allowing the addition of a resistive load either to the inspiratory or the expiratory limb. When the resistive load was added to the inspiratory side, the activity of the diaphragm increased without change in the post-inspiratory activity. When the resistive load was added to the expiratory side, the post-inspiratory activity of the diaphragm, which normally counters the expiration, was greatly reduced. However, changes in the pattern of breathing did not seem to depend only on increased airways resistances. The T_I/T_{TOT} relationship and f_R did not vary. The reflex control of the ventilatory timing from bronchopulmonary afferences (Clark and Von Euler,1972; Winning and Widdicombe, 1976) appeared to be unaltered despite the increase in gas density. On the other hand, increases in V_T and V_T/T_I are principally observed at sea level when chemoreceptors are stimulated either by hypoxemia (Jannes et al.,1979) or by hypocapnia (Grimaldi et al. 1981). An increase in energy expenditure associated to an impairment of pulmonary gas exchange, as suggested by Ghoutwa (1971), could possibly explain an increase in the respiratory control output.

ACKNOWLEDGEMENTS

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References will appear in PROCEEDINGS.
Figures follow.

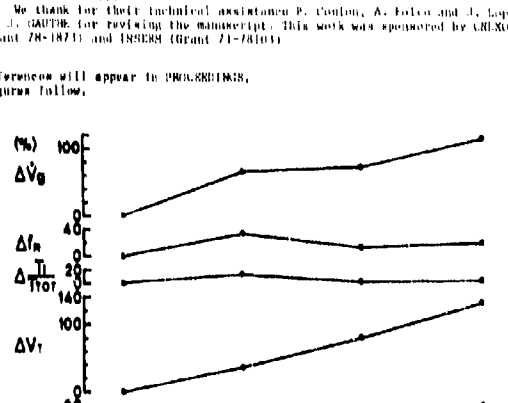

Figure 1.

Relative changes in ventilatory variables measured in 4 rats exposed to high pressures of oxygen-helium and using an identical compression procedure, during steps at 300, 600 and 900 msw. Minute volume of ventilation ($\dot{V}_E$), respiratory frequency (f_R), ratio between durations of inspiration and the total breath (T_I/T_{TOT}), tidal volume (V_T) and mean inspiratory flow rate as function of depth.

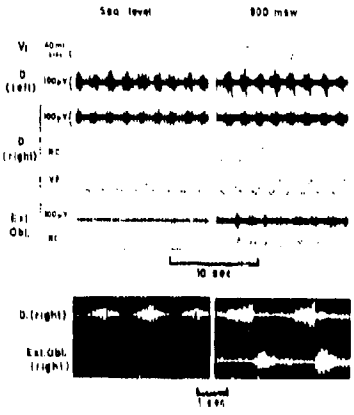

Figure 2.

The upper part shows simultaneous graphic recordings of the spirogram ($\dot{V}_E$), electrical activities of the right and left cupolae of the diaphragm (D) and an abdominal muscle (Ext. Obl.) obtained in an awake cat at sea level, then during a step at 900 msw. The muscular activities were processed by a resistance-capacitance integrator (RC) and by a voltage-to-frequency integrator (VF). The lower part details patterns of discharge of the right diaphragmatic cupola and right external obliquus.

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PHYSIOLOGICAL RESPONSES TO IMMERSION AT 31 ATA (SEAHAWK IV). H. Matsuda, S. I. Hong, H. Nakayama, H. Arita, Y. C. Lin, J. Claybaugh, C. Lundgren and R. H. Smith. Japan Marine Science and Technology Center, Yokosuka, Japan; State University of New York at Buffalo, Buffalo, New York; U.S.A.; University of Hawaii and Tripler Army Medical Center, Honolulu, Hawaii, U.S.A.; Texas University, Isohara, Japan.

Cardiorespiratory and renal responses to a 2-hour head-out immersion in thermoneutral water (34-35°C) were studied in 4 male divers before (predive control at 1 ATA air), during (2 subjects each on the 7th and 11th day at 31 ATA) and after (postdive control at 1 ATA air) a 14-day dry saturation dive at 31 ATA (SEAHAWK IV), conducted at the Japan Marine Science and Technology Center in July-September, 1979.

Each experiment consisted of three periods: 1 hr pre-immersion, 2 hr immersion, and 1 hr post-immersion. At zero time, the subject emptied his urinary bladder and started the pre-immersion period while seated by the wet pot inside the hyperbaric chamber. The chamber gas temperature was maintained at 28°C at 1 ATA (both pre- and postdive) and 31.5°C at 31 ATA. The skin (at 10 sites) and rectal temperatures were measured every 15 min. An impedance cardiograph was taken at 30 and 40 min, while the minute volume ($\dot{V}_E$), oxygen consumption ($\dot{V}_{O_2}$), and CO_2 output ($\dot{V}_{CO_2}$) were determined at 45-50 min. The vital capacity and its subdivisions were determined at 50 min. At 55 min, a venous blood sample was obtained, followed by a urine collection at 60 min. The subject then entered the wet pot and lowered himself to the neck in a sitting position for 2 hrs during which time the various measurements described above were repeated each hour, except for the skin temperature and venous blood sampling. At the end of 1 hr immersion, the subject came out of the wet pot briefly to urinate and then reimmersed immediately. At the end of 2 hr immersion, the subject came out of the wet pot and urinated immediately, following which a venous blood sample was obtained. During the 1 hr post-immersion period, all measurements were repeated except for the venous blood sampling. Overall, there were minor variations in the rectal and mean skin temperature during the 4 hr period, but all subjects felt quite comfortable throughout the experiment and there were no consistent changes in $\dot{V}_{O_2}$ as related with these body temperature fluctuations. In fact, the $\dot{V}_{O_2}$ tended to be slightly lower during immersion as compared to the pre- and/or post-immersion periods. These findings indicate the absence of any cold stress.

The vital capacity (VC) (at 1 ATA and 31 ATA) decreased during immersion by approximately 400 ml and remained low throughout the immersion period at both pressures. During post-immersion, the VC returned to the pre-immersion level. Although the expiratory reserve volume (ERV) was higher at 31 ATA as compared to 1 ATA (ATPV-500 ml,BTPS), it decreased upon immersion by approximately 1.1 l(BTPS) at both pressures. The pattern of change of the inspiratory capacity (IC) was essentially opposite to that of the ERV.

The heart rate during pre-immersion was slightly lower at 31 ATA than at 1 ATA but was the same at both pressures during immersion or post-immersion. Although the thoracic impedance ($\Delta Z/Z_0$) decreased by 6% during immersion in all experiments, it was lowest at 1 ATA predive, and increased at 31 ATA and at 1 ATA postdive, in that order. On the other hand, the opposite trend was observed for the calculated stroke index and cardiac index. Although the latter variables increased significantly during immersion, they were highest at 1 ATA predive and decreased at 31 ATA and at 1 ATA postdive, in that order (fig. 1).

As expected, a significant diuresis and natriuresis developed during immersion in all experiments (fig. 2). At 1 ATA predive, the urine flow increased from about 1 ml/min during pre-immersion to 4.3±0.8 (SE) during the first hour and to 6.5±1.6 during the second hour of immersion, and then decreased to 1.4±0.8 ml/min during post-immersion. However, the magnitude of the increase in urine flow during immersion was approximately 8% less at 31 ATA and 60% less at 1 ATA postdive, than at 1 ATA predive. The above increase in urine flow during immersion was accompanied by a marked reduction in urine osmolality. Thus, at 1 ATA predive, the urine became hypotonic (256 mOsm/kg) during the second hour of immersion and then returned to hypertonicity during post-immersion. However, the urine collected during the second hour of immersion was still slightly hypertonic (454 mOsm/kg) at 31 ATA, and was considerably

hypertonic (571 mOsm/Kg) at 1 ATA postdive. It should be pointed out that the pre-immersion urine flow and osmolality were quite comparable in all three experimental condition (i.e., 1 ATA predive, 31 ATA, and 1 ATA postdive). The creatinine clearance did not change during immersion at 1 ATA predive, but tended to decrease at 31 ATA (from 136±12 to 124±10 ml/min) and at 1 ATA postdive (from 145±26 to 111±6 ml/min). It is, therefore, possible that the observed difference in the immersion diuresis is at least in part due to a difference in the glomerular filtration rate.

Despite such a marked difference in the degree of immersion diuresis, there were no differences in the rate of excretion of Na, K, urea, and total osmotic substances under the three experimental conditions. This indicates that the fractional reabsorption of osmotic substances is reduced at 31 ATA and at 1 ATA postdive. Such a maintenance of natriuresis in the face of attenuation of diuresis during immersion at 31 ATA and at 1 ATA postdive led to a significant difference in the free water clearance. The latter value was around +2.0 ml/min during pre-immersion in all experiments, and increased to +1.4, -0.5 and +1.0 ml/min during the second hour of immersion at 1 ATA predive, 31 ATA, and 1 ATA postdive, respectively. This indicates that the free water reabsorption during immersion is less inhibited at 31 ATA and at 1 ATA postdive than at 1 ATA predive. Overall, the magnitude of diuretic response was negatively correlated with urinary ADH excretion ($r = -0.36$; $p<0.025$). However, the natriuresis was not always accompanied by a reduction in urinary aldosterone excretion. Although the underlying mechanisms for this phenomenon can not be proposed, it may be related to the fact that the degree of intrathoracic blood pooling during immersion (as indicated by the thoracic impedance, the stroke index, and the cardiac index) was lower at 31 ATA and 1 ATA postdive than at 1 ATA predive. These findings also suggest that the adequate stimulus for the inhibition of the renin-aldosterone system (which is considered to be primarily responsible for immersion natriuresis) during immersion is different from that for the inhibition of ADH (which is considered to be primarily responsible for immersion diuresis).

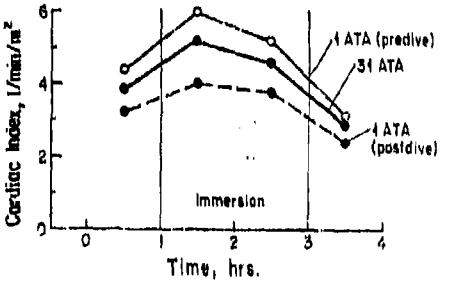

Fig. 1: The effect of head-out immersion on cardiac index at 1 ATA air (pre- and postdive) and 31 ATA He-O2. The cardiac index was calculated from the values of heart rate and stroke volume (derived from the thoracic impedance). Each point represents the mean of 4 subjects.

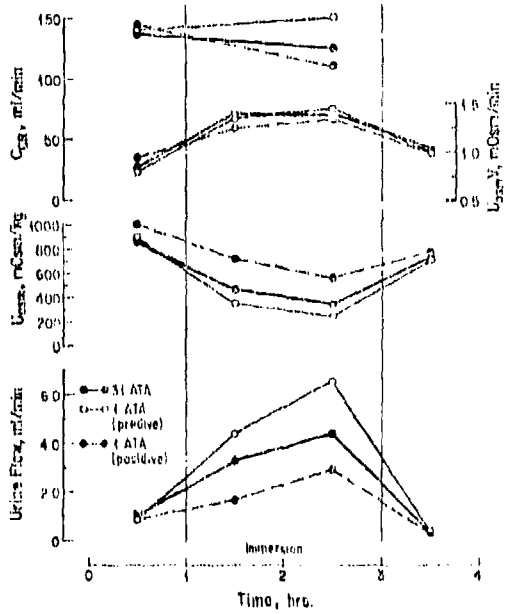

Fig. 2: The effect of head-out immersion on C_{CH_2O}, urine flow, $U_{Na}V$, $U_{K}V$, and $U_{urea}V$ (urinary excretion rate) at 1 ATA air (pre- and postdive) and 31 ATA He-O_2. Each point represents the mean of 4 subjects.

THE EFFECT OF WATER TEMPERATURE ON VITAL CAPACITY DURING HEAD-OUT IMMERSION. David J. Kurss*, Claes J.G. Lundgren and Arvid J. Pasche. Hyperbaric Research Laboratory, Department of Physiology, State University of New York at Buffalo, Buffalo, New York 14214.

Immersion may reduce the vital capacity(VC).The mechanism has traditionally been ascribed to hydrostatic effects, in particular to intrathoracic blood pooling (3). However, during lung volume measurements in immersed subjects we noticed a tendency for VC to recover during exercise. A reduction in intrathoracic blood pooling secondary to warming and vasodilation in peripheral tissues was a possible mechanism that we considered for explaining this observed increase in VC.

As a way to illuminate this hypothesis we tested the influence of different water temperatures on VC in the immersed condition. Various maneuvers were subsequently performed to manipulate the blood distribution in connection with immersion.

Methods: Between 3 and 9 subjects were studied in different experiments. An upright, sitting position was assumed throughout all procedures. While non-immersed a bathrobe was worn and during head-out immersion the subjects wore swim trunks. Air temperature ranged between 18° and 23°C and water temperature was 20°, 35° or 40°C, controlled within ±0.25°C. In some experiments inflatable tourniquets were placed as proximally as possible on upper arms and thighs. Vital capacity was recorded repetitively at 2.5 minute intervals. When tourniquets were used the desired pressure was achieved within a few seconds. A pressure of 250 torr was used for arterial stasis and 60 and 90 torr (compensating for difference in depth of immersion) was applied on arms and legs, respectively, for venous stasis. The Valsalva maneuver was performed at 80-90% of VC and was immediately followed by a rapid full inspiration and VC measurement.

Results: The results of VC measurements in 9 subjects are shown as normalized mean values ±S.E. in Fig 1. Immediately upon immersion there was a fall in VC to approximately 94% of the pre-immersion value, with no significant difference between any two water temperatures. However, within 2.5 min, and for the remainder of the immersion period, the VC was clearly affected by water temperature. The mean values of all measurements in the latter period are shown for each temperature in Fig 1. As a measure of conservatism in the interpretation of data, mean values of measurements spanning 12.5 min were used for the immersion period. There was no significant difference between pre- and post-immersion VC. The mean VC in 35°C water was 94.6±0.2 of pre-immersion control values ($p < 0.004$). In 20°C water the VC went down from the pre-immersion level to a mean value of 91.1±0.6 ($p < 0.005$). This differed significantly from the 35°C level ($p < 0.025$) as well as from the 40°C level ($p < 0.005$). It may be noted that there was a gradual fall in VC in 20°C water from 92.7±1.5 to 90.5±1.6 ($p < 0.01$). Remarkably, the 40°C VC value at 98.0±0.2 was not significantly different from the pre-immersion result, and it was higher than the 35°C value ($p < 0.025$). While all subjects were comfortable in thermoneutral condition, i.e. 35°C water (cf. 2), some felt overly warm in 40°C water and all felt cold and shivered in 20°C water. In three subjects immersed in 10° water and wearing wet suits there was no subjective feeling of cold and no shivering. Yet, their mean VC went as low as 88.9±1.4. This was close to their VC (89.3±0.5) when wearing swim trunks in 20°C water.

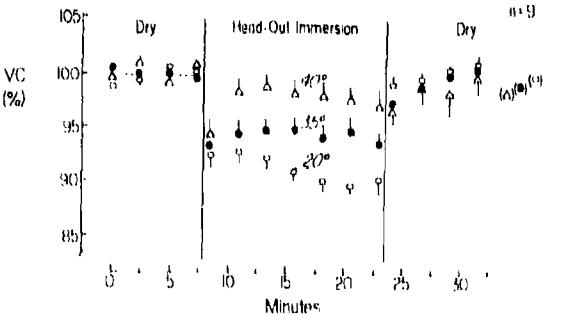

Fig 1 Vital capacity (VC) in the sitting position during pre-immersion (dry) and head-out immersion in water at 20°, 35° and 40°C. Results were normalized to means of pre-immersion value and mean ±S.E. are given for 9 subjects. Dashed lines indicate mean values for measurements obtained during related time span.

As shown in Fig 2, the application of arterial stasis to the extremities before immersion in 20°C water largely prevented the decline in VC otherwise seen. The mean VC in 4 measurements was only reduced to 98.0±0.1 (p = 0.05). However, upon release of the arterial stasis there was a rapid reduction in VC to 92.1±0.5 at the preceding level ($p < 0.005$). After this, venous stasis was applied and the VC values climbed to a mean of 91.6±1.1, which was higher than during the preceding period ($p < 0.01$) and lower than during the pre-immersion control period ($p < 0.01$). Following the release of the venous stasis the VC fell significantly ($p < 0.01$), accepting a value of 86.5±1.1 of pre-immersion control ($p < 0.005$). Taking a Valsalva maneuver allowed the subjects to increase their mean VC to 97.5±2.2 from the preceding 90±1 level ($p < 0.01$). Returning to the non-immersed situation the mean VC increased but, at 96±1.1, did not quite attain the pre-immersion level ($p < 0.05$).

*Authors' names in alphabetical order

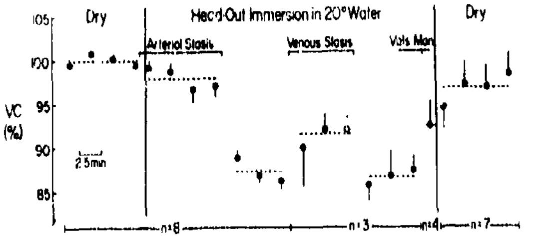

Fig 2 Vital capacity (VC) in the sitting position during non-immersion (dry) and head-out immersion in 20°C water. The effects of periods of arterial and venous occlusions by tourniquets on arms and legs and the Valsalva maneuver are shown. The values are means ±S.E. from 3 to 8 experiments (see abscissa) in 3 or 4 subjects. Dashed lines indicate mean values for measurements obtained during related time span.

Discussion: The immediate 6-7% reduction in VC upon immersion was unaffected by widely differing water temperatures (20°, 35° and 40°C). The lowering of the lungs' capacity to hold air was presumably caused by a sudden redistribution of blood from the peripheral into thoracic vessels. This notion gains some support by the fact that arterial stasis on the extremities prevented this first drop in VC in 20°C water. The slight reduction (2%) in VC despite the use of the arterial stasis may be ascribed to blood movement not prevented by the tourniquets. The VC in water of neutral temperature (35°C) remained at the initial immersion level. The mean reduction of 5.4% is in good agreement with 14 other studies yielding an average VC reduction of 5.1% (cf. 2). It may therefore be concluded that, after the initial redistribution of blood due to hydrostatic immersion effects, there were no further major adjustments in blood distribution in the 35°C water. However, such adjustments apparently occurred in the cool and the warm water. After the initial 7.5% drop in VC in 20°C water there was a further reduction by 2.2% during the immersion period. The nature of this slower change is still open to speculation. In addition to the hydrostatic effect on VC evident in 35°C water there was probably an element of cold vasoconstriction in 20°C water accounting for part of the large, and increasing, drop in VC. The possibility cannot be excluded, however, that some of the VC reduction toward the end of the exposure to 20°C water was caused by lessening of neuro-muscular performance.

The crucial role of blood redistribution for the observed effects is further borne out by the gains in VC achieved by the application of venous stasis and the Valsalva maneuver (4.3% and 6.0%, respectively). The effect of the venous stasis is to allow blood to accumulate distal to the tourniquets. The increased intrathoracic pressure generated by the Valsalva maneuver forces blood out of the chest cavity (cf. 4). After the initial drop in VC by 5.7% upon immersion in 40°C water the VC rapidly recovered almost to the pre-immersion level. In all likelihood this reflected, after the initial increase in intrathoracic blood volume, a redistribution of blood from the chest cavity to peripheral vessels which were subject to thermoregulatory vasodilation.

Remarkably, when the subjects were protected by a wet suit and comfortable in 10°C water the loss in VC was the same (11.1%), and equally rapid, as it was (10.7%) when they were naked and shivering in 20°C water. It is therefore reasonable to conclude that both that part of the intrathoracic blood redistribution which depends on peripheral cold vasoconstriction and that caused by hydrostatic effects of the immersion were of the same magnitude in the two conditions.

It follows from the present observations that when lung volumes are measured during immersion, and possibly non-immersion, the subject's thermal situation should be considered. In addition, to the extent that intrathoracic blood pooling has secondary effects on cardiorespiratory function, e.g. causing air trapping, changes in compliance (2, 5) and cardiac output (1), these effec may also be modified by changes in thermal stress. One should also note that warm water immersion, presumably through peripheral vasodilation, almost completely counteracted the hydrostatic effect evidenced throughout the neutral temperature immersions. This indicates that in high temperatures the external hydrostatic load during immersion may be overcome by intravascular hydrostatic forces. A new piece of evidence is presented demonstrating that physiologically significant cooling may occur in the suited immersed subject in the absence of subjective sensations and shivering (cf. 6).

References and acknowledgements will appear in PROCEEDINGS.

OXYGEN SUFFICIENCY AND UTILIZATION WITHIN THE CELL

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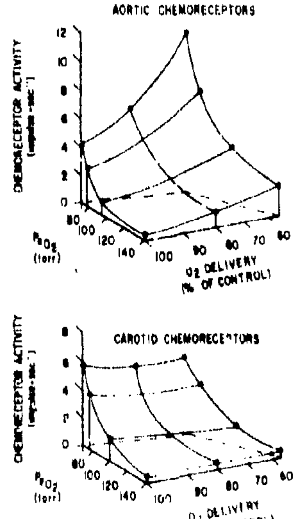

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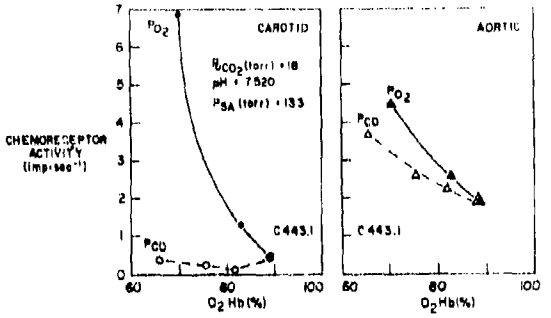

Fig. 5. The relative effects of arterial O_2 delivery on aortic body and carotid body chemoreceptor activity at various levels [illegible]

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HETEROGENEITY OF CAPILLARY DISTRIBUTION AND CAPILLARY CIRCULATION IN MAMMALIAN SKELETAL MUSCLES. E.M. Rankin, S.D. Gray, L.R. Dodd and B.D. Lin, Department of Human Physiology, School of Medicine, Univ. of Calif., Davis, CA 95616, U.S.A.

In evaluating effects of blood flow and arterial oxygen content on tissue oxygen supply, distribution of perfused capillaries is often represented by a mean capillary density or average intercapillary distance, in a field of uniform oxygen utilization capacity per unit mass (3,5,7). The morphology of many mammalian skeletal muscles suggests that neither capillaries nor oxygen utilization capacity are evenly distributed. At least 3 fiber types are present in most muscles: FG (fast glycolytic), FOG (fast oxidative/glycolytic), SO (slow oxidative). These differ in size, contraction velocity, capacity for oxygen uptake and susceptibility to fatigue (1). Capillary supply to FOG and SO fibers is greater per unit fiber area, leading to clustering of capillaries around groups of these fibers in cross-sections of muscle (6). Uneven perfusion of the capillary network may lead to additional inhomogeneities within the diffusion field (4). Unless the distances between perfused capillaries are inversely proportional to the rates of oxygen uptake by intervening muscle cells, efficiency of oxygen transport will be reduced.

Honig and his colleagues measured distances between blood-perfused capillaries of rat gracilis muscles by intravital microscopy. They reported variation from 0.1 to 3 times the mean values (4). The present study seeks to examine systematically, for representative mammalian skeletal muscles, the spatial distribution of (i) all capillaries, and (ii) perfused (or well-perfused) capillaries, and to relate their patterns of distribution to the arrangement of muscle fiber types.

I. Distribution of all capillaries. Lower leg muscles of 2-kg female New Zealand white rabbits were used, because of the clear histochemical distinction between fiber types and staining of all capillaries by reaction for alkaline phosphatase (2). Measurements were made photomicrographically, on matched fields of serial frozen sections, of the following parameters: (i) fractional areas of each fiber type present (ii) mean fiber "diameter" (total area ÷ number of fibers)$^{1/2}$ (iii) capillary density (number of capillaries ÷ total area in mm^2) and (iv) individual and mean intercapillary distances (ICD) within the field. ICD's were measured by drawing lines from each capillary to surrounding capillaries on a tracing of the field, to form a network of closed triangles between all capillary points, with no lines crossing between points. Connecting line lengths were measured with a millimeter scale. An example of such an assay is shown in Fig. 1-I-A.

Measurements were made on 6 mixed muscles: medial and lateral gastrocnemii, sartorius, plantaris, extensor digitorum longus, anterior tibialis, containing from 29 to 69% FG fibers, 24 to 57% FOG, and 0 to 19% SO, and on the soleus muscle, 89-100% SO fibers, 0-11% FOG. Mean fiber diameters ranged from 48 to 70μm. Capillary densities fell between 221 and 613/mm^2. Mean intercapillary distances ranged from 76 to 47μm, inversely in relation to capillary density. Mean ICD's measured from the assay of interconnecting lines were almost exactly equal to ($\pi/4$ × capillary density)$^{-1/2}$. Individual values were distributed approximately as log-normal curves (Fig. 1-I-B) with logarithmic standard deviations (S_{log}) between 0.19 and 0.22 (about 95% of all values lay between 1/4 and 4 times the means).

Location of tissue mass with respect to diffusion distance from the nearest capillary was evaluated by plotting contour intervals for each section at multiples of 1/2 mean ICD (Fig. 1-I-C). For mixed muscles, 64 to 73% of section area lay within 1 unit diffusion radius (ICD/2), 19 to 26% between 1 and 1.5 units, 4 to 12% between 1.5 and 2 units, and less than 3% beyond 2 units. Groups of FOG and SO fibers tended to lie in or near the innermost contour interval. For soleus, the closest lying area was slightly larger, 74-78% than for the mixed muscles and the two outer areas slightly smaller, 2-4% and less than 1%, respectively.

II. Distribution of perfused capillaries. To distinguish "open" capillaries in the total population, the lower leg was perfused with india-ink (diluted 1/2, dialyzed vs Ringer's, heparinized) for periods ranging from 3.5 to 90 seconds before freezing and sectioning the muscles. Perfusion pressures and flows were comparable to arterial pressures and blood flows just before perfusion, which was started immediately upon clamping the artery, via a T-cannula previously inserted. Ink spots were counted on a serial section counterstained with eosin, in fields matched to those used for counting total capillaries. Capillary densities, intercapillary distances, etc. for the ink-filled capillaries were measured as described above.

Table I. Fraction of ink-filled capillaries: mean ± SD (N fields counted).

PERFUSION	3.5 sec	7.5 sec	15 sec	30 sec	60-90 sec
Med. gastroc.	.18±.22(39)	.34±.17(28)	.39±.17(28)	.74±.21(26)	.74±.16(29)
Soleus	.12±.17(19)	.23±.09(13)	.26±.18(13)	.67±.23(13)	.80±.21(13)

The progression of ink filling with perfusion duration for medial gastrocnemius and soleus muscles is shown by table 1. Filled fraction (fo) for individual fields varied widely, particularly for short perfusions, but the means increased regularly with time. The small increment in fo between 7.5 and 15 sec suggests these durations complete the filling of a population of open or well-perfused capillaries. fo after 15 sec perfusion was taken to represent this population in resting skeletal muscles (fig. 1-II-A).

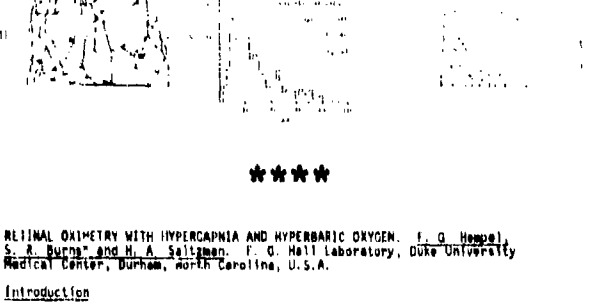
The fraction of open capillaries according to this definition fell between 0.25 and 0.54 for four fields of medial gastroc and was 0.12 and 0.38 for two fields of soleus. Mean ICD's for open capillaries were inversely related to fo, and ranged from 71 to 133um. Distribution was still close to the log-normal pattern (fig. 1-II-B) and variability was increased as fo fell. For medial gastroc, S_{log} ranged from 0.24 to 0.32, for soleus, 0.24 to 0.31 (S_D = 0.30 is equivalent to 95% of individual values falling between 1/8 and 8 times the mean). Tissue areas at different diffusion distances from open capillaries were computed with respect to multiples of total capillary ICD/2, in order to provide an anatomically fixed reference for variable fo. Figure 1-C (I and II) shows a pair of contour maps for all capillaries and for ink-filled capillaries of one muscle field with fo = 0.40. Fractional section area within the ICD/2 contour of open capillaries was diminished in proportion to the reduction in fo, observed values lying between 13 and 46%. The area beyond twice the basic distance was increased to 10% at fo = .54, 20-30% at fo = .38 to.43, 51% at fo = .26 and 61% at fo = .12. If the distribution patterns represented by these sections are fixed in time, tissue volume lying beyond the unit diffusion radius (ICD/2, equivalent to radius of a Krogh cylinder) must determine the critical parameters for O_2 supply to these resting muscles.

The contour maps for both gastrocnemius and soleus muscles show "islands" of well supplied cells surrounded by "seas" of tissue remote from open capillaries. In mixed muscles, the islands are clustered around groups of FOG and SO fibers. However, groups of these cells are represented proportionally in the remote areas. Although the arrangement of the entire capillary bed is related to the organization of muscle fiber types in mixed muscles (3,6), the distribution of open capillaries appears to result from characteristics of the vascular supply to muscle fiber bundles, and is not associated with localization of the different fiber types.

(Supported by USPHS Grant HL 17998).

References will appear in PROCEEDINGS.
Figure 1 follows.

Figure 1. Distribution of total capillaries (Row I, alkaline phosphatase stain) and open capillaries (Row II, ink on eosin counterstained adjacent section) in the same 0.4 mm² field of a cross section of rabbit medial gastrocnemius. This is a relatively "red" part of the muscle, with 74% FG fibers, 12% FOG and 14% SO. Column A, map of intercapillary distances, B distribution of intercapillary distances, C contour maps at intervals of ICD/2 (total caps = 2, total capillary density = 613/mm², capillary/fiber ratio 1.44, the fraction of ink-filled capillaries 0.40). Mean ICD(total = 47μm, ICD/2 = 11μm). The distribution curves are approximately log-normal, with log standard deviations of 0.20 and 0.24, respectively. Fractional tissue areas within radius contours of 23μm, between 23 and 35, between 35 and 46 and beyond 46μm are as follows: total capillaries .26, .19, .04, .01. Open capillaries .46, .21, .10, .07.



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RETINAL OXIMETRY WITH HYPERCAPNIA AND HYPERBARIC OXYGEN. F. Q. Hempel, S. R. Burns* and H. A. Saltzman. F. O. Hall Laboratory, Duke University Medical Center, Durham, North Carolina, U.S.A.

Introduction

Under hyperbaric conditions it is theoretically possible to raise the PO_2 of arterial blood to a level where oxygen dissociation from hemoglobin does not take place in the tissue and hemoglobin remains saturated in transit. The retina in particular lends itself to studies of this kind because of its high rate of oxygen extraction and its unique optical access. Using a reflectance oximetric technique and pressures into the hyperbaric region, we have investigated the effect of increasing the partial pressure of inspired oxygen on the hemoglobin saturation characteristics in the posterior pole of the rabbit eye, more specifically the choroid. Because essentially all of the oxygen supplied to the retina of this animal comes from the choroid our methods made it possible to determine that oxygen tension at which the oxygen needs of the retina were met by physically dissolved oxygen as we monitored the progression from mixed arterial plus venous blood to blood that had fully oxygenated hemoglobin. Furthermore, because hyperbaric experimentation permits the addition of sequentially greater carbon dioxide pressures without subtraction of oxygen, the oximetric effect of increasing hypercapnia combined with a fixed normoxic or hyperoxic respiratory state could be, and was, investigated as well. Finally, the effect of these unusual respiratory conditions on retinal function has not been fully explored before, nor has function been correlated with the oxygenation state of the circulating blood. Additional observations were therefore made on the electroretinographic (ERG) activity which accompanied these gas changes.

Methods

Instrumentation for this study is illustrated in Figure 1. The system is composed of a dual-wavelength spectrophotometer coupled to a conventional fundus (retinal) camera by means of fiber optics. The fundus camera was focused on the retina just below and nasal to the optic disc. Monochromatic light from the spectrophotometer entered the eye through a dilated pupil, was reflected from the fundus, and was received on a photomultiplier tube. The sample, or oxygenation-dependent, wavelength for the oxy-deoxyhemoglobin transition was set at 577 nm. Absorbance was monitored relative to a reference at 586 nm, with the latter wavelength representing an isosbestic extinction point for these hemoglobin species. Each monochromatic beam was flashed onto the retina at 30 Hz, and the arithmetic difference between the intensity of the reflected light, 577-586, was displayed on a chart recorder along with a readout of the reference (586 nm) beam. Variations in the reflected reference light were used as an indicator of the relative blood volume in the retinal field. The entire optical apparatus was installed in a large walk-in hyperbaric chamber with appropriate penetrations through the chamber wall for electrical recording.

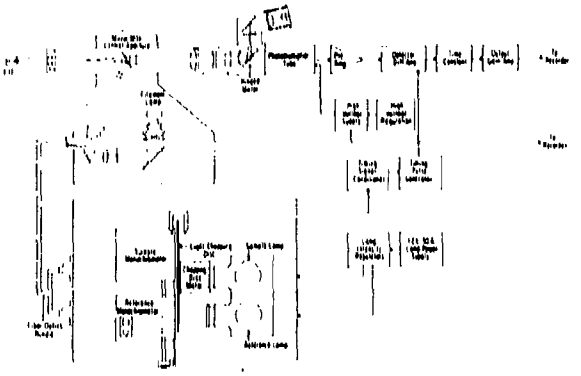

FIG. 1

Schematic diagram of the instrumentation system for oximetric studies of the retina. The supply voltage to the photomultiplier tube is held constant by a regulation circuit during the interval the 586 nm light is opened to the eye by the mechanical chopper. Adjustments in this high voltage are indicative of changing blood volume.

Rabbits were deeply anesthetized with repeated doses of pentobarbital and a tracheal tube was inserted. Paralysis was induced with gallamine triethiodide (Flaxedil) and the animals were artificially respired with a minute volume producing air-equilibrated arterial PO_2 and PCO_2 values of /#11 and 20.6±7.0 Torr, respectively. Animals were secured in a stereotaxic headholder. Gases were administered from premixed cylinders via the respirator.

The electroretinographic signal was evoked by a light flash delivered to the opposite eye and recorded from it with a corneal electrode of the lc vacuum type used clinically. The e responses were likewise displayed on a chart recorder and stored on tape for further analysis.

Results

The oximetric reaction of choroidal blood to increasing tensions of pure oxygen is summarized in Figure 2A along with the corresponding changes in vascular volume which occurred. For these data, six animals were ventilated with oxygen in progressively greater fractions, up to 4 ata. Zero oxygen measurement represents the oximetric response after 2 minutes of nitrogen ventilation. A characteristic oxygen saturation curve of hemoglobin is generated as the data are plotted up to 0.84 ata O_2, and the additional observation was made that 2.0 and 4.0 ata O_2 leads to small but distinct hemoglobin oxygenation increases. A decrease in regional blood volume accompanied each oxygen increment, so the possibility existed that a reduction in choroidal blood flow also had occurred. The net effect of this vasoconstrictive response would be an incomplete oxygen saturation in venous blood. Accordingly, oximetric changes were also monitored while vasodilation was induced in the animals by ventilating them with increasing CO_2 fractions in gas mixtures with a constant inspired oxygen fraction of 21%. Results of these experiments are shown in Figure 2B. With each CO_2 increase up to 5% CO_2 at 4 ata (equivalent to 20% CO_2 at sea level) there is a fall in the relative oxygenation of hemoglobin parallel with a substantial blood volume increase. A higher CO_2 fraction, 7.5% at 4 ata, elicits a shift in the 577-586 signal toward greater oxygenation and, at the same time, the choroidal blood volume drops.

Electroretinographic signals from the fellow eye during the hypercapnic series show that the c-wave, generated in the receptor-pigment epithelial layers, is relatively unaffected by progressively greater CO_2 pressures, while the a-wave is reduced by an average of 32% while breathing the highest (7.5% CO_2) fraction. This CO_2 tension, equivalent to 30% CO_2 at 1 ata, led to a virtually extinguished b-wave (average amplitude = 3% of control) in the 6 animals tested. Pure oxygen at pressures up to 4 ata produced no significant changes in the ERG during the short exposures employed here.

Discussion

The present results clearly indicate the reactivity of the choroidal vasculature to high oxygen pressures and to hypercapnia in the form of a decrease in blood volume with the first condition and a large increase in blood volume in the latter respiratory state. A moderate vasodilatory response of the choroidal circulation to carbon dioxide has been shown before (2), but the vasoconstriction (decrease in blood volume) due to hyperbaric oxygen is a unique observation in what was formerly believed to be a passive system without autoregulation (1). Oxygenation was virtually complete in hemoglobin at 0.84 ata pure O_2 (equivalent to air at 4 ata) yet small increments in oxygen saturation were observed in 2.0 and 4.0 ata

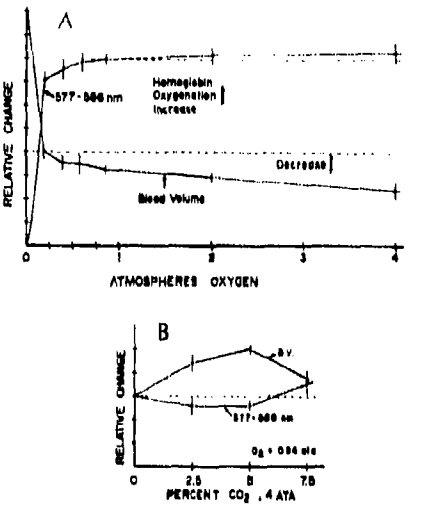

FIG. 2

A. Increase in oxygen saturation of choroidal hemoglobin and decrease in blood volume with atmospheres inspired oxygen. Blood volume is shown relative to control level (dashed line) measured with 0.2 ata oxygen inspiration. B. Relative effects of increasing percentage of carbon dioxide at 4 ata on blood volume (BV) and hemoglobin saturation (577-586 nm). Ordinate at same scale as A, above. Dashed line indicates level while breathing air, no added CO_2, at 4 ata. Points in both A. and B. figures are means of 6 trials, ± standard errors.

oxygen pressures suggesting that oxygen-induced vasoconstriction limits choroidal blood flow sufficiently to prevent full arterialization of the choroidal blood. A similar effect has been noted in the cerebral circulation where venous oxygen partial pressures do not reach a hemoglobin-saturating magnitude in 4 ata oxygen (3). In this sense, it was not possible to achieve a partial pressure of oxygen in the retinal blood so high that some oxygen extraction from hemoglobin did not take place. In short, the provision of oxygen totally from physically-dissolved oxygen was unattainable.

A decrease in oxygen saturation was noted when the carbon dioxide percentage in the inspired gas was raised from zero to 5%. At 4 ata, the carbon dioxide level reached an equivalent of 20%. It was shown in Figure 2A that the oxygen inspired in these experiments, 0.84 ata, was enough to all but completely oxygenate hemoglobin. That this oxygen saturation fell is apparently a consequence of the Bohr shift, because concomitant blood volume increases presumably indicated a higher flow rate and a higher likelihood of the blood's remaining oxygenated in transit. Paradoxically, very high carbon dioxide levels, 7.5% CO_2 at 4 ata, caused a drop in choroidal blood volume to an intermediate position along with raising the relative saturation of hemoglobin with oxygen. No reasons for these effects of very high hypercapnia are immediately apparent, but we may postulate that no further Bohr shift occurs, and that blood flow remains high while the hypercapnia reduces the oxygen needs and extraction by the retina. Consequently, the arterial-venous oxygen difference would be smaller under these extreme conditions.

The striking effects of hypercapnia on the electroretinogram are likewise unique. Only with chemical uncoupling in the retina such as that following aspartate administration (6) has abolition of the b-wave combined with preservation of the a-wave been seen. Extreme hypercapnia (7.5% CO_2) has the most depressant effect. The survival of the a- and c-waves clearly indicates that the hypercapnic block of synaptic function or glial cell activity takes place at a point afferent to the receptor cell layer, because this region gives rise to these waves while the b-wave arises in the inner layers (4, 5). Hypoxia due, for example, to a Bohr shift can be ruled out because of the high oxygen saturation measured in hemoglobin simultaneously, and because hypoxia typically reduces the c-wave first (personal observations).

In summary, vasoconstriction limits the attainment of fully arterialized choroidal blood even with arterial PO_2 values greater than 2000 Torr, and the hypercapnia necessary to offset the vasoconstriction surely depresses retinal oxidative metabolism. Thus, achieving an equality between oxygen provision by the plasma and oxygen consumption in the retina is unlikely in the living eye.

Supported by Grants EY 01953 and HL 07896 from the National Institutes of Health.

References will appear in PROCEEDINGS.

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A MECHANISM FOR THE BENEFICIAL EFFECT HYPERBARIC OXYGEN ON STAPHYLOCOCCAL OSTEOMYELITIS. J.T. Hader and G.L. Brown*. University of Texas Medical Branch, Galveston, Texas, U.S.A.

Hyperbaric oxygen (HBO) therapy is frequently used as adjunctive therapy in chronic osteomyelitis. Previously we demonstrated that HBO used as the sole treatment modality was as effective as antibiotic (cephalothin) in the treatment of experimental Staphylococcus aureus osteomyelitis. Although HBO inhibits growth of most microorganisms including S. aureus, inhibition occurs at oxygen tensions higher than those found in tissue under standard HBO conditions. Our in vitro growth curves and kill curves using cephalothin and S. aureus under standard HBO conditions were identical to those obtained under ambient conditions. Since HBO did not per se inhibit or kill this strain of S. aureus, another mechanism was sought.

MATERIALS AND METHODS

A 16 gauge needle was inserted percutaneously into the left tibial metaphysis of a New Zealand White Rabbit. One tenth ml 5% sodium morrhuate, 0.1 ml *S. aureus* (3×10^6 organisms), and 0.1 ml sterile saline were injected. The needle was removed, the infection was allowed to progress 1-4 weeks, a period during which osteomyelitis becomes well established radiographic criteria.

Measurement of Blood Flow and Intramedullary Oxygen

The animal was anesthetized and a small hole was drilled into the shaft of the normal right tibia and the infected left tibia. If a bone fractured, the study was aborted. A 16 gauge Teflon coated mass spectrometer probe was inserted through the hole into the intramedullary canal, directed toward the tibial metaphysis, and the osteotomy sealed with bone wax. The partial pressure of oxygen and argon were measured by a mass spectrometer (Chamtron, St. Louis, Missouri). Data was utilized from 6 rabbits that completed the entire study.

A tracheostomy was performed through which appropriate gases were administered. I) The animal was breathing ambient air. The oxygen tensions were those found in normal and osteomyelitic bones under ambient conditions. II) The inspired gas was changed from ambient air to 80% argon and 20% oxygen. This gas mixture was administered for 30 minutes and was the "argon wash-in phase". The argon-oxygen mixture was changed back to ambient air, allowing the accumulated argon to be "washed-out" of the tissues. The rate of argon "wash-out" allowed comparison of blood flow between normal and osteomyelitic bone. III) After these measurements, the animal was pressurized to 2 absolute atmospheres (ATA) in a small hyperbaric chamber. The inspired gas was changed to 100% oxygen and the oxygen tension in normal and osteomyelitic bone measured. IV) The chamber was decompressed to ambient conditions. A repeat argon "wash-in and wash-out" was accomplished. Blood flow between normal and osteomyelitic bone was compared after hyperbaric oxygen exposure.

Bone pH measurements were obtained from normal and osteomyelitic bone by placing a tissue pH probe into the intramedullary canal and directed into the tibial metaphysis area.

Phagocytic Killing of *S. aureus* Under Different Oxygen Tensions

S. aureus was grown overnight in trypticase soy broth, washed, and resuspended in Hanks balanced salt solution (HBSS). Rabbit peritoneal polymorphonuclear leukocytes (PMN) were harvested 4.5 hours after intraperitoneal injection of 0.1% glycogen, washed thrice, and resuspended in HBSS.

Three tubes were prepared for each time point (all studies were performed in duplicate). To the first tube was added 1×10^6 *S. aureus*, 3×10^6 PMN, and 10% pooled human serum (opsonin) to a total volume of 1 ml. Two control tubes were prepared for each time point - one without PMN and the other without opsonin. HBSS and heat inactivated fetal calf serum were substituted for PMN and opsonin, respectively. A small aliquot was taken from each tube, added to sterile water and the number of colony forming units (CFU) of *S. aureus* determined.

The tubes were tumbled for 30 minutes at 4°C to provide optimal bacterial attachment to the PMN. The contents of each tube were then decanted into a polyethylene culture dish (15 x 10mm). The resulting suspension was approximately 1 mm thick as optimal oxygen penetration was insured. Five different atmospheric conditions were studied. Dishes were placed in a 37°C incubator (pO_2 = 150 mmHg, ambient conditions) or in a 37°C chamber where the oxygen tension was 21 mmHg (oxygen tension found in osteomyelitic bone under ambient conditions), 45 mmHg (oxygen tension found in normal bone under ambient conditions), 109 mmHg (oxygen tension found in osteomyelitic bone under our HBO conditions), or 760 mmHg (100% oxygen). At least six separate experiments were performed for each chamber oxygen tension. A parallel ambient experiment was run for each chamber oxygen tension experiment. After 1 or 2 hours a dish, representing each original tube was removed from the incubator or the chamber. An aliquot was taken from the plate, added to sterile water, and the number of CFU of *S. aureus* determined. The percentage of original inoculum was then calculated. The viability of the PMN was examined by the exclusion of trypan blue dye.

The data was analyzed by the Student's unpaired t-Test.

RESULTS

Oxygen tensions in normal and osteomyelitic bone are shown in Figure 1. The partial pressure of oxygen in osteomyelitic bone under ambient conditions was 20.9 ± 1.7 mmHg, whereas the oxygen tension in normal bone was 44.7 ± 0.7 mmHg ($p < 0.001$). When the animals were placed under hyperbaric conditions, the oxygen tensions increased in both the osteomyelitic bone (104.0 ± 6.8 mmHg) and normal bone (121 ± 18.7 mmHg). This difference was significant statistically ($p < 0.001$).

Perfusion was decreased in osteomyelitic bone and was not acutely increased by HBO in either the normal or infected bone. The intramedullary pH was likewise decreased in osteomyelitic bone as compared to normal bone.

The phagocytic killing data are expressed as the percentage of surviving *S. aureus* (Figure 2). The control tubes (*S. aureus* plus opsonin without PMN and *S. aureus* plus PMN without opsonin) showed a percentage of surviving *S. aureus* greater than 100% under all 5 oxygen tensions.

Phagocytic killing occurred only when *S. aureus*, PMN, and opsonin were in the in vitro test system. The greatest survival (least killing) of *S. aureus* occurred at an oxygen tension of 21 mmHg (76.9 ± 8.5% and 80.2 ± 7.8% at 1 and 2 hours, respectively). The increasing oxygen tensions resulted in progressively decreasing survival (greater killing) of *S. aureus*: 45 mmHg - 59.7 ± 2.1% (1 hour) and 56.2 ± 4.3% (2 hours), 109 mmHg - 55.6 ± 5.0% (1 hour) and 45.2 ± 4.1% (2 hours), 150 mmHg (ambient) - 46.2 ± 2.4% (1 hour) and 28.2 ± 2.9% (2 hours), and 760 mmHg - 45.2 ± 5.2% (1 hour) and 19.6 ± 6.8% (2 hours).

Comparison of the differences in percent of survival of *S. aureus* at 2 hours using any two oxygen tensions (21 mmHg, 45 mmHg, 109 mmHg, 150 mmHg, and 760 mmHg was significant ($p < 0.001$) except the difference between 45 mmHg and 109 mmHg, and 150 mmHg and 760 mmHg ($p < 0.1$).

The viability of the PMN under all five oxygen tensions was greater than 95% at 2 hours as shown by the exclusion of trypan blue dye.

DISCUSSION

Osteomyelitic bone in this model has a decreased blood flow, decreased pH, and a markedly reduced partial pressure of oxygen. The oxygen tension in osteomyelitic bone (20.9 ± 1.7 mmHg) was significantly decreased compared to normal bone (44.7 ± 0.7 mmHg).

Hyperbaric oxygen failed acutely to influence blood flow in osteomyelitic bone. However, HBO increased the oxygen tension to supraphysiologic levels in osteomyelitic bone (104.0 ± 6.8 mmHg).

Phagocytic killing of this *S. aureus* was markedly reduced under oxygen tensions found in osteomyelitic bone. At decreased oxygen tensions a problem exists in the ability of the phagocytic or intracellular killing mechanisms to handle pathogenic organisms. Since the cause of this difficulty is not clear from our studies, we have used the broad term phagocytic killing to describe any breakdown in the process from ingestion to intracellular killing of *S. aureus*. However, other investigators have shown *S. aureus* to be ingested normally but not totally killed under anaerobic conditions. Adequate molecular oxygen appears to be necessary for effective intracellular killing of this *S. aureus*.

Under the oxygen tensions found in osteomyelitic bone treated with HBO, the phagocytic killing of this *S. aureus* returns to normal when compared to the phagocytic killing found under the oxygen tensions in normal bone. Interestingly, there is a tendency in the literature to equate phagocytic killing under *ambient conditions* to "*normal* phagocytic killing", instead of phagocytic killing under oxygen tensions found within normal tissue. We feel phagocytic killing under tissue oxygen tensions is a more valid representation of "normal phagocytic killing". Phagocytic killing can be enhanced by further increasing the oxygen tensions. Currently, the optimal oxygen tensions for phagocytic killing appear to be between 150 - 760 mmHg. However, phagocytic killing at higher oxygen tensions are yet to be explored.

Thus, intramedullary oxygen tension in osteomyelitic bone is insufficient to support normal phagocytic function. Reduced phagocytic cidal activity may explain both the chronicity of this infection and the effect of HBO. HBO is effective in staphylococcal osteomyelitis by increasing the intramedullary tensions to levels where phagocytic killing may proceed optimally.

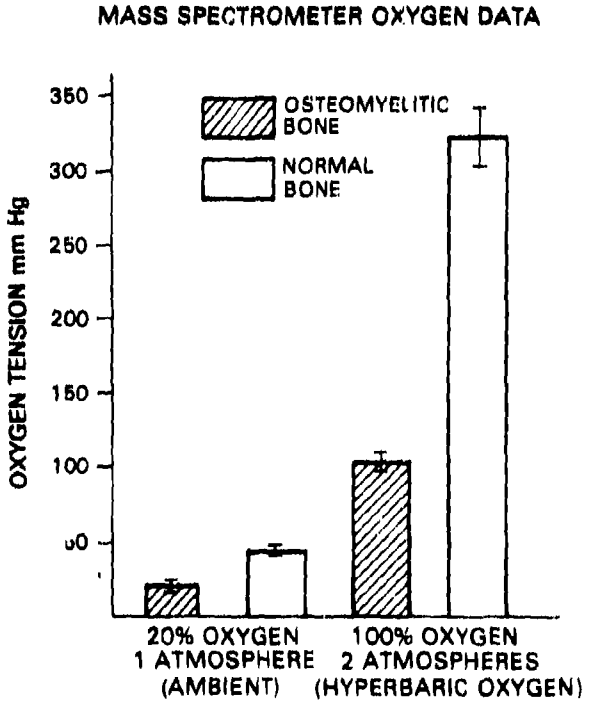


Figure 1. Oxygen tensions in normal and osteomyelitic bone under ambient and hyperbaric oxygen (100% at 2 absolute atmospheres). The oxygen tensions were measured intermittently from normal and osteomyelitic bone by a mass spectrometer. Brackets denote ± SEM.

OXYGEN SUFFICIENCY AND UTILIZATION WITHIN THE CELL

SESSION X

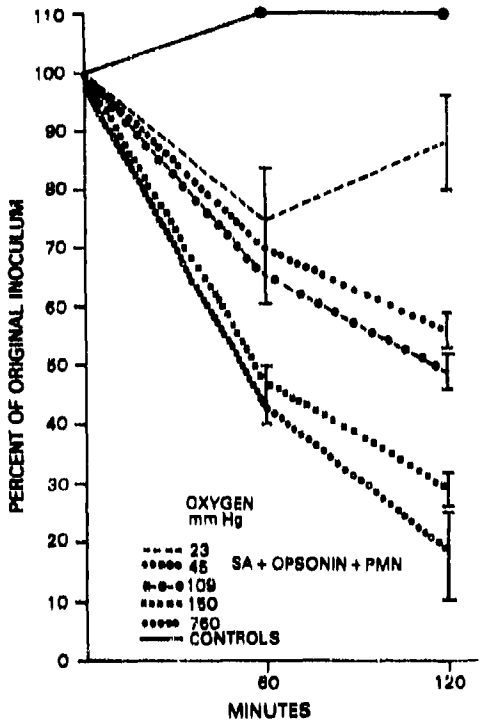


Figure 2. Phagocytic killing of S. aureus (SA) by rabbit peritoneal leukocytes (PMN) with opsonin (10% serum) under different oxygen tensions. The results are expressed as the percentage of the original inoculum of SA. Controls represent SA plus opsonin without PMN or SA plus PMN without opsonin under the different oxygen tensions. Brackets denote ± SEM.

METABOLISM AND THERMAL PHYSIOLOGY

SESSION XI

AN ANALYSIS OF HEAT STRESS UNDER HYPERBARIC CONDITIONS. R. E. Nevitt, Naval Submarine Medical Research Laboratory, Groton, Connecticut 06340, U.S.A.

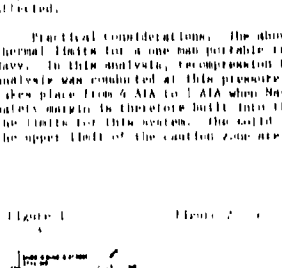
The recognition of hyperthermia as a potential hazard during hyperbaric operations, until recently, has been totally ignored. The diving medical and scientific community were abruptly notified of such a danger when two North Sea divers lost their lives as a result of hyperthermic stress, and it was but a few months later that a seminar was convened to discuss "Thermal Problems in Diving: Hypothermia-Hyperthermia" (1). During this seminar several participants revealed that virtually no information on hyperthermia during diving existed and stressed the need for experimentation in this area. Furthermore, a recent evaluation of a portable recompression system (PRSS) at the Naval Submarine Medical Research Laboratory showed that the most serious problem observed was that of thermal stress (2,3). Upon completion of the tests, it was recognized that 1) the observed thermal stress might produce a significant safety problem and might compromise the adequacy of decompression treatment in a tropical climate, and 2) predictions of thermal stress under these conditions would have to be based on theory only, since no human laboratory experimentation under such conditions has been performed. Especially lacking in this regard was the effect of pressure on man's ability to dissipate heat by evaporative mechanisms.

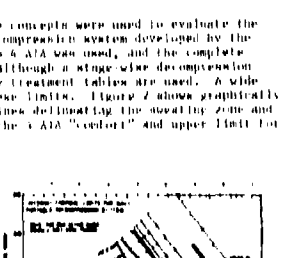
Thermal heat exchange between the skin surface and the environment can be expressed in terms of radiative and convective losses (the sensible losses) and the evaporative losses (the insensible losses). The sensible and insensible losses are governed by the differences in mean skin temperature and ambient temperature, and saturated skin vapor pressure and ambient vapor pressure respectively, so that

$$H_{sk} = (h_r + h_c)(\bar{t}_{sk} - \bar{t}_a) + w h_e (P_{a,sk} - P_a) \qquad \text{Eq. 1}$$

where H_{sk} = total heat loss via skin (Btu/ft²/hr), h_r, h_c, and h_e = radiative, convective, and evaporative heat transfer coefficients respectively (Btu/ft²/hr/°F); $\bar{t}_{sk}$ = mean skin temperature (°F); t_a = operative temperature (°F, in this analysis t_a = ambient temperature = mean radiant temperature); w = skin wettedness (dimensionless); $P_{a,sk}$ = saturated skin vapor pressure (torr), P_a = ambient vapor pressure (torr). This equation may be plotted on a psychrometric chart (temperature on the abscissa and water vapor on the ordinate) and will describe a straight line that passes through the two points $[P_{a,sk}, \bar{t}_{sk}]$ and $[P_a, t_a]$ with a negative slope of $[(h_r + h_c)/w h_e]$ (1). This unique concept was extended to flight and space to include the hypo- and hyperbaric environments (4). For this analysis, Nevitt's hyperbaric example was expanded to include pressures of 1, 2, 4, and 7 atmospheres absolute (ATA), helium pressures of 10 and 30 ATA, and water immersion. The resulting figure (figure 1) shows for the first time a comprehensive graphical presentation of the narrowing "window" with increasing pressure of the comfort to heat stress limits. The dashed lines represent the limit for comfort where skin wettedness (w) = 0.06 and regulation of heat exchange by sweating begins. The solid lines represent the upper limit for evaporative regulation where w = 1 (skin fully wet with sweat). Each line was constructed according to Nishi (4) with the following conditions and constants: subject in undressed at rest (metabolic rate 58.2 W/m²); velocity of gas (v) = 0.1 m/sec; t_{sk} = 94.6°F at comfort and 96.5°F at maximum sweating; h_r, h_{fr} and t_r (the radiative heat coefficient) and respiration considerations were taken from Nishi (4). Man's thermophysiological behavior in a warm to hot hyperbaric environment, graphically depicted in figure 1, has the following features: a) as pressure increases the thermal "window" from comfort to heat stress greatly narrows. At 1 ATA and 80% RH this window has a span of 20°F, which is reduced to about 0° at 50 ATA in a helium atmosphere. As the density of gas increases to a theoretical limit of 1 (water environment) this window has narrowed to about 1°F (5); b) the comfort limits (above which the environment becomes uncomfortably warm) are very near the low comfort limits (below which the environment becomes uncomfortably cold) described in the hyperbaric literature; c) the most significant decreases in limitation take place at the lower pressures (1 at high relative humidity, the upper limits for evaporative regulation are little affected by pressure. At mild to low relative humidity, these upper limits are greatly affected.

Practical considerations. The above concepts were used to evaluate the thermal limits for a one man portable recompression system developed by the Navy. In this analysis, recompression to 6 ATA was used, and the complete analysis was conducted at this pressure although a stage-wise decompression takes place from 6 ATA to 1 ATA when Navy treatment tables are used. A wide safety margin is therefore built into these limits. Figure 2 shows graphically the limits for this system. The solid lines delineating the sweating zone and the upper limit of the comfort zone are the 1 ATA "comfort" and upper limit for





evaporative regulation lines shown in Figure 1 (w = 0.06 and 1 respectively). The line delineating the sweating zone and lower limit of the caution zone is for w = 0.7. At points above this line a considerable amount of heat stress is incurred with profuse sweating and much discomfort experienced by the divet ot subject. Rectal temperature and heart rate will rise, but will reach a plateau. The danger zone, points above the w = 1 line, signifies that thermoregulation by sweating has reached a limit and that internal body temperature and heart rate will continue to rise until collapse. Body heat storage will increase at a rate computed from the metabolic heat production and the combined radiative and convective losses or gains. Rate of body temperature rise is then easily computed from standard physical characteristics. A body temperature of 39.5°C was chosen to represent that point where heat stroke occurance will be greater than 1 in 100 (6). Hourly ingestion to reach this temperature were plotted in Figure 2. A physician or technician in the field can now, knowing the ambient temperature and relative humidity predict the thermal load expected and take the necessary pre auttionary measures. This chart must be termed "interim", since it is based on an analysis using physical principles and empirical evidence collected at 1 Ata. Human hyperbaric experiments are presently underway to verify these predictive methods, and the results will be reported. Extension of this graphical method to include variables of work rate, clothing, gas velocity, and cold at increased barometric pressures will also be reported.

References will appear in PROCEEDINGS.

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CONTRIBUTION OF METABOLIC AND RESPIRATORY HEAT TO CORE TEMPERATURE GAIN AFTER COLD WATER IMMERSION. M. J. Cosa, P. A. Hayes and J. B. Morrison. Department of Kinesiology, Simon Fraser University, Burnaby, B.C. Canada and Admiralty Marine Technology Establishment, Hants, U.K.

Accidental hypothermia is a serious problem in cold air and water exposures. Inhalation warming is an attractive procedure for the treatment or prevention in remote or closed environments. It supplies heat directly to the core area, is readily portable and can be easily and safely administered. At present, a strong controversy regarding the effectiveness of this technique is evident in the literature, and both animal and human studies are at variance (Hayward and Steinman, 1975; Lloyd, Mitchell and Williams, 1976; Pavlin Berbein and Chaney, 1976; Marcus, 1978; Morrison, Cosa and Hayward, 1979; Auld, Light and Norman, 1979). Disagreement exists over the quantity of heat delivered, its distribution and its significance relative to metabolic heat production.

To determine the relative contribution of metabolic heat and respiratory heat to core temperature change, ten male subjects were cooled by immersion to the neck to 11.1°C water. Subjects were no clothing and maintained a sitting posture with a minimum of movement. Rectal, tympanic and skin temperatures were recorded. Subjects were removed from the water at a rectal temperature of 35.0°C, dried thoroughly and placed in a sleeping bag for rewarming. Ventilation, respiratory gas fractions and inspired and expired gas temperatures were then measured for a period of 60 minutes. Warming commenced 5 minutes after the signal to leave the water was given. All subjects were rewarmed on three occasions, once by metabolic heat alone (shivering), once by inhalation warming with spontaneous breathing of saturated air at 47°C (control) and once by inhalation warming with ventilation regulated at 60 l/min by respiting a controlled fraction of CO2 (hyperventilation). In this manner, rewarming data was obtained for three distinct levels of respiratory heat exchange.

There were no significant differences between the three treatments in the absolute values of rectal, tympanic or skin temperatures measured at the commencement of rewarming (to). Core temperatures continued to decline after leaving the water and afterdrop was not arrested until after the rewarming treatment was well established. All temperature data were normalized relative to the temperature at the start of rewarming. The mean response of the ten subjects to each treatment was then calculated.

The magnitude of the afterdrop in rectal temperature was reduced by both the active rewarming treatments in comparison to shivering ($p < 0.05$). The time taken to recover to initial temperature log was also shortened ($p < 0.05$) from 23 minutes for shivering to 15 minutes for control and 10 minutes for hyperventilation. After 60 minutes of rewarming, hyperventilation and control gave greater net gains in rectal temperature (1.5 and 1.1°C) than did shivering (0.84°C). The afterdrop of tympanic temperature was small (ca. 0.2°C) in all treatments and there were no significant differences. Shivering took longer to arrest afterdrop and to recover initial temperature ($p < 0.01$) than the other two treatments. The subsequent rate of rewarming was greatest for hyperventilation which recorded a net temperature gain of 1.7°C compared with 1.4°C for the other two treatments.

There were no significant differences between procedures in the change of mean skin temperature although shivering recorded a slightly larger rise (9.1°C) than either control (8.5°C) or hyperventilation (8.7°C). Subjects shivered vigorously in the early stages of rewarming, recording a mean oxygen uptake of 1.5 l/min BTPD. Thermogenesis decreased rapidly in response to skin and core temperature changes to a mean value of 0.1 l/min at t = 60 mins. Metabolic heat production was substantially reduced by inhalation rewarming ($p < 0.05$) from 218 Kcal when shivering to 181 Kcals (control) and 147 Kcals when hyperventilating. The fall in metabolic heat production was greater than the corresponding respiratory heat gain which increased from a loss of 10 Kcals when shivering to gains of 20 Kcals (control) and 60 Kcals (hyperventilation).

As differences between treatments in the absolute values of mean skin temperature were small (<1.0°C) and not significant, it is concluded that the fall in metabolic heat production in response to the two inhalation rewarming procedures must result from more rapid temperature gains at the central cold receptors. This conclusion is supported by the relative measure of rectal and tympanic temperatures. Calculations show that, on average, for each kcal of respiratory heat supplied 1.4 Kcals of metabolic heat were forfeited. The fact that respiratory heating enhanced the recovery of core temperature implies that respiratory heat must be more efficient than metabolic heat in both arresting afterdrop and raising core temperature.

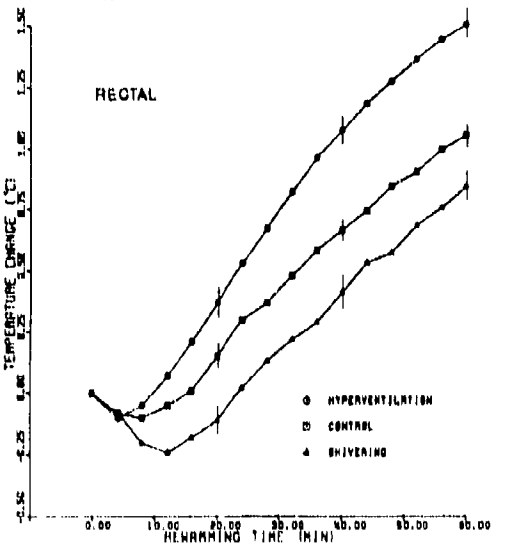
In order to quantify the above tendency, the fraction of total heat input donated to core temperature gain was calculated using a core mass of 46% body weight (Burton 1935) and rectal and tympanic temperature changes at t = 60 minutes. Results indicate that the percentage of total heat donated to the core increased from 11% in shivering to 16% in control and 23% in hyperventilation. Assuming that the fraction of metabolic heat donated to the core does not change significantly between treatments it can be theorized that in order to produce the core temperature gains recorded with inhalation rewarming approximately 40 to 60% of respiratory heat contributed to core temperature gain. Thus although the absolute magnitude of respiratory heat was small, its efficiency as a source of core heating was estimated to be 3 to 5 times greater than that of metabolic heat production.

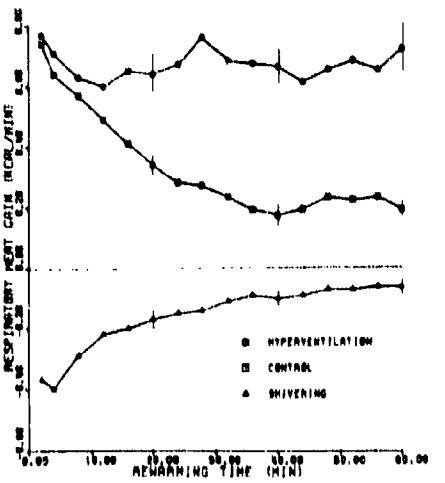
Respiratory heat loss is estimated to be 5 to 10% of metabolic heat production in normal air environments. In divers breathing oxygen-helium mixtures the greater thermal conductivity, specific heat and density can result in substantial respiratory heat losses. Had the present study been repeated at 30 ATA the estimates of Webb (1975) predict that respiratory heat losses for the shivering procedures would have been approximately 70 Kcals, or 40% of metabolic heat production. Respiratory heat gain from inhalation rewarming would also have been enhanced.

This study disagrees with the findings of Lloyd, Mitchel and Williams (1976), Marcus (1978), and Auld, Light and Norman (1979), and supports those of Pavlin, Bornbein and Chaney (1976), Hayward and Steinman (1975), and Morrison, Cosa and Hayward (1979). It is difficult to explain the disparity of results among authors, but the following factors may contribute to differences reported. Rewarming rates are sensitive to variation of absolute body temperature and composition (Morrison, Hayward and Cosa, 1980), and therefore comparative data must be closely matched. As the respiratory heat gain is largely condensatory in nature, the effectiveness drops rapidly when inspired gas is not 100% saturated. Finally, as shown, the contribution of respiratory heat can be partly hidden by a concomitant drop in metabolic heat production.

In conclusion, this study indicates that whilst inhalation warming in a normal air environment provides 10% of total body heat input it is more efficient in terms of heat delivery to the core than shivering thermogenesis. Inhalation warming is shown to be a practical method of treating or preventing hypothermia. The potential benefits of this treatment will be enhanced when breathing oxygen-helium gas mixture at increased pressure.

References will appear in PROCEEDINGS.
Figures 1 and 2 follow.





THE METABOLIC AND THERMAL STATUS OF DIVERS DURING SIMULATED DIVES TO 55 BAR. M.F. Garrard, P.A. Hayes, R.I. Carlyle and M.J. Stock[1]. Physiological Laboratory AMRE, Fort Road, Alverstoke, Gosport, Hants, UK and [1]St George's Hospital Medical School, Cranmer Terrace, Tooting, London, UK.

Many studies into the metabolic and thermal status of divers in helium have been invalidated by the inability to make physiological measurements against a stable dietary background. During successive duplicate dives to 31 and 43 bar, and single dives to 49 and 55 bar, divers were fed a constant and controlled dietary intake for the whole of the dive duration (maximum 28 days). This made possible a comprehensive series of nutritional, metabolic and thermal measurements free from dietary intake error. With a known intake and measured output true metabolic balances were computed for energy, nitrogen, water, calcium, magnesium, zinc and phosphorus. Many of the earlier findings pertaining to nitrogen metabolism and some of the associated metabolic and hormonal changes have been previously reported (Carlyle et al, 1978a,b). Accumulated data from all these dives provide evidence for specific metabolic changes brought about by the hyperbaric environment. An increased urinary urea can account for the negative nitrogen balance observed deeper than approximately 30 bar. This increase is thought to arise from a specific stimulation of protein catabolism via thyroxine (T_4), probably released from peripheral stores. There were only minor disturbances in carbohydrate metabolism and no significant modification of fat metabolism or balance of the ions. There was no indication of thermogenic stimulation, and basal metabolic rate, core and mean skin temperatures remained unchanged down to 55 bar and back. The resting energy expenditure was maximum at maximum depth on the 43 bar dives, followed by a significant decrease during the early phases of decompression (Carlyle et al, 1980). Circulatory levels of T_4 are significantly raised at depth, as are those of noradrenaline, yet triiodothyronine (T_3) and T_3 resin uptake levels remained unchanged. The levels of thyroid stimulating hormone were unpredictable with no conclusive pattern emerging, whereas reverse T_3 (rT_3) followed the T_4 result, being maximum during the early phase of decompression. Therefore it appears that there is an increased conversion of T_4 to rT_3 with no complementary increase in the T_4 to T_3 conversion. As well as increased T_4 release from peripheral stores, T_4 biliary clearance may be reduced, thus further increasing the circulatory levels, which in turn indicates an alteration in the conjugation of thyroxine to form sulphates and glucuronides in the liver. Thyroxine is also known to influence the peripheral nervous system and raised levels will shorten the reaction time of stretch reflexes. In accord with the observed hyperbaric hyperreflexia (Harris, 1978). Minor changes in the acuity of peripheral temperature perception were observed when cooling the skin from high temperatures. No significant changes were found in the perception of warmth. The loss of cool sensation displayed a time dependence, reaching a maximum at the end of each dive, followed by a slow recovery over the next month (Hayes, 1979). An analysis of water balance showed that evaporative mass flux from the skin is markedly reduced with depth. A decrease in the measured evaporative losses (down to 28% of the 1 bar capability at 45 bar) can be correlated with the change in evaporative mass transfer coefficient (reduced to 15% of the 1 bar value); the latter being inversely related to the density. The body appears to compensate by a concomitant diuresis as seen from the calculated water balance (Carlyle et al, 1979). Direct measurements of regional heat loss using surface plate calorimeters in comfortable conditions demonstrate the importance of body orientation and position with regard to the magnitude and distribution of local heat loss (Hemplemann et al, 1978c). No gross disturbance of the thermoregulatory response to submaximal exercise was observed down to 55 bar. However, the rise in skin temperature and level of heat discomfort were minimised by maintaining absolute humidity at a low level (10 mg l^{-1}). Maximum comfort levels of warmed and humidified gas (0.4 bar O_2) inspired via a mouthpiece remained relatively unchanged over the range 0.26 bar at 40 ± 2 °C. The comfort level in air (1 bar) for the same three divers was 42 ± 2 °C. Following cold water diving (4 °C) to the limits of peripheral endurance the positive heat gain imparted to the body varied from 7.5 to 19 W in air (range 40-50 °C) through 4.5 to 10.5 (9 bar), 3.9 to 11.5 (10 bar), up to 8.9 to 40.4 W (26 bar). Transfer rates are high at the onset of rewarming as Q is high but fall with the loss of thermogenic stimulus. Whereas some 86-88% of the heat transfer is attributable to latent heat of condensation at 1 bar, only 4-5% of the total heat transferred comes from the water vapour at 26 bar. The expired temperature and consequently the heat gain must to a certain extent also depend upon gas heat content and following more drastic cooling it is anticipated that the transfer rates could be higher.

References will appear in PROCEEDINGS.

MOLECULAR AND CELLULAR EFFECTS OF HYDROSTATIC PRESSURE

, STUDY OF THE SPECIFIC ACTION OF "PER SE" HYDROSTATIC PRESSURE ON FISH CONSIDERED AS A PHYSIOLOGICAL MODEL. L. Bachelard, A. Delaunay and A. Sallon. Laboratoire de Physiologie, Faculté de Médecine - 9759 BREST Cedex - FRANCE.

As a waterbreather, the fish can be submitted either to the specific action of "per se" hydrostatic pressure (when compressed in a closed vessel chamber entirely filled with water) or to the influence of both pressure and hyperbaric inert gas tensions (when compressed in an open aquarium where bubbling achieves gas equilibration of the water and fish body compartments (1&2)).

Physiological modifications were observed in vels exposed to a 101 ATA hydrostatic pressure : increased activity, hyperventilation, increased metabolic rate, haemodynamical changes, tachycardia, ECG disorders (1&2). Pressure threshold for the appearance of these modifications is greater at slow compression rate (10 ATA.hr $^{-1}$) than at rapid compression rate (10 ATA.min $^{-1}$). So, these specific excitatory reactions of the vel to "per se" hydrostatic pressure have been compared to High Pressure Nervous Syndrome described in mammals (3).

There are two phases during "per se" hydrostatic pressure exposure : first, the above described excitatory phase below about 60 ATA and then a phase of inhibition of ventilation, metabolism, EEG activity, leading to the death of the fish at greater pressure. Fish lethality under "per se" hydrostatic pressure varies according to specific experimental pressure value and exposure duration. As an example, Table I gives the duration of pressure exposures which are lethal for trout at a temperature of 15°C.

The combination of the action of certain anaesthetic drugs (Methane tricaine sulfonate (MS 222 ®), Pentobarbital ®) on the one hand with the action of "per se" hydrostatic pressure on the other reveals in some cases a reversal effect of anaesthesia under pressurization, but there were also observed in other cases either a strengthening of anaesthesia or a lack of interaction. The influence of pressure on narcotic potency depends upon 1) the nature of the drug, 2) the values of temperature and/or pressure, 3) the species of fish and 4) the physiological process which is considered as the criterion of "anesthetic depth (EEG activity, evoked visual potentials, ventilatory activity... (3, 1&2).

Another interaction between "per se" hydrostatic pressure and anaesthetic drug action was observed when anaesthetized trout showed a better tolerance to "per se" hydrostatic pressure than untreated fish.

Taking into consideration that certain inert gases exhibit narcotic properties, it was interesting to investigate the occurrence of a possible influence of Nitrogen or Helium on hydrostatic pressure action. For this investigation the fish is a suitable model because the density of ventilated liquid and hence ventilation is unaffected by the nature and pressure of the tested inert gas. The hyperbaric devices were modified in order to reach total pressures of 150 ATA which is composed of a given partial pressure of inert gas and the complementary "per se" hydrostatic pressure. 150 g rainbow trouts were submitted to experimental conditions enumerated in Table 2. In all cases the O_2 partial pressure was initially (i.e. before pressurization) 1 ATA, the temperature 15°C and the compression rate 10 ATA.min $^{-1}$. The visual observation of spectacular activity indicated the survival times at maximal pressure (Table 1 and 2).

The saturation of water with helium at 100, 120 and 150 ATA gives a prolongation of survival relative to "per se" hydrostatic conditions (Table 1). Considering this criterion, helium acts in the same way as certain narcotics because it antagonizes the lethal action of hydrostatic pressure. This result was also confirmed by ECG and evoked visual potential recordings.

The saturation of water with nitrogen leads to a greater lethality than that of "per se" hydrostatic pressure (Table 1). So, nitrogen saturation strengthens the toxicity of "per se" hydrostatic pressure. Table 2 shows that if the nitrogen dosage is limited to 10 ATA, the total pressure of 150 ATA (including 1 ATA O_2, 10 ATA N and 139 ATA "per se" hydrostatic pressure) gives survival times superior to those derived under the effects of 150 ATA hydrostatic pressure alone. Table 2 indicates that the compression stage where the 10 ATA nitrogen dosage is administered influences the results. The most suitable moment of N_2 administration for the survival of trout corresponds to the pressurization stage at 10 ATA. The addition of nitrogen may either increase or decrease the tolerance of trout exposed to hydrostatic pressure, according to the amount and moment of the administration of nitrogen. A 10 ATA partial pressure of nitrogen can reverse the action of "per se" hydrostatic pressure.

In order to interpret the above results, two quantitative studies were performed : first, a study of heart rate values recorded in vels under various temperature and pressure values (4&); and second, a quantitative analysis of recovery time from pentobarbital anaesthesia in trout submitted to hydrostatic pressure (2&). The conclusions were in accordance with JOHNSON and EYRING (5&), proposing that "per se" hydrostatic pressure acts by modifying the kinetics of chemical reactions which limit the rate of biological processes. Two kinds of pressure impact can be considered : either a structural change in one or more elements of the limiting chemical reaction (enzyme substratum, solvent, activated complex) ; or a structural (and hence functional) change in membranes and/or protoplasm, leading to concentration changes in substratum supplying intracellular chemical reactions.

Recent results concerning the influence of inert gas on "per se" hydrostatic pressure reinforce this second interpretation because the inert gases do not directly participate in any reaction, but the gases act as narcotic drugs by dissolving into certain structures of the cell. The lethal action of pressure would result in the blockage of a vital process at molecular level, and then the dissolved inert gases would change the molecular structures and so lighten (in the case of 10 ATA nitrogen dosage or 150 ATA helium) the blockage or strengthen it (in the case of 139 ATA nitrogen).

TABLE 1

Mean survival time (N = 10) of trout exposed to various hyperbaric conditions

Condition \ Pressure (ATA)	100	120	150
"per se" hydrostatic pressure	60 min	12 min	< 2 min
Helium pressure	20 hr	6 hr	3 hr 45
Nitrogen pressure	60 min	0	0

TABLE 2

Conditions	survival time
"per se" hydrostatic pressure	2 min
10 ATA N then 140 ATA "per se" hydrostatic pressure	2 min 40
30 ATA "per se" hydrostatic water then 10 ATA N then 110 ATA "per se" hydrostatic pressure	22 min
50 ATA "per se" hydrostatic water then 10 ATA N then 90 ATA "per se" hydrostatic pressure	55 min
80 ATA "per se" hydrostatic water then 10 ATA N then 60 ATA "per se" hydrostatic pressure	10 min

Mean survival time (N = 10) of trouts submitted to a total pressure of 150 ATA obtained by combination of 10 ATA of Nitrogen and the complementary hydrostatic pressure.

References will appear in PROCEEDINGS.

OSMOTIC FRAGILITY OF ERYTHROCYTES: EFFECTS OF HYDROSTATIC PRESSURE AND PENTANOL

A. C. Hall and A. G. Macdonald
Department of Physiology, University of Aberdeen, Marischal College, Aberdeen, U.K.

Introduction

Although hydrostatic pressure has been shown to order lipid bilayers and dissociate protein polymers, the effects being enhanced at low temperatures, its actions on the mechanical properties of cell membranes are difficult to predict.

The red blood cell is an excellent system for investigating this problem since when a population of erythrocytes is subjected to an osmotic stress the amount of haemolysis, measured spectrophotometrically, is determined by the mechanical state of the cells: the more fragile the cells are the more haemolysis occurs. The problem of the heterogeneity of cell ages in the cell population can be overcome by lysing the hypotonic NaCl serum ion which gives approximately 50% haemolysis (called H50 Na Cl). In this way only the mature erythrocytes are studied.

Pressure equipment has been constructed which enables the injection of this hypotonic solution into an erythrocyte suspension equilibrated at a selected experimental temperature and pressure. After the osmotic shock is given, the unlysed cells are fixed, decompressed and the haemoglobin which has been released by lysis subsequently measured. It represents the stress the cells have undergone at pressure.

Results

Fig 1 shows that high pressure increases the osmotic fragility of human red cells. The H50 was found for a given blood sample at the experimental temperature and atmospheric pressure and the amount of haemolysis produced was then normalised to 50%. At all pressures red cell fragility is greatest at 5°C and there was no significant difference between the slopes at 21° and 37°C. The cells are therefore maximally stabilised as the temperature is raised to 21°C. The disorder - effect of the higher temperature apparently does not confer any increase in osmotic stability at high pressure. Therefore lowering the temperature from the physiological level does not increase the pressure effect in a simple linear manner.

Fig 2 shows the results obtained from bovine red cells at 37°C equilibrated at pressure and then subjected to an osmotic shock of H30 Na Cl only or with 100 mM pentanol. The data show that the fragility of bovine red cells at 5°C under pressure is not significantly different from human red cells under the same conditions and therefore pressure would appear to be having the same effect. It should be noted however that at atmospheric pressure the situation is different; bovine red cells are more fragile with an H30 of 0.43% compared to 0.12% Na Cl.

Addition of pentanol to the H30 Na Cl solution gives an anti-haemolytic effect of about 60%, a well known phenomenon due to an increase in critical haemolytic volume (Vc) and not to an osmotic protection. Above 100 ATM the fragility of pentanol-treated cells is affected by pressure to the same extent as untreated cells, and thus it seems likely that pressure is also increasing fragility by the same mechanism in each case. There is a significant and interesting change in slope at 100 ATM for the pentanol treated cells. Interestingly from 1 to 100 ATM the anti-haemolytic effect of pentanol is increased compared to the untreated controls by pressure. Pressure enhances the protective effect even though it is likely that pressure order any disordered state in the bilayer component of the membrane caused by pentanol (Fig 2).

Fragility by extrapolation, at about 175 ATM cells treated with 100 mM pentanol should be as fragile as untreated cells at atmospheric pressure.

Discussion

Pressure may increase red cell fragility either by an effect on the ionic balance of the cell in such a way as to increase the initial cell volume (Vo) and hence fragility, or by a direct action on the cell membrane, thus a 100 Vo and no decrease in Vc.

Pressure may increase passive ion permeability and or inhibit the active trans-port of cations. This would increase Vo and thus make the cells apparently more fragile. However if red cells are incubated at 5mM ATM and 0% for 30 minutes and then the haemolysis performed at atmospheric pressure no additional haemolysis over unpressurised control was noted, thus it seems unlikely that a pressure induced ionic imbalance can explain the results presented here.

It is much more probable that pressure increases fragility by a direct effect on the cell membrane. Pressure may "order" the lipid bilayer component and decrease the membrane surface area, both by the enhancement at low temperatures. This would decrease the Vc and decrease the Vc. On the other hand high pressure might disassociate the membrane protein network in a way similar to the pressure depolymerization of actin, tubulin and other structural polymers. The protein network, which is attached on the inner surface of the red cell membrane, is highly specified and seem to have a major role in maintaining cytoskeleton shape (Ungar, 1979). Thus pressure may disrupt the cytoskeleton, thereby Vo and hence fragility.

We favour this interpretation because liposomes made from red cells show different sensitivity to pressure, in terms of osmotic fragility. Almost all (95%) have been shown to be red cell membrane vesicles. The temperature dependence, however, is similar to that of intact cells, in that fragility is increased at low temperatures. Thus the bilayer ordering effects of low temperature do not contribute to membrane stability.

The observation that pressure above 100 ATM does not affect the anti-haemolytic effect of pentanol strongly suggests that each have different sites of action. Thus we suggest that pressure alters the cells' Vo by acting on the protein network underlying the membrane, whereas pentanol protects the cell by increasing Vc, perhaps by association primarily with the lipid components. Pentanol may equally interact with the protein component however, but this is not manifest in the experiments above 100 ATM. The effect of pentanol below 100 ATM is difficult to interpret at present.

The early experiments by Ebbecke (1930) which showed that red cells become more spherical at pressures up to 2000 ATM may also be explained by pressure causing an extensive disruption to the protein network. Presumably the cytoskeleton is only partially irreversibly denatured in view of Hawbritch's (1937) findings that such cells were more fragile in subsequent experiments. At these high pressures there may be additional problems of interpretation due to temperature changes on compression and alterations to red cell ionic balance.

References will appear in PROCEEDINGS.
Figures 1 and 2 follow.

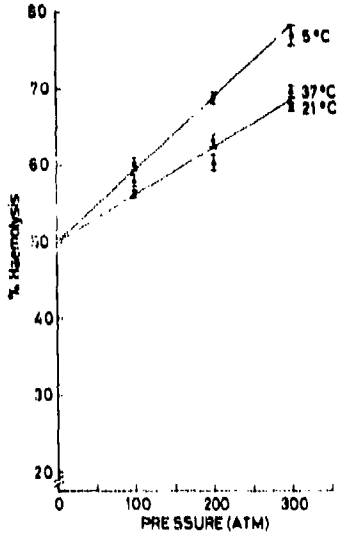


Fig 1. Human red cells equilibrated at 5°C, 21°C and 37°C (●) and at pressure subjected to an osmotic shock of H30 Na Cl.

Means ± S.d., for a minimum of 5 experiments.

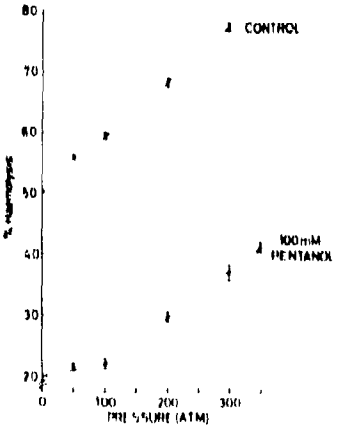


Fig. Bovine red cells equilibrated at 5°C at pressure subjected to an osmotic shock of H30 Na Cl only (●) and with 100 mM pentanol.

Means ± S.d., for a minimum of 5 experiments.

A MATHEMATICAL ANALYSIS OF HIGH PRESSURE AND ANAESTHETIC EFFECTS.

H.J. Halsey*, Angela F. Mott*, G.C. Spicer* and Bridget Wardley-Smith*,
Divisions of Anaesthesia* and of Medical Computing*, Clinical Research Centre,
Watford Road, Harrow, Middx. England.

The pressure reversal of anaesthesia is a well established phenomenon in both amphibians and mammals. Since 1972 the quantitative data on the decrease of anaesthetic potency with increasing pressure have usually been analysed in terms of the critical volume hypothesis. This predicted that there should be a universal linear relationship between the percentage increase in anaesthetising partial pressure of any agent and the increase in pressure of helium which is used as the "inert" gas (Miller et al, 1973). It has been demonstrated that the use of high pressure helium is equivalent to hydrostatic pressure (Miller et al, 1969) although in mammals helium does appear to have a weak inherent anaesthetic potency (Halsey, 1974).

There has been a considerable discussion as to whether the universal linearity prediction of the critical volume hypothesis is proven. It does appear to be established in experiments with newts (Miller et al, 1973) but the data in mammals are controversial. For example, detailed studies with nitrogen and argon in mice indicate that pressure reversal is non-linear (Smith et al, 1979), but this is disputed by one group of workers (Miller and Wilson, 1978). There have been fewer studies with intravenous agents but there does appear to be agreement that their degree of pressure reversal is different from that for the inhalational agents (Halsey et al, 1978; Miller and Wilson, 1978). However, these last two studies disagree as to whether the reversal is linear.

The issue of universal linearity is particularly important because it is the major prediction of the unitary critical volume hypothesis. One alternative is the multi-site hypothesis which postulates different molecular sites with limited degrees of occupancy, (Halsey et al, 1978). In view of this importance it seemed appropriate to attempt to analyse the available data in terms of a mathematical model. We formulated three specific questions:

1. Are the percentage increases in anaesthetic requirements unequivocally non-linear when all experimental errors are included?

2. If they are non-linear, do they fit a mathematical model based on a simple saturation of the molecular sites - analogous to the Langmuir adsorption isotherm (Glasstone, 1946)?

3. Alternatively, do they have to be fitted to a more complex model which would predict additional effects as the pressure is increased?

In our preliminary analysis we have used the data obtained for the pressure reversal of the intravenous agents (Halsey et al, 1978) because the individual values of the variables were available to us. In these experiments anaesthetic potencies were determined in terms of infusion rates under steady state conditions. Technical limitations prevented us from directly measuring the anaesthetic concentrations in the serum while the animals were at pressure.

However, the potencies of the agents are expressed as percentage increases relative to the control period at normal pressure rather than as absolute values. We were concerned about the theoretical possibility of the rates of metabolism or excretion changing with pressure. We therefore established a stable and defined level of anaesthesia and measured waking times after the infusion was switched off. For all the agents so far studied there were no significant differences in the waking times between the control and high pressure conditions.

In answer to the first question we have established that the pressure reversal curves based on all the individual data values for althesin, thiopentone, propanidid and ketamine are significantly non-linear.

The departures from a linear relation between ambient pressure and inhibition of anaesthetic effect suggested that an expression of the following form might be suitable:

$$y = \frac{p}{a + bp + cp^2} \qquad (1)$$

where y = % inhibition of anaesthetic potency, p is pressure above atmospheric and a, b, c are constants.

This curve has a maximum at $\frac{a}{c}$ and declines to zero at $p = 0$ and ∞.

When a and b are positive and $c = 0$ it is identical to a Langmuir adsorption curve. The functional changes in y and p are related by the equation:

$$\frac{dy}{y} = \frac{dp}{p} - \frac{dl}{l} \qquad (2)$$

where $l(p) = a + bp + cp^2$ in the present case.

The values of the parameters (with their standard errors) for the four anaesthetics were as follows:

	a	b	c
Althesin	1,6[illegible] (0,560)	-0,0[illegible] (0,0[illegible])	0,[illegible]1 (0,[illegible])
Thiopentone	2,[illegible] (0,[illegible])	-0,[illegible] (0,[illegible])	0,[illegible]6 (0,[illegible])
Propanidid	4,8[illegible] (1,[illegible])	-0,[illegible] (0,[illegible])	0,[illegible] (0,[illegible])
Ketamine	,8[illegible] (1,[illegible])	-0,[illegible] (0,[illegible])	0,[illegible] (0,[illegible])

The standard errors indicate that in some cases the parameters are not significantly different from zero. However in all cases fitting the parameters in turn resulted in a decrease in the deviation.

It will be seen that the b's are negative and these quadratics have imaginary roots. Their values are everywhere positive. Consequently the effect of pressure in the second form of Equation (c) is always opposed to that in the first.

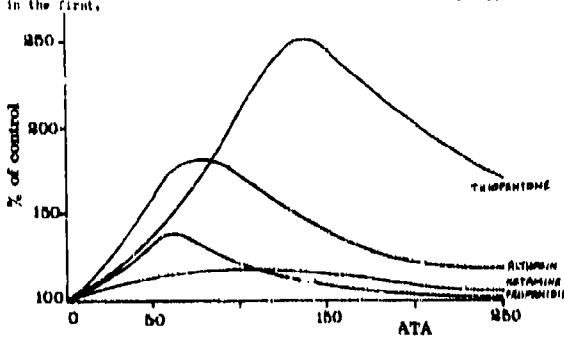


Fig. 1 illustrates the computed curves up to a pressure of 250 atm. It is interesting that the observed data which it limits to a maximum pressure of 50 - 100 atm predicts curves with maxima in all cases. For example although the thiopentone indicated an apparent upswing in the pressure reversal curve of the observed data, the computed curve has a maximum at 145 atm.

If the term in p^2 is omitted and the data are fitted to the equation

$$y = \frac{p}{a + pb}$$

the values of a and b (with their standard errors) are:

	a	b
Althesin	1,55 (0,[illegible])	-0,[illegible] (0,[illegible])
Thiopentone	2,[illegible] (0,[illegible])	-0,[illegible] (0,[illegible])
Propanidid	0,[illegible] (0,[illegible])	-0,[illegible] (0,[illegible])
Ketamine	2,[illegible] (0,[illegible])	-0,[illegible] (0,[illegible])

Again, all the b's are negative and the equation is not a Langmuir curve but one which increases steadily with pressure and becomes infinite at a pressure given by $p = a/b$.

What seems to be happening is that the data are essentially concave upward at lower pressures and cannot be fitted to an adsorption type of model. The other implication is that the antagonistic effect is not infinite at all pressures but goes through a minimum at a pressure $p_{min} = b/2c$.

The differences between the parameters for the different agents is in accord with them acting at different sites. However, the nature of the results is so consistent in the four separate series of experiments that we believe it must reflect some underlying general mechanism at these sites. A possible mechanism is that there are two sites operating, one of which is strong at low pressures and dies away and another which is weak at low pressures and increases with pressure. Alternatively the combination of these two effects predicts that the inhibition would first decline with pressure and then increase. This at first might appear unlikely but it is known that the effects of pressure on the unfolding and folding of proteins can behave in this way. Lipids are compressible but do not show this biphasic response to pressure (see Halsey et al, 1978). Thus the mathematical analysis of our data for the intravenous agents provides unexpected support for the postulate that the sites of action of at least some anaesthetics are hydrophobic areas of proteins.

References will appear in PROCEEDINGS.

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[illegible]

pressure to biological systems, especially to gas-breathing animals, and this use is based on the view that helium is non-narcotic. Another proposed biological use for high-pressure helium has to do with the retrieval and culture of bacteria from the deep sea. Jannasch and Wirsen (2) have developed apparatus for isobaric retrieval and transfer of deep-sea samples. Taylor (9) has explored the possibility of using compressed helium for pressure chambers which would have a gas phase and in which barophilic bacteria could be attached or otherwise manipulated without the need for an all-liquid environment. Clearly, it is desirable for this sort of use that helium be without specific biological effect. However, the results of our experiments over the past few years do not support the view that helium pressures are equivalent to hydrostatic pressures. Instead, it appears that helium has significant effects on microbial growth.

The data in Fig. 1 show differences in the effects of hydrostatic pressure and of helium pressure on the extent of growth of Saccharomyces cerevisiae. A similar picture was obtained for growth rate. It is readily apparent that hydrostatic pressure is a more potent growth inhibitor than is helium. This finding basically agrees with the findings of Macdonald (4) for tetrahymena cell division. S. cerevisiae is very sensitive to pressure compared with the bacteria with which we have worked previously, and so it was possible to carry out these experiments with compressed helium from commercial tanks of the gas. Taylor (9) used a vessel in which helium from a 100-atm source was further compressed to 300 atm. P_{50} data for a marine bacterium IP-6 show that 500 atm helium was much less inhibitory for growth than was 500 atm hydrostatic pressure. In essence, it appears that helium to a degree reverses the growth inhibitory action of hydrostatic pressure. In our experiments, the difference between helium pressure and hydrostatic pressure could not be related to air contamination of the helium since addition of even as much as 0.5 atm oxygen to the cultures through the vehicle of FC-80 fluorocarbon liquid did not reduce the sensitivity of the organism to pressure.

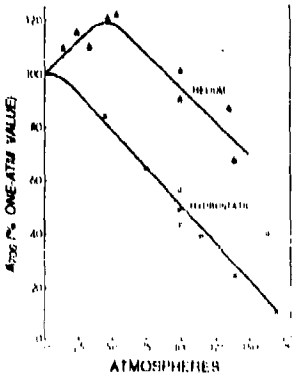

Fig. 1. Comparative effects of hydrostatic pressure (○) and helium pressure (▲) on growth of S. cerevisiae. Cultures were inoculated with a 4% inoculum of an overnight culture in tryptone-glucose-Marmite medium (•) plus 10% of ampicillin per ml and incubated at 24°C. For application of hydrostatic pressure, cultures were placed in plastic syringes of the type we have used previously and compressed in standard pressure chambers (6). For application of helium pressure, the cultures were placed in flasks containing stirring bars coated with teflon, and the flasks were placed in standard pressure chambers. The chambers were connected to cylinders of compressed helium and pressurized. The cultures were stirred initially with the stirring bars to speed up gas transfer. In these experiments, air was not flushed out of the chambers, and air removal was found not to affect the experimental results. For absorbancy determinations, cultures were decompressed slowly to avoid excessively cooling, warming, and immediately recompressed. Absorbancy was measured with a Beckman DU spectrophotometer set for 700 nm light. The values indicated are maximal absorbancy values.

As mentioned, helium is generally considered to have negative narcotic potency, and it antagonizes the narcotic actions of nitrous oxide. In contrast, we found that helium acts to potentiate or enhance the growth inhibitory actions of nitrous oxide for bacteria (7). The data presented in Fig. 2 indicate that helium also enhances the inhibitory effect of nitrous oxide on yeast growth.

Other experiments indicated a similar enhancing effect for nitrous oxide inhibition of growth of Tetrahymena thermophila. Even though high-pressure helium enhances growth inhibition, hydrostatic pressure acts to reverse the inhibition, as it acts to reverse narcotic responses. As shown in Fig. 2, 16.4 atm of nitrous oxide almost completely suppressed growth of S. cerevisiae. However, application of 200 atm hydrostatic pressure to the culture nearly completely reversed the effect of nitrous oxide, even though 200 atm hydrostatic pressure alone also almost completely stopped growth. Here the antagonistic actions of nitrous oxide and hydrostatic pressure are clear. At a lower level of 3.4 atm of nitrous oxide, it was possible by application of 100 atm hydrostatic pressure to obtain better growth than at one atm in the absence of nitrous oxide.

It seems clear from the data presented that the growth responses of yeast to pressure imposed by use of compressed helium are very different from those to purely hydrostatic pressure. Other work in this laboratory has led to the same conclusion for bacterial cells and tetrahymena. The net conclusion is that helium must have specific effects on cell growth separate from those due simply to pressure. Previously, Kalaises et al. (3) showed that helium can enhance

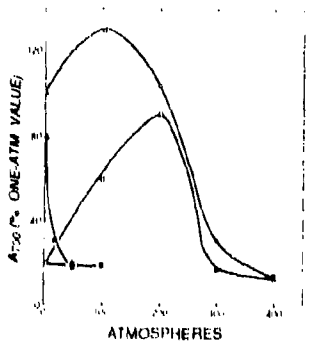

Fig. 2. Potentiation by helium and reversal by hydrostatic pressure of the growth inhibitory action of nitrous oxide for S. cerevisiae growing in tryptone-glucose-Marmite medium at 24°C. Data are presented for the effects of hydrostatic pressure on cultures exposed to 3.4 atm nitrous oxide (○) or 16.4 atm nitrous oxide (□) and of helium pressure on cultures exposed to nitrous oxide at pressures of 10 (▲) or 16.4 (■) atm. Cultures were exposed to helium-nitrous oxide mixtures as described previously (7). Cultures were exposed to nitrous oxide at high hydrostatic pressure by placing them in gas-tight syringes (Glenco Scientific Co.) with the proper amount of nitrous oxide and then compressing the syringes in standard pressure chambers. The amounts of nitrous oxide required were calculated by use of the Ostwald coefficients presented by Wilhelm, et al. (10). The abscissa scale indicates pressure in addition to that due to nitrous oxide.

iron uptake by bacteria in iron-deficient media. This enhancement was not important in our experiments with complex, iron-sufficient media. However, it is still clear that helium must specifically affect some reactions involved in the growth of cells.

ACKNOWLEDGMENTS

This work was supported by the U.S. Office of Naval Research under contracts N00014-75-C-0616 and N00014-76-C0001. We thank Gary Bender for technical assistance.

References will appear in PROCEEDINGS.

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EFFECTS OF HELIUM-OXYGEN HYPERBARIC EXPOSURES ON CEREBRAL, CARDIAC AND ADRENOCORTICAL RESPONSES OF RATS. J. Abraini and J. Risse, Centre d'Etudes et de Recherches Physiologiques Appliquées à la Marine Nationale, Toulon Naval, France.

Rats variously exposed to 6 ATA of either pure oxygen or nitrogen-oxygen mixtures, modifications of brain energetic adrenergic substrates have been reported. In order to study more precisely the specific modifications due to nitrogen hyperbaric exposures, we measured brain concentrations of glucose, lactate and glycogen in rats exposed to various hyperbaric situations. Ketonemia and lactatemia have been also recorded because of their possible interference with brain levels.

MATERIAL AND METHODS.

Groups of 15 male rats (mean weight = 300 g, water and food at libitum) have been used. Each exposure has been preceded by a decompression of the pressure chamber with a nitrogen-oxygen mixture, the effluent gas being the same than the one used during the corresponding hyperbaric exposure. During the experiments, the temperature was regulated at 22°C, CO_2 maintained between 200 and 2 000 ppm and CO_2 removed. In order to avoid any decompression induced artefacts, animals were sacrificed at pressure by deep freezing in liquid nitrogen the 4 diving profiles used are shown in figure 1.

In order to study adequate compression less hyperbaric exposure effects, in the acute (5 min, 6 ATA) one rat/week sacrificed just after the end of compression (series N_2-6, 6 ATA), the other at the end 30 minute (series N_2-30, 6 ATA) as usual.

Compression to 11 ATA has been used to evidence the correlation between nitrogen narcosis and biochemical modifications (series N_2-6, 11 ATA).

Compression to 21 ATA with an O_2-N_2 oxygen mixture has been done for studying the effects of fast compression combined with a different diluent gas (series He-6, 21 ATA).

Glucose, lactate and glycogen have been measured according to classical enzymatic techniques.

RESULTS.

Series N_2-30, 6 ATA, N_2-6, 6 ATA, 2 min and 6 min, 11 ATA behave normally during the exposure (convulsive clonic hand, ataxia, N_2-30, 11 ATA) except specific nitrogen narcosis behaviour (hyperexcitability and aggressivity).

The blood glucose values (figure 2, expressed in percent of controls) decreased very much in the acute series (-25 to -40%, significant), increased in the series N_2-30, 6 ATA (+20 and +40 %) and N_2-6, 11 ATA. In all series, no change in brain glucose values was noted, whereas in the 6 ATA series brain glycogen and lactate have been increased.

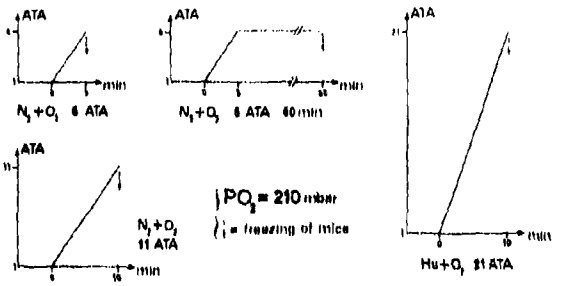


FIGURE 1 : Hyperbaric exposures profiles .

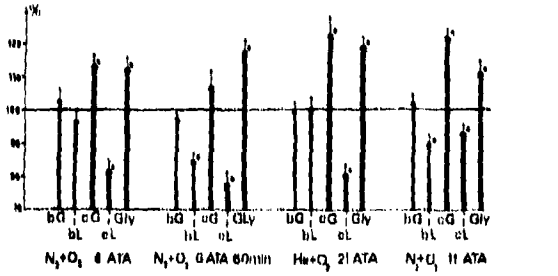


FIGURE 2 : Effects of different normoxic hyperbaric exposures on blood glucose, lactates cerebral glucose, lactate and glycogen expressed as percent of controls . ($p<0,05$) .

DISCUSSION :

We have checked that these modifications are not artefacts caused by different freezing rate in animals sacrificed at depth .

Blood lactate :

All the N_2+O_2 exposures induced a decrease in lactatemia . No modification has been observed in series Hu+O_2 21 ATA of this experiment . However, complementary tests show that generally Hu+O_2 induces the same decrease of lactatemia , such a modification has been also reported with pure oxygen (HBO) . Therefore this alteration has to be considered independant of the inhaled gas mixture .

Brain glucose :

The increase in brain glucose in series N_2+O_2 6 ATA is greater than the one observed in series N_2+O_2 6 ATA 60 min . This modification seems to be transient or diminished during the sojourn at depth . The increase in brain glucose is greater in series N_2+O_2 11 ATA and Hu+O_2 21 ATA : this modification seems to be linked with pressure and can be observed with both nitrogen and helium . This increase is not related to an increase in glycemia . It is not also triggered by activation of glycogenolysis : brain glycogen increases in all series .

Brain lactate :

In all exposed series, brain lactate was significantly decreased . No variation is equivalent whatever can be the pressure, respiration rate and diluent gas (the same decrease has been reported in HBO) . It is interesting to note that brain lactate decrease is less important in animals which showed nitrogen narcosis (N_2+O_2 11 ATA) .

Brain glycogen :

Brain glycogen was significantly increased in all series . Comparison between brain glycogen and diving profiles suggests that this modification is linked with both time and pressure . However, in series N_2+O_2 11 ATA, the increase is less important but still significant . Previous works showed that an increase in brain glycogen induced by pharmacological or physiological factors is often due to an increase in brain glucose . It may be the case in our observations .

CONCLUSION

Different normoxic hyperbaric exposures induced in the brain an increase in glucose and glycogen associated with a decrease in lactate . These modifications apply to animals with both normal nitrogen narcosis or HBOs, whatever the inhaled gas mixture is . We are now working on different hypotheses :

Increase in facilitated transport of glucose which could be due to a gas concentration gradient during compression ; such a phenomenon has been reported after intravascular injection of hyperoncotic solutes .

Decrease of glucose consumption, other metabolites such as lactate and glutamate being used as a fuel (such a modification is induced by acute hypercapnia) .

Decrease in neuronal glycolytic activity, secondary to a decrease in functional activity, either by direct metabolic effect of gaseous pressure or by modification of interneuronal action .

Complementary studies are running on glucose transport and utilization . They could help a better understanding of the situation .

TOXIC EFFECTS OF OXYGEN ON THE FUNCTIONS OF PULMONARY CYTOCHROME P-450

G.M. Gerlach, A. Sybert, A. Knoblauch, S. Brennan, M. Peale and J.E. Sylvester
The Johns Hopkins Medical Institutions, Baltimore, Maryland, U.S.A.

Cytochrome P-450, because of its negative redox potential, should be sensitive to oxidative damage and pulmonary cytochrome P-450 should be especially affected because of the high tissue PO_2 in the lung. In order to test this hypothesis we measured the effect of exposure to 100% O_2 on two functions of the pulmonary cytochrome, that of tissue carrier for CO and that of extrahepatic drug metabolism.

For several years we have been investigating the possibility that cytochrome P-450 might act as a tissue carrier for O_2 and CO in the lung and placenta. (J. Appl. Physiol. 43: 880-885, 1977, J. Appl. Physiol., 39: 725-734, 1975, Research Topics in Physiology Vol. I Ed. D.J. Davies and C.G. Barnes, Academic Press, New York, 1978 Pgs. 197-251). One particularly striking phenomenon demonstrated by these experiments is that of saturation kinetics for CO transport. In these experiments, which are described in detail in the 1977 paper, we observed that steady state diffusing capacity of the lung for CO (DLCO) measured in anesthetized, paralyzed dogs, ventilated at constant tidal volume and frequency was affected by the inspired CO concentration used in the measurement. We found that as inspired CO concentration was increased, the magnitude of the DLCO increased, reached a maximum and then decreased. The maximum DLCO was observed at an alveolar CO level of approximately 100 ppm. In the above mentioned paper we demonstrated that this sort of change in DLCO would be caused by a tissue carrier and a sigmoid dissociation curve similar to that of cytochrome P-450. Furthermore, we demonstrated that the maximum DLCO should occur at the P50 of the carrier. This is evidence that cytochrome P-450 may be the carrier since the P50 of the cytochrome is near 100 ppm. In 7 dogs we found that after exposure to one to two hours of 100% oxygen that the manifestation of saturation kinetics was completely abolished, i.e. the DLCO did not change as the CO concentration was varied. The manifestation of saturation kinetics returned in most animals after 2-3 hours after cessation of hyperoxia. Administration of large doses of reduced glutathione did not prevent the action of oxygen, possibly because this substance could not penetrate cell membranes and affect intracellular oxidative events. We interpret the results as indicating that the function of the carrier is abolished, perhaps reversibly by a change in redox state brought about by hyperoxia.

We also measured the effect of hyperoxia on the o-demethylation of P-nitroanisole in isolated blood perfused rabbit lungs. This reaction is known to be mediated by cytochrome P-450. The rate of metabolism, measured in a group of control rabbits was 2.67 ± .33 μg/hr./g.dry wt. (mean ± S.E.). In rabbits exposed to 100% O_2 from 12 to 24 hrs. no metabolism was observed. The lungs exposed to hyperoxia were grossly normal and showed normal compliance. At the time of writing this abstract, dose response relationships for shorter exposures as well as the effects of antioxidants are being carried out.

(Supported by DSPP's Grants HL 18442, HL 07100 and the Porter R. Francis Foundation)

STUDY ON DEFINITION OF MAXIMUM PERMISSIBLE GAS FLOW IN LUNGS DURING DECOMPRESSION. J. Parc, J. le Chuiton. Commission d'Etudes Pratiques d'Intervention sous la Mer. 83800 TOULON NAVAL, FRANCE.

1.- Experimental approach

Deep saturation profiles set up by CEPIS-MER (Undersea Operations Practical Studies Committee) and used for human diving have always been calculated from results obtained and lessons learned in animal experimentations at heavy depths (500 to 1 000 meters) carried out on miniature Pittman-Moore pots.

A very high acceleration in breathing has been frequently observed during continuous decompressions after 24 hours dives at 750 to 1 000 meters. Breathing frequency would then rise from 12 respirations per minute up to very high values reaching 200 respirations per minute. Interruption of decompression would entail return to normal within 10 to 30 minutes

To explain this finding, we imagined a saturation of the pulmonary barrier at permissible gas flow level ; the flow of gas crossing the barrier being higher than the maximum capacity of the lungs.

When decompression has not been interrupted, the inert gas excess unable to cannot out will produce a certain amount of bubbles responsible for serious accidents through arterial embolization.

This study aims to define the maximum gas flow able to cross the pulmonary barrier without affecting the barrier's efficiency.

2.- Definition hypothesis

Since the body is proportionally composed of aqueous, adipose and fibrous tissues with different solubility characteristics with respect to inert gases, each type of tissue will take charge of a certain mass of inert gas as a function of gas distribution in the body for a particular dive within time and depth limits and shall afterwards take responsability for a definite gas flow within decompression limits.

3.- Definition procedure

3.1.- Define mass of gas in each type of tissue from the total mass of the tissue and the inert gas solubility coefficient given to the tissue.

3.2.- The overall value of gas masses thus obtained is equal to the overall mass of inert gas dissolved in the body for each time-depth values.

3.3.- Compute decompression table, using classical supersaturation coefficient while concurrently calculating overall flow of inert gas with flow variations during the decompression preliminarily selected.

3.4.- A dual graph may then be plotted showing decompression profile and inert gas flow variation curve.

4.- Results

4.1.- Human dives lasting 30 to 90 minutes at 70 to 150 meters have thus been defined and experimented.

4.2.- The examination of curves has shown that :

- the flow factor seems to prevail at the heaviest depths.

- the supersaturation coefficient seems to prevail at the lowest depths.

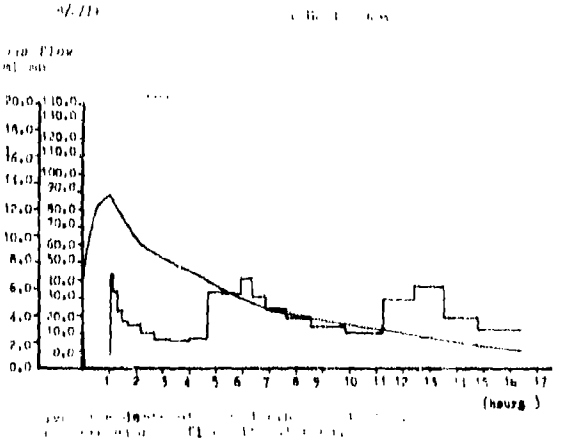
- Example : (See graphs)

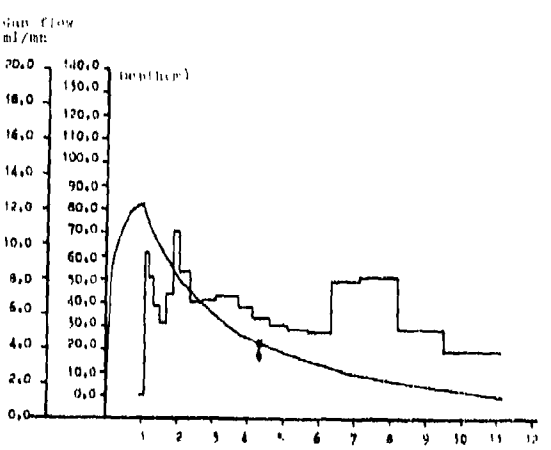
Two 60 minutes dives at 150 meters, one of which induced accidents.

5.- Conclusions

The hypothesis considering maximum permissible inert gas flow through pulmonary interface does not yet seem representing decompression as a whole since the supersaturation coefficient appears primordial at end of decompression.

However, this approach shall perhaps permit linking the various definition hypothesis based on supersaturation coefficients and their variations, pressure gradients and, possibly, distribution.





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EVALUATION OF DECOMPRESSION TABLES BY A MODEL DESCRIBING BUBBLE DYNAMICS IN TISSUE. S. Meisel, Y. Talmon, and D. Kerem. Dept. of Chemical Engineering and Dept. of Physiology & Biophysics, Faculty of Medicine, - Technion, Israel Institute of Technology, Haifa, Israel.

Decompression following a hyperbaric exposure may cause formation of gas bubbles in tissue and blood. It is widely accepted that this gas phase is the cause of marginal symptoms of decompression sickness. It has been suggested that the formation of bubbles could also occur during symptomless decompression carried out by following conventional diving tables, in which case the bubbles are termed 'silent'.

We believe that bubble formation and its dynamics are the key to a correct rationale in computing decompression tables. To pursue this concept further, we have developed in this paper a mathematical model which describes bubble dynamics in tissue, in relation to environmental parameters characteristic of a dive, such as bottom time and depth.

We assume that a gas phase is already present in the tissue undergoing decompression and probably exists as nuclei even under normal conditions due to the heterogeneous nature of the tissue. This gas phase is considered to be finely-dispersed in the tissue as minute bubbles, that grow upon decompression by physical expansion and inward diffusion of inert gas from surrounding supersaturated tissue. At the same time blood flowing in capillaries absorbs inert gas from the tissue. Bubble evolution will eventually take place due to surface tension, tissue elasticity and inherent unsaturation (Hills and LeMessurier, 1969) which establish a tension gradient favoring inert gas efflux.

We surmise the bubbles to be spherical and so dispersed as to be considered situated in an infinite medium of perfused tissue. Perfusion is taken into account as a uniformly distributed mass sink. A mass balance on the bubble yields (after deleting a convective term found to be of minor significance) an expression that can be written in a dimensionless form as:

(1)

where τ ($\tau = \frac{Dt}{R_{b_0}^2}$), a dimensionless pressure of inert gas in tissue, is defined as $\tau = \frac{P-P_a}{P_{t_0}-P_a}$, P denotes pressure and the subscripts a and o denote arterial and initial. We also define the following dimensionless variables:
a dimensionless time,

where t is time, D is the diffusivity and R_{b_0} is initial bubble radius.
the ratio of bubble radius to its initial value,

and a dimensionless radial-coordinate,

Dimensionless perfusion modulus is,

where λ and Q are the solubility coefficient and perfusion rate. The subscripts b and t denote blood and tissue related parameters.

The dimensionless pressure in the bubble is given by:

where the three terms in the numerator stand for the inherent unsaturation, tissue elasticity and the surface tension. K is the elastic modulus of the tissue and γ is the surface tension.

An expression for ΔP, in the case of air breathing, is obtained from Hills and LeMessurier (1969)

Eq.(1) is transformed into a cartesian form, a solution to which is found in Carslaw and Jaeger (1959). The solution is then substituted in the dimensionless Fick's law, and the result, an expression for bubble radius rate of change, is finally given by:

$$\frac{dr}{dt} = (P_o - P_a)\frac{r_o^2}{r^2}\left(\frac{P_a}{P_B}\right)\left\{\omega^{-1/2}(1+\frac{c}{\sqrt{\pi t}})-\phi_H(1+\frac{2}{c}\sqrt{\frac{\pi}{\pi t}})\right\}$$

Numerical integration of the above equation yields $\varepsilon(\tau)$ for a step change in alveolar inert-gas tension, assuming steady state values of P_o-P_a.

This model can predict the behaviour of a decompressed bubble for various depths and saturation fractions (f_s), for different breathing gas mixtures, and can be used for the evaluation of decompression tables.

Our basic assumption is that marginal symptoms become overt when pressure in a semi-rigid tissue exceeds a critical value. If F_n is the concentration of nucleates in tissue and δ is the added pressure of the gas phase volume then we have (Nims, 1951):

$$\delta = \frac{\phi}{\beta} F_n K \varepsilon R_n^3$$

If the critical δ for inducing symptoms is 11 mmHg (after Inman and Saunders, 1942) then ε_{cr} can be easily estimated.

Thus, bubble radius change following a stage decompression can be calculated and symptoms can be expected when ε exceeds ε_{cr}.

To illustrate this procedure we present two figures. Fig.1 shows the pattern of bubble radius change after a saturation exposure (f_s=1) at 30 m. The decompression profile includes stops at 7 m and 3 m with more time spent at the shallower stop. This is typical of conventional decompression tables.

Fig.2 shows equivalent patterns after a 30 m exposure at saturation fractions of 0.3, 0.15 and 0.05. The issue of a proper uptake function was avoided by simply choosing f_s values. The first stages of the decompression reveal bubble resolution because of the low degree of supersaturation, but upon further decompression bubble growth takes over. It must be kept in mind that the saturation fraction values relate to the first decompression stop only and require adjustment if the surface is considered as the reference.

The model also predicts, in agreement with empirical findings that more time spent in deeper stops results in a shorter total decompression time. Thus, a smaller maximal bubble radius is obtained when time is partitioned in favor of deeper stops. Further applications of this model include evaluation of therapeutic recompression profiles with and without oxygen breathing and optimization of decompression profiles.

References will appear in PROCEEDINGS.

Figures 1 and 2 follow.

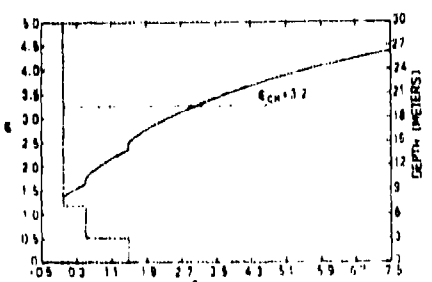

Fig.1: Change of bubble size (solid curve) for a given decompression profile (dotted line) after saturation at 30 meters.

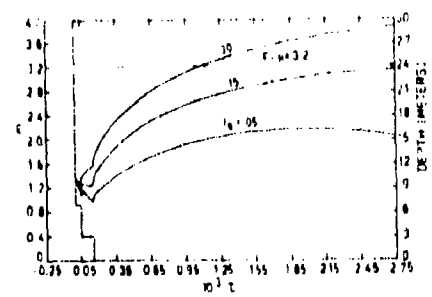

Fig.2: Change of bubble size (solid curves) for a given decompression profile (dotted line) after various degrees of saturation (f_s) at 30 meters.

COMPUTER SIMULATION OF DIFFUSIVE GAS MIXING IN THE LUNG AT 10 ATA. H.D.Van Liew, Department of Physiology, State University of New York at Buffalo, Buffalo, NY, 14214, U.S.A.

Gas-phase diffusivity is inversely proportional to gas density, so diffusive mixing of air molecules within the lung can be expected to be slowed by a factor of 10 when a person breathes air at 10 ATA. However, it is known that people tolerate 10 ATA of air without signs of severe gas exchange impairment. In experiments at 9.5 ATA (5), a heavy gas, SF_6, was clearly not as well mixed as it had been at 1 ATA, but the decrease was far less than the approximately 10-fold decrease of diffusivity. Why is pulmonary function so insensitive to diffusivity changes?

With the aid of a computer, one can simulate diffusive mixing of gas in a container of any shape. Simulations for the branched airway system of the human lung at normal pressures (2,3,4) have shown the following: a) The several most peripheral (lower) generations along any path are essentially at diffusion equilibrium with each other because the airways are so short. The lower airways and the alveoli they serve account for almost all of the lung volume. b) At some location along the airways, there is a steep concentration gradient between the well-mixed gas in lower airways and unmixed inspired gas in upper airways. c) During inspiratory flow, convection pushes the gradient region peripherally. d) Whenever flow slows or stops, diffusive discharge from upper to lower airways causes the gradient region to move mouthward.

In a hyperbaric air environment, the outcome of these processes can be expected to change because diffusivity is less and because convection becomes relatively more important in diffusion/convection interactions. In this communication, we report on simulations of diffusion in the lung at 10 ATA, with special emphasis on the efficiency of gas exchange per breath as judged by the amount of inspirate that remains in the functional residual capacity (FRC) after expiration.

METHODS

For our program (4), we used the morphometric equations "A" of Weibel (6) to generate a lung of desired size, then divided this lung into 24 compartments, one for the trachea and one each for the sum of all airways in each of 23 generations of branching.

The simulation of diffusion alone consists of allowing an indicator gas to move between the gas volumes (alveoli plus airways) of adjacent compartments. The rate of movement between a compartment and its neighbor is caused to be directly proportional to summed cross-sectional area of all airways in that generation, to gas-phase diffusivity, and to the concentration difference between the compartments; rate is inversely proportional to length of the airways in the generation. This process occurs between each pair of compartments for a short time interval. At the end of the interval, the new concentration in each compartment is calculated and then another interval is allowed to occur.

The simulation of diffusion plus convective addition of inspired gas into the lung consists of the above process plus addition of an appropriate amount of indicator gas during each time interval into the particular generation in which diffusive conductance just equals the desired convective flow in effect, all the gas that enters the appropriate compartment by convection can leave it by diffusion. For computations presented here we used diffusivity of O_2 in air.

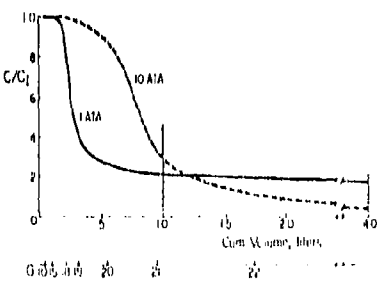

Figure 1. Position of indicator gas concentration (relative to inspired concentration) vs cumulative volume inside the lung at 1 and 10 ATA after 1 sec of constant inspiratory flow. Midpoints of the various generations of branching shown on lower axis.

RESULTS AND DISCUSSION

Figure 1 shows the indicator gas concentration inside a lung that originally contained no indicator. The computations are for the end of 1 sec of inspiration of indicator at a constant flow rate of .5 l/sec, equivalent to the end of a 1.0 l inspiration. The concentration is displayed as a profile of C/C$_I$ (concentration relative to inspired concentration) vs cumulative lung volume along the airway starting at the top of the trachea. The gas to the left of the vertical arrow will be exhaled, and if there were no further mixing, its profile would be traced out in the exhaled gas for the 1 ATA simulation. About 200 ml of high concentration indicator would be exhaled on the upper airway "dead space" followed by a sloping "alveolar plateau" that has C/C$_I$ of .28 to .22. The gas in the FRC (1 liter) to the right of the vertical arrow is almost level at C/C$_I$ of about .18. The dashed 10 ATA profile in Fig 1 is considerably different: there is no plateau in the gas that will be exhaled and there is a gradient from C/C$_I$ of .28 to .06 within the FRC. The computations showed that for the 1 ATA case of Fig. 1, 500 ml of indicator was in the FRC whereas the value was only 245 ml at 10 ATA.

In a real breath there must be a slowing, stopping, and reversing of flow at the end of inspiration. We simulated the additional mixing that occurs in the transient state before expiration is allowed to occur as it would during a breathhold. Results are shown in Fig. 2. For the 1 ATA case, after only .2 sec of this "breathhold mode" of diffusive mixing, the steep gradient has moved mouthward so that a better dead space estimate would be about 150 ml. The process is much slower at 10 ATA; in 1 sec, the profile is still to the right of the beginning 1 ATA profile. It would take about 4 sec at 10 ATA to match the .2 sec profile for 1 ATA. A dead space estimate after 1 sec at 10 ATA would be about 400 ml. In the 10 ATA case, the mouthward movement, slowed by low diffusivity, is nonetheless aided by the fact that the profile is in the higher numbered generations that have large conductance (summed cross sectional area of

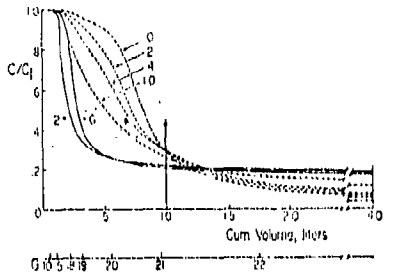

Figure 2. Change of the profiles during a breathhold in the inspiratory position. Solid curves = 1 ATA. Dashed curves = 10 ATA. Times shown in sec. Profiles from Fig. 1 are labelled zero.

the airways divided by their length); at 1 ATA, .2 sec in the breathhold mode adds 48 ml to the FRC at the end of the flow phase, whereas the same duration at 10 ATA adds slightly more, 53 ml.

CONCLUSIONS

The computations on which Fig. 2 is based show that if the breathhold mode is .2 sec, the fraction of inspired gas to exchange into the FRC is .80 at 1 ATA and half as great at 10 ATA. A 1.0 sec pause at end-inspiration could compensate for about half of the effect of the 10-fold decrease of diffusivity, or a doubling of ventilation with the .2 sec breathhold mode could completely compensate.

These computations estimate the minimal gas exchange. Measured profiles during expiration in man at 9.5 ATA (5) were not as far to the right as the dashed curves of Fig. 2. As suggested by Engel *et al.* (1), convective mixing due to heart action probably increases the effective diffusivity; if so, the profiles of Fig. 1 and 2 would be moved slightly to the left for 1 ATA (because the profiles are already in small-volume, low-conductance upper airways) and markedly to the left at 10 ATA (because the profiles are in high-conductance airways where additional mixing can have a large impact on amount of gas exchange.) (Supported in part by NIH Grant HL-14414.)

References will appear in PROCEEDINGS.

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MORE RECENT EXPERIMENTS ON BUBBLE FORMATION IN SUPERSATURATED GELATIN. D. E. Yount, C. J. Yeung, and T. D. Kunkle, Department of Physics and Astronomy and Department of Physiology, University of Hawaii, Honolulu, Hawaii 96822 U.S.A.

Previous experiments on bubble formation in supersaturated gelatin (Yount and Strauss 1976; Yount, Yeung, and Ingle 1979) were carried out mainly with rectangular pressure schedules consisting of a rapid compression, equilibration of the sample at some increased pressure, and a rapid decompression. This simulates a dive profile from which pressure-reduction limits can be determined as a function of saturation pressure. For this class of schedules, the results *in vitro* are quite similar to those *in vivo*, and a mathematical model developed to describe bubble nucleation in gelatin (Yount 1979a) has been found to be in remarkably good agreement with decompression data obtained from rats and humans (Yount 1979b). More specifically, isopleths of constant bubble number in gelatin are similar to those in rats (Watt and Lin 1979), and they are also of the same mathematical form as the lines of constant aftereffective dose in rats (Berghage et al, 1976) and as the pressure reduction limits in humans (Berghage et al, 1976; Hennessy and Hempleman 1977).

The gelatin experiments reported here extend the isopleths of constant bubble number into a new pressure region, thereby simulating conditions that would be experienced, for example, by humans exposed to high altitudes or to isobaric countercurrent diffusion. The new region can also be explored by using slow compressions or stepped compressions which permit a significant rise in the dissolved gas tension τ while the ambient pressure P_{amb} is still increasing. Thus, whereas conventional rectangular schedules satisfy the condition that the initial compression is greater than or equal to the final decompression, the reverse is true for the schedule shown in Fig. 1.

The new pressure region can be characterized mathematically by the inequality

$$P_{ss} \geq P_{crush} \qquad (1)$$

where

$$P_{ss} = (\tau - P_{amb})_{max} \qquad (2)$$

is the maximum supersaturation achieved during decompression and where

$$P_{crush} = (P_{amb} - \tau)_{max} \qquad (3)$$

is the maximum over-pressure or crushing pressure achieved during compression. For the schedule shown in Fig. 1, the supersaturation is given by

$$P_{ss} = P_s - P_f \qquad (4)$$

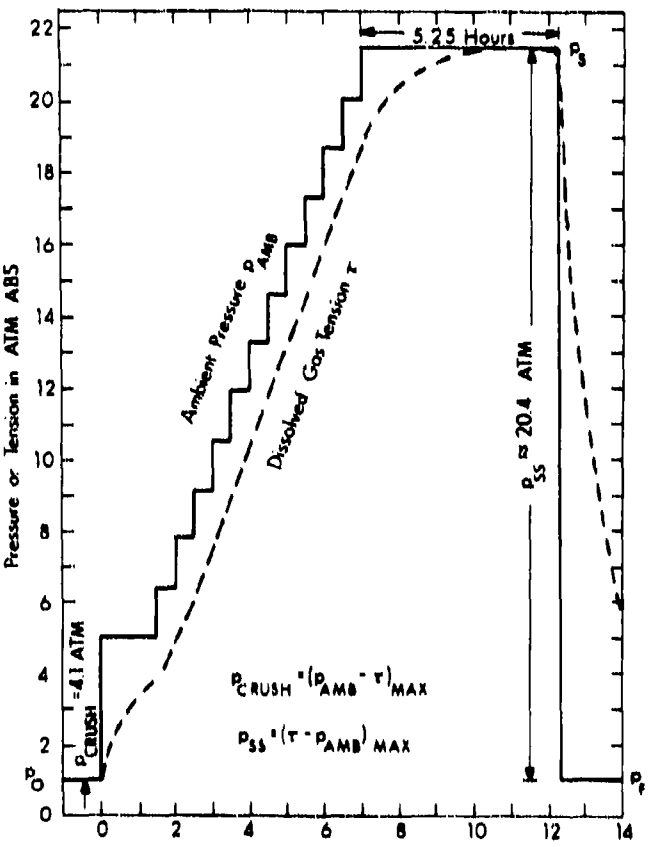
where P_s is the saturation or equilibration pressure and P_f is the final pressure at which the bubble counts are made. By design, the maximum over-pressure P_{crush} occurs on the first step and is simply the magnitude of the initial compression.

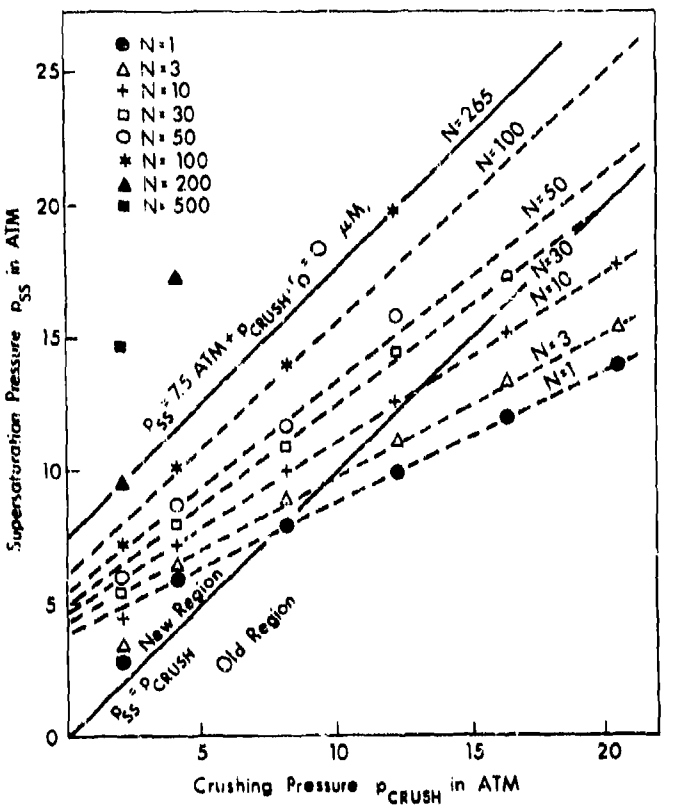
Our interest in the variables P_{ss} and P_{crush} is due in part to the experimental observation (Yount and Strauss 1976) that bubble counts in gelatin subjected to a rectangular pressure schedule depend only upon these pressure differences and not upon the absolute pressures per se. Furthermore, since P_{crush} is determined by a control agent in the test specimen while P_{ss} has the inherent restrictions that the latter is certain, whether there is a bubble the largest will grow in a short

The isopleths for constant bubble number N in gelatin are shown in Fig. 3. The measured points extend well into the new region defined by Eq. 1, and over much of this region, the dashed lines calculated from nucleation theory (Yount 1979a) give an accurate description of the data. Additional agreement with the aberrant points at large P_{ss} and large P_{crush} can be obtained by taking into account the skin thickness of the spherical gas nuclei that are the main initiators of bubble formation in gelatin. There remains, however, a surplus of nuclei at $P_{crush} = 2$ atm which is activated when the maximum supersaturation P_{ss} is much larger than the maximum over-pressure P_{crush}. The "surplus" nuclei are easily crushed and must therefore be gas-filled and rather fragile. Furthermore, their size distribution differs markedly from that of spherical gas nuclei. A class of objects that might fit this description is gas-filled crevices in suspended dust particles.

An important implication of this work is that supersaturation tolerances are much lower for humans exposed to high altitude or to isobaric countercurrent diffusion than they are for dives terminated by a decompression. Similarly, the prophylactic effects of a large initial compression can be inadvertently squandered through the initiation of any dive profile which allows a significant increase in the dissolved gas tension τ before the maximum depth is reached. Finally, in dives of long duration, the regeneration of gas nuclei *in vivo* may erase the effects of a large initial compression and produce a condition analogous to that specified by Eq. 1.

References will appear in PROCEEDINGS.
Figures 1 and 2 follow.





MICROBIOLOGICAL STUDIES ON ACUTE OTITIS EXTERNA IN SATURATION DIVERS

S. R. Alcock, Department of Bacteriology, Medical School, University of Aberdeen, Scotland.

Otitis externa is the major infection problem associated with diving (8,10). It is probably the commonest cause of morbidity during saturation dives, and in this environment the symptoms are frequently incapacitating (3, 9, 10).

A critical factor in the pathogenesis of the disease appears to be the relative proportions of gram-positive and gram-negative bacteria in the ear canal. The normal flora is predominantly gram-positive, mainly staphylococci and corynebacteria; that in otitis externa is predominantly gram-negative, mainly Enterobacteriaceae and Pseudomonas aeruginosa (5, 11). Hydration of the skin of the ear canal probably predisposes to colonisation and overgrowth by gram-negative bacteria (12, 6). P.aeruginosa is the gram-negative species most often implicated in overt disease (5, 11).

During 1974-75 two saturation dives in the North Sea were terminated because of incapacitating otitis externa, and others were disrupted. P.aeruginosa was consistently isolated from the ears of divers with otitis. This paper describes data obtained during seven subsequent dives which were subjected to microbiological monitoring and control.

METHODS

CHAMBER COMPLEXES

Two complexes (Fig. 1, T & R) situated on different ships were studied at different times. Individual chambers were named after their diameter in millimetres. Each chamber had an 'S.A.S.' area which contained the lavatory, shower and wash-basin for that chamber and was very cramped. In the R complex this area was separated from the rest of a chamber by an air lock (usually open during the dives). In the T complex there was no separation in the 1500 chamber and only a loose fitting screen in the 2,500. The main living chamber was the 2,500 in both complexes, and housed 6 - 7 divers. An atmosphere of oxyhelium (FO_2 400 m bar) was recycled over 7 - 8 mins. through tanks of silica gel, carbon and soda-lime.

DIVE MONITORING AND CONTROL

Four dives (T_1 - T_4) lasting a total of 54 days and involving 25 divers were monitored in the T complex, and three dives (R_1 - R_3) lasting a total of 65 days and involving 31 divers were monitored in the R complex. Work was at a depth of 75 - 85 metres, and divers spent 4 - 8 hours each day on the sea bed for about 9 of every 14 days in saturation.

The divers' ears and the chamber complex were swabbed before each saturation and at least every 2 days thereafter. Divers were not admitted to the chambers if gram-negative bacilli were isolated from their ears in the pre-dive screen. During a dive, divers from whose ears gram negative bacilli had been isolated were treated every 8 hours with ear drops containing gentamicin sulphate 0.3% w/v and polymyxin B sulphate 0.5% w/v. Infected divers were decompressed as soon as operational needs allowed.

During the first two dives in the T complex 'Savlon' (I.C.I.) 1/200 was used to disinfect the chamber, thereafter 'Panacide' (dichlorophen, B.D.H.) 200 parts/10^6 was used. A high standard of general and personal hygiene was enforced during the dives.

In the R system only, divers routinely used prophylactic ear drops containing boric acid, alcohol and glycerol.

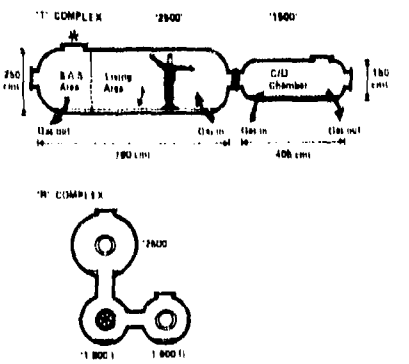


Fig. 1. Arrangement of pressure chambers in the T & R living complexes. Symbols: A, false floor overlying bilge and heating elements; c/d, compression/decompression; ⊕, diving bell keys on here.

MICROBIOLOGICAL TECHNIQUES

As described by Alcock (1977)[1].

RESULTS

DIVERS' EAR SWABS

The pattern of data illustrated in Fig.2 is representative of that obtained in all of the dives studied. Many divers had used prophylactic and/or antibiotic ear drops during previous dives. They entered saturation with either normal (60%) or no detectable ear flora. Thereafter gram-negative bacilli were isolated from the ears of 39 (67%) of the 58 divers studied. The ears of 85% of infected divers became colonised with gram-negative bacilli within the first 6 days of the dive. An absence of detected ear flora in the pre-dive screen did not predispose to infection.

P. aeruginosa was isolated at some time from 84% of infected divers, and was the first isolation of gram-negative bacilli in 50% of cases. Non-pseudomonad gram-negative bacilli isolated from divers ears (and from the chambers) contained a high percentage of members of the Enterobacteriaceae.

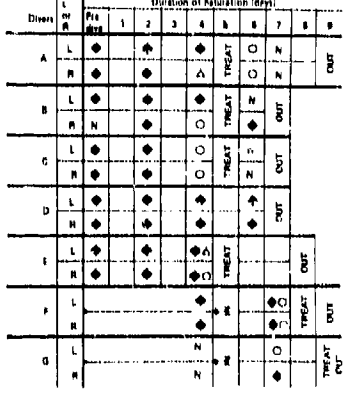


Fig. 2. Ear flora of divers during the dive, R_2. Symbols: ◆, normal gram-positive flora; Δ, non-pseudomonad gram-negative bacilli; ○, P.aeruginosa; N, no bacteria isolated; L, left; R, right.

Seven divers never entered the water but remained in the chambers as tenders. Three became infected, two with P.aeruginosa. Actual diving, with direct wetting of the ear canal, was thus not essential for infection. Divers using the T and R complexes suffered a similar incidence of ear infection, suggesting that the prophylactic ear drops used by the R complex divers were not effective.

Five (25%) of the T complex divers and five (15%) of the R complex divers developed ear pain. Gram-negative bacilli were isolated from the ears of all these divers, and P.aeruginosa from eight of them. The pain developed within 0 - 4 days of taking the ear swab from which gram-negative bacilli were first isolated. It was never incapacitating.

Twenty-one divers (54% of all infected divers) did not start decompression for five or more days after taking ear swabs from which gram negative bacilli were first isolated. All but two of them were treated, and only one (who was treated) suffered pain. These data, combined with the finding that only two of all treated divers suffered pain, suggest that, with treatment, infected divers can remain in saturation and incur little risk of pain.

CHAMBER SWABS

During the dives 377 swabs were taken from the main living (2,500) chambers of the T and R complexes. The 'S.A.S.' regions of these chambers (lavatory, wash basin, shower, and the adjacent chamber) showed heavy contamination with P. aeruginosa and other gram-negative bacilli within 1 - 2 days of starting a dive, and continuously thereafter. Elsewhere, only scattered isolations were made, the gas regeneration systems remaining particularly clear. In the first dive studied, the man's bedding showed a mixed flora of gram-negative bacilli after 4 days in saturation. In subsequent dives bedding was changed every 2 - 3 days. Daily disinfection with 'Savlon' or 'Panacide' failed to reduce contamination of the 'S.A.S.' areas to acceptable levels.

Limiting sampling of diving suits and hoods showed scattered contamination with P. aeruginosa and other gram negative bacilli; washing with 'Panacide' did not eliminate this.

SEROTYPES OF PSEUDOMONAS AERUGINOSA

Isolations of P.aeruginosa from dives T_1 and R_1 - R_3 were serotyped (4) and phage typed (2) at the Central Public Health Laboratory, Colindale, London.

Chamber contamination with P.aeruginosa was not detected before dive T_1, the diver (H) entered with two strains (of serotypes 11 and 2b/5c) in his ears, and he was not removed for 3 days. The 2b/5c strain later became predominant in his ears, but type 11 strains accounted for 16 of 18 isolations of P.aeruginosa from the ears of the other 4 divers; for 11 of 12 isolations from the chamber and for 4 of 4 isolations from the diving suits. The remaining strains isolated were of type 2b/5c. The phage typing results indicated that all strains of each serotype were indistinguishable. No other strains of P.aeruginosa were isolated from any source during the dive. Although initial chamber contamination may not have been detected, the evidence suggests strongly that diver H introduced the infection.

The 46 chamber isolations made during R_1 - R_3 were almost equally divided between 3 serotypes (Nos. 3, 11 and 6), but 91% of the 45 ear isolations were of only two of these (Nos. 3 and 11). Pseudomonas aeruginosa was isolated from the ears of 17 divers and only three were colonised with type 6 strains. Before the start of R_1, P.aeruginosa of serotype 11 was isolated from the 2,500 chamber, and by day 15 of this dive serotypes 11, 3 and 6 were widely distributed in the chamber complex. Once established, this pattern of contamination remained consistent throughout the rest of R_1, and throughout R_2 and R_3.

The data from the R complex point to the chambers as a possible reservoir of infection during and between the dives. The data from T_1 do not contradict this view and point to a single diver as the probable source of organisms which, in this dive, caused both ear infection and chamber contamination. Both sets of data suggest that in a saturation environment certain serotypes of P.aeruginosa are more likely than others to colonise the ear canal.

CONCLUSION

A characteristic pattern of diver infection and chamber contamination was consistently observed in the 7 dives studied. The control measures employed did not prevent colonisation of the ear canal with gram-negative bacilli, but they did control the operational problem which precipitated the study - incapacitating ear pain.

The results are relevant not only to the problem of otitis externa in divers, but also to the general microbiology of confined, hyperbaric environments. There appears to have been no comparable microbiological survey of saturation dives under commercial conditions.

Further investigations have been undertaken in two areas: the properties of P.aeruginosa grown in vitro under hyperbaric conditions (7), and the possibility that, in a saturation environment, certain serotypes of P. aeruginosa are more pathogenic than others.

References will appear in PROCEEDINGS.

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AN EPIDEMIOLOGICAL STUDY OF FATAL DIVING ACCIDENTS IN TWO COMMERCIAL DIVING POPULATIONS. M. I. Bradley. Naval Medical Research Institute, Bethesda, Maryland, U.S.A.

The distribution of fatal diving accidents in commercial diver populations in the Gulf of Mexico and in the British sector of the North Sea has been examined, and the factors that influence or determine that distribution are discussed. Recommendations for safer diving practices are presented and areas where research is needed are suggested.

There are an estimated 900 commercial divers in the United States who work in the Gulf of Mexico. From 1968 to 1975 there was an average of 7.2% deaths per year in this group of divers, an average annual fatality rate of 2,39 per thousand per years. About 200 commercial divers work in the British sector of the North Sea. From 1972 to 1976, there has been an average of 3.17 deaths per year in this group, with a fatality rate of 4.8/1,000 per year. The incidence of fatal diving accidents for each year in these two diver populations is presented in Table I. From this data it is apparent that commercial diving operations in the North Sea are more hazardous than those in the Gulf of Mexico. In the Gulf, the highest fatality rate occurred from 1968 to 1970; in the North Sea the peak period was from 1974 to 1976. These periods corresponded to the introduction of new diving techniques and heightened diving operations. It is noteworthy that in recent years there has been a substantial reduction in mortality in both areas.

Most accidents involve multiple factors that are mutually dependent. To understand the causes of accidents requires identification and analysis of interactions between variables that affect safety. One can respond to behavior by modifying accidents to an application of epidemiologic methods. Such an analysis is ideal only in terms of interaction between "host factors," "agent factors," and "vector factors." Host factors are the characteristics of the persons suffering injury; environmental factors refer to circumstances and objects that predispose or contribute to injury; and agent factors are the agents capable of producing injury.

Host factors

Age and experience: the average age of the divers who died in the Gulf of Mexico was 41, ranging from 24 to 64 years; the mean age of the diving fatalities in the North Sea was 36.5 years, with a range of 21 to 60 years. Because the data were inadequate, the degree of experience could not be assessed for either group; however, the average age of the North Sea fatalities, in which 77 of the divers were between 20 and 29 years of age, suggests a lower level of diving experience.

Health of a diver: the nature of diving requires that divers be in good health. Nevertheless, a small number of diver fatalities seem to be associated with conditions that contribute to, or are a result of, the accidents in the Gulf, and in two of the cases in the North Sea, medical conditions that contributed to the fatality were present.

Behavioral factors. Behavioral dysfunction in diving is a major contributor to diving accidents in all diving populations. Behavioral dysfunction in divers may take the form of poor judgement, careless and panic have been recognized as important contributors to fatal diving accidents. In 8 of the 9 GOM accidents and 10 of those in the North Sea, poor judgement or panic on the part of the diver was cited.

Summary of host factors. The "typical" commercial diver involved in a fatal diving accident is most likely to be in poor health. He is probably somewhat inexperienced as a diver. Poor judgement on his part or panic are associated contributors to the accident.

Environmental factors

Environmental factors that can contribute to a fatal diving accident are varied. They include, but are not limited to: oxygen at depth, [illegible] currents, [illegible] equipment failure, [illegible] and [illegible] and [illegible] water.

Depth of dive. In the Gulf, the mean depth of dive in which there was a fatality was 190 ft (range 90-310 ft). The mean depth of dive in which there was substantially greater [illegible] than in the Gulf. The [illegible]

these accidents according to depth is presented in Table 2. The majority of fatal diving accidents in the North Sea occurred during dives in excess of 200 ft. In both the Gulf and North Sea, episodes of unexplained diver unconsciousness or unaccountable actions have been contributory to accidents occurring during dives of 300 ft and greater.

Breathing gas. Compressed air was the breathing gas in use during the majority (67%) of fatal accidents in the Gulf. Helium-oxygen mixtures were most commonly (63%) in use during fatal North Sea accidents.

Cold. Cold was mentioned as a contributory factor in 11% of the North Sea fatalities. It was not a factor in any of the Gulf accidents.

Sea state. Heavy sea states were considered to be a factor in 15% of the North Sea accidents; all of these accidents occurred on the surface. In none of the Gulf accidents were bad weather conditions considered to be a factor.

Equipment failure. Severed or fouled hoses occurred in 33% of the fatalities in the Gulf and in 11% of the North Sea accidents. In 11% of the North Sea deaths, a diving bell was dropped; in another 19% of the North Sea fatalities there was some form of equipment failure, usually concerned with the underwater breathing gear.

Capability of others. In 33% of the Gulf fatalities and in 72% of the North Sea accidents there was some form of judgmental error by the diving supervisor, tender, or bellman.

Summary of environmental factors. There is considerable influence of environmental factors in commercial diver fatalities. Deeper dives carry a greater risk. Cold and sea state contribute heavily in the North Sea. However, the most important environmental factors present in fatal accidents are equipment failure and diving supervisor/tender errors during the conduct of the dive. Improved equipment selection, maintenance, and operation, together with adherence to expert, safe operating and emergency procedures would appear to offer the greatest possibility for reducing accidents.

Agent Factors

Agent factors are those agencies that constitute the direct causes of injury. The distribution of agent factors in these two populations is given in Table 1. In both groups, drowning was the most common proximate cause of death. Decompression sickness/air embolism and asphyxia were next in order.

Summary

Commercial diving is a hazardous occupation, nevertheless, the fatality rates are not as high as for other high risk occupations, such as anthracite mining, in the United States. In recent years, there has been a significant downward trend in mortality rates in the commercial diver populations in the North Sea and the Gulf of Mexico.

The interactions of host factors, environmental factors, and agent factors in commercial diving fatalities has been examined. The contribution of environmental factors to diving fatalities appears to be the greatest problem and the most amenable to change. Research into the cause of diver unconsciousness and unexplicable actions occurring at depths below 300 ft is needed.

Acknowledgments

Naval Medical Research and Development Command, Work Unit No. M0099.PN, 003.AD65. The opinions and assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

The superb editorial assistance of Miss B. M. Matzen is greatly appreciated.

Table 1

Frequency of fatal diving accidents by cause for diving

Gulf of Mexico								
Year	1968	1969	1970	1971	1972	1973	1974	1975
No. of deaths	5	1	5	1	1	1	2	1
North Sea								
Year	1971	1972	19	19	1975	1976	1977	1978
No. of deaths	1	1	2	5	1	1	1	2

Table 2

The distribution of fatal diving accidents according to depth

Dive Depths (ft)	Gulf of Mexico (%)	North Sea (%)
Surface	17	16
1-100	33	16
101-200	11	16
201+	39	52

Table 3

The distribution of causes of death in diving accidents

Cause	Gulf of Mexico (%)	North Sea (%)
Drowning	44	63
Decompression sickness/ air embolism	28	19
Asphyxia	17	7
Trauma	11	0
Other	0	11

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DRUG THERAPY OF DECOMPRESSION SICKNESS. B. Broussaile, Centre d'Etudes et de Recherches de Biophysiologie Appliquée à la Marine, B.P. 610, 83003 Toulon Naval, FRANCE.

This question has not been a field of intensive research since the report done in october 1978 at the EUBS'8 meeting in Luxembourg.

Let us recall the biological syndrome linked to the presence of decompression induced bubbles. It is essentially :

- anoxia
- microcirculation disturbance, with plasma leakage
- platelet aggregation and hypercoagulation
- interstitial oedema
- shock
- veino and broncho constriction.

Symptomatic therapy of these different disorders is mainly based on physiopathological and pathological considerations derived from animal experiments.

Clinical control for the efficiency of this therapy is difficult, due to the small incidence of decompression sickness and its polymorphism. A statistical study including controls is uneasy. On the other hand, such a therapy is never used alone, but in combination with recompression and oxygen therapy, the efficiency of which have been already demonstrated. Our opinion is finally based mainly on clinical appreciation.

In a general way, the efficiency or at least the inocuity of a therapy should have been demonstrated, before recommendation.

The use of plasma expanders in order to restaure blood volume and microcirculation is the only one generally agreed on.

Intravenous infusion should be started as soon as possible, in the same time than normobaric oxygenation during evacuation towards an Hyperbaric Therapy Centre, and continued during hyperbaric therapy.

Its efficiency is demonstrated by animal experiments (recently : WELLS and al, 1978) : infusion either of crystalloïdic solutes (Ringer lactate) or of macromolecular solutes (Dextran) re-established microcirculation, and a better way, associated with recompression (which is not efficient alone on this model).

In human therapy, the advantages of the different solutes, and the chronology of infusion are discussed.

For some authors, prior infusion of Ringer lactate is preferable (MOLLER, 1979), recommends 5 to 8 ml/kg of weight/hour) in order to obtain a faster filling of the vascular bed (CHIROSTI, 1978) but this solute does not stay a long time in the circulation.

Dextran solutes are preferred by most practicians. Their high osmotic pressure is not a disadvantage, due to plasmatic leakage and interstitial oedema existing in decompression sickness. SCHIFFMAN, 1978, recommends the use of both solutes : Ringer lactate and Dextran.

- The use of corticoids is more discussed. At pharmacological dosages, their efficiency on experimental animal decompression sickness has not been demonstrated (HARING et coll.). In heavy doses, secondary effects cannot be neglected, and their efficiency has been evidenced exclusively in preventive therapy. Even if they have been recommended due to their possible protective action on cellular anoxia, the use of corticoids does not seem to be justified.

Among antiagregant drugs, Aspirin is largely used, even if the efficiency on platelet aggregation during decompression sickness has not been evidenced.

But PHILP (1979) has recently demonstrated that in man, prior Aspirin administration prevents the decompression induced platelet drop, and other biological modifications such as increase of cholesterol, transaminases and triglycerides are less than compared to non protected controls. However, Aspirin does not reduce the incidence of decompression sickness.

Dipyridamol (Persantin ®) being without action, one may suggest that Aspirin acts on an other mechanism, who it could be a decrease in prostaglandin synthesis (this has been confirmed by WELL and al., 1978, who evidenced the protective effect of indomethacin on pigs). In females after decompression occurs in dogs

On the other hand, clinical practice reports the very good results obtained by Aspirin, on the field of intravenous infusion of lysin salicylate (MOREIWIJ ...'8, and GISMER short abstract, mainly on head induced pathologies and interaction on circulating platelet number).

Influence on injection of 1 g of lysin salicylate (Aspegic ®) seems to be recommended, both clinical appreciation, less efficient, and which can be taken as of in the occurrence of hyperplatelet (... a vomiting crisis of MENIERE-like syndrome)

Amongst the other anti-platelet drugs, we demonstrated ourselves that Sloctegolin (Sermion R) was a blocking antiaggregating drug, lessens decompression induced platelet stop, when injected preventively in animals.

The vasodilatating action of this drug, added to its antiaggregative effect should make it to be listed among the drugs proposed.

Up to now, only Aspirin is to be recommended.

- The use of anticoagulants (heparin) is more and more discussed, even if they are certainly active.

HALLENBECK'S (1979), PALMER'S (1977) and WOLKIEWIEZ'S (1979) studies demonstrating the importance of spinal cord haemorrhages, should send heparin users carefull when an isolated or combined neurological syndrome exits.

- As for as vasodilatating drugs are concerned, no new point has come to complete what we already published at EUBS'S meeting in Luxembourg, in October 1978.

Experimentally, BALLDIN (1978) evidenced the preventive effect of terbutalin

- The protective effect of diazepam (Valium R) on hyperoxic seizures has been experimentally demonstrated by HANSEN (1979) during hyperbaric oxygen therapy at 3 ATA, but WOLKIEWIEZ who never uses diazepam during oxygen therapy at 2,7 ATA, never evidenced seizures. This last pressure is probably sufficiently efficient, and certainly less hazardous.

The preventive use of diazepam is therefore still discussed.

CONCLUSION

Few new facts has been reported since the report presented at EUBS meeting in 1978.

The doubt on efficiency and inocuity of vasodilatators, anticoagulants, high dose corticoïds and diazepam seems most important.

The only agreement is on the use of plasma expanders, and namely Dextran, and at a lesser degree, on the efficiency of Aspirin.

References will appear in PROCEEDINGS.

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DECOMPRESSION SICKNESS IN A COMMERCIAL DIVING POPULATION. M.H.Symons and I.A.Booth. Humber Diving Research Facility, London, England.

There are many different proprietary decompression tables in use in the UK Sector of the North Sea. The vast majority of companies use tables of United States Navy origin. Others use tables derived from independent laboratories, or produced within the company. There is considerable uncertainty as to the true incidence of decompression sickness amongst the commercial diving population. However, many believe that the incidence is far higher than the 2% value frequently quoted for the US Navy tables. We have tried to obtain, by means of questionnaire analysis, some idea of the numbers of men who have experienced decompression sickness and to relate the pattern to the types of diving they have performed. This analysis is based upon the answers to the first 200 questionnaires received from divers who presented themselves for medical examination for fitness to dive in the U.K. Sector.

The mean age of the whole population studied was 36.1 yrs (5.4 SD. The population was divided into sub-groups, according to the divers experience of different modes of diving. It was found that the group who had performed air diving only, without surface decompression, formed the youngest group with a mean age of 25.7 yrs (3.7 SD (n=27), whereas those with oxy-helium experience were the oldest with a mean age of 39.4 (5.6 SD. 17% divers were included in this latter group. No significant differences were found between any of the sub-groups with respect to years of experience as a commercial diver. In an analysis of the oxy-helium experienced men, no correlation was found between maximum depth achieved during their career and their age or number of years as a professional diver.

Of the 54 divers who had performed air diving only, without surface decompression, only one had experienced decompression, a cutaneous manifestation. In a group who had performed air diving only, but with surface decompression procedures, (n=63), 21 had suffered decompression sickness. The difference between the surface decompression group and the non-surface decompression group is significant, $p \leq 0.05$. 8 of the men in the former group had experienced skin bends, 9 reported limb bends, 11 reported 'niggle' and 3 men said that they had suffered Type 2 decompression sickness.

172 men studied had performed either oxy-helium 'bounce' diving or saturation diving. 80 of these divers reported having had decompression sickness, of which 69 were limb bends. The incidence was correlated with maximum depth and their commonest diving depth. The data is shown below.

Table 1. Relationship between maximum depth and incidence of DCS of any kind.

Depth Range (m)	Number of men	Number with DCS	%
50 - 100	60	20	34
100 - 150	58	20	35
150 - 200	53	32	60

Table 2. Relationship between commonest diving depth and history of DCS in the oxy-helium group of divers.

Depth Range (m)	Number of men	Number with DCS
0 - 50	74	33
50 - 150	89	60
150 - 200	7	0

There is a good correlation with maximum depth, but no significant correlation with commonest depth.

An analysis was also made of the number of type 1 cases experienced by the men and it was found that this did not correlate significantly with, either their age or the number of years of experience as a professional diver. A study of the sites of bends showed that in all groups the upper part of the body was more affected than the lower, and also that the right side of the body was more commonly affected than the left.

Of the 172 mixed gas divers, 60 had experienced niggles (39%) and of this group 28 admitting to not always reporting them. The above results suggest that the number of men experiencing decompression sickness in the UK Sector of the North Sea is greater than one would have expected from the low reported incidence of decompression sickness on the U.S. Navy tables.

In particular, surface decompression appears to carry a significantly increased risk of decompression sickness. The correlation between the reported maximum depth dived and the history of having experienced decompression sickness supports the theory that the incidence of decompression sickness increases with the depth of the dive. The high incidence of men who consistently do not report minor manifestations of decompression sickness, suggests that retrospective analyses of decompression data may yield an artificially low figure for the true incidence of decompression sickness.

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AN EVALUATION OF CARDIOPULMONARY RESUSCITATION TECHNIQUES FOR USE IN A DIVING BELL. Roy Myers, M.D., and Mark F. Bradley, M.D. Maryland Institute for Emergency Medical Services, University of Maryland, Baltimore, Maryland U.S.A.; Naval Medical Research Institute, Bethesda, Maryland U.S.A.

Divers who lose consciousness while operating out of a diving bell require rescue and resuscitation. The small size of these bells and the configuration of the bell interior, with its skirt and center hatch, pose special problems in delivering cardiopulmonary resuscitation (CPR). Because of these conditions in the bell, it is impossible to place the unconscious diver in the supine position usually used for CPR.

A commercial diving company has devised an operational scheme that is purported to be effective in the resuscitation of a diver who is retrieved into a bell. Review of this scheme made us seriously doubt its effectiveness. Therefore, we evaluated the diving company method together with other CPR procedures that might be used in the bell.

METHODS AND MATERIALS

The effectiveness of two groups of individuals acting as resuscitators and one mechanical CPR system were evaluated. The first group of resuscitators was comprised of three CPR instructors, who were highly experienced with resuscitation procedures. The second group consisted of five individuals who had received CPR instruction and certification. (Divers having recent CPR training might be considered to have equivalent capability to the second group.) Lastly, we evaluated a gas-driven CPR machine, which delivers both compression and ventilation.

To test the efficacy of CPR methods, we employed two models. The first was a recording mannequin used for training individuals in CPR (Resusci-Anne: Laerdal Medical Corporation). With this device we measured a) the compression pressure, b) the location where the compression pressure was applied, c) the tidal volume achieved during ventilation, and d) the duration of effectively sustained CPR. The second phase of the study employed fresh human cadavers before autopsy. We assessed the adequacy of cardiac compression by monitoring radial arterial blood pressure. The cadavers were ventilated by machine with constant, appropriate tidal volumes. All procedures on the cadavers were done both with and without medical antishock trousers. The medical antishock trousers stimulated to some degree the increased central venous return that would occur during immersion in water.

Six combinations of subject positions and resuscitation techniques were studied:

1) Subject supine on a firm bed with the resuscitator providing compression and ventilation from above.

2) Subject upright with the back against a firm surface and compression administered by hand to chest with the resuscitator in front of the subject.

3) Subject upright with compression administered by pulling the subject's chest onto the head of the resuscitator.

4) Subject upright with compression administered by pulling the subject's chest against the knee of the resuscitator.

5) Subject upright with the back against a firm surface and compression administered by pushing against the subject's chest with the resuscitator's knee.

6) Subject upright with the resuscitator standing behind the subject, arms around the subject and fist compressing the subject's chest (a modified Heimlich manoeuver).

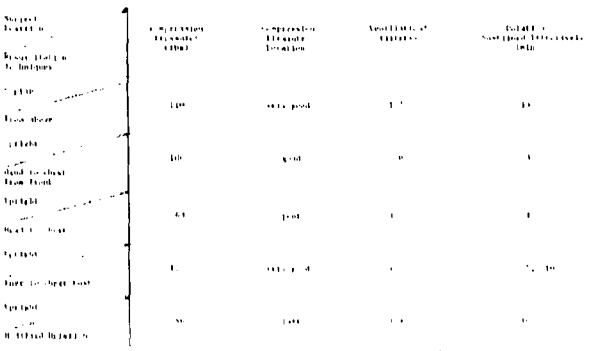
RESULTS AND DISCUSSIONS

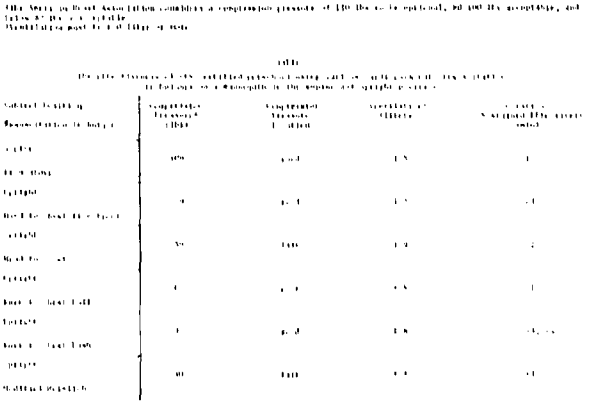
Mannequin Subjects

The efficiency data of the CPR instructors with the resuscitation mannequin in the supine and upright positions with various resuscitation techniques is presented in Table 1. The efficiency data for the CPR certified resuscitators is given in Table 2.

With the subject in the supine position, the instructors were more consistent in providing adequate ventilation and pressure generation and showed less deterioration in performance over time, especially after 10 minutes had elapsed.

In all of the upright positions, adequate ventilation was very difficult to achieve because we had to hyperextend the subject's head to maintain an open airway. The rigid collar of the diving company design did not provide adequate hyperextension. We have therefore developed a collar of different design, which did provide enough hyperextension to adequately ventilate the subject in the upright position.





Most of the resuscitation techniques with the subject in the upright position failed to attain adequate compression pressure or good pressure location and could be sustained for periods of less than three minutes before the resuscitator was exhausted. The least-effective techniques were the hand-to-chest (#2), head chest (#3), and pulling the subject knee-to-chest (#6).

the modified Heimlich technique (#4) was least tiring for the resuscitator but was generally ineffective in generating adequate compression. Technique (#5), with the mannequin seated with its back against a firm surface and chest compression administered by pushing with the resuscitator's knee, was acceptable.

Cadaver Subjects

The results obtained by testing the various chest compression techniques on supine and upright human cadavers generally substantiated the findings from the mannequin phase of the study. Again, the supine position proved to be the best for providing adequate blood pressure of 140/44. With the subject in the upright position, the next most effective technique depended on the relative size of both the victim and the resuscitator. When the resuscitator was larger than the subject, compression of the cadaver's chest from behind (a modified Heimlich maneuver) resulted in adequate arterial blood pressure (120/70) and the least fatigue to the resuscitator. However, when the subject's size was equal or larger than that of the resuscitator the knee-chest position was more effective and a blood pressure of 130/0 was produced. With this technique, the resuscitator's knee compresses the subject's chest, while the subject is supported by the resuscitator's hand on the shoulders and the back is against a firm support. In equal or larger sized subjects the modified Heimlich maneuver produced a blood pressure of 90/0, which is unacceptable. Attempts to perform chest compression on a truly suspended upright cadaver (as in a safety diving harness) by pulling the chest onto the resuscitator's knee or head was rapidly exhausting (one to two minutes) and produced an unsatisfactory arterial blood pressure of 60-90/0.

The use of medical antishock trousers produced an elevation of systolic blood pressure about 25 mm Hg above systolic blood pressure when trousers were not used. Nevertheless, their use did not substantially increase arterial blood pressure to acceptable levels in the upright position.

Finally, a gas-driven CPR machine, which delivers both compression and ventilations, was evaluated on the mannequin and cadavers in the supine and upright positions. For subjects in the erect sitting position, the device provided adequate and even compression and ventilation and required little energy expenditure from the individual doing the resuscitation.

In summary, we have found that the collar as developed by the diving company does not adequately hyperextend the head in the mannequin to maintain an open airway. Secondly, we found that the head/knee-to-chest resuscitation technique advocated by the diving company produces grossly inadequate compression pressures and rapidly exhausts the resuscitator. Thirdly, resuscitation cannot be performed with the subject suspended by a harness at the back of the neck. Finally, using either a modified Heimlich maneuver or a push-with-the-knee against chest technique, we have shown that marginally satisfactory resuscitation can be performed for short periods with the subject in the sitting position. This finding leads us to recommend that modifications to bell interiors be undertaken so that manual resuscitation can be done in the supine position. As an alternative, bells could be outfitted with a gas-driven mechanical cardiopulmonary resuscitator to be used with the subject in a seated position with a backboard.

Acknowledgments

Naval Medical Research and Development Command, Work Unit No. MR099.PN00J.3061. The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large. The authors are grateful to Ian Gordon of Maryland Institute for Emergency Medical Services for help and advice on use of the mannequin and gas-driven resuscitator and to Mrs. B. Watson for editorial assistance.

MOLECULAR AND CELLULAR EFFECTS OF HYDROSTATIC PRESSURE

MOLECULAR AND CELLULAR EFFECTS OF HYDROSTATIC PRESSURE: A PHYSIOLOGIST'S VIEW, A. G. Macdonald, Physiology Department, Marischal College, Aberdeen University, Aberdeen, Scotland.

Our understanding of the effects of hydrostatic pressure at the cellular level is advancing rapidly in some areas and not at all in others. Scattered along a very broad and primarily advancing front there are scenes of activity, and dormancy, and the purpose of this paper is to outline the whole in a way which makes sense to the non specialist and which might also stimulate further activity from the specialists in the field. This symposium provides us with the opportunity to reflect on the significance of pressure physiology in the technology of human diving and in contemporary biology generally.

I shall make use of the cellular physiologist's traditional view of cell organization to impose some order on an otherwise fragmented collection of pressure studies. The cell is, above all else, an entity defined by its bounding plasma membrane, whose fundamental and paradoxical role is to act as a highly selective barrier. It is therefore natural to ask first, how does pressure affect cell membranes? The answer is, in many different ways. Recent experiments with human erythrocytes have demonstrated that pressures of 30 atm or more affect the ionic regulation of the cell, leading to an increased steady state concentration of internal Na^+. Pressure in some way disturbs the normal relationship between the Na^+ pump and intracellular sodium levels. Other experiments with the same cell suggest that pressure increases the passive permeability to ions, a conclusion reached in previous studies with frog skin and neurones. Much remains to be clarified but it may be true, and certainly premature, to suggest that the effects of model membrane pressure on the ionic regulation carried out by cell membranes are mild, but widespread in tissue cells and may be surprisingly significant in human physiology at extreme depth. It is obvious from experiments with humans and experimental animals that prolonged hyperbaric exposure does not cause severe problems of ionic regulation but nevertheless cellular regulatory processes are probably altered and possibly adapt to pressures in excess of 20 atm.

Another major role of the bounding cell membrane is inter cellular communication, both by rapid electrical and slower chemical means. The long overdue investigation of neuronal excitability under pressure is now getting into its stride yielding fundamental data which are exceedingly difficult to interpret. Action potentials in such diverse preparations as the squid giant axon, neurones in central ganglia in the snail Helix, and in the peripheral nervous system of the rat and frog are all slowed (broadened) by pressures within the 200 atm range. The point of interest should now shift to measuring how pressure affects channel conductance and gating mechanisms. Such measurements are technically demanding, the molecular targets are still quite obscure, and interpretations of the results will not be easy.

The physiology of synapses under pressure has considerable importance in leading to an understanding of how pressure affects the activity of the integrated organism, and the hyperexcitability and other symptoms in human divers in particular. Spontaneous action potentials have been reported in pressurized crustacean axons, and could conceivably be an example of how integrated functions are upset by pressure, but most workers would probably envisage a major role for synapses in the origin of the high pressure nervous syndrome. A preliminary generalization is that pressure depresses excitatory transmission and for hyperexcitable mechanisms we might look for disturbances in the interneuronal traffic. Nevertheless isolated synaptic preparations are rewarding targets for pressure experiments, especially as some appear to be remarkably sensitive.

The cell interior is the environment which supports 'soluble' enzyme chemistry and complex processes such as the highly structured chemistry of contraction and other types of motility, some of which are involved in synaptic function. Interestingly research into muscular contraction under pressure is now apparently dormant whereas once it was an active field. Considerable progress is being made in understanding how enzymes respond to high pressure, a significant problem in deep sea biology no less than deep diving physiology. Generally pressure acts by disturbing enzyme-substrate or ligand interactions and the inhibition is no more likely than the stimulation of enzyme activity. The difficulty of transferring conclusions from _in vitro_ experiments to real life is well known and particularly difficult in the case of human physiology. The details of the reaction conditions, in particular the rate-determining step, are critical in determining how pressure affects a given reaction. In view of the adaptive changes which appear in the ionic regulation of erythrocytes under pressure it is clear that new steady state levels of critically important ions and other regulating molecules may be found in pressurised tissues _in vivo_. One wonders how confident we should be of predicting how cells _in vivo_ will actually behave under pressure, a point which includes both the metabolic and the excitable behaviour of cells.

Another major aspect of cell organization to be considered is inheritance, gene expression and differentiation. It has been remarked that the peculiarly stable nature of DNA and the way moderate pressures act on molecules in solution leads to no interaction of interest between the two. However the dynamic aspect of macromolecular synthesis, and all that follows, is sensitive to pressure, and in human diving the whole question of the reversibility or otherwise, of pressure symptoms has to be considered as thoroughly as possible. In the complex machinery of protein synthesis a variety of sites have been shown to be susceptible to several hundred atmospheres pressure. Genetic effects of pressure have so far only been demonstrated in microorganisms subjected to similarly high pressures.

From the difficulties of predicting whether cells under pressure _in vivo_ will behave in the same way as cells _in vitro_, and the reversibility of pressure effects, I wish to move on to the problem of predicting the effects of pressure in specific physiological processes. It is surely a sign of maturity in a field of research that testable predictions become more realistic and a theoretical approach to the subject emerges. Although certain molecular interactions are known to be particularly sensitive to pressure it is generally difficult (that is impossible!) to predict the pressure-sensitivity of a given physiological process. The reason is that the presence of known pressure labile molecular targets are obscured by a multitude of other unknown intervening factors. Nevertheless with sufficient knowledge of simple systems it ought to be possible to predict their sensitivity to pressure, and in at least one case this has happened recently.

Much of the cells biochemical activity, including ionic regulation, is carried out by membrane-bound enzymes. The activity of these enzymes is often determined by the state of the surrounding bilayer lipids. It was first assumed, and then confirmed, that the bilayer component of cell membranes would become more ordered under pressure. It was also predicted that bilayer membranes would undergo a phase transition to the gel state under pressure, a process since demonstrated in numerous studies with model membranes and to a lesser extent in natural membranes, in which it has been shown that pressure raises the phase transition temperature[dT/dP] in accordance with the Clausius Clapeyron relationship. The effect of membrane lipids on enzyme activity is manifest in Arrhenius plots which show a break at a characteristic temperature, generally held to be the temperature at which the lipids undergo a phase transition. It was accordingly predicted that such enzymes would respond to high pressure with a shift in the temperature at which the break in the Arrhenius plot occurs. Two independent studies have demonstrated that membrane bound ATP ases do in fact respond to pressure in this way, and there is some agreement between the dT/dP for the Arrhenius break point and typical dT/dP values for the phase transition temperature of membrane lipids.

So simple is this prediction that some may argue that it has little significance, whilst others may quarrel from the [illegible] of the quantitative agreement between prediction and result. Another reservation is that although the thermodynamics of phase transition and break point phenomena may be fairly well understood the kinetic aspects are not at all resolved. We do not know if a break point in an Arrhenius plot is shifted by high pressure to a higher temperature because of transition behaviour in the boundary lipid, which determines the activity of the enzyme in question, or because lateral phase separation takes place [illegible] the enzyme activity to come under the influence of other interaction.

The behaviour of enzymes and other proteins such as ion channels and receptors in a lipid membrane phase at high pressure is an interesting problem. Much of our knowledge of how pressure affects proteins only applies to proteins in aqueous solution, in which changes in hydration involving either ionic interactions or hydrophobic effects, cause a major volume change and thereby determine the pressure sensitivity of the molecule. What happens to proteins, which are immersed in a hydrocarbon lipid bilayer and complex[illegible]? Does the solvent lipid exert a dominant effect on the mobility and conformation of the protein? Does boundary lipid behave like solvent lipid or does it behave as part of an [illegible] lipoprotein complex? These questions are highly relevant to the kinetics of the action potential, the functioning of synapses and the activity of many membrane bound enzymes. In short, our understanding of how pressure affects physiological processes [illegible] [illegible] from the broad and thermodynamic to the detailed level already achieved in the study of the kinetics of soluble enzymes.

Finally we should not [illegible] the obvious fact that in practical diving physiology we are always concerned with high partial pressures of inert gases, and rarely with pure hydrostatic pressure. It has previously been acceptable to regard inert gases as extremely weak and their [illegible] [illegible] that simplification tenable. Hyperbaric nitrogen perturbs spin labelled erythrocyte bilayers in ways which are contrary to predictions based on either "anaesthetic mechanisms" or a simple isotropic compression of the bilayer. With inert gas effects as with hydrostatic pressure we have to move on from gross thermodynamic models to a more detailed, kinetic level of analysis.

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[illegible] HYPERBARIC CONDITIONS [illegible]
[illegible]
[illegible] Michaud, Laboratoire de [illegible]

Previous observations led to the assumption that [illegible] hydrostatic pressure and/or hyperbaric inert gases [illegible] biological effects [illegible] on certain molecular structures. We tried to verify this hypothesis [illegible] simple biological models: 1) a [illegible] one of the smallest and simplest DNA viruses, and 2) a more complex DNA virus (Herpes Simplex Virus [illegible] and HSV2).

I - MATERIAL and METHODS

I.1. Preliminary experimentation was carried out in order to study the [illegible] effect of hyperbaric exposure on the cells used for virus development (primate Vervet monkey kidney cells, [illegible] diploid cells and Hela cells), in order to investigate "per se" hydrostatic pressure. [illegible] tissue culture [illegible] plastic tubes were completely filled with Eagle's medium, [illegible] at the [illegible] [illegible] pressure to the enclosed liquid. [illegible] tubes were incompletely filled with the [illegible] [illegible] the surface contact with the hyperbaric [illegible] hyperbaric chamber supplied by [illegible] helium [illegible] the rate of 1 ATA/min [illegible] In each case, an experimental pressure of [illegible] ATA. After decompression, the cells were submitted to optical and [illegible] [illegible].

I.2. Experimentation on Polyoma virus. At 37°C the tissue culture of primary Vervet monkey kidney cells was inoculated with [illegible] virus. After 1 hour the cells were rinsed and the tubes filled with Eagle's medium. The infected tissue cultures were then submitted to hyperbaric conditions as described for the preliminary experimentation.

The hyperbaric experimentation was carried out under [illegible] [illegible] [illegible] After decompression, [illegible] virus [illegible] [illegible] and compared to control samples kept at the same temperature but under barometric pressure.

I.3. Experimentation with Herpes Simplex Virus (HSV1 and HSV2) [illegible] Vervet monkey cells, inoculated with [illegible] submitted to "per se" hydrostatic pressure [illegible] and [illegible] ATA. In each case at a temperature of 37°C, [illegible] estimated 48 hours after decompression [illegible] carried out in order to look for possible changes in cell and [illegible] and virus morphogenesis.

II - RESULTS

II.1. During preliminary experimentation [illegible] reproduction ability of Hela cells [illegible] after recompression. [illegible] examination of Vervet monkey cells also showed that hyperbaric conditions did not modify the various intracellular structures.

II.2. Polyoma Virus. Whatever the [illegible] [illegible]

II.3. Herpes Simplex Virus. [illegible]

III - DISCUSSION

The preliminary experiments showed that the cells which were used for various cultures did not change under hyperbaric conditions. Thus effects of "per se" hydrostatic pressure and/or hyperbaric inert gases (He, N_2) were therefore due to a direct damaging of the virus development.

With respect to the Echo 11 virus, there was for each pressure value a linear relation between the logarithm of virus titration and the inverse ratio of absolute temperature. This relation leads us to consider that the kinetics of virus multiplication are the result of a biological process which obeys the same law as chemical kinetics (JOHNSON and EYRING, 1970). According to this interpretation, the effect of pressure on virus development appears in the plot log (titration) = A.(1/T) + B as a change in slope A and ordinate at the origin B. Hence several hypotheses can be suggested :

1) Pressure may modify the nature of the chemical reaction limiting the virus synthesis.

2) Pressure may modify the structure of one or several elements in the limiting chemical reaction (substratum, enzymes - replicase, polypeptidase, phosphorylase - activated complex).

3) In addition, pressure may alter a structural compound of the virus (capsid proteins, nucleic acid,...).

4) Hyperbaric inert gas pressure (N_2, He) modifies the structure of the virus compounds by a specific action which in some cases antagonizes the specific action of "per se" hydrostatic pressure. The effects of inert gases may be related to the amount of dissolved gas stored within the virus structures and their consequent effect on virus development depends upon the complexity of the virus (size, number of macromolecules).

5) Although the host cells appeared morphologically undamaged after decompression, functional changes may develop under pressurization. LANDAU (1972) demonstrated changes in the protein synthesis under hydrostatic pressure. So, the synthesis of interferon and various viral proteins may be modified under hyperbaric conditions.

The present investigation and results are in accordance with the previous assertions. Moreover, the results may take on applied interest because it is important to know the risks involved and the evolution of viral disease during prolonged human saturation dives.

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EFFECT OF HYDROSTATIC PRESSURE ON ACTIVE TRANSPORT, METABOLISM AND THE DONNAN EQUILIBRIUM IN HUMAN ERYTHROCYTES. J. P. Inglefinger, R. S. Yang, P. A. Martin, C. S. Campbell and S. J. Hong.

The specific aim of the investigation described here was to evaluate the effect of moderate hydrostatic pressures on sodium pumping, Na-K-ATPase activity, glycolysis and the Donnan equilibrium using the human erythrocyte as a model.

1. Sodium transport: Active and passive sodium transport was studied at pressures ranging from 1-400 ATA. Briefly the experiments were performed as follows. Human erythrocytes were incubated in ^{24}Na, washed and suspended in a tracer-free medium. The suspension was placed in a cylinder with no gas phase. One end of the cylinder consisted of movable piston and pressure was transmitted to the suspension via this piston during incubation in a hyperbaric chamber. At various times the pressure was released and an aliquot of the suspension was sampled. The cells and medium were separated and the radioactivity appearing in the medium was measured. If one knows the total radioactivity in the suspension and the radioactivity appearing in the medium at known times, the rate constant for sodium release can be computed. Active and passive sodium components can be distinguished by performing the measurements in the presence and absence of ouabain, a cardiotonic steroid, which inhibits active sodium transport. The logarithm of the fraction of sodium remaining in the cell, $(1 - \frac{A}{A_\infty})$ is plotted against time.

The slope of the line obtained from such an experiment is the rate constant for the sodium release. In the first series of experiments, cells were allowed to release sodium at ambient pressure for the first 30 min., after which the chamber was pressurized to 150 ATA and the sodium release was measured at that pressure. The rate of release declined about 40 %. Approximately 2/3 of the total sodium release is by active transport. In a second experiment, the chamber was pressurized from the beginning and the pressure was released at 30 min. Sodium transport was depressed at pressure but returned to near control levels when the pressure was released. In a control experiment, a pressure pulse was delivered, before each sample was taken, to determine if the pulse on sodium transport was observed. This insured that the effects of rapid compression and decompression did not affect the results. These experiments demonstrated that hydrostatic pressure inhibits the sodium pump and that this inhibition is reversible. Figure 1 shows the inhibition of sodium flux over the entire pressure range tested, 1-400 ATA. The effect of pressure is reproducible, rising very steeply between 1 and 10 ATA and then reaching a plateau. These results are remarkable in that the onset is at pressures well within the range encountered by divers, and not less than those encountered by some divers normally.

2. Na-K-ATPase activity: In these series of experiments, attempts were made to determine whether the inhibition of sodium transport observed at pressure could be explained by inhibition of Na + K ATPase. To this end, cells were lysed and washed free of hemoglobin. The washed membrane yielded a ghost with erythrocyte membranes tightly containing the Na-K-ATPase. The membranes were incubated in a balanced salt solution containing the ions $^{24}Na^+$, K^+, Ca^{++}, Mg^{++}, Cl^-, ATP, CO_2 and Tris, pH 7.4 at 0°C, in the cylinder described above at pressures ranging from 1-150 ATA. ATPase activity was measured by chemically determining the amount of phosphate hydrolyzed from ATP during a 10 min incubation. The kinds of hydrolysis measured were: first, total hydrolysis, and second, the amount of hydrolysis that occurred in the presence of ouabain. This second measurement yields Mg-ATPase activity and represents nonspecific ATPase activity of the membranes. It probably represents nonspecific cleavage of ATP by many enzymes. The difference between total and Mg ATPase activity can be attributed to the Na-K-ATPase. Figure 2 shows the total, Mg- and Na-K-ATPase activities as functions of applied pressure. Control ATPase activity measured at 1 ATA is expressed as 100%. It shows that total and Mg-ATPase activities exhibit a biphasic response to pressure; they are both activated by low pressure and then return to control or less-than-control levels at higher pressure. On the other hand, the Na-K-ATPase exhibits a monotonic activation by pressure. Moreover, the activation of the enzyme has roughly the same pressure sensitivity as the inhibition of sodium transport. These experiments indicate that pressure inhibition of sodium transport cannot be attributed to inhibition of the Na-K-ATPase which in fact is activated by pressure. One hypothesis is that pressure uncouples the Na-K-ATPase and the sodium pump in some manner. Clearly further experimentation is necessary to prove or disprove this notion.

3. Metabolism: The aim of the third series of experiments was to evaluate metabolism and thus ascertain if the inhibition of transport could be attributed to decreased metabolism of glucose. In these experiments ATP, ADP, glucose, pyruvate and lactate were measured in red cell suspensions incubated 2.5 hours at pressures from 1 to 140 ATA. There was a consistent and significant increase in ATP at all pressures tested, while ADP levels declined, as might be expected. The ATP/ADP ratio is always greater than control at increased pressure. The redox state, indicated by the pyridine nucleotide ratio, gives some indication of the overall state of metabolism. This ratio can be computed from the lactic dehydrogenase equilibrium. Little or no change in this ratio was observed at any pressure level. This indicates that no dramatic deviation from normal steady state is occurring at pressure. Glucose utilization is uninfluenced by pressure but lactate production is diminished at some pressures. It is well known that the rate of glycolysis is dependent on available ADP. One source of ADP is that generated by ATP utilization. Furthermore, one source of ATP utilization is active sodium pumping. One possibility is that lactate production is reduced because of decreased ATP utilization by the pump and therefore decreased availability of ADP, probably at the phosphoglycerate kinase step. Alternatively, the increase in ATP may allosterically inhibit phosphofructokinase and therefore diminish the flow of substrate to lactate. In any event, it would appear that the effects of pressure on glycolysis are not primary but rather secondary to an inhibition of active sodium transport.

4. The Gibbs-Donnan equilibrium: The erythrocyte is in Donnan equilibrium with respect to anions; therefore, the distribution of anions across the membrane is related only to the concentration and net charge of the impermeant species inside the cell. In the erythrocyte, these are principally hemoglobin and 2,3-DPG. Thus any alteration in the charge of these molecules, either by hydrogen ion titration, ligand binding or conformational change will be directly reflected in the distribution of anions, i.e., a change in the Gibbs-Donnan equilibrium. We determined that 5-120 ATM pressure of either N_2 or He changes the equilibrium chloride distribution ratio (r) progressively from 0,64 ± 0,5 to 0,82 ± ,03, n = 4, p <,05. This means that pressure alters the net charge on impermeant anions within the erythrocyte. This result cannot be explained by pressure-induced alterations of membrane properties since Cl^- is at equilibrium. One must then assume that the charge has changed as the result of titration (pH), ligand binding, a change in protein conformation, or some combination of these events. Although changes in pH may be brought about by alteration in metabolism, erythrocyte metabolism is relatively uninfluenced by pressure. It is likely then that pressure is acting by altering hemoglobin conformation or ligand binding. Pressure is known to affect ligand binding in hemoglobin solutions. In addition to providing fundamental information about the effect of pressure on hemoglobin charge, the effect of pressure on the anion distribution ratio also provides information regarding the effect of pressure on red cell function, viz O_2 transport. A change in r must be accompanied by a change in pH (OH^- ions are also in Donnan equilibrium), either as a cause or effect, and therefore will influence O_2 dissociation. It has been reported that pressure influences O_2 dissociation in hemoglobin solutions.

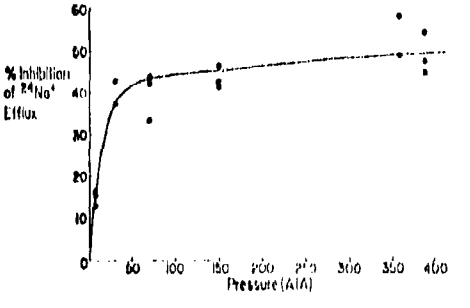

Figure 1. Inhibition of $^{24}Na^+$ efflux from human erythrocytes as a function of pressure. Percent inhibition was computed as 100-[(rate constant at pressure/rate constant at 1 ATA)x100]. Rate constants were determined from experiments described in the text. Individual experiments are shown.

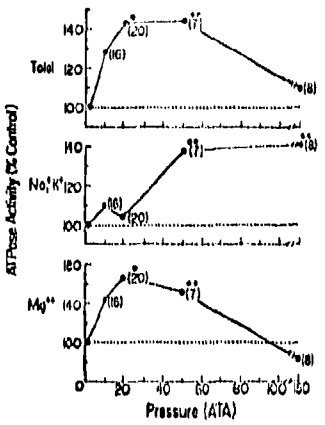

Figure 2. Total, Na^+, K^+-, and Mg^{++}-ATPase activities as a function of pressure (see text for details). The interrupted line parallel to the X axis is the control (100%). The means of the numbers of experiments indicated in parentheses are shown. p values were calculated using the t-test for paired experiments.
* $p < 0.5$ ** $p < 0.01$

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EFFECTS OF HIGH HYDROSTATIC PRESSURES ON Na^+ TRANSPORTS ACROSS ISOLATED GILL EPITHELIUM OF SEA WATER ACCLIMATED EELS *Anguilla anguilla* L.

André J. R. Péqueux
University of Liège, Laboratory of Animal Physiology, Liège, Belgium.

When applied to isolated non perfused gills of sea water acclimated eels *Anguilla anguilla* L., hydrostatic pressure is known to induce changes in tissue Na^+, K^+ and Cl^- contents (Péqueux and Gilles, 1977 ; Péqueux, 1979). In sea water (SW) used as the incubation medium, application of pressure steps higher than 250 atm has indeed been shown to bring about a severe increase of the tissue Na^+ and Cl^- contents and a decrease of K^+.

Preliminary experiments done in SW have established that both an inhibition of Na^+ active extrusion processes and an increase of the passive Na^+ entrance along the concentration gradient contribute to the pressure induced increase of the tissue Na^+ content (Péqueux, 1979). However, both events resulting in a similar final effect in SW cannot be discriminated easily.

The experiments reported in this paper were therefore initiated in order to bring more insight to the nature of the effects of pressure on the various transport processes involved in Na^+ transfers at work in gill epithelium.

Isolated gills from European silver eels *Anguilla anguilla* L. acclimated to SW were incubated at atmospheric pressure and under high hydrostatic pressures in a pressure vessel designed to avoid the presence of any gas phase (see description and details in previous papers: Péqueux 1976a and b; Péqueux and Gilles 1977). At the end of incubation period, gill filaments were cut off the gills, they were blotted on filter paper, weighed and dried at constant weight in an oven at 110°C for dry weight measurements. Inorganic ions were extracted after treatment with HNO_3 0.1N for 48 hours. Na^+ and K^+ determinations were done by flame photometry and Cl^- content was estimated with a Buchler-Cotlove chloridometer. Results were expressed in μEq/tissue wet weight. Ions fluxes were estimated by measuring net changes of the total tissue ions contents. Compartmental analysis and radioactive efflux measurements were done on the basis of typical wash out experiments of pieces of gill tissue pre-loaded for 45 minutes in radioactive saline (traced Na^{24}). All Results were expressed in μEq/gDW on basis of the results of viability of the incubation medium.
The so-called "saline isotonic to the blood" contained 170 mM/l NaCl, 5 mM/l KCl, 2.6 mM/l CaCl$_2$, 0.1 mM/l $MgSO_4$, buffered at pH 7.6 by means of 10 mM/l Tris buffer.

Experiments carried out at atmospheric pressure in that physiological medium where the concentration gradient across epithelium is considerably reduced or even abolished, have shown the tissue water and ions content to remain constant for more than 40 minutes incubation (Table I). In opposition to results obtained upon incubation in SW, application of active transport inhibitors like ouabain, 2-4(dinitrophenol (DNP) and anoxy does not result in any significant effect (Table I). It can therefore be reasonably concluded that, in such conditions, the activity of "pumping" mechanisms is extremely reduced and practically undetectible. The same holds true in respect of diffusional movements from environment towards blood along concentration gradients.

Results of Table I show that, in identical incubation conditions, application of a pressure step of 500 atm induces a mean increase of tissue Na^+ content of about 25% (individual data sometimes attaining 40%). Concomitantly and despite the absence of detectible active components, there is a decrease in K^+ content (21%). On the contrary, Cl^- content does not appear to be significantly modified. Upon decompression, Na^+ and K^+ contents have been observed to resume their initial level within more or less 30-40 minutes which indicates that pressure induced variations are fully reversible.

In consideration of the conclusions drawn from experiments done at atmospheric pressure, pressure induced increase in Na^+ content can be reasonably ascribed essentially to an effect on Na^+ passive permeability. This is moreover in full agreement with the results obtained by incubating gills in Na^+-free sea water (Péqueux, 1979), be it in also with the drop in tissue K^+ content which occurs at 500 atm when the supposed Na^+/K^+ coupled transport is considered as being ineffective. Up to now, that question of the K^+ movements is far from being solved even at atmospheric pressure. According to Bellamy (1963), the gill epithelium is little permeable to K^+ ions. On the other hand, a K^+ lack in the surrounding medium severely disturbs the maintenance of the blood Na^+ balance (Maetz, 1964; Kamiya and Utida, 1968). That K^+ ions may be involved in active exchange processes against Na^+ appears therefore as very likely but the importance and the exact modalities of the procedure remain to be established. At this step of investigation on pressure effects and in consideration of the results presented in this paper, it thus seems to be more reasonable to consider that high pressures act by enhancing the passive K^+ permeability. In that view, it is worth noticing that conclusive evidences concerning the pressure sensitivity of K^+ passive movements have been obtained with human red blood cells more accessible to experimentation under pressure. It has indeed been demonstrated that the net K^+ efflux from human erythrocytes essentially to be considered as passive, increases gently but almost linearly with the pressure until 600-700 atm, while above that pressure range, a very pronounced increase in membrane K^+ permeability occurs (Péqueux, Gilles, Pilwat and Zimmermann, 1980; Zimmermann, Pilwat, Péqueux and Gilles, 1980).

If the results presented in table I appear that active transport activity falls to negligible values when gills are transferred into physiological medium, they also suggest that passive diffusion from outside towards body fluids is very low too. This could be compared to observations done by Motais *et al.* (1966) that Na^+ or Cl^- influx is instantaneously reduced to very low levels when sea water acclimated fishes are suddenly transferred into fresh water. In such a latter case, Motais *et al.* (1966) interpreted such flux readjustments in terms of the possible occurrence of exchange-diffusion processes.

Investigations on the effects of pressure on exchange diffusion appears as almost impossible without the help of isotopic tracers. In consideration of the difficulty to obtain a functional perfused preparation of fish isolated gill, furthermore susceptible to work under pressure, it has been projected to adapt the wash out method of a Na^{24} pre-loaded piece of tissue to the peculiar pressure technics in use.
By that method, three compartments respectively A, B and C have been identified in gills incubated at atmospheric pressure in isotonic saline (table 2). Compartment B has been considered as corresponding to the Na^+ fraction contained in cells epithelium while A and C respectively to the outside facing extracellular spaces and to the body fluids reservoir.

Table 1. Effect of 15 minutes pressure application on Na^{24} efflux from a washed out fraction of isolated gills from a decerebrated eel Anguilla anguilla.

Compartment	% of radioactivity in compartment	Rate constant k_i	Half life time
A	[illegible]	[illegible]	[illegible]
B	[illegible]	[illegible]	[illegible]
C	[illegible]	[illegible]	[illegible]

Data result from graphical analysis of complex exponential curves. Mean ± SEM of [illegible] experiments.

The effects of 15 minutes pressure application on Na^{24} efflux have been investigated after 15 minutes pre-washing in order to avoid any interference due to Na^{24} from compartment A. Pressure effects were evaluated by comparing ions content and radiosodium content of gill epithelium before and after pressure application.

Results of table 1 show a slight but not yet significant increase of tissue Na^{+} content measured by flame photometry in gills submitted to 500 atm. Concomitantly there is less radioactive Na^{+} remaining in compressed gills than in controls incubated at atmospheric pressure and specific radioactivity in compressed gills appears as significantly lower ($0.01 < P < 0.02$). On the other hand, much more radioactivity has appeared in incubation medium under pressure than at atmospheric pressure. By comparison with control data, Na^{24} efflux indued increased of about 140% at 500 atm.

According to observations and conclusions reported above, the possibility of a pressure induced increase of active Na^{+} efflux cannot be considered. Such an effect should induce a decrease in tissue Na^{+} content in opposition to what occurs and, moreover, pressure steps of that magnitude are known to directly inhibit active transport processes (Péqueux, 1979; Péqueux and Gilles, 1977-1978). It is therefore more reasonable to consider that the observed increase of radiosodium efflux without concomitant decrease of tissue Na^{+} content reflects a pressure induced stimulation of exchange-diffusion processes Na^{+}-Na^{+}. As increased exchange-diffusion Na^{+}-Na^{+} does not result in any net variation of tissue Na^{+} content, the pressure induced increase of tissue Na^{+} content thus appears effectively as essentially due to an effect on the Na^{+} passive diffusion from the environment towards body fluids.

When isolated gills are incubated under pressure in sea water, it is thus very likely that, in addition to both inhibition of the Na^{+} pump and enhancement of Na^{+} passive permeability contributing to pressure induced changes of tissue Na^{+} content reported in previous papers (Péqueux and Gilles, 1977; Péqueux 1979), exchange-diffusion Na^{+}-Na^{+} must be pressure activated too although it does not result in net variation of tissue Na^{+}. Experiments with isotopic tracers are under investigation in order to test that hypothesis.

Table 2. Effect of pressure and [illegible] on tissue Na^{+} content and Na^{24} efflux from a [illegible] gill preparation.

Incubation conditions	Na^{+} content μEq/g wet tissue	Na^{24} content cpm/mg wet tissue	Influx μEq/cm²/h
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]

Main results of two experiments in which pressure and [illegible] were applied to gills [illegible].

Results presented in this paper also corroborate the idea that pressure acts differently and selectively on Na^{+} and Cl^{-} transports in agreement with observations reported previously and subsequent conclusions on the relationships binding both mechanisms (Péqueux and Gilles, 1977; Péqueux, 1979). Results of table 1 indeed show that tissue Cl^{-} content remains unaffected by 1 hour pressure application. That observation obviously does not implicate that all possible components of Cl^{-} transports are insensitive to pressure. Experiments using radioactive Cl^{-} are now carried out in order to bring more insight to that question.

By now on, it is clear that hydrostatic pressure affects the functioning of biological membranes by modifying selectively their properties of passive and active ions transport in a way depending of the magnitude of the applied pressure.

At the present time, little can still be said as to the molecular aspect of pressure induced disturbances but several evidences prompt us to explain such effects in terms of phase transitions in the lipidic components of the membrane affecting the conformation of the enzyme proteins associated with the active processes and of the pathways specifically involved in passive ions transports.

It appears as evident that knowledge of how hydrostatic pressure affects membrane processes is a fundamental problem in biology of marine organisms and is essential to a thorough understanding of underwater physiology. According to that view, investigations on the effects of hydrostatic pressure on ions transport across fish gill epithelium might contribute efficiently to development of undersea biomedical sciences.

References will appear in PROCEEDINGS.

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A QUANTITATIVE DESCRIPTION OF PRESSURE-INDUCED ALTERATIONS IN IONIC CHANNELS OF THE SQUID GIANT AXON. Brij B. Shrivastav, Lawrence J. Parmentier and Peter R. Bennett. J.G. Hall Environmental Laboratory, Duke University Medical Center, Durham, N.C., U.S.A.

The effects of increased hydrostatic pressure on animals are many and varied. These effects on different organs are manifestations of physicochemical and structural changes in individual cells and their relationship to each other. In the nervous system these effects are apparent in terms of a generalized hyperexcitability known as "High Pressure Nervous Syndrome" (HPNS). In general HPNS develops as tremors in the pressure range of 70 ATA. Further increase in pressure to 90-100 ATA produces convulsions and respiratory distress, eventually resulting in muscle contraction, paralysis and death.

An action potential is a transient but specific change in membrane polarization, which is brought about by breakdown of membrane permeability barriers for sodium and potassium ions. This results in an inward flow of Na^{+} and an outward flow of K^{+} down their respective electrochemical gradients. The ions are thought to flow through specific transmembrane pathways, referred to as ionic channels, which are embedded in the lipid matrix of the membrane. Hodgkin and Huxley (J. Physiol., 117:500-544, 1952) provided a quantitative description and plausible explanation for the observed changes in membrane conductances, as first measured in the giant axon of the squid, by a set of equations which defined the opening and closing of the ionic channels as both voltage and time dependent. From these equations it is possible to calculate rate constants for the channel opening mechanisms. It is then possible to apply Eyring rate theory to pressure-induced changes in these rate constants to determine aspects of the free energy changes which are involved in both the normal functioning of these channels and the alterations of that normal functioning which are brought about by the exposure to increased pressures. At constant temperature the rate processes of any reaction at different pressures are related as follows:

$$\frac{k}{k_0} = \exp \frac{-V^{\ddagger} (P_2 - P_1)}{RT}$$

where k_1 and k_2 are the forward rate constants at pressure P_1 and P_2. R and T have the usual meaning. From this equation any change in the volume of activation ($V^{\ddagger}$) for a reaction will be reflected in the alteration of the reaction rate at increased pressures. By measuring the rate of channel opening at different pressures it is possible to calculate $V^{\ddagger}$ and determine the effects of the free energy involved in control mechanisms of the ionic channel.

Single giant axons between 500-800 µ in diameter from the squid Loligo were cannulated and hung vertically inside a high pressure chamber designed for electrophysiological experimentation. Axial wire electrodes were inserted longitudinally down the axon and the fiber was voltage clamped using conventional voltage clamp techniques. In separate experiments either sodium or potassium currents were blocked pharmacologically to allow each current to be studied in isolation. In experiments where pressure and temperature were both varied current due to neither ion was blocked. The temperature was controlled to within ± 0.2°C by a cooling coil inside the chamber, and the chamber was filled with mineral oil. Data was collected at 1, 100 and 150 ATM and normalized to 8.5°C for analysis.

Figure 1 shows three superimposed action potentials from one axon at 1 ATM and 8.5°C, 100 ATM and 8.5°C and at 100 ATM at 13.5°C. With the temperature held constant 100 ATM of pressure caused a decrease in both the rise and fall of the action potential. When the temperature was slowly increased 5° while the pressure was held constant both the rate of rise and fall were increased. This suggests that pressure and temperature are both primarily operating on the kinetics of the current gating mechanisms.

Families of ionic currents are shown in Figure 2. It can be seen that pressure is slowing the rising phase of both sodium and potassium currents without appreciable change in the steady state potassium currents.

Increased pressure had no effect on the maximum value of the potassium conductance, $\bar{g}_K$, and on the steady state value of the activation parameter for the potassium conductance, n_∞. However pressure increased the time constant, τ_n for the rise of the potassium currents and thus decreased the rate constant, α_n. The increase in τ_n at 100 and 150 ATA was 17.5 ± 1.7 and 29.6 ± 5.5% (mean ± SEM) respectively.

Pressure had no effect on the maximum sodium conductance, $\bar{g}_{Na}$, and on the steady state value of the activation parameter for the sodium conductance, m_∞. Also pressure did not change the curve h_∞ (Fig. 1B). However, like the potassium channel system, pressure increased the time constant τ_m and thus decreased the rate constant α_m. This increase of τ_m at 100 and 150 ATA was 17.7 ± 6.5 and 21.1 ± 5.2 (mean ± SEM) respectively. Using values of α_n at 1, 100 and 150 ATM, the volume of activation ($V^{\ddagger}$) for the opening of the potassium channel was calculated. The mean values of $V^{\ddagger}$ at 100 and 150 ATA were 60.1 ± 6.2 and 114.1 ± 7.2 ml-mol (mean ± SEM) respectively. Similarly, the changed values of τ_m were used to calculate the volume of activation, $V^{\ddagger}$, at 100 and 150 ATA were 28.1 ± 6.1 and 45.0 ± 5.5 ml-mol (mean ± SEM) respectively. The values of $V^{\ddagger}$ both for the sodium and potassium channel are not different at 100 and 150 ATA. This suggests that the volume of activation both for the sodium and the potassium channel systems is independent of pressure. The $V^{\ddagger}$ for breakage of the hydrogen bonds is about 44 ml-mol. Similarly the $V^{\ddagger}$ for the formation of ionic bonds and hydrophobic bond

action is about (21.5, and 417.0 ml/mol respectively. By comparing values of $\Delta V^{\ddagger}$ for these non-covalent bonds and the $\Delta V^{\ddagger}$ values associated with the opening of the sodium and potassium channels, it would appear that opening of the potassium channel is associated either with the breakdown of about 7 to 8 hydrogen bonds or the formation of about 2 ionic bonds or hydrophobic interactions. Similarly the opening of the sodium channel seems to involve either breakdown of 5 to 6 hydrogen bonds or formation of 1 to 2 ionic bonds or hydrophobic interactions. Since pressurization will affect the rate of any chemical reaction which itself involves a volume change, living systems which are subjected to high hydrostatic pressures can be expected to experience altered rates of function. The results presented here demonstrate both the usefulness of using the altered state of pressure to study basic membrane reactions and the types of dysfunction which can be produced by this variable. Changes in membrane kinetics such as are described here may prove to be a significant factor in the etiology of certain pressure related medical problems such as occur in the High Pressure Nervous Syndrome.

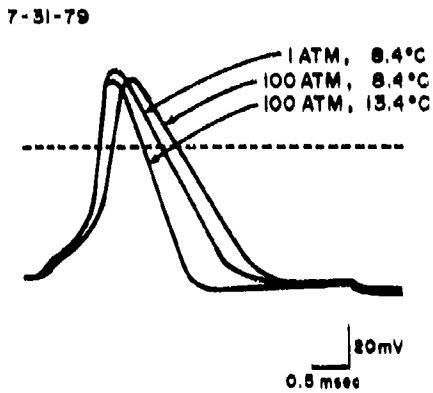

Figure 1. Three superimposed action potentials at varying pressures and temperatures. Increased pressure (100 ATM) slowed both the rising and falling phases of the action potential. A subsequent rise in temperature (5.0°C) restored the rising phase to control level while overcompensating for the falling phase.

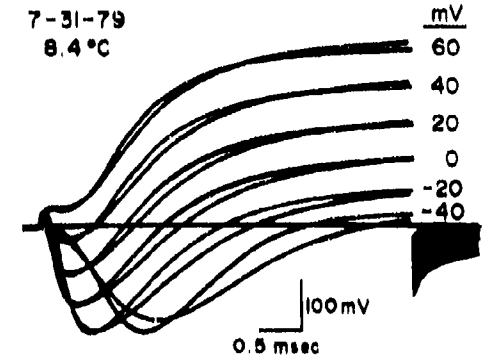

Figure 2. Superimposed ionic currents measured at 1 ATM and 100 ATM. Increased pressure slowed the rising phase of both the sodium and potassium currents. Temperature was maintained constant. The slow kinetics at 100 ATM result in less accumulation of potassium in the Hodgkin-Huxley space to responsible for the increase in the steady state current seen at the positive depolarizations.

TRANSIENT VERSUS STEADY STATE EFFECTS OF HIGH HYDROSTATIC PRESSURE. K. T. Wann, A. G. Macdonald, A. A. Harper and N. L. J. Ashford, Dept. of Physiology, Marischal College, University of Aberdeen, Aberdeen, AB9 1AS, U.K.

Introduction

The effects of high hydrostatic pressure on the electrical activity of a variety of excitable tissues have been described (see Wann and Macdonald, 1979). In this paper we draw attention to the fact that in many of these experiments previously described, pressure is probably affecting various cellular activities simultaneously and one major consequence of this is that the cell's electrical response to pressure is not a simple one. In particular we wish to distinguish between the transient and steady-state effects of pressure. Pressurization also produces small transient temperature changes (~1°C) in the experiments to be described. These complicate the interpretation of any transient changes in electrical activity produced by high pressure, and are therefore discussed where relevant.

The studies discussed here have been performed with *in vitro* preparations. Much of the data have been obtained using neurones of the suboesophageal ganglionic mass of the snail (*Helix pomatia* or *aspersa*), but we also describe experiments recording miniature end-plate currents (MEPCs) from the sartorius muscle of the frog *Rana temporaria* or *pipiens*. Our methods have been described elsewhere (Wann et al, 1979). In all of the studies hydrostatic pressure was used (the compression medium was light mineral oil) and the compression rate was usually $5 \times 10^5 N.m^{-2}$ steps applied at five minute intervals or $10.3 \times 10^5 N.m^{-2}$ steps every minute. In a few experiments a "pump" compression to $104 \times 10^5 N.m^{-2}$ in five minutes was employed.

Results

Hydrostatic pressure produces marked changes in the electrical characteristics of *Helix* ganglion cells. Over the pressure range $1-758 \times 10^5 N.m^{-2}$ depolarization and a concomitant reduction in input resistance are observed. What is additionally significant is that the initial depolarization is greatest within seconds of applying the pressure step and during a five minute period at pressure the resting membrane potential partially reverts to its precompression value. This effect will be referred to as accommodation and is in the wrong direction to be caused by the small temperature increment which accompanies pressurization. This behaviour is most commonly seen at higher pressures ($> 104 \times 10^5 N.m^{-2}$) and the time constant of the accommodation is typically 2-3 minutes at temperatures of 18°-22°C. The steady state depolarization observed (after five minutes at the new pressure) is variable and in quiescent cells a maximum depolarization of approximately 15 mV is produced by $758 \times 10^5 N.m^{-2}$. It should be noted that on compression at lower temperatures (< 7°C) the transient resting membrane potential changes are absent and the steady state depolarization is close to that observed with compression at higher temperatures.

Of considerable interest is the finding that the changes in input resistance with pressurization show no such transient effects: pressure simply reduces the input resistance. We conclude that although the depolarization of *Helix* ganglion cells is produced by an increase in the somatic membrane permeability, secondary changes in the cell may be responsible for the accommodation behaviour. One possibility is that small changes in ionic balance occur which offset the primary effect of pressure on the resting membrane potential.

Higher pressures ($> 104 \times 10^5 N.m^{-2}$) produce variable effects on the threshold of *Helix* ganglion cells. One type of behaviour is significant to this discussion. Pressure often depresses the excitability and again the effect is greatest initially on compression, and excitability can return to precompression values during a 1-2 minute stay at pressure. The temperature increment associated with pressurization to 100 and may contribute to this behaviour, although we believe that pressure ($104-154 \times 10^5 N.m^{-2}$) does produce a genuine transient reduction in excitability of *Helix* ganglion cells.

In experiments with cells which are not isolated from synaptic input it might be argued that the effects of pressure on excitability may be due to altered synaptic bombardment of the impaled cell. However pressure depresses fast excitatory synaptic transmission in *Helix* neurones without any transient or "rebound" effects.

In view of these effects of pressure on resting membrane potential, input resistance, threshold and synaptic transmission it is not surprising that the firing pattern of many ganglion cells is altered in an erratic way by pressure. We distinguish two types of behaviour.

Firstly, high pressure can convert a rhythmically discharging firing pattern into a periodic bursting pattern. There is a gradual transition with increased pressure from one type of activity to the other. The total spike output of the cell remains at about control value.

Secondly, the firing frequency of cells which are synaptically driven is decreased by high pressure ($> 5 \times 10^5 N.m^{-2}$) the firing pattern does however remain regular. In this case however both transient and steady state effects are observed (Fig 4). The interesting finding is that the time course of the rebound effect approximately follows the threshold changes described above and also on decompression transient "overshoot" effects are observed. This behaviour is reminiscent of the effects of hydrostatic pressure on ciliary frequency (Peters and Kitching, 1977; Cosgles and Holwill, 1977).

Directly the firing frequency of pacemaker cells is increased by high pressure ($> 5 \times 10^5 N.m^{-2}$). Again the firing remains rhythmic and is characterized by an initial rise in frequency followed by a decline to the steady state level. In this case the changes in firing frequency seem to follow the resting membrane potential changes described above.

Finally, high pressure may have a variety of effects on the firing pattern of a nerve cell. Figure 2 shows the complicated response of an unidentified Helix nerve cell to three pressure steps. When pressure is first applied ($1-52 \times 10^5 N.m^{-2}$) there is little effect initially, then the frequency declines to below control value (A). A second pressure step ($52-104 \times 10^5 N.m^{-2}$) accelerates firing transiently then the frequency of firing drops further below the control level (B). Note however that the frequency returns to the level prior to the second pressure step after 5 min (beginning of C). The third pressure step from $104-156 \times 10^5 N.m^{-2}$ halts firing transiently then the frequency returns to the level recorded before the third step.

This experiment illustrates that high pressure can produce a variety of transient effects and that these can be either excitatory (B) or depressant (C).

A final and general point is that the magnitude of the transient effects described for all parameters is a function of the rate of compression and the magnitude of the pressure step. Thus transient effects are more pronounced if compression is applied rapidly and the compression step is large.

Parallel experiments with the amphibian neuro-muscular junction have confirmed that transient responses to compression are not simply confined to electrophysiological measurements with Helix nerve cells. The processes controlling transmitter release are very sensitive to pressure and at $104 \times 10^5 N.m^{-2}$ the quantal release of transmitter is almost entirely abolished. In some experiments m.e.p.p. frequency does rebound to 70% of the precompression value after about 12 minutes at $104 \times 10^5 N.m^{-2}$. A small pressure step ($10 \times 10^5 N.m^{-2}$) applied at this time can partially offset this recovery.

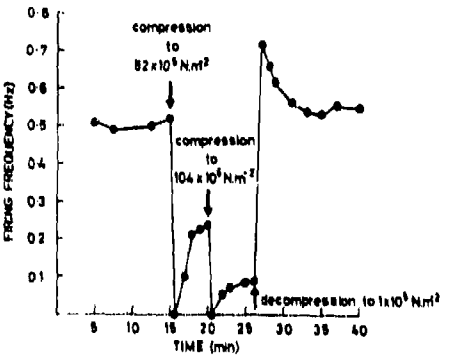

Fig 1 The effects of hydrostatic pressure on the firing frequency of a Helix ganglion cell. Overshoot effects were observed on application of both compression steps and also on decompression.

Discussion and Conclusion

We have reported here several transient effects of pressure on the electrical properties of two preparations. Previously it has been observed that high pressure can produce transient changes in ciliary beating (Pease and Kitching, 1939), cardiac beating frequency (Landau and Moreland, 1952; Gerbagon and Hogan, 1977) and the evoked luminescence of Cunctorterus (Nie et al, 1958). In attempting to interpret such behaviour we must be aware of the possibility that small temperature fluctuations associated with compression and decompression will complicate the picture. In the more recent experiments we can be more certain of such temperature changes (e.g. Gerbagon and Hogan, 1977).

How do we explain the transient effects produced by pressure? It has been suggested that the changes in ciliary activity following pressure application are explicable if it is assumed that the activity is dependent on the rate of two consecutive reactions (Johnson, Eyring and Stover, 1974). With regard to the excitable behaviour of the cells used in the present study it is difficult to be precise concerning the cellular reactions controlling activity. It has been argued that the discharge frequency of invertebrate neurones can be modulated by the activity of the PFK-FDP and substrate cycle (Chaplain, 1979). If such membrane-bound enzymes were pressure susceptible then irregular discharge patterns might be produced by pressure. Clearly the resting membrane potential behaviour at pressure must also control the activity of the cell. Whether the membrane potential response with pressure can be explained on metabolic grounds is uncertain at present. It may be simplest to view all the effects of pressure as direct structural changes of the membrane. The time course of the transient changes in activity must eventually provide some clue to their basis. Whatever their explanation, it is important that such non steady-state behaviour be recognised and in particular we can imagine that it must complicate studies where the rate of compression is being examined.

We should ask why in certain cases pressure produces no transient effects. For example the input resistance of Helix ganglion cells is a simple function of pressure. Additionally, in voltage-clamp experiments with Helix ganglion cells we find little evidence of transient effects, pressure simply reducing both peak inward and steady-state outward currents, (Harper, Macdonald and Wann, 1977). At the amphibian end-plate the principal effect of high pressure is to prolong the m.e.p.p. decay and again no transient changes in this parameter are observed (Ashford, Macdonald and Wann, 1979). Perhaps in these experiments the processes being studied are rate limited by one factor at pressure e.g. the microviscosity of the membrane lipids. If pressure affected the microviscosity in a simple way then transient changes in these parameters might not be produced.

It is, we believe, important to distinguish these transient and steady-state effects of pressure. In our experiments we have used simple in vitro preparations. If our observations are applicable to more complex systems however then it is clear that the analysis of the effects of high pressure on central nervous system function or whole animal behaviour will be made all the more complicated.

Acknowledgements

This work was supported by S.R.C. grants to K.T.W. and A.G.M. Their support is gratefully acknowledged.

References will appear in PROCEEDINGS.
Figures 1 and 2 follow.

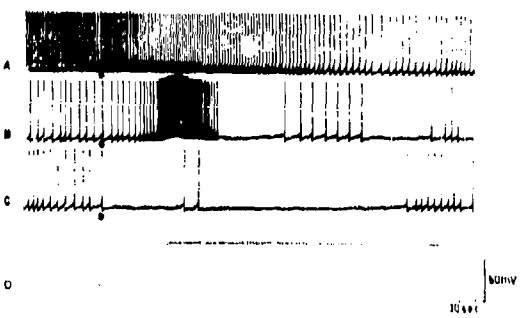

Fig 2 The effect of high hydrostatic pressure on the discharge frequency of a Helix ganglion cell. The pressure steps were applied at the times indicated by ↓. The steps were (a) $1-52 \times 10^5 N.m^{-2}$ (B) $52-104 \times 10^5 N.m^{-2}$ (C) $104-156 \times 10^5 N.m^{-2}$. The pressure "profile" for each step is shown in D.

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THE EFFECTS OF HIGH PRESSURE ON INDIVIDUAL CHOLINOCEPTIVE RECEPTOR MOLECULES
ARE FUNCTIONAL. J. F. Bentley, J. Braswell, P. Wasserman and D. V. Miller, Department of Anesthesia and Pharmacology, Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts, U.S.A.

Although it has long been known that high pressure may cause excitability in whole animals, it is only in recent years that extensive electrophysiological studies have been undertaken to attempt to elucidate the underlying mechanisms. To obtain a complete understanding these physiological effects must be supplemented by biochemical work which often provides complementary information not available to physiological techniques. This is particularly true of receptor controlled ion channels where biochemical techniques alone can answer questions about the role of pressure in modulating receptor occupancy. Such techniques, when developed, may be applied to the central nervous system, where pressure has its most pronounced effects, as readily as to the peripheral nervous system. The basis of such biochemical techniques is the filtration binding assay. In this paper we describe a novel apparatus for carrying out such assays under partial pressures of gas. The ATA of inert gases, and apply it to examine the effects of such variables on the stability, number, affinity, ligand binding kinetics and partial agonist response of the nicotinic acetylcholine receptor (AChR).

AchR rich membranes were prepared at high pH, by osmotic lysis from the Electrophorus electric organ by differential and sucrose density gradient centrifugation following homogenization of the electric tissue. Total binding of labelled agonists and antagonists to these membranes was removed using glass fiber filters to separate the membrane bound and free ligands. A Boyer-Severs isotherm relationship was used to determine what fraction of the binding was specifically to AchR.

Equilibrium assays were carried out at 4°C thermostated, 8 inch (4 atm) pressure chamber. Twelve filter units were mounted on a rack such that each filter could be driven to a point on which the base of the unit could be connected to either pressure from which or vacuum (release). AchR antigen and ligands could be introduced under inert gas pressure to each filter unit.

Initially AchR membrane suspensions were exposed to up to 300 ATA of helium at 25°C for an hour, slowly decompressed and finally the AchR concentration assayed. This treatment had no effect showing any effects of pressure to be reversible. Next AchR was pre-mixed with either tritiated acetylcholine or d-tubocurarine under conditions where about half the receptors were occupied by ligand. Helium pressures of up to 300 ATA progressively decreased the proportion of receptor occupied by either the agonist or antagonist. The cause of this decrease could be either loss of receptor sites or a reduction in affinity, that is an increase in dissociation constant, K_d. Accordingly, AchR was equilibrated with a wide range of ^{3}[H]-acetylcholine concentrations so that complete binding curves at constant pressure could be obtained. At 5 ATA helium the binding deviated from mass action in the direction of positive co-operativity; Hill analysis yielded a coefficient of 1.5. Determination of the binding curve at 275 ATA of helium did not significantly change this value, but the K_d increased from 15nM at 5 ATA to 21nM at 275 ATA. The Hill plots of this data are shown in figure 1. Thermodynamic analysis suggests that this is equivalent to an apparent volume change of about -60 ml/mole. This value should be interpreted with caution, however, as the kinetics of ^{3}[H]-acetylcholine binding are biphasic. A fast initial phase corresponding to acetylcholine binding is completed within seconds, whilst a slower second phase takes minutes and is caused by a slow conformation change of the receptor. Unfortunately, the fast phase cannot be studied at pressure yet because our mixing time is too long. However, preliminary experiments suggest that the kinetics of the slow phase are not greatly affected, implying that the decrease in overall affinity at pressure is determined by a reduction in the fast rate constant. We are currently completing a rapid mixing device which, together with cooling the chamber, should allow the slow and fast steps both to be studied. Data on this aspect will be presented.

A second parameter that can be studied in AchR membranes is their cation permeability following addition of an agonist. This is possible because the preparation contains partly sealed membrane vesicles. These may be loaded with a radioactive cation by pre-incubation, for example with ^{86}RbCl. The external radioactivity can be removed by exclusion chromatography, and then the radioactivity released on addition of agonist assessed by filtration. At 4° the proportion of ions released by the agonist carbachol is dependent on concentration and dose response curves can be obtained. Great difficulty is encountered in doing this experiment at pressure, however, because of the additional time required and the inherent leakiness of the vesicles. Preliminary data suggest that the maximum carbachol stimulated permeability is not reduced by pressure, and that the dose-response curve is not shifted dramatically.

Thus the effects of helium pressure on this post-synaptic membrane can be studied in detail. Our data suggest that function is not dramatically effected at pressure, which implies that the pressure induced conduction failure at the neuromuscular junction reported by several workers is not post-synaptic in origin.

Of particular interest to diving physiology is the effects of other inert gases and their mixtures. Work on nitrogen and argon is proceeding and results will be presented. At present we have shown that volatile anaesthetics change the binding of ^{3}[H]-acetylcholine in the opposite direction to helium and that these effects are additive (i.e. they oppose each other).

These agents appear to act on the slow phase of the binding kinetics and this distinguishes them from pressure, which probably acts on the fast phase. Volatile agents block the permeability response non-competitively and preliminary results suggest this block is relieved by helium pressure. Thus the post-synaptic membrane does seem to provide a model of the in vivo observation of pressure reversal. Detailed results can be obtained and mechanisms evaluated. The methodology is directly applicable to other neurotransmitter systems and should be useful in elucidating the aetiology of the high pressure nervous system.

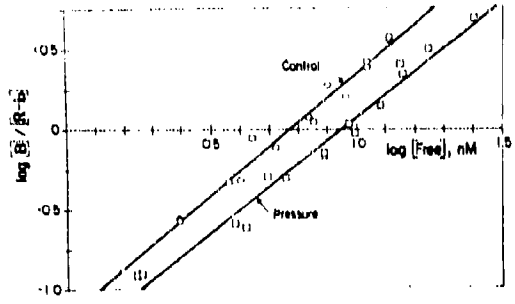

Figure 1. The effect of helium pressure (275 ATA) on the specific binding of ^{3}[H]-acetylcholine to receptor rich membranes isolated from the electroplaque of Torpedo californica. B is bound acetylcholine, R is the total receptor binding sites. The ordinate is the free acetylcholine concentration. The slope of this Hill plot and the dissociation constant are respectively 1.5 ± 0.06 and 15 ± 2.1 nM for the control and 1.4 ± 0.07 and 23 ± 1.2 nM at pressure.

THE EFFECTS OF GENERAL ANAESTHETICS ON POST-SYNAPTIC RESPONSES. H. J. Little and W. D. M. Paton, Department of Pharmacology, University of Oxford, South Parks Road, Oxford, OX1 3QT, England.

Introduction

For many years the basis of the action of general anaesthetics has been thought to lie in their direct interference with synaptic transmission. This conclusion is based on the fact that synaptic transmission is depressed by lower concentrations of anaesthetics than are required to affect axonal conduction. (Larrabee and Posternak, 1952). However, no single action of anaesthetics on the synaptic transmission has yet been identified which could adequately explain their anaesthetic action in vivo. It is possible that the reason for this is that general anaesthetics do not all act by the same mechanism. However, the excellent correlation of anaesthetic potency with lipid solubility and the fact that the in vivo general anaesthetic actions of all agents are reversed by high pressure suggests that there is some common mechanism in their production of anaesthesia. It may be that the reason a common basis has not been found is that many studies have been restricted to one particular group of anaesthetic drugs. In order to determine the relevance of the actions of general anaesthetics on an isolated system it is important to compare the relative potencies of a wide range of anaesthetic agents and also to determine the effects of high pressure. It would be expected that if the effects of the anaesthetics studied are involved in the production of anaesthesia then they would be reversed by high pressure.

Recent work in this laboratory has explored the actions of anaesthetics on transmitter output to determine whether this could provide a common mechanism. The guinea-pig ileum was used as a model preparation and the output of acetylcholine was measured. It was found first that there were some radical differences between the actions of anaesthetics; certain gaseous anaesthetics - nitrous oxide, argon, nitrogen, sulphur hexafluoride, carbon tetrafluoride increased the acetylcholine output whilst urethane, octanol and phenobarbitone decreased it. In addition, it was found that whether the anaesthetics increased or decreased the transmitter release the changes were not reversed by high pressures of helium (190 atm). From these results it was concluded that the effects of anaesthetic action on transmitter release, as far as could be determined from their actions on this peripheral tissue, would not provide a common element in their in vivo actions.

This suggested that the important site for anaesthetic action might be post-synaptic. There have been many suggestions recently that general anaesthetics act by affecting the control of ion permeability of the cell membrane. Several mechanisms can be envisaged by which they could prevent the changes in the conformation of membrane proteins which occur during synaptic transmission.

We are currently investigating the effects of anaesthetics on the actions of agonists which cause different conductance changes within the post-synaptic membrane. Two preparations are being used for these studies, the guinea-pig ileum, which provides a direct comparison with the studies on transmitter release, and the rat superior cervical ganglion. The latter preparation responds to nicotinic, muscarinic, noradrenergic and GABA-ergic agonists, with different conductance changes involving sodium, potassium and/or chloride.

Methods

The effects of general anaesthetics on the responses of the guinea-pig ileum to substance P, acetylcholine, potassium chloride and electrical stimulation have been compared and also their effects on the development of desensitisation to these agonists. The ileum was suspended in an organ bath in Krebs solution at 37°C, continuously bubbled with 95% O_2, 5% CO_2. Solid or liquid anaesthetics were added to the Krebs solution after control dose response curves had been established. The dose response curves were then repeated in the presence of the anaesthetics, and on control preparations to which anaesthetic had not been added. The responses on the control preparations were reproducible throughout.

Desensitisation was investigated by adding repeated doses of concentration of each agonist which produced a nearly maximal response and then repeating these doses in the presence of the anaesthetic.

Apparatus has been designed and built in which the surface potential changes in the ganglion caused by the addition of agonist drugs can be recorded from inside a pressure chamber. The method is an adaption of that of Brown and Marsh (1975). The ganglion is continuously superfused with Krebs solution and solutions of the drugs are added automatically at intervals by means of a switching system triggered from outside the chamber. The potential changes are recorded using Ag/AgCl electrodes positioned at either end of the ganglion. Potential changes down to 0.05 mV can now be recorded satisfactorily from inside the pressure chamber. At present, the effects of high pressure helium on the responses to the agonists are being tested and then the effects of pressure on the actions of the anaesthetics will be investigated.

Results

The anaesthetics which have been studied on the ileum are urethane, octanol, pentobarbitone and phenobarbitone. The volatile agents are currently under investigation. Octanol, (0.25 and 0.5 mM) urethane (50 and 100 mM) and pentobarbitone (0.2 and 0.4 mM) decreased the responses to acetylcholine, substance P, potassium chloride, and to electrical stimulation. The maximum responses in each case were decreased, as were the gradients of the log dose response curves. Of considerable interest was the observation that the acetylcholine responses were depressed less than those to substance P, potassium chloride or electrical stimulation.

Phenobarbitone, which differs from pentobarbitone in being proportionally more anticonvulsant and sedative rather than general anaesthetic, was used at the same molar concentrations as of the latter in order to have a direct comparison. The responses to acetylcholine, substance P, potassium chloride and electrical stimulation were not greatly affected by these concentrations (0,2 mM and 0,4 mM) of phenobarbitone.

The doses of substance P were given at 3 min intervals during the dose response curves, since no desensitisation occurs when this time schedule is used. To determine whether the greater effect of anaesthetics on substance P response compared with those to acetylcholine was due to increased desensitisation their effects were tested on repeated administration (at 3 min intervals) of a concentration of substance P (200 mg) which produced a just submaximal response. No desensitisation was found in the presence of the anaesthetics in these tests.

In view of the suggestion (Young and Sigman, 1979) that an increase in desensitisation may contribute to general anaesthesia this phenomenon was further investigated by repeating this concentration of substance P at 1 min intervals and also a corresponding concentration of potassium chloride at 1 min intervals. (The time intervals between the successive sets of doses were sufficient to exclude non-specific desensitisation).

In the absence of anaesthetic the decrease in response amplitude to substance P after 10 doses at 1 min intervals was 12%. Urethane and octanol increased this change to 59% and 100% respectively but with pentobarbitone it was only 10%. No significant depression of the responses to potassium chloride were seen either with or without the anaesthetics.

Discussion

These results showed that the general anaesthetics tested so far depressed the post-synaptic responses to all the agonists, while phenobarbitone appeared to have a different effect. It has been suggested previously that anaesthetics have a selective effect on changes in sodium permeability, (Theorell, 1956; Narkov, 1975).

The response to acetylcholine on the ileum involves increases in permeability to Na^+ and K^+ (Bolton, 1971). The responses to substance P by the ganglion cells of the myenteric plexus have been shown to be due to a decrease in potassium conductance (Grafe, Mayer and Wood, 1979) and it is likely that it has the same effect on the smooth muscle. (The responses to substance P are not antagonised by hyoscine). These results provide a direct comparison between the effects of the agonists on the same tissues and show that, in contrast to the previous results, acetylcholine responses were less depressed than the others. An increase in the desensitisation to acetylcholine has been suggested by Magazanik (1976) for several drugs including the barbiturates and long chain alcohols, and by Sigman and Young (1979) for volatile anaesthetics. The present results show that, while octanol and urethane clearly increased the desensitisation to substance P this is not an action common to all general anaesthetics as it was not seen with pentobarbitone.

The main conclusion to be drawn from the results thus far is that they are compatible with the theory that an action common to all general anaesthetics is a depression of post-synaptic responses but there is no selectivity for sodium conductance changes. Current work is being directed towards determining the effects of high pressure on post-synaptic responses and the effects of anaesthetics on these using the method developed for the ganglion preparation.

References will appear in PROCEEDINGS.

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PHARMACOLOGICAL INVESTIGATION OF THE HIGH PRESSURE NEUROLOGICAL SYNDROME: BRAIN MONOAMINE CONCENTRATIONS. S.G. Daniels, A.R. Green*, D.H. Kollin**, R.J. Lever, M.J. Little, M.H. Paton & D.H. Smith***. University Department of Pharmacology, South Parks Road, Oxford, U.K., *Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford, U.K., **Physical Chemistry Laboratory, South Parks Road, Oxford, U.K., ***Much of the work described in this abstract was carried out by Dr. D.H. Kollin while on leave from the San Francisco Medical Center, supported by U.S.P.H.S. Grant 5F-1 - GM - 15521 and ONR N 00 14 - 75 - C 0728.

Introduction

In man the effects of high pressure, known collectively as the high pressure neurological syndrome (HPNS) are typically, tremor, nausea, dizziness and deficiencies in the performance of psychomotor tests. HPNS is a serious limitation to man's ability to function under the pressures commonly encountered in commercial diving (Halsey, Bettis & Smith, 1975). Furthermore it is possible that the same serious signs observed in animals may affect man at only slightly deeper depths. In mice tremors are observed in the range 10-50 ATA, convulsions in the pressure range 20-100 ATA followed by death at 120-140 ATA (e.g. Brauer, 1975). The pressure at which these signs first appear is dependent on compression rates. The work described in this abstract arises from the effect of reserpine reported by Brauer, Beaver & Sheehan (1975), and is concerned with the effects of high pressure on the levels of monoamine neurotransmitters in the central nervous system and on the manner in which the high pressure neurological syndrome may be modified by drugs which selectively deplete the different transmitters.

Methods

In the investigation male CD1 mice were used in the weight range 20-30g. The high pressure experiments were performed in a 1,6 l hyperbaric chamber in which one mouse contained in a restraining cage with a rectal thermal probe inserted, could be observed and the observations recorded using a closed circuit television system. The rectal temperature was maintained between 36,5 and 38, 0°C by adjusting the chamber temperature (usually in the range 15-25°C). The chamber gases were mixed with a fan powered by an inductor motor and containers of soda lime and activated charcoal were used to remove carbon dioxide and effluent gases. Prior to compressions the chamber was flushed with pure oxygen.

Four behavioural end-points were employed. Mild tremors were characterised by intermittent twitching of the neck and back muscles during which the mouse often adopted a hunched posture. Coarse tremors were defined by a shivering of the whole body during which the animals found coordinated movement difficult. Convulsions were characterised as seizures of sufficient severity so as to prevent the animal righting itself. They were often followed by a brief period of respiratory failure. Death was defined by a complete absence of movement for a one minute period. A minimum of six animals were used for each treatment group.

Following the experiments the brains were rapidly removed and kept at -20°C until analysis, which was performed within two weeks. The brains were homogenized in acidified butanol. Following centrifugation the supernatant fluid was divided into two fractions. One was used to analyse 5-hydroxytryptamine (5-HT) and its metabolic product 5-hydroxyindole acetic acid (5-HIAA) by the fluorometric method of Curzon and Green (1970). The other fraction was used for the analysis of dopamine (DA) and noradrenaline (NA) using the fluorometric assay of Chang (1964). Owing to the damage induced by decompression the brain weights of animals killed after compression were some 10% less than the corresponding value for animals which had not been subjected to pressure.

Experiments were performed to assess the role of stresses arising from the restraint of the animals in the chamber. Periods of restraint of 30 min or 6,5 hours (periods similar to those involved in fast and slow compression experiments respectively) had no effect on the levels of DA, NA or 5-HT see Table 1. However, a marked increase of 5-HIAA level was observed indicative of higher 5-HT turnover. This is consistent with other reports of increased 5-HT turnover following stress induced by immobilization (Curzon & Green, 1971). Slow compression at 1 ATA/min, or rapid compression at 15 ATA/min, had no effect on DA or NA. Both, however, elevated brain 5-HIAA above the levels induced by restraint alone. Rapid compression also caused a small but statistically significant increase in 5-HT levels which was not observed on slow compression.

Results

Reserpine: Following reserpine pre-treatment (4 mg/kg given i.p. approximately 24 hours before experimentation) brain monoamine concentrations were markedly reduced compared with comparable (vehicle injected) controls. After the application of high pressure 5-HT concentrations increased but was still low when compared to the control animals. Both 5-HIAA and DA concentrations increased on the application of high pressures after reserpine. There were no differences between the NA concentrations of reserpine-treated animals compared with those exposed to both reserpine and pressure. Reserpine markedly reduced the onset pressures of the characteristic signs of HPNS (Table 1).

p-Chlorophenylalanine (PCPA): Doses of 300 mg/kg were given i.p. approximately 72, 48 and 24 hours before experimentation and, as expected of a tryptophan hydroxylase inhibitor, were found to decrease 5-HT and 5-HIAA concentrations while not affecting DA or NA. No significant change in the concentrations was observed after pressurization, nor did PCPA affect the onset of HPNS.

α-methyl-p-tyrosine (αMPT): Animals were treated i.p. with 350 mg/kg at approximately 18 and 16 hr before experimentation. αMPT is a tyrosine hydroxylase inhibitor and produced a significant decrease in NA and a modest decrease in DA whilst 5-HT and 5-HIAA concentrations were not affected. αMPT produced no effects on the onset pressure of the observed signs of HPNS.

PLA-63: Animals were treated i.p. with 50 mg/kg about 1 hr before experimentation. PLA-63 inhibits dopamine β-hydroxylase producing a marked decrease in brain NA concentrations and a rise in brain DA. 5-HT concentrations are not affected and PLA-63 does not modify the increase in 5-HIAA observed on pressurization, though after pressure treatment the NA concentrations become even lower than with PLA-63 alone. Animals treated with PLA-63 exhibited the signs of HPNS at lower onset pressures.

Nitrogen: Partial pressures of nitrogen in the range 30-40 atm act as a general anaesthetic and cause the signs of the HPNS to occur at significantly higher onset pressures (Lever, Miller, Paton & Smith, 1971). The associated change in the brain amine levels are at present under investigation.

Discussion

The effect of immobilization stress in these experiments, possibly heightened by pressurization, is reflected in the increased 5-HIAA concentrations suggesting increased 5-HT turnover. Reserpine, PCPA and αMPT or PLA 63 were unable to prevent this increase. The effects of stress on brain catecholamines appears to be small.

The fact that the decreases in 5-HT and 5-HIAA after PCPA were not associated with changes in the HPNS suggests that 5-HT does not play a major role. However, the changes in 5-HIAA concentrations at high pressure were smaller than at atmospheric pressure, suggesting less decrease in 5-HT release, and it would be worth testing this aspect more stringently.

The inhibition of NA and DA synthesis by αMPT did not affect the HPNS although the greater depletion of these amines by reserpine clearly lowered all the thresholds. This difference may have been due to the fact that reserpine inhibits the reuptake mechanism into storage granules and this might have a different effect from those of synthesis inhibitors such as PCPA and αMPT (Carlsson & Hedberg, 1974). The lack of effect of αMPT on the HPNS might suggest that the catecholamines are not involved. However, αMPT did not cause a significant decrease in DA at pressure. (Reserpine caused less depletion of DA at pressure but the effects on NA were unchanged; suggesting a possible effect of pressure on DA metabolism).

The decrease in the onset pressures for the HPNS signs caused by PLA63 on first sight appear to be linked to reduced NA concentrations. However, this is not consistent with the results obtained using αMPT, which also decreased NA concentrations. It is possible that the increased DA concentrations induced by PLA63 may play a part in mediating the effects of this drug on the HPNS.

The results reported here are in keeping with the suggestion by Brauer et al (1976) that the catecholamines are involved in the behavioural changes induced by high pressure. At the same time it has not been possible consistently to associate the HPNS changes with effects on any particular amine. Despite this it is of interest to note that the greater effects of reserpine and of PLA63, compared with those of αMPT, on the HPNS parallels the effects of these agents on electroshock and pentylenetetrazol convulsions (e.g. Killam & Frey, 1971). This supports the idea that there is some common factor in the production of convulsions by these techniques and by pressure and, together with the present results, suggests that more than one neurotransmitter is involved.

References will appear in PROCEEDINGS.
Table 1 follows.

Table 1. HPNS Onset Pressures and Brain Amine Levels

Experimental Conditions/ Compression Rate	Treatment	Fine Tremor	Coarse Tremor	Convulsions	Death	5HT	5-HIAA	DA	NA
Untreated						0.66±0.02	0.43±0.02	1.11±0.07	0.14±0.01
Restrained 20 min						0.70±0.03	0.85±0.03	1.29±0.05	0.18±0.02
Restrained 2.5 hr						0.63±0.04	0.54±0.04	1.16±0.07	0.16±0.03
Rapid		0	0	7.2±5	13.9±11	0.18±0.03	0.83±0.05	1.09±0.07	0.13±0.01
Slow		7.4±6	9.3±5	10.2±4	12.8±6	0.82±0.02	0.70±0.05	1.08±0.04	0.16±0.02
	Vehicle no pressure					0.66±0.06	0.48±0.04	1.20±0.06	0.11±0.02
	Reserpine no pressure					0.27±0.02	0.58±0.04	0.13±0.07	0.04±0.01
	Vehicle slow compression	7.0±3	8.4±2	10.1±3	12.4±6	0.64±0.07	0.70±0.07	1.72±0.09	0.11±0.01
	Reserpine slow compression	4.0±3	4.2±3	4.7±4	6.4±3	0.18±0.04	0.84±0.07	0.82±0.14	0.03±0.01
	Halvic n 2 no pressure					0.61±0.02	0.48±0.02	1.21±0.06	0.11±0.02
	PCPA no pressure					0.19±0.02	0.18±0.02	1.09±0.08	0.16±0.01
	Halvic n 2 slow compression	6.0±2	8.0±1	10.2±4	13.5±5	0.54±0.03	0.70±0.02	1.29±0.14	0.12±0.02
	PCPA slow compression	6.0±1	8.2±1	9.5±5	12.7±6	0.40±0.03	0.12±0.02	1.18±0.11	0.18±0.02
	Halvic n 2 no pressure					0.59±0.03	0.35±0.01	1.16±0.06	0.11±0.01
	pMPT no pressure					0.61±0.04	0.14±0.04	1.07±0.07	0.03±0.01
	Halvic n 2 slow compression	5.1±5	8.0±2	8.8±4	12.3±5	0.55±0.02	0.64±0.04	1.07±0.07	0.09±0.01
	pMPT slow compression	6.2±5	8.8±4	10.1±3	12.4±1	0.59±0.70	0.74±0.04	0.82±0.11	0.02±0.01
	Vehicle no pressure					0.64±0.02	0.33±0.02	1.34±0.02	0.13±0.02

Treatment	Fine Tremor	Coarse Tremor	Convulsions	Death	5HT	5-HIAA	DA	NA
FLA 63 no pressure					0.66±0.02	0.40±0.02	1.51±0.11	0.07±0.01
Vehicle slow compression	5.8±5	8.0±5	9.1±5	12.6±6	0.65±0.02	0.54±0.10	1.22±0.07	0.12±0.02
FLA 63 slow compression	5.0±3	6.5±3	7.4±2	8.8±7	0.66±0.01	0.56±0.01	1.47±0.07	0.04±0.01

Results show mean ± SEM of six or more observations. Ø - rate of change too rapid to observe accurately. Slow compression = 1 atm/min. Rapid compression = 15 atm/min.

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PREVENTION OF HPNS : THE POSSIBLE USE OF STRUCTURAL ISOMERS OF ANAESTHETICS. Bridget Wardley-Smith and M. J. Halsey. Division of Anaesthesia, Clinical Research Centre, Harrow, Middlesex, United Kingdom.

Mammals exposed to increased ambient pressures exhibit first uncoordinated tremors around 90 atmospheres absolute (ATA) then convulsions, respiratory distress and finally death as the total pressure is raised to 100-140 ATA. These changes are encompassed in the High Pressure Neurological Syndrome (HPNS) (Hunter and Bennett, 1974). There have been a number of human studies at pressures up to 60 ATA (Lambertsen, 1976) but the physiological perturbations of high pressure are now the major limiting factors in diving to sea depths greater than 500 m (50 ATA). Low concentrations of a variety of anaesthetic substances have been demonstrated to ameliorate some of the adverse effects of high pressure in amphibians (Miller, 1972; Halsey and Wardley-Smith, 1975). However, only a limited range of gaseous anaesthetics have been studied in animals (Brauer et al, 1974b) and the underlying mechanisms of action are unknown. Nitrogen (as 'Trimix') has been used experimentally in man (Bennett et al, 1974) but as yet the overall results are not entirely satisfactory.

Our recent experiments to investigate the interaction of pressure and anaesthetics in rats have led us to postulate that the molecular receptors for anaesthesia and HPNS may be separate (Halsey, Wardley-Smith and Green, 1978). One aspect of the data on which this hypothesis is based is that although all the anaesthetics were antagonised by pressure, there were considerable differences in their ability to provide protection against HPNS. For example, Althesin and Ketamine were both effective in preventing HPNS even in sub-anaesthetic doses, whereas methohexitone actually potentiated the tremors and convulsions seen in HPNS in the rat.

However, although some anaesthetics have no effect on HPNS, (e.g. thiopentone), no compound unrelated to an anaesthetic has yet been found to have any significant effect in preventing it (Wardley-Smith and Halsey, 1979). It thus seemed possible that a non-anaesthetic compound with a close structural relationship to an anaesthetic might prove useful in the treatment of HPNS. The steroid anaesthetic alphaxalone (the main component of Althesin) has several non anaesthetic isomers with only small structural changes : since alphaxalone is effective in preventing HPNS these compounds seemed appropriate to study for anti-HPNS activity.

METHODS

Adult, male Sprague-Dawley rats, 250-400 g were used in all experiments. The lateral tail vein was cannulated to permit infusion of drugs at pressure from a pump which was externally controlled. Temperature was measured via a colonic thermistor and was maintained at 37 ± 0.5°C.

We used tremor as a means of assessing the severity of HPNS : it has been shown to have a reproducible onset pressure (Brauer et al, 1974a) and any improvement subsequent to drug administration can be easily detected. The method we have developed for measuring tremor will be described in detail elsewhere. Briefly, it consists of a small strain gauge either taped directly onto a rat anesthetised in a rodent "hammock" (Doncaster, 1972) or a strain gauge incorporated into a small cage in which the rat is restrained only by taping its tail. Both systems gave an excellent signal indicating onset of tremor, but the signal from the cage allowed detailed analysis of tremor frequency, and more recent experiments have used this technique only. After preparation under halothane anaesthesia, the restrained rat was placed in the pressure chamber and allowed to wake up. Once a suitable control reading had been obtained, 0.4 ATA oxygen was added and compression with helium at 3 ATA/min was commenced. The signal from the strain gauge was continuously recorded on magnetic tape and was observed on an oscilloscope.

We compared the effects of 'Althesin' (9 mg/ml alphaxalone dissolved in Cremophor RL); Δ16-alphaxalone (30 mg/ml sonicated in Cremophor RL); 3β hydroxy-alphaxalone (10 mg/ml sonicated in Cremophor RL) and Cremophor RL alone as a control. Once tremor had become moderate to severe, each compound was infused for up to 2 minutes. As well as continuous recording, the animals were constantly watched to detect any observed change in tremor. After each infusion, all animals were carefully observed to ensure that time-adaptation to pressure did not eliminate tremor (Brauer et al, 1975).

RESULTS

Both methods of monitoring tremor gave a good and point for detecting the onset of tremor and, conversely, reliably detected any improvement in HPNS, as shown by tremor being attenuated or abolished.

We found that the frequency of the tremor was consistent between different animals, varying from 11-14 Hz. The threshold for tremor onset (ATA - s.e.m.) was 56.1 - 1.9.

Results of the generalised effects on tremor of infusing alphaxalone or its isomers are shown in Fig. 1. Alphaxalone was the most effective, but anaesthesia occurred very shortly after tremor had ceased. 3β hydroxy- and Δ16-alphaxalone both reduced the severity of tremor, but were not as effective as alphaxalone. Neither isomer had any anaesthetic effect. Once tremor had returned, usually about 3 min after the initial drug infusion, a second dose was given: Δ16-alphaxalone was still effective, but 3β hydroxy-alphaxalone had no effect on tremor during second or subsequent doses, suggesting that its metabolised form blocked the HPNS receptor.

However, although the isomers of alphaxalone improved HPNS as shown by a reduction of tremor, both observed and recorded, they were not totally effective as shown in Fig. 2. It can be seen that although the severity is greatly reduced, the basic frequency of the tremor is still present. This appears as a higher frequency signal superimposed on the respiratory signal.

DISCUSSION

The use of structural isomers of anaesthetics is an approach which may make it possible to distinguish between separate molecular receptors for anaesthesia and HPNS, and thus to enable a drug to be found which is safe and effective in treating HPNS. Isomers of an anaesthetic already shown to be effective in preventing tremor could have considerable potential as a pharmacological approach. Our results so far are encouraging, but a number of further questions remain. It has been suggested that the isomers of alphaxalone are non-anaesthetic simply because they do not reach the molecular receptor for anaesthesia. The fact that we found the isomers only partially effective in preventing tremor could be due to an insufficient concentration at the molecular level, but the existence of any effect on HPNS demonstrates at least a partial concentration of the drug being available to the receptor. It is possible that isomers of the newer water soluble steroids would be more suitable, since a much greater reservoir of the drug should be available to the molecular receptor, and we are currently investigating other anaesthetics with active isomers to test this idea further.

Attempts to find a drug not related to anaesthetics to treat HPNS have so far not been successful. A study in which we screened anticonvulsant drugs for anti-HPNS activity in mice showed that only those compounds which were anaesthetic at higher concentrations, e.g. diazepam, were of any value. Non anaesthetic anticonvulsants such as phenytoin were completely inactive against HPNS in our preparation (Wardley-Smith and Halsey, 1979). This provides further support for the concept of some interaction between anaesthesia and HPNS receptors. However, it seems certain that the receptors are not identical in view of the considerable variation between different anaesthetics in their ability to prevent HPNS in rats (Green, Halsey and Wardley-Smith, 1979).

This idea of linked receptors is not inconsistent with other experiments in intact animals, which have demonstrated that the area of the brain affected by anaesthetics and pressure is the same (Angel, Halsey and Wardley-Smith, 1974) i.e. in the somatosensory pathway leading to the cerebral cortex. These experiments looked at the reduction of the evoked somatosensory cortical response by urethane followed by its recovery on increasing ambient pressure. These data also suggest that the effects of pressure, both alone and on anaesthesia, are not due to a general excitation, such as might be mediated by catecholamine release.

It is thus of potential importance to understand more about the precise receptors for anaesthesia and HPNS, since the separate sites would allow the possibility of a drug entirely effective in treating HPNS without undesirable anaesthetic "side effects". Hopefully, the study of inactive isomers of anaesthetics shown to be of value in ameliorating HPNS will continue to provide promising results.

References will appear in PROCEEDINGS.
Figures 1 and 2 follow.

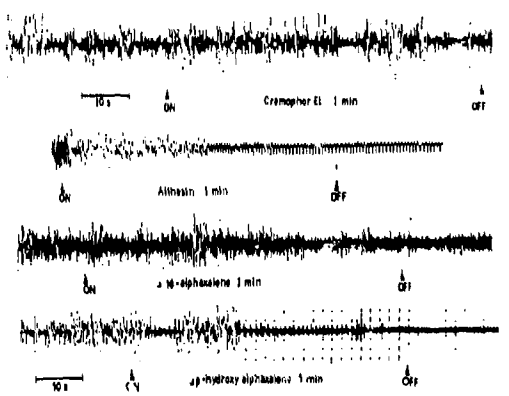

Figure 1

Traces obtained from a strain gauge taped beneath a rat showing the effect of Althesin and its isomers on tremor. Cremophor EL had no effect. Althesin abolished tremor but resulted in anaesthesia after 30 mi. Δ16- and 3β-hydroxy-alphaxalone were both partially effective in attenuating tremor.

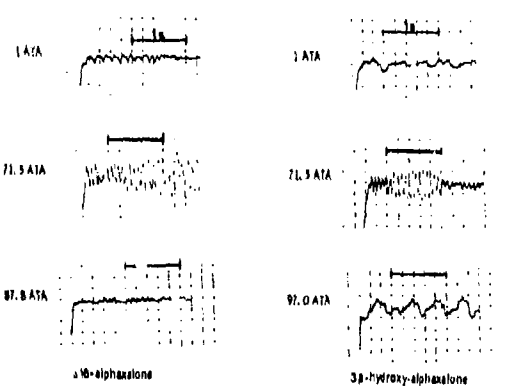

Figure 2

Detailed results for the non-anaesthetic isomers of alphaxalone. Each trace is the signal from the strain gauge built into the structure of a small cage. Top tracent control at 1 ATA; middle tracent untreated tremor; bottom tracent immediately after administration of Δ16-alphaxalone or 3β-hydroxy-alphaxalone. Note in bottom right trace small tremor signal superimposed on the respiratory signal. Time bar in each trace represents 1 s.

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RAPID COMPRESSION WITH TRIMIX (He-O_2). P.B. Bennett, R. Coggin, J. Roby and J.R. Stilley. I.G. Hall Laboratory, Duke University Medical Center, Durham, North Carolina, U.S.A.

The High Pressure Nervous Syndrome (HPNS) provides a formidable limitation to the ability of man to dive to very great depths. Rapid compression to pressures greater than 16 ATA (500 ft) induces dizziness, nausea, vomiting, tremors, fatigue and somnolence with deterioration in performance and changes in the EEG activity which at sufficiently high pressures causes convulsions in animals. Thus human divers to 46 ATA (1500 ft) or 60.5 ATA (1950 ft) even with operationally unrealistic 1 to 2 days compression may result in HPNS detrimental to work efficiency. Various strategies therefore have been utilized to ameliorate the signs and symptoms including selection of less sensitive divers, slow exponential compression with stages for adaptation, use of excursions from less deep saturation and the use of narcotics. Over several years the I.G. Hall Laboratory has specifically studied the addition of nitrogen to helium-oxygen to control the HPNS in a number of human deep dives and animal studies which will be discussed here and related to the current "Atlantis" project of deep trimix research in divers at 45.6 ATA (1500 ft).

In 1959 it was noted that application of pressure to tadpoles anesthetized with alcohol caused pressure reversal of anesthesia and the tadpoles resumed swimming. More recently a number of workers have noted that narcotic gases added to the breathing mixture of animals significantly raised the pressure (Pc) at which convulsions occurred. Gray early work at the I.G. Hall Laboratory which defined Pc as the occurrence of overt convulsions together with EEG spike and wave activity found no change from the 111 ATA (1700 ft) in 60 rats whose colonic temperature was maintained normal. Mean tremor thresholds, however, were increased for 10 rats from 55 ATA (1750 ft) with 10% N_2 in He/O_2 to 81 ATA (2662 ft) and with 20% N_2 to 108 ATA (3567 ft). At 40% N_2 the rats were anesthetized and still showed EEG seizure activity but not overt convulsions at the slightly lower pressure of 99 ATA (3210 ft). These differences may be due to the method of addition of narcotic. In the Rostain papio papio study N_2 was added throughout compression rather than initially, as with the above experiment. Other factors such as method of compression, species or temperature differences also may be concerned. Again it should be noted that although pressure reversal does appear valid it has not proved possible to apply pressure (or helium) and "wake-up" a truly anesthetized air breathing animal. Most of the studies were made with poikilothermic mice utilizing righting response as the measurement. Due to such problems with animal models, a continuous series of human studies of the potential value of helium/nitrogen/oxygen mixtures (Trimix) in controlling HPNS in man has been made at this Laboratory.

Thus in 1971 4 divers were compressed in 20 min to 21 ATA (720 ft) with 25% N_2 in He/O_2 and later in 13 mins with 18% (5.6 ATA) N_2 in He/O_2 to 31 ATA (1000 ft). Control exposures were made also to the same depths in He/O_2 alone and to 7 ATA (200 ft) compressed air with the same N_2 partial pressure. Decompression using 0.8 ATA O_2 required only 4 days. A battery of neurophysiological and performance tests were given. The N_2 suppressed the nausea and dizziness and the intention and postural tremors noted with helium alone. Psychomotor performance markedly improved with nitrogen present but some decrement in intellectual performance remained. The EEG showed little change. Subjectively, two subjects were HPNS-sensitive and preferred Trimix, whereas the other two reported that nitrogen narcosis reduced their efficiency.

Computations of the correct percentage of nitrogen necessary to negate the effects of helium pressure based on interactions with lipid monolayers suggested 10% as optimal. Accordingly in 1974 a further 5 divers were compressed to 31 ATA (1000 ft) in 11 mins breathing 3.2 ATA N_2, 0.5 ATA O_2 and the remainder helium. Tests were made of postural tremor, EEG, psychomotor and intellectual performance, and subjective sensations. One diver worked underwater for 40 mins wearing closed circuit breathing apparatus in water at 56°F (14°C). Decompression using 0.8 ATA O_2 took 4 days. The performance tests showed no signs of decrement due either to narcosis or HPNS. No tremor or EEG changes were noted and there was no nausea, dizziness or fatigue. Two further satisfactory dives were made to 31 ATA with 10% N_2 also to test 5-day decompressions at the lower 0.6 ATA O_2.

To verify whether or not rapid compression with Trimix to pressures greater than 31 ATA (1000 ft) would be equally successful, joint studies were made with the R.N. Physiological Laboratory. Compression was made to 40.6 ATA (1312 ft) by 2 divers breathing 6% N_2, 0.5 ATA O_2 and the remainder helium. The lower nitrogen percentage was chosen initially to reduce the potential risk of N_2 narcosis. Dizziness, lightheadedness, nausea, tremors and marked fatigue occurred, which indicated little or no protection from HPNS.

A further dive, a week after the successful 6 day decompression involved the same nitrogen percentage but a slower compression rate of 1 ft/min to a final depth of 1 hr 40 mins to 40.6 ATA. During a 30 min stage at this depth the divers were fit and well. However, during further compression from 40.6 ATA (1312 ft) at 1 ft/min the dive was aborted at 47 ATA (1521 ft) due to the presence of undue HPNS with marked tremors, fatigue, nausea and dizziness. Although previous evidence suggests this would have diminished with time at depth, it was evident that the 5-6% Trimix even at the slower rate was ineffective in preventing HPNS.

With the new 109 ATA (3,600 ft) pressure chamber facility at the I.G. Hall Laboratory in 1978, a series of deep Trimix dives called "Atlantis" was initiated. The two primary objectives are first to establish the relationship between a given partial pressure of nitrogen and the rate of compression required to prevent HPNS; and secondly, to determine the effects of inspired gas density, hydrostatic pressure and narcosis on various respiratory and circulatory parameters. These include the dyspnea reported by many deep divers and arterial blood gases during rest and exercise. Two experimental dives per year are planned with variables changed one at a time to study two nitrogen percentages and three rates of compression, utilizing mostly the same highly trained subjects.

Atlantis I began on April 19, 1979 with 4 subjects compressed with 5% N_2 in He/O_2 in the very fast time of 12 hrs 20 mins to 460 m (1509 ft / 45.6 ATA) where they spent 4 days of extensive performance, neurophysiological and pulmonary function experiments (Fig. 1). Decompression was designed for just over 7 days but at 0.5 ATA O_2 rather than the previously successful use of 0.6 ATA. However, due to "bends" at 150 ft, after augmented oxygen breathing, recompression was made to 220 ft with a 24 hr hold. Successful decompression with 0.6 ATA O_2 was made at 1 ft/hr to the surface.

Measurements at subjective speed at 460 m showed increased tendencies toward withdrawn, depressed, tense, excited, drowsy, lethargic, clumsy, incompetent behavior which had improved by day 2 for most moods except for lethargy and drowsiness which were prevalent throughout the time at maximum depths. Sleep quality was poor during the time at maximum depth which may have added to some of these impressions.

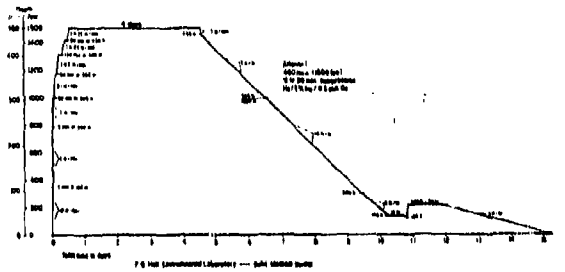

Fig. 1. Profile for fast decompression Trimix dive to 460 m by 3 subjects at F.G. Hall Laboratory April 19th, 1979.

Immediately after compression the divers experienced HPNS with various degrees of nausea and fatigue. Intellectual performance tests throughout day 1, due to both compression rate and pressure (Fig. 2) indicated a decrement almost twice that of the second and subsequent days, when the residual effects probably were due to hydrostatic pressure alone. Return to normal values occurred during decompression by 31 ATA (1000 ft). The psychomotor tests were less affected in general, but showed a similar biphasic depression of ability especially in the Bennett Hand-Tool task which on day 1 was depressed by 44% but by days 2 to 4 by only 20%.

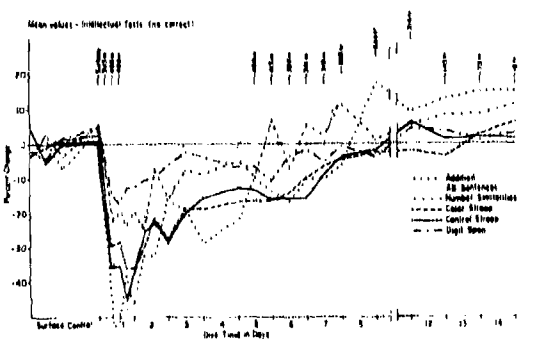

Fig. 2. Mean percentage change in intellectual performance tests of three subjects compressed rapidly to 460 m during Atlantis I whose profile is shown in Fig. 1.

Postural tremor was absent but intention tremor was marked which caused difficulty with arterial cannulation although this was accomplished satisfactorily in all three subjects. By day 2 although the divers appeared normal and completed all tasks satisfactorily nevertheless they tended to act more slowly.

The EEG showed an increase in all frequencies on day 1, with Δ and θ activity reaching peaks +85% and +60% above normal respectively, and α, β frequencies increased to +10% and +10%. By day 2, the α and β fast frequencies had fallen to between -10 and -30% below normal, but the Δ and θ remained +20% above normal.

Although the divers were able to function well after day 2, clearly signs and symptoms of HPNS were present. In March 1980 the same profile will be repeated with the same subjects with 10% replacing 5% N_2. A direct comparison will then be made between the efficacy of 10% with respect to 5% N_2 in controlling HPNS in extremely fast compressions to 1000 ft. It should be possible too to differentiate which aspects of HPNS are ameliorated by Trimix, such as postural tremors, and which not, for example, EEG changes. Certainly it would appear from the results of Atlantis I the Trimix may select which signs or symptoms of HPNS are prevented. This may be related to recent evidence which suggests that pressure and narcosis (anesthesia) act at different sites of the central nervous system so that a unitary antagonism indeed may not be likely.

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THE EFFECT OF HIGH PRESSURE ON COOPERATIVE LIPID-PROTEIN INTERACTIONS. R. A. Galla and J. R. Trudell. Stanford University Medical Center, Stanford, California 94305, U.S.A.

Application of high helium pressure (100 ATA) to bilayer membranes of dipalmitoylphosphatidylcholine results in a 7.5°C increase in the lipid phase transition temperature and dramatic changes in membrane fluidity and lateral compressibility (1). This effect is due to a large difference in densities between the solid and fluid phases of the phospholipids. High pressure always moves an equilibrium toward the more dense phase. However, the importance of these changes to biological systems depends on the extent to which membrane proteins interact with and have their function modified by phase changes in the phospholipids. This paper describes the effect of high pressure in a membrane containing highly cooperative protein-phospholipid clusters.

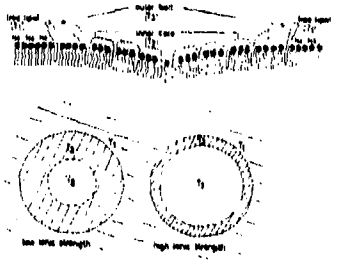

Figure 1: Proposal for the domain structure of the polymyxin-phosphatidic acid complex. A separation into three areas of different lipid properties has been established (from Reference 2).

Very recently (2,3), a cooperative lipid-protein interaction was reported between dipalmitoylphosphatidic acid membranes and polymyxin, a decapeptide antibiotic, carrying five positive charges which can interact with a negatively charged membrane. A hydrophobic tail anchors the molecule within the lipid matrix. Binding of this peptide causes a phase separation in the lipid matrix. Domains of protein bound lipids are formed that exhibit a lower phase transition temperature than the remaining pure phosphatidic acid bilayer. This effect is due to an expansion of the lipid matrix in the protein-lipid cluster. A model was proposed (Figure 1) where an inner core of peptide-bound lipid is surrounded by an annular ring of less tightly-bound lipid. This outer domain is surrounded by the free phosphatidic acid matrix (2).

High pressure applied to a membrane containing these phase separations leads to dramatic changes in the lipid organization. One result is a loss in the cooperativity of the binding process at 100 ATA. A rigidification of the lipid matrix is associated with the loss of cooperativity. A second result is a change in the relative area of the three domains after exposure to high pressure. At low protein concentration (r_b < 2 Mol%) pressure causes membrane-solvated protein (T_1 in Figure 1) to enter into the inner domain structure by increasing the annular ring (denoted by T_3 in Figure 1).

At high protein concentration (3 Mol% < r_b < 7 Mol%) pressure causes reorganization of the cluster proportions. The size of the inner core (T_2 in Figure 1) diminishes, whereas the annular ring (T_3 in Figure 1) increases. The protein solvated in the free lipid matrix (T_1 in Figure 1) is redistributed into the other two protein-containing domains, leaving pure phosphatidic acid in T_1 of Figure 1.

Our experiments show clearly that pressure causes dramatic effects on lipid-protein interactions, especially on the so-called boundary lipids in the surroundings of a protein molecule. Enzymatic reactions in a biological membrane are known to be controlled by cooperative processes. Our experiments give a measure of the effect of pressure on these processes. Moreover, lipid composition affects the activity of membrane-bound protein assemblies (4). The results presented here show that high pressure alters lipid-protein interactions which could lead to an alteration in the biochemical function of membrane components.

References will appear in PROCEEDINGS.

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CURRENTS IN A VOLTAGE-CLAMPED VERTEBRATE NEURON AT 1 ATMOSPHERE PRESSURE. Joan J. Kendig, Ph.D. Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305 U.S.A.

Introduction In previous studies on both vertebrate and invertebrate axons, we have identified and characterized a phenomenon which may be related to the high pressure nervous syndrome (HPNS) (Kendig, et al., 1978a). Some axons, when exposed to high pressure, generate repetitive impulses in response to a single stimulus. As pressure increases, the train of repetitive impulses becomes longer; eventually these axons begin to generate impulses spontaneously in the absence of a stimulus. As in the case with HPNS, pressure-induced repetitive impulse generation is inhibited by anesthetic agents (Kendig, et al., 1978b). The phenomenon can be observed at moderate pressures well within the range associated with HPNS, ie, from 80-100 atmospheres (atm). In these studies extracellular recording techniques were employed; analysis of the basic mechanism was therefore necessarily limited. In our present studies we have turned to a voltage-clamped preparation, in which membrane ionic currents can be directly monitored.

Methods The node of Ranvier of bullfrog (Rana catesbiana) sciatic nerve axon was arranged for voltage clamping by a technique similar to that previously described (Hille, 1967; Courtney, et al., 1978). In adapting the preparation to the hyperbaric chamber, some modifications were made in configuration of the electrode-solution contacts and in physical placement of the electrodes. These modifications did not alter the properties of the node as determined in our previous studies at normobaric pressure (Kendig, et al., 1979). The solution in contact with the external surface of the node and the following composition: NaCl 114 mM, KCl 2.4 mM, $CaCl_2$ 2 mM, HEPES buffer 10 mM; pH was adjusted to 7.3. The solution at the cut ends of the internodal segments was 120 mM KCl buffered with 20 mM HEPES, pH adjusted to 7.3. Particular care was taken to exclude the possibility of artifacts introduced by temperature changes. Temperature was monitored by a precision thermistor in the pool containing the node, and controlled by a thermoelectric device beneath the clamp chamber. Temperature in the pool containing the node was maintained at 10° C, the standard temperature for experiments of this type on the amphibian node of Ranvier. Measurements were made when the temperature was within 0.25° C of the control value; during changes in pressure transient temperature excursions were limited to 2° C. Temperature was also monitored by a microthermistor in the gas phase above the node. The same constraints of limited excursion and return to control temperatures were observed for temperatures in the gas phase as in the solution. The nodal mem-

brane was held at a transmembrane potential at which 40% of the sodium channels were inactivated (h = .6), usually 80-90 mV. Sodium and potassium channel function was measured by imposing depolarizing test pulses of variable magnitude and duration, and monitoring the transient inward and steady-state outward currents carried by sodium and potassium ions respectively. The pressure chamber was similar to the one used in our previous studies (Kendig, et al., 1975). Compression was carried out by admitting helium from a high pressure cylinder, the gas phase above the Ringer's solution consisting of one atm. air and the balance helium. Pressures ranged from one to 100 atmospheres. Compression was carried out as rapidly as temperature control permitted; 45 minutes to one hour was required to raise the pressure to 100 atm. Partial decompression was carried out successfully in some cases, and complete decompression to normobaric pressure in a few. Pressure effects were reversible on decompression.

Results. This report concerns itself primarily with a finding which may account for the repetitive impulse generation observed in our earlier studies. On compression, there was a consistent, pressure-related shift. In the current required to maintain the transmembrane potential at its preset level of 80-90 mV. The direction of the shift corresponded to the generation of an inward current. Its magnitude varied considerably among preparations, ranging from barely discernible to 4 nA at 100 atm. The current was stable at periods up to 20 min at any given pressure. The current level was restored to control value on decompression.

The current was depolarizing; modes held in the current-clamped rather than voltage-clamped mode showed a pressure-related depolarization on compression. Current-clamped modes depolarized to the point of block of action potential generation did not completely regain the control resting potential on decompression, however there was a return toward control values. In the early stages of compression-related depolarization, there was a decrease in threshold for action potential initiation.

Analysis of the basis for the inward current is not yet complete. One possibility considered was that it might be due to a decrease in potassium permeability, by analogy with the pacemaker depolarization of cardiac tissue. However blocking potassium channels by application of external tetraethylammonium chloride (TEA) or substitution of CsF for the KCl in contact with the cut internodes did not prevent the appearance of the inward current.

Discussion. The identity of the ion responsible for the current is not yet established. If, as seems likely, potassium is not involved in its generation, then an increase in permeability to sodium is a possible candidate. A pressure-related increase in ion conductance has recently been reported in invertebrate preparations (Parmentier, et al., 1979).

Is this inward current responsible for the repetitive activity observed in other axons? A depolarizing shift in membrane potential, with accompanying inward current, will produce repetitive activity in axons capable of generating trains of impulses in response to a prolonged stimulus. Repetitive activity was not observed in the present study; the large myelinated nerves used in these experiments are probably motor neurons, which in vertebrates do not support multiple responses to a constant stimulus. A similar depolarizing current, however, would have produced repetitive activity in the invertebrate axons used in the previous studies (Kendig, 1978a). It is tentatively proposed that a pressure-responsive inward current is the basis for the pressure-generated repetitive impulse activity. The evidence linking these phenomena to HPNS is indirect but plausible; repetitive impulse generation has been linked to some drug-induced convulsive phenomena, and could well be responsible for the seizure activity associated with HPNS. The lower threshold for action potential generation observed at moderate pressures would also contribute to a pressure-induced increase in excitability which might well be involved in HPNS.
References and Acknowledgements will appear in PROCEEDINGS.

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DIFFERENTIAL EFFECTS OF PRESSURE ON THE MAMMALIAN CENTRAL NERVOUS SYSTEM. E.G. Kaufmann, P.B. Bennett, and J.L. Impeyj Jr. U.S. Naval Laboratory, Duke University Medical Center, Durham, North Carolina, U.S.A.

Perhaps the most striking influence of high pressures on biological processes is experienced in alterations of function of excitable tissues, such as changes in muscle tension (Cattell and Edwards, J. Cell. Comp. Physiol., 11:1-9, 1937), or the excitability and conduction velocity in nerve fibers (Grundfest, Cold. Spring Harbor Symp. Quant. Biol., 4:179-187, 1936). In the intact animal, the underlying biophysical and biochemical alterations affect the basic elements of the nervous system - membranes, synapses, and so forth, and translate into a complex chain of events which manifest themselves in symptoms called the High Pressure Nervous Syndrome (HPNS; Bennett, Ocean Industry, 3:28-34, 1968). In animals, this process has been observed to culminate in generalized seizures when pressures are sufficiently high.

Anatomically, the brain is known to be organized into regions subserving specific functions. Even though extensive reciprocal connections exist between regions serving different as well as similar functions, epileptic seizures have been thought to frequently occur as a result of sudden changes in a limited region of the central nervous system, often in the cerebral cortex. Both possibility must also be considered in the case of seizures of hyperbaric origin. The aim of this paper is to examine the results of a series of experiments in the mammalian nervous system and arrive at some estimate of the anatomical structures or systems most affected by exposure to high pressure.

The cerebellum. Electroencephalographic recordings show that the typical spike-and-wave pattern of pressure-induced seizures can be recorded almost concurrently from every structure so far examined: the cortex, hippocampus, red nucleus, cerebellum, reticular formation and vestibular nuclei (Cromer et al., Undersea Biomed. Res., 3:401-408, 1977; Kaufmann, et al., Undersea Biomed. Res., 3:91-107, 1977 and 5:63-70, 1978). We found the participation of the cerebellum particularly interesting, because a number of authors had suggested that the cerebellum is involved in terminating or modulating seizures (Cooke and Snider, Epilepsia, 3:69-78, 1955). Its destruction was also believed to contribute toward the vestibular symptoms of HPNS (Cromer, et al., Undersea Biomed. Res., 1:411, 1974). When we compared the effects of pressure in normal rats with those on rats with cerebellar ablations, we found modest changes in the convulsion threshold pressure: normal animals seized at 99 bars, while cerebellectomized rats seized at 89 bars and sustained about twice the number of seizures. The fact that HPNS convulsions were aggravated by cerebellar removal is consistent with the hypothesis that one of the effects of pressure is a decrease in cerebellar inhibitory tone. However, the relatively modest change in seizure threshold, although statistically significant (p < .05), and the similarity of other HPNS signs in both groups, suggests that the fundamental processes resulting in HPNS proceed in substantially unaltered fashion despite extensive removal of a major structure of motor control. This was not entirely unexpected: since pressure is uniformly applied to the entire organism, neural functioning would still be altered in the enormous pool of cells which constitute the remainder of the CNS. When excitability reaches a critical level, it tends to do so simultaneously throughout the pool, and hence the seizures. The capacity of the cerebellum to modulating this pervasive process seems to be relatively small.

Most studies on tissues in vitro require relatively high pressures, above 200 bars, to affect parameters such as action potential amplitude and conduction velocity, or membrane resistance and capacitance. The question then arises as to the size of the neuronal pool necessary to bring about a maximal response, or seizures at the lower pressures (100 bars) usually effective in intact mammals. One way this question can be addressed is to examine the progression of HPNS in limbs served by the distal portion of a transected spinal cord, thus eliminating all influences from the higher centers.

The spinal cord. Long ago Ebbecke (Pflüg. Arch. ges. Physiol., 217: 785-789, 1930) reported that high pressure continued to evoke spontaneous contractions in the hindlimbs of spinalized frogs, but this finding could not be verified in liquid-breathing spinal mice (Kylstra, Science, 158:793-796, 1967). We performed the experiment in rats breathing a helium-oxygen mixture. Twenty Wistar rats were implanted with EMG electrodes over the frontal cortex and allowed to recover. In 16 the spinal cord was transected at levels T7-T11, and 4 served as unoperated controls. The animals were allowed to recover for three days, during which time spinal withdrawal reflexes recovered so that clearly defined responses were evident to painful stimuli. In three of the spinalized animals, spinal nerves L2-L5 were sectioned after exiting the intervertebral foramina, thus totally denervating one hind limb.

On the day of the experiment, the animal was suspended in a whole-body sling with all limbs hanging free and secured inside a 208 liter pressure vessel. Needle EMG electrodes were placed in both hind limbs and one fore limb. Compression took place in a He-O$_2$ atmosphere at 1 bar/min, to a maximum pressure of 120 bars.

Symptoms of HPNS (tremors and myoclonic jerks) in the fore limbs of spinalized animals were indistinguishable from those of intact animals; becoming progressively more intense with increasing pressure. This pattern was also observed caudal to the lesion, but at a much lower intensity.

In all animals, increased EMG activity was usually evident at about 90 bars; onset of visible symptoms progressed from mild fasciculations at 90-95 bars, to tremors and myoclonic jerks, and seizures between 90 and 110 bars (Fig. 1). Limbs whose spinal nerves had been sectioned, on the other hand, remained flaccid throughout the pressure exposure. Activity profiles reconstructed by computing the area encompassed by EMG records at 10 bar intervals revealed fluctuation of intensity with increasing pressure, suggesting that the effects of pressure do not progress in a linear fashion throughout a given exposure. No evidence was seen that the threshold for pressure effects at the spinal level is different from that in the brain. Furthermore, it is evident that the neuronal pool of the spinal cord is sufficient

Fig. 1. A sudden burst in the electromyogram (EMG) of both hindlimbs (L.H. EMG, R.H. EMG) of a rat with a complete cord transection reveals a spinal seizure at 98 bars of pressure. No change is seen in the EMG. Intense tremor in the frontal limbs (F. EMG) continues uninterrupted. Lowest trace indicates 1 sec intervals.

to sustain massive, synchronized discharges but that the peripheral neuromuscular system has a much higher threshold. These results are consistent with the concept that pressure affects identical neural elements in the same way regardless of where they happen to be located. The expression, or the consequences of these effects, however, depends on the organization of these components, and this can be demonstrated further by means of evoked potentials recorded at different points of a pathway.

The visual pathway. We chose for this study the geniculo striate pathway of the guinea pig. Stainless steel electrodes were implanted in the optic chiasm (o.c.), lateral geniculate nucleus (l.g.n.) of the thalamus, and the striate cortex (s.c.x.). The position of each electrode was functionally localized by recording its characteristic response to photic stimulation. After several days of recovery, short latency responses of the l.g.n. (< 10 msec) and s.c.x. (< 50 msec) were recorded to electrical stimuli (10-100 µA, .02-.05 msec duration) applied to the o.c. Amplified, 32 response sequences were summed by means of a signal averaging computer. Responses to pressures up to 100 bars He-O$_2$ in 10 bar increments were compared with responses at surface, at a variety of stimulus intensities. Interpretation of the presynaptic and postsynaptic components of the evoked potentials was based on classical criteria.

At the l.g.n., exposure to pressure resulted in virtually no changes in the latency of either the presynaptic or postsynaptic components of the evoked responses (Fig. 2A), which correlated nearly perfectly with small excursions of temperature recorded during the experiments. Occasionally, the postsynaptic responses also showed a decline mimicking the effects of synaptic fatigue. While the effects of pressure on the evoked responses recorded at the

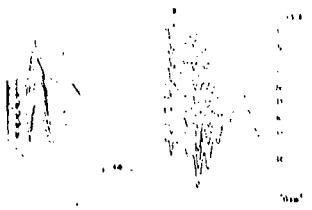

Fig. 2. Evoked potentials recorded at the lateral geniculate nucleus (A) and the striate cortex (C) of a guinea pig between bar (S) and 60 bars pressure, in $He-O_2$. (1) presynaptic, tract component; (2) postsynaptic, radiation response. See text.

LGN were modest, the effects at the cortex were dramatically different. Latency changes were very similar to those seen at the geniculate, and correlated with temperature. The amplitudes of the postsynaptic cortical response, however, increased as early as 10 atm, and attained values of up to 300% with increasing pressure, until the seizure threshold was reached (Fig. 2B). Following a period of post-ictal depression, the process of amplitude augmentation was again repeated. By varying the stimulus intensity and duration, it was possible to compare response thresholds at surface and at bars pressure. Again the effects of pressure at the lateral geniculate were modest. At the cortex, amplitude changes were a function of the stimulus intensity, becoming greater for strong than for weak stimuli and suggesting a change in excitability at the cortical element.

These results support the concept that the excitatory effects of high pressures depend on organization of the local neuronal circuitry, such as the presence of recurrent excitatory collaterals, and perhaps the size of the neuronal pool available for their expression. At the same time, the occurrence of seizures in the spinal cord, as well as the paroxysmal spike-and-wave pattern simultaneously recorded throughout many structures in the brain provide strong evidence that HPNS does not have a focal origin in the CNS. Peripheral nerves, the neuromuscular junction, and muscle fibers themselves, on the other hand, are more resistant to pressure, as evidenced by the total lack of activity in our denervated limb preparations. Their participation in the evolution of the complete syndrome in the intact relatively minor and depends largely on input from the central motor pathways. At the highest levels of the nervous system, such as the cortex, pressure may enhance tendencies for synchronized neuronal discharges, as reflected by the progressive augmentation of delta and theta activity in the electroencephalogram (Bennett and Towse, Electroenceph. clin. Neurophysiol., 31:181-191, 1971; Knauth et al., Undersea Biomed. Res., 4:181-232, 1977) and the greatly facilitated cortical evoked responses seen in our studies. It is likely that synaptic processes, such as the time course of release of transmitter substances at presynaptic terminals, their action at the postsynaptic membrane, and their inactivation or re-uptake play a prominent role in the events leading to high pressure hyperexcitability.

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SOMATIC EVOKED POTENTIALS IN MONKEY DURING SATURATION DIVES (To 31 ATA) AND DECOMPRESSION. M. Bugnard, K. Sekiya, Y. Figarol, J. Bontoux, C.L.S. Photogravure Hyperbare, CNRS, Faculté de Médecine Nord, Bd. Bourdet, 13001 MARSEILLE, France; M. Imperial, Bat Méditerranée, 247 Lyon.

Bioelectrical changes due to high pressure environment are well known, especially in man (Bennett et al., 1969; Lincoln et al., 1969-1971; Bennett et Towse, 1971; Bontoux et Bugnard 1972; Bennett et al., 1975) and monkey (Bontoux et al., 1970; 1972). The modifications are the most common peculiarities of the H.P.N.S. (High Pressure Nervous Syndrome). Our present interest is different in that it is focused on event-related potentials in monkeys : deep saturation dives - an effect of many significant modifications in electrical neural evoked potentials (N1-P1-N2) and their recovery cycles.

Method

Four experiments were performed on four adults Papio papio maintained in long stays in restraining chairs. The four dives went down at depths of 500 msw (Comanche III, Corsair VIII and Corsair V) and 200 msw (Dive IV). Four sessions, and Pi3 + Ar-O2 with Helium were used for the three dives. There was no difference in the two gases.

Ipsilateral and contralateral SEP resulted from electrical stimulation of the 3rd digit nerve at the axial nerve of peripheral level (electric current, one millisecond constant voltage shocks ; three seconds period for repetition). Active stimulus was given in order to obtain a direct neurological response. A fixed level on the Nelson was 1.5 x response, at the threshold, at maximal value, at all high maximum values, such specific level of motor excitation as used to indicate : an unknown but constant level of the neighbouring motor fibres to contract in the specific nerve. After that any variation in SEP will be due to a non-neurological variation, granted the sensors in the same valley being constant. All were recorded from the Rolandic areas, through monopolar electrodes at the level, yielding the shortest cortical latency evoked electrode placed at the vertex. The signals recorded using bipolar lead derivation (Cz-Fpz), (Cz-Oz) and (Cz-Pz). Neural nerve SEP were notified at their maximal amplitude, with respect to the two monkeys.

Results

1. Under 1 ATA normal SEP with two components, from ipsilateral to their primary positive component and their contralateral latency. Duration : 1) The latencies of the SEP signal is 10-15 ms. Positive to negative amplitude, positive and negative to the stimulus presence. But the SEP wave shape is constant in a given animal, and constant at depth.

2. The latency of the cortical evoked potentials does not change at 9 ATA, 10-15 ms, and 1 msec.

3. The latency of the final wave is 10 msec with increasing pressure.

4. For constant level of nerve excitation, the amplitude of the N or P waves, with respect to the base line of the SEP increases at depth. Such facilitation was powerfull in Animals who displayed some fetal episode (x6 in some case). Facilitation precedes the crisis, and persists after ; then facilitation slowly subsides. N (40/60) wave undergoes especially strong facilitation. A startle response (60 ms latency in neck muscles) is common. N 200 wave developps at depth as a SEP due to a strong nerve stimulation (M maximal level).

5. The recovery cycle of the various waves at depth displays modulate but lasting initial inhibition, without later facilitation.

6. Sural SEP are similar to sciatic SEP, in their form and depth variations. The ipsilateral evoked potentials from sciatic or sural excitation are similar to the contralateral one in their shape and variations at depth.

7. The polysynaptic reflexes (in Anterior Tibialis) due to a sural train of shocks undergoes high facilitation. The monosynaptic reflexes (in Hoffmann) do not display such high facilitation - not reciprocal inhibition.

8. A difference was not found between SEP elicited in environment with and without nitrogen.

DISCUSSION

Given the data and theory of Creutzfeld (1974) about cortical mechanisms for SEP, and from the review by Wolfe and Perl (1975) on the ascending pathways for cortical input, we conclude the following for SEP at depth :

1. Lemniscal pathways functions are not impaired at depth, neither in velocity of the impulses, nor in synaptic capacity of transmitt (punct-synaptic linkage at cortical or thalamic or cortical level).

2. The increase of the cortical P (15/10) waves at depth could be understood as either an increase of the incoming thalamic-cortical volley and/or an increase of the cortical excitability - true excitation or desinhibition. Such variation can be due to local cortical modification, or to thalamic modification of the thalamic-cortical relations.

3. The increase of the N (40/60) waves could be in line with the preceding hypothesis, due to the dendritic superficial network of the pyramidal cells.

4. Understanding of the later P (80/150) wave (inhibitory activity), the N (200) wave (due to group II afferents ?) require physiological analysis of the cortical activity.

5. The startle response appears to be due to the facilitation of specific and coupled foci in reticular nuc. and cortical sensori, area (Bunet et al., 1971).

6. The facilitation of the polysynaptic reflexes supports either a reduction of presynaptic inhibition or a facilitation of interneurons without complexum change of motoneurons excitability (Wilkie et Wilson, 1977). A presynaptic disinhibition could also explained, in part, SEP facilitation. Presynaptic and postsynaptic variations could be due to direct effects of the pressure, or to indirect effect, through modifications of the supraspinal controls.

Such hypothesis req. the experimental analysis (electrophysiology and pharmacology) at depth.

7. SEP facilitation at depth (like visual evoked potentials at depth) to under consideration as a signal for neurophysiological defect at depth, and expected to provide a sensitive index of such defect.

References will appear in Proc.EPUB.8.

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DIFFERENTIATION OF THE TWO PHASES OF THE CONVULSIVE STATE OF THE HPNS IN VERTEBRATES. R. W. Beaver, R. W. Beaver, H. W. Collier, W. P. Moncrief, Jr., and R. D. McCall. Institute of Marine Biomedical Research, University of North Carolina, Wilmington, North Carolina, USA.

Despite the mass of exploratory work, the biophysical changes giving rise to the series of events collectively designated as the high pressure nervous syndrome (HPNS) remain unclear. While a considerable consensus has been offered that changes in membrane properties, partly reversible by various anesthetic agents, occur at the presumptive giving rise to the HPNS, there is little question that numerous other changes in enzyme function and in the properties and mode of activation of contractile elements of tissues begin to occur in the same pressure range. Furthermore, the nature of the intracellular events involved in giving rise to the observed incoherent and behavioral changes has remained obscure. Indeed, it would now appear that available evidence does not favor the original view that high pressures entail increases in excitability of any of the neuromuscular components of the CNS. That evidence there is appears to favor the view that even the early stages of the HPNS reflect central and not peripheral changes and, furthermore, that at least the early stages of the HPNS involve changes at supraspinal level. While investigators contemplating these questions have tended to think of the various convulsive high pressure nervous syndromes as successive manifestations of a common underlying change in CNS function, there is at present no real assurance that this hypothesis should be accepted in preference to the possibility that different biophysical effects of pressure are involved in at least some of the various discrete manifestations which are produced, together under the term HPNS.

Of all the manifestations of the HPNS, the convulsive stage has received the most extensive exploration in the widest variety of species and under the widest range of compression conditions. Evidence has accumulated which has served to indicate that the convulsive stage of the HPNS is a type of at least two successive different neurological events, which are interconnected by various degrees in different species.

The most extensive information is currently available for one species, namely the rhesus monkey. In this species, if the initial compression is carried out at a relatively rapid rate, the HPNS develops predominantly as a tonic seizure, sustained tonic hyperextension with outward rotation of forearms and hands with extension of lower limbs, and tonic extension of upper limbs, trunk and neck muscles. This tonic seizure is usually followed by a clonic seizure.

events respond quite differently to manipulation of the compression conditions and to drug pretreatment. The observed differences are summarized in Table 1. In addition to the differences observed in the adult animal, studies of maturation of newborn mice reveal quite different time courses in the progressive change in susceptibility to the two seizure types. Since both seizure types are recognizable in the majority of the mouse strains examined to date, it has been possible to undertake studies concerning the genetics of susceptibility to Type I and to Type II seizures. Here again, the data reveal striking differences in genetic control of Type I and Type II convulsions. Finally, radioautographic studies utilizing deoxyglucose to detect regions of enhanced glycolysis presumably associated with localized paroxysmal activity in the brain of animals during Type I and Type II seizures reveal striking differences: Type II seizures involve large areas of the cortex and mid-line structures of the thalamus, while Type I seizures are represented primarily in lower portions of the brain, including in particular portions of the ascending reticular formation and the ventral raphé components of the upper brain stem, as well as the posterior hypothalamus up to about the level of the optic chiasm (Fig. 1).

Taken together, the data indicate that Type I seizures represent a unique paroxysmal event, the properties of which distinguish it from virtually all convulsants that have been explored to date but which show a number of traits which suggest some kinship with seizures evoked by agents commonly considered as acting primarily upon presynaptic or post-synaptic inhibitory activity in the CNS. Type II convulsions, on the basis of this evidence, cannot be considered as a generalization of Type I convulsions, but rather appear to represent a discrete neurologic event superimposed upon the Type I convulsions, usually at high pressures.

Under certain circumstances, threshold pressures for Type I convulsions can be increased to the point where they coincide with thresholds for Type II convulsions, giving rise to compound convulsions with some of the characteristics of either. A particular case in point here is the effect of slow compression: It would now appear that the extent to which HPNS Type I convulsion thresholds can be increased in the mouse by slowing the compression rate is limited by the point at which Type I convulsion thresholds intersect the level of pressures at which Type II convulsions are elicited. An interesting situation has been observed in the Sprague-Dawley rat where the HPNS seizure seen in the adult reveals complex characteristics, which partake to some degree of those in both types of seizures observed in the mouse. The nature of this event is clarified by observations in the juvenile rat: up until about the age of twenty days, two distinct seizures can be recognized in this species as in the mouse. Pharmacologic, clinical, and kinetic characteristics strongly suggest that here again, the first seizure corresponds to Type I seizures in the mouse, while the second corresponds to Type II seizures. Convulsion thresholds for these two events converge after the twentieth day of age and, from approximately the twenty-ninth day of life on, give rise to the compound seizure characteristic of the adult.

Recognition of the differences between Type I and Type II seizures invites reconsideration of the results of comparative studies of HPNS convulsions published previously. For this purpose, we suggest that it is permissible to tentatively equate clinically observed "clonic" seizures with Type I seizures, and "tonic" seizures with Type II seizures. Using these criteria, the 25 species examined in all can be subdivided into five categories: mouse-like - with a succession of Type I and Type II seizures; rat-like - compound seizures; animals showing Type II seizures only; plus an additional two less well defined categories for which to date either only Type I seizures have been observed, or for which the first seizure may be of either Type I or Type II. Of the 15 mammalian species examined, nine fall in the first category, three in the second category, and none in the third category. Three species fall into the last, indeterminate categories. Among the ten species of lower vertebrates and two birds, five show Type II seizures only, one may have shown compound seizures and four fall into the two indeterminate categories. Table 2 shows a summary of the mean convulsion thresholds for each of these groups, together with the appropriate standard deviation of the mean where adequate numbers are available to permit calculating this statistic. Perusal of the Table shows that among the three major types of seizures, mouse-like seizure patterns show the lowest convulsion threshold pressure; animals showing compound seizures have substantially higher convulsion thresholds; and animals showing only Type II seizures have convulsion thresholds substantially higher than either of the other two. The data suggest the possibility that part of the association of low HPNS convulsion thresholds with large anddightlyencephalized brains may prove to be attributable to the fact that in birds and lower vertebrates Type II seizures, rather than Type I seizures, often are the first and only manifestation of the convulsive stage of the HPNS. Differences observed within the order of mammals may be attributable in part to the prevalence of compound seizures among rodents, and in part to substantially higher susceptibilities of primates to HPNS convulsions when subjected to relatively rapid compression. These considerations may resolve the apparent conflict between observations revealing a progressive decrease in HPNS susceptibility during maturation of newborn rodents on the one hand, and the increase in HPNS convulsions susceptibility on the phylogenetic scale with increasing brain development on the other hand.

The data also pose the question of what one might expect to encounter in primates and ultimately in man. It has been shown repeatedly that HPNS convulsions observed as motor seizures in various primates, are generally, but not invariably, associated with electrical seizures in leads taken from the skull or the brain surface - a feature not associated with Type I seizures in the mouse; on the other hand, HPNS convulsions in squirrel and Rhesus monkeys are not associated with any recognizable changes in heart rate, while Type II seizures in the mouse, as well as the compound seizures in the rat, are associated with a transient bradycardia more pronounced in the former than the latter. Again, in the mouse diphenylhydantoin does not protect against Type I seizures, but markedly enhances Type II convulsion thresholds. To date, this drug has not been found to elevate HPNS convulsion thresholds in the monkey. In general, Type II convulsions are less dependent upon compression rate than Type I convulsions. In the two primates studied from this point of view, HPNS convulsion thresholds have been found to be highly susceptible to compression rate changes. Altogether, we consider, therefore, that it is likely that the HPNS convulsions observed in the squirrel monkey and in the macaque represent a neurologic event most nearly comparable to Type I seizures in the mouse. The ultimate resolution of this question will have to be deferred until radioautographic experiments will have provided a mapping of enhanced metabolic activity in the monkey brain comparable to what is now known for the mouse and the rat.

Taken together, the data available to date indicate that the convulsive phase of HPNS involves two distinct neurologic events, the first of which seems likely to involve interference with inhibitory activity in the CNS and to involve a series of deep structures extending from the brain stem to ventral and lateral structures in the diencephalon. The data furthermore suggest that this is also the event responsible for HPNS convulsions in primates and provide a basis for further detailed investigation of what is now a well defined neurologic entity.

Table 1
Differences Between Type I and Type II HPNS Seizures in Mice

Criteria:	Type I:	Type II:
Clinical	Clonic Burst	Tonic/clonic sequence
EEG	Little change	4 to 5 Hz spike and wave; post-ictal silence
Heart Rate	No change; no atropine effect	80-90% decrease; atropine blocked
Compression rate Dependence	Very ($\bar{k}$ = 11)	None (k = 0 or negative)
Strain differences	Marked	Few and small with one exception
Phenobarbital	Protects	Protects to a much greater degree than Type I
Diphenylhydatoin	Sensitizes	Markedly protects
Trimethadione	Sensitized early; Protects slightly late	Protects early, no effect late
Reserpine	Sensitizes, esp. at low compression rate	Little effect
Ontogenetic	Mature more resistant than newborn	Little change from birth to maturity
Spinal animal	No seizures below transection	Seizures also in isolated part of spinal cord
Mortality	None	29%

Table 2
HPNS Seizure Types and Convulsion Threshold Pressures
In 15 Species of Mammals and 10 Species of Birds and Lower Vertebrates

Type	Mammals %	Mammals $\bar{P}_c$ (atm)	Birds and Lower Vertebrates %	Birds and Lower Vertebrates $\bar{P}_c$ (atm)
I & II	60	77.6±4.9	0	-
Compd.	20	96.7±3.9	10	108
II Only	0	-	50	136.4±19.5
I Only	13	77.7	20	84 and 150
I or II	7	65	20	107
Mean $\bar{P}_c$ (atm)		81.4±3.9		124.3±11.2

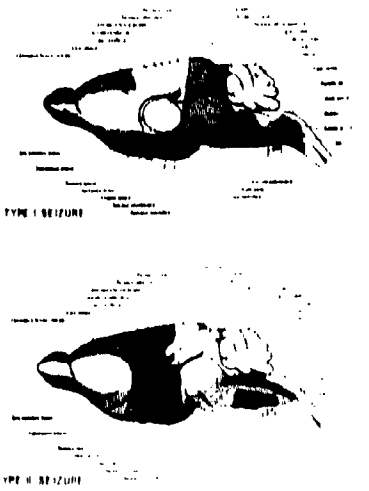

Figure 1 Distribution of relative densities in brains of CD-1 mice following 14 deoxyglucose injection immediately after either Type I or Type 2 HPNS seizure

ENERGY AND BODY FLUID BALANCE DURING A 14-DAY DRY SATURATION DIVE AT 11 ATA (SEAHORSE IV). H. Nakayama, S. F. Bang, J. Claybaugh, R. Patsel, Y. Ishii, T. Ohta, K. Shirai* and K. Matsuda. Japan Marine Science and Technology Center, Yokosuka, Japan; State University of New York at Buffalo, Buffalo, New York, U.S.A.; University of Hawaii and Tripler Army Medical Center, Honolulu, Hawaii, U.S.A.; Nagoya University, Nagoya, Japan; Iwai University, Ichihara, Japan; University of Occupational and Environmental Health, Yahata-Nishiku, Japan.

Comprehensive studies on the energy and body fluid balance of 4 divers were conducted during the course of a 14-day dry saturation dive at 31 ATA held in July-September, 1979 at the Japan Marine Science and Technology Center. In this dive, the chamber temperature at 11 ATA was maintained at about 31.5°C and the P_{O_2} at 0.4 ATA.

A. Energy Balance: The daily caloric intake amounted to 2,800 - 3,000 Kcal throughout the dive; the body weight decreased by 700 gm over a 14-day period at 31 ATA, and gradually returned to the predive level during 12 days of decompression. The average O_2 consumption at rest showed no significant change at 31 ATA but decreased slightly during the decompression and postdive (1 ATA) control periods. The R.Q. values remained at around 0.85 throughout the dive. The rectal temperature decreased by 0.2-0.5°C during the 3 day compression period but returned to the predive level following the completion of compression. On the other hand, the mean skin temperature decreased from the predive level of 33.7±0.1°C (SE) to 32.2±0.18°C during compression, leveled off at about 32.0°C during exposure to 31 ATA, and then gradually returned to the predive level during decompression.

A venous blood sample was obtained periodically from each diver at 6:30 a.m. during the dive and was centrifuged inside the chamber. Subsequently, all serum samples were analyzed by a 20-channel SMAC (Sequential Multiple Analyzer plus Computer).

The glucose level increased from 89mg predive to about 130mg during the second week at 31 ATA, followed by a return to the predive level during decompression. The triglyceride level also increased transiently from 95mg predive to 148mg on the third day at 11 ATA. There were no significant changes in the level of cholesterol, uric acid and bilirubin (total and direct) during the dive. On the other hand, the levels of various intracellular enzymes (e.g., LDH, alkaline phosphatase, SGPT, and SGOT) increased continuously during compression and the early 31 ATA period, and then leveled off. During decompression, only the LDH level returned to the predive level.

B. Body Fluid Balance: With the onset of compression, the urine flow began to increase significantly. The daily urine flow increased from 1,419±77 ml predive to 1,900-1,990 ml throughout the 31 ATA period, and then gradually decreased to the predive level during decompression (Fig. 1). Although the above increase in urine flow was accompanied by a reduction in urine osmolality (from 770 to about 650mOsm/kg$_{H_2O}$, the osmolal clearance was consistently higher (10) at 11 ATA, as compared to the predive period. An increase in the excretion of Na, K, and urea was largely responsible for the observed increase in osmolal clearance. The glomerular filtration rate (estimated by endogenous creatinine clearance) decreased by 10-20 at 11 ATA. It is thus evident that the observed hyperbaric diuresis is primarily due to an inhibition of tubular reabsorption of both solutes and water. There were at least 50% increases in the fractional excretion of filtered water, Na, K, urea and total osmotic particles at 31 ATA, as compared to the corresponding predive values. The calculated free water clearance (urine flow minus osmolal clearance) remained at about +2,200 ml/day throughout the dive. Therefore, the standard free water clearance (free water clearance/osmolal clearance) decreased significantly, indicating that the free water reabsorption from the collecting duct must also be reduced at 31 ATA.

Perhaps the most important finding in the present dive is an observation that the pattern of diurnal variation in urine flow changed significantly at 11 ATA. When the daily urine flow was measured over 4 successive intervals (0700-1200, 1200-1500, 1500-1900, and 1900-0700 hr next morning), the only difference in urine flow between 1 and 31 ATA was observed in the overnight sample (obtained during 1900-0700 hr). In other words, the observed increase in daily urine flow at 11 ATA could be accounted for mostly by the corresponding increase in urine flow at night (Fig. 2). In fact, despite the overall increase in daily urine flow at 11 ATA, the urine flow during the daytime tended to decrease toward the end of the 11 ATA period. This hyperbaric nocturia was not accompanied by an increase in the creatinine excretion but was associated with a marked increase in the excretion of osmotic substances. However, the overnight negative free water clearance tended to decrease at depth, indicating that the free water reabsorption is also suppressed at night at 11 ATA. Although the mechanism for this hyperbaric nocturia is not clear at present, it is important to point out that the divers had to wake up at least once at night to urinate, thereby disturbing their sleep pattern.

As stated earlier, a marked increase in urine flow was observed with the onset of compression, which appears to be responsible for the development of a mild dehydration (see below). This compression diuresis was most marked during compression to 21 ATA from 11 ATA. This compression diuresis was not accompanied by any increase in creatinine or osmolal clearance over the level observed during the corresponding time of the predive day. This again indicates that the free water reabsorption is somehow suppressed during compression.

Despite the presence of a sustained diuresis, the daily water input (including the estimated "water of oxidation") decreased from 4,000 ml predive to about 2,700-2,800 ml at 11 ATA. At 1 ATA air, the total sensible water output (urine and fecal water) was 1,600 ml/day, giving a sensible water balance of 1,400 ml/day. The latter value corresponded well to the measured insensible water loss (1,300 ml/day). At 31 ATA, a combination of decreased water input and increased sensible water output led to a 50% reduction in sensible water balance. However, a corresponding reduction in insensible water loss (to about 650 ml/day) was observed. These findings, together with the fact that the body weight decreased only slightly (see above), indicate that the overall water balance was fairly well maintained at 11 ATA. In fact, the serum protein concentration as well as the blood hemoglobin content, the erythrocyte count and the hematocrit ratio showed a transient increase only during the early period at 31 ATA, after which they returned to predive levels.

These findings indicate that the diuresis observed during a prolonged exposure to 11 ATA may be attributed to 1) an inhibition of insensible water loss, and 2) an inhibition of the tubular reabsorption of solutes and water at night. However, a possible mechanism underlying the hyperbaric diuresis can not be proposed until a complete analysis of urinary ADH, aldosterone and prostaglandin E is completed.

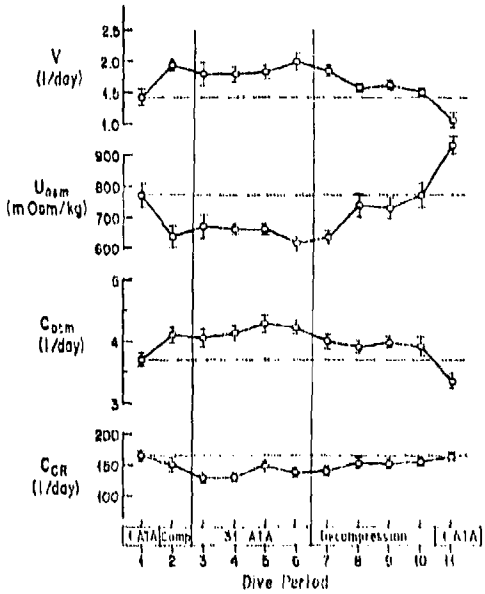

Fig. 1: Urine flow (V), urine osmolality (U_{osm}), osmolal clearance (C_{osm}) and glomerular filtration rate (C_{CR}) during various periods of SEAHORSE IV. Each point represents the mean (±SE) of 4 subjects.

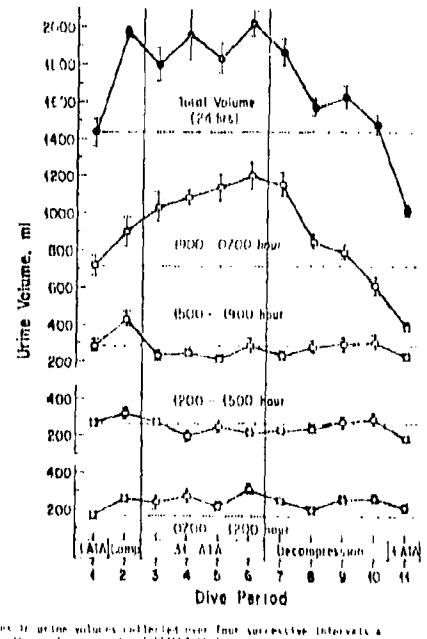

Fig. 2: Changes in urine volumes collected over four successive intervals a day during various periods of SEAHORSE IV. The total daily urine volume (shown in Fig. 1) is also given on the top of the figure for comparison. Each point represents the mean (±SE) of 4 subjects.

A COMPUTER MODEL DESIGNED TO MAKE RAPID PREDICTIONS OF DIVER TEMPERATURE CHANGES. S. Selbrack and L. Elsom. Department of Physiology, University of Aberdeen, Aberdeen, AB9 2AS, Scotland.

Whenever an accident to a diving system results in the loss of heat supplies almost the first question to be answered is "how long can the diver survive?". This question has been put to the medical support teams in Aberdeen and with North Sea temperatures at about 5°C the only quick answer can be "not long". It would be very useful to have a rapid and accurate means of estimating the rate of change of body temperature from whatever facts are known about environmental conditions. This paper reports a computer program designed to give a rapid on-line estimate of rate of change of body temperature following sudden changes in environmental conditions. The program can also be used to produce tables relating body temperatures to a wide range of environmental conditions and to aid in the design of emergency heat sources.

The computer model uses basic physical equations to describe the heat exchange processes between the body and the environment. A model designed in this way, once it has been proved to be in agreement with experimental measurements, can be used to predict over a much greater range of conditions than can a model designed to use empirical relationships derived from a necessarily limited range of conditions.

Evaluation of the physical parameters for helium mixtures has been, wherever possible, by reference to basic thermodynamic principles in the absence of reliable experimental values. Some of the physiological parameters have been "best estimates" drawn from the literature and some have been measured.

Before a theoretical model can be used with confidence it must be shown to describe accurately conditions which are known the model must be in agreement with whatever experimental measurements have been made. The data to test this model was collected from 52 divers taking part in training and experimental dives to depths to 300 metres. Rectal temperatures and six body surface temperatures were monitored for periods up to 7 hours. Environmental conditions were monitored throughout and divers' activity and subjective assessments of thermal comfort recorded.

A total of 178 data sets were used to relate chamber and body temperatures to depth. The following relationships were derived for divers known to be in thermal balance.

Day-time chamber temperatures increase by 0,05°/0,1°C for each metre increase in depth from 28,2°C in heliox on the surface. Night-time chamber temperatures increase by 0,00?/0,1°C metre from a surface value of ?7,0°C.

Mean body temperatures (Tb) were calculated according to the relationship:

Tb = 0,67 · rectal temperature + 0,33 · mean skin temperature.

The linear relationship between day-time mean body temperature and depth shows an increase of 0,001?°C metre from a temperature on the surface of 35,9°C and night-time mean body temperature shows an increase of 0,003?°C metre from a surface value of 35,03°C. The day-time temperatures are better fitted by a curved line and show a minimum mean body temperature at 90 metres.

Day-time rectal temperatures change by 0,001?°C metre from an average and level value of 36,62°C but there is a statistically significant fit only with a second order line and this analysis shows minimum rectal temperatures at 120 metres. Night-time rectal temperatures change at a rate of 0,002°C metre from 37,09° on the surface.

The agreement between the computer model and these results will be shown and predictions made by the model will be presented.

COMPARATIVE EFFECTS OF VARIOUS PROTECTIVE AGENTS UPON ACUTE CEREBRAL HYDROXYL OXYGEN TOXICITY IN MICE : PARTICULAR INTEREST OF SOME BENZODIAZEPINES. P. Brug*, P. Jeanny**, A. Chaumont*, D. Guillot** and P. Broussolle*. * Centre d'Etudes et de Recherches Biophysiologiques Appliquees a la Marine, B.P. 610, 83800 Toulon Naval, FRANCE and ** Laboratoire de Physiologie, Faculte de Medecine, 13385 Marseille, Cedex 4, FRANCE.

Under definite and reproducible experimental conditions, the aim of this work was to compare the efficiency of various agents against CNS induced convulsion previously studied from one hand, and on the other hand, with very recently studied other protective agents.

The high level of protection observed in recent experimental data, i.e. in the presence of propanidol, gamma hydroxy butyrate and various benzodiazepine warrant the opportunity for the use of these drugs against CNS seizures in man.

Briefly, each protective agent given I.P. in 0,9% w/v NaCl 10 ml.kg^{-1} prior to CNS exposure (6 ATA ; 120 min) was tested on 20 2/3 male Swiss mice. Control animals received equivalent volume of 0,9% NaCl (or other vehicle) and were exposed concomitantly to CNS with treated animals. The various observed parameters were : first pre-convulsive signs ; clonic and tonic-clonic fits ; gasps and death. Methods and statistical treatment of results were described previously, (BRUG et al ; Mol. Aero. Spat. Med. Subaq. Hyperbat ; 1976, 15, 2001).

Results :

1°) Metabolic agents : Succinate (1 mM.Kg^{-1}) and glutathione (12 mM.Kg^{-1}) caused a significant protection, but glucose (5 g.Kg^{-1}) plus xanthine (2 · 1,5 Kg^{-1}) had a weak effect.

2°) Antioxidants and redox system intermediates : the satisfactory protection induced by 2,6 di-tert-amethylhydroquinone (10 mg Kg^{-1}) could be at least partially explained by its used vehicle, i.e. propylene glycol ; Menadione (vit. K$_3$) plus Ro bisulfite (each at 0,2 mM.Kg^{-1}) showed good protective effects.

3°) Cations : Ca chloride effectively protected against convulsions, but at a sub-toxic dose of 600 mg.Kg^{-1} ; further, Mg lactate (2 mM by I.V. in rabbit de 2,5 mM.Kg^{-1}) exhibited clearly a higher protection than Ca (1 mM), but unfortunately both metals were shown to have a marked toxicity ; Co(II) protective effect was further increased in the presence of sodium hydroxybutyrate (0,2 mM.Kg^{-1}) or acetonitrile.

4°) Alpha and beta receptor blockers and serotonin : Hydergine^R (1 mg Kg^{-1}, I.P.) and Papaverine (6 mg.Kg^{-1}) significantly delayed the onset of convulsions ; these findings are in keeping with a cerebral protection which may be secondary to a peripheral, extra cerebral predominant vasodilatation ; Serotonin (0,05 mM.Kg^{-1}), which does not cross the blood brain barrier, appears as a very effective protector ; in addition, propranolol (10 mg Kg^{-1}) was also clearly effective against CNS toxicity and was devoid of undesirable behavioural side effects.

5°) Phenothiazine and uretides : the two phenothiazines i.e. chlorpromazine (5 mg.Kg^{-1}) and levomepromazine (5 mg Kg^{-1}) caused a low protection, as did the two anticonvulsant uretides, i.e. diphenylhydantoin (50 mg Kg^{-1}) and phenobarbitone (5 mg Kg^{-1}).

6°) Drugs acting on G.A.B.A. metabolism : the weak protective action afforded by G.A.B.A. itself required a high concentration of this amino acid (500 mM.Kg^{-1}), further GABA transaminase inhibitors (AOAA ; dipropyl acetic acid) showed also little beneficial action, in contrast with Ethosuximide which, more lastly afforded a powerfull protection, and significantly increased the GABA cerebral level (KRAUSKOPF and WATSON, Mol. Aero. Spat. Med. Soc. Hyperb., 1978, 68, 851).

7°) Drugs acting on central monoaminergic systems : Pargyline (1 mg Kg^{-1}), a powerfull monoamine oxydase inhibitor, significantly delayed the occurrence of all examined symptoms, in contrast with alpha methyl p tyrosine (200 mg Kg^{-1}), an inhibitor of tyrosine hydroxylase which promoted very little, but significantly the occurrence of convulsions.

Among drugs believed to act specifically upon dopaminergic system, gamma hydroxybutyrate (1,25 mM.Kg^{-1}), an inhibitory agent of mitochondrial tyrosine aminotransferase, exerted a strong protection, as its precursors did (i.e. gamma butyrolactone and 1,4 butanediol).

Haloperidol (10 mg.Kg^{-1}), a blocker of dopaminergic receptors (with a 3 hours prior CNS), delayed all CNS induced symptoms, but surprisingly enough, apomorphin chlorhydrate (5 mg Kg^{-1}), an activator of dopaminergic receptors appeared as weakly active in preventing convulsions.

8°) Benzodiazepine drugs : Diazepam (Valium^R ; 1 mg Kg^{-1}) and clonazepam (Rivotril^R ; 0,1 mg Kg^{-1}) were found to protect very markedly the mice against the CNS convulsions and pulmonary oedema. In addition, at the therapeutic low doses used, spontaneous motor activity seemed well preserved. We have very recently studied some other benzodiazepines mainly used in the clinic in regard of their anti anxiety properties, as Tetrazepam (Myolastan^R, 0,5 mg Kg^{-1}), oxazepam (Seresta^R, 1,2 mg Kg^{-1}), clotiazepam (Veratran^R, 0,5 mg Kg^{-1}), bromazepam (Lexomil^R, 0,5 mg Kg^{-1}) and the B-Adelocarpate (Lysanxia^R, 0,5 mg Kg^{-1}), all these drugs afforded a net, potent and long-term protection against CNS induced convulsions and pulmonary toxicity was also significantly delayed.

Discussion

In mice, various pharmacological agents such as propanidol, gamma hydroxy butyrate and some benzodiazepines (e.g. diazepam, clonazepam) possess powerful protective effects against acute CNS toxicity. Keeping in mind that caution is certainly still needed in extrapolating from these preliminary data, we hope that this work gives a proposal to the use of these pharmacological agents to man submitted to CNS.

Indeed, it must be remembered that the benzodiazepine receptors in human brain corresponds to that of rat brain in affinity, with specificity and regional distribution (SCHLICHTER and SEALE, Commun. Psychopharmacol., 1978, 2, 171).

Considering the various chemical heterogeneity among the protective agents studied herein, as well as on the interactions, the intimate mechanism of CNS induced toxicity remains largely to be solved. Nevertheless the overall potency towards CNS induced convulsions established, the encouraging effects the possible involvement in some respects of the GABA ergic synapses, often considered as the primary site of action of benzodiazepines (see also GABA ergic pathways connects the oscillations with the substantia nigra, mediating postsynaptic inhibition of striatal dopamine neurons). In vivo the fact that diazepam and clonazepam clearly delayed (1 h) the occurrence of CNS induced decline of endogenous dopamine as well as the CNS induced convulsive fits, support the view that dopamine could be involved likewise in the clinic (see : Proc. 22nd Neurosci. CNRS et al ; in : Int. Symp. on Hyperbaria and Oxygen Toxicity, DENNER, 1979, Abstracts). It should be mentioned that bilateral destruction of the recently described dopaminergic mesencephalical pathway induces a decrease of convulsive activity in the rat (DAVIDAR et al ; Brain Res., 1979, 161, 261) which is also a major pre-mortem symptom observed under CNS.

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EFFECT OF EXCESSIVE OXYGEN UPON THE CAPABILITY OF THE LUNGS TO FILTER GAS EMBOLI.

B.D. Butler and B.A. Hills, Marine Biomedical Institute and Dept. of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

The pulmonary circulation, situated between the heart and systemic beds, has a secondary role as a filter for blood-borne particles carried in venous blood. The effectiveness of this filter has been established (Heinemann & Fishman, 1969) and reviewed extensively by Chan and Yang (1969). The ability to trap venous microbubbles down to 22 μm under normal conditions is evident, (Butler & Hills, 1979). However, impairment of the filtering ability by overloading the vessels with gas infusions (Oyama & Spencer, 1971, Mandelbaum & King, 1963 and Butler & Hills, 1979) or by the use of vasodilators (Butler & Hills, 1979) or by chronic exposure to oxygen (Hills & Butler, 1978) has been reported.

Prolonged ventilation on high concentrations of oxygen may lead to a progressive sequellae of pulmonary pathological events often including oedema, atelectasis, airway inflammation and pulmonary hypertension. The extent of pathology and progression to acute pulmonary damage is dependent upon both the partial pressure of oxygen breathed and the duration of the exposure. Numerous investigators have examined the various hemodynamic, biochemical and cardiopulmonary changes associated with pulmonary oxygen toxicity as discussed in the excellent review by Clark and Lambertsen (1971). The use of oxygen for the treatment of various infections and traumatic illnesses, including decompression sickness, is widespread. However, recognition of the limits and hazards is essential.

This study was conducted to examine the effects that pathologic changes caused by hyperbaric oxygen exposures can have upon the ability of the pulmonary circulation to serve as a physiological filter for venous air micro-emboli.

Materials and Methods.

Eight dogs of either sex (20-24 kg) were mildly sedated, but not to a level of surgical anaesthesia, with sodium pentobarbital (Nembutal, 15 mg/kg I.P.).

Once the animals were sedated they were placed in an experimental pressure chamber, which was then flushed with 100% oxygen for approximately thirty minutes, until the oxygen percentage exceeded 95%. At this time the pressure was increased with 100% oxygen to 2 ATA. The animal remained at this depth for 17 hours. Chamber gas was routinely monitored using a medical gas analyzer (Perkin Elmer 1100) for fluctuations in the carbon dioxide and oxygen levels, flushing with 100% oxygen as required.

Following the 17-hour exposure on 100% oxygen to 2ATA, the animals were returned to ambient pressure and anaesthetized with sodium pentobarbital (30 mg/kg I.P.). The animals were intubated and the endotracheal tubes connected to a no. litre Douglas bag which was inflated with 100% oxygen such that they remained breathing oxygen throughout the experiment. The animals were allowed to respire spontaneously.

The right femoral artery was cut down for placement of a blood catheter for monitoring blood pressure into the thoracic aorta while a Swan Ganz thermodilution catheter was placed in the pulmonary artery via a cut-down in the right femoral vein. Cardiac output was obtained using the thermodilution technique. Once inserted, all of the catheters were allowed to back fill with blood and were then slowly flushed with degassed heparinized saline (1 u-ml sodium heparin) so as to avoid any inadvertent introduction of bubbles. Needle electrodes were placed in standard lead positions I or II for electrocardiographic recording. A chest-band strain gauge was placed around the animal's thorax for monitoring respiration. End tidal carbon dioxide was measured by mass spectrometry (Perkin Elmer Medical Gas Analyzer 1100). Arterial and venous blood pressures were recorded using standard blood pressure transducers. Blood gas and pH values were determined from mixed venous and aortic blood samples using a standard blood gas analysis system (Radiometer).

Arterial Doppler monitoring was implemented by transcutaneous placement of 8 mHz probes over the left femoral or popliteal arteries and the right carotid artery. The transceived signal from the doppler recorder (Sonicaid 8V1000) was filtered and amplified for recording. The right carotid artery often required dissection for proper placement of the probe. The Doppler probes were held in position with bar clamps which were suspended independently of the surgical cradle, thus preventing artefacts from gross body movements. Once the surgical procedures were completed, control measurements were taken for a 45 minute to allow stabilization. All physiological parameters were continuously recorded on a six channel strip chart recorder. Methods for the production and calibration of microbubbles used for air embolism studies have been previously reported, Butler & Hills (1979).

Following the control periods, either microbubbles of gas boluses were infused into the right ventricle. The volumes and bubble diameters are presented in the table. Infusion rates were controlled by an infusion pump at either 1.0 ml/min, 0.1 ml/min. When the experiments were complete, the animals were killed with an overdose of sodium pentobarbital and an immediate autopsy performed. Lungs were then removed for standard histological analysis.

Results.

In four out of eight animals embolized in this study, Doppler signals from arterial bubbles were recorded. (See Table). Microbubble sizes ranged from 14 μm to 61 μm while total gas volumes ranged from 0.1 ml to 3.25 ml for microbubbles and 10 ml for bolus infusions. Relevant changes in physiological parameters have been recorded. Mean arterial pressures decreased from 147 mm Hg to 107.3 mm Hg or by 27.44% from control. Control values are from post-oxygenation, pre-embolization conditions. Pulse pressure and heart rate changes were relatively minor, 3.25% and 1.05% respectively; while cardiac index and stroke volume decreased significantly - 48.21% and 52.43% respectively. Mean pulmonary artery pressure showed a significant decrease to 24.56% from control while mean pulmonary-wedge pressures dropped to a value of 2.03% from control. Total pulmonary vascular resistance increased by 19.15% from control, while breathing frequencies increased by 31.56%.

The physiological changes for the four animals in which no arterial Doppler signals were recorded showed no significant changes from control values.

Discussion.

The use of the Doppler technique for detecting arterial bubbles has been rigorously tested in a previous study (Butler & Hills, 1979); so the results indicate that excessive exposure to oxygen can cause the lungs to release trapped venous bubbles. However the effect is variable, as seen in the Table, and does not appear to be primarily associated with the size of bubble filtered from the venous return to the heart and lungs. The release phenomenon is unlikely to be a primary effect of oxygen but is more likely to arise from the pathological changes induced by the oxygen. Indeed, these were observed upon microscopic examination although there was no obvious correlation between the pathology and the ability of the particular lung to pass or trap venous bubbles. The exact pathway of the bubbles in passing from venous to arterial systems is obscure and would warrant a much more extensive study.

The delay in the appearance of arterial bubbles following venous embolization (10-30 mins.) is similar to times recorded when other factors are used to compromise the lung as a bubble trap (Butler & Hills, 1979). This indicates that the mechanism could be more complex than simple filtration and may involve edema, a humoral factor or a physical agent such as a surfactant whose level is known to be changed by oxygen poisoning. (Brannolte et al., 1976).

Whatever the mechanism, however, it is very serious in diving to find that excessive exposure to oxygen can facilitate the release of venous bubbles into arterial blood, especially when so many otherwise asymptomatic venous bubbles are regularly detected in routine dives. Although this study does not permit us to estimate how much exposure is too much, it does suggest monitoring pulmonary toxicity closely during a decompression and considering the state of the lungs most carefully when prescribing additional oxygen therapy for treating a case of "limit bends".

Reference will appear in Proceedings, table follows.

TABLE

Arterial Doppler Detection of Intravenously Infused Microbubbles following Oxygen Exposure (17 hours on 100% O_2 at 2 ATA.

Dog	Weight (kg)	Sex (M/F)	Bubble Diameter* (um)	Arterial Doppler Detection	Total Gas Volume Injected Prior to Detection (ml)	Infusion Rate (ml/min)
1	[illegible]	M	14	+	.4 Micro-bubbles in Air Bolus	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
4	[illegible]	[illegible]	[illegible]	[illegible]	0.25 Micro-bubbles in Air Bolus	[illegible]
[illegible]	[illegible]	M	[illegible]	[illegible]	1.4	[illegible]
6	[illegible]	[illegible]	22	+	[illegible]	[illegible]
[illegible]	[illegible]	M	[illegible]	[illegible]	.9	[illegible]
[illegible]	[illegible]	M	[illegible]	[illegible]	[illegible]	[illegible]

* Mean Diameter

SEM OBSERVATIONS OF OXYGEN TOXICITY IN GUINEA PIGS EXPOSED TO CONTINUOUS 100%, 85%, OR 75% OXYGEN AT 1 ATM. A. J. McKee and M. L. Bradley. Naval Medical Research Institute, Bethesda, Maryland, U.S.A.

The histopathological changes resulting from exposure to toxic levels of 100% oxygen are well documented in man and many experimental animals. However, there are many aspects of the toxic syndrome that are not fully understood or resolved at this time. One such area is presently being investigated in our laboratory. We are studying the pathologic effects and/or benefits of continuous oxygen breathing at various intermittent oxygen-air schedules. This report is designed to describe the scanning electron microscopy (SEM) observations of the comparative role of development and severity of pulmonary oxygen toxicity in guinea pigs exposed to continuous 100%, 85%, or 75% oxygen breathing or air at 1 ATM. The study was conducted on young (150-300 gm) guinea pigs divided into four groups: group 1 (exposed to air), group 2 (exposed to 100% oxygen), group 3 (exposed to 85% oxygen), and group 4 (exposed to 75% oxygen). The exposure times ranged from 24 hr to 174 hr. At predetermined times during the exposures, a pair of animals was removed from the exposure chamber. Both animals were anesthetized by intraperitoneal injections. One animal was immediately prepared for histopathological examinations (light microscopy, SEM, transmission electron microscopy) by intratracheal infusion of Karnovsky's fixative (3% paraformaldehyde and 5% glutaraldehyde) buffered with sodium cacodylate at a pH of 7.2. The specimens were critical-point dried and coated with a heavy metal (gold/palladium) prior to SEM examination. The second animal was used to obtain the quasistatic air pressure-volume curves on the lungs prior to preparation for histopathological studies.

The measurement of the air pressure-volume curves revealed that after 20 hr of 100% oxygen exposure there was a 25% reduction in lung compliance. In animals breathing 85% and 75% oxygen similar decreases in compliance were noted at 95 and 100 hr, respectively. Cyclical inflation and deflation of the lungs of animals with moderate oxygen toxicity caused some increase in compliance. These mechanical changes were interpreted as reflecting an increase in the surface tension and closure of air spaces.

The first pathologic changes were observed in animals exposed to 100% oxygen for 48 hr. Gross lesions included mild hyperemia and focal pin-point hemorrhages on the surface of the lung. Portions of the lung appeared atelectatic. Histopathologic lesions observed by light microscopy included focal congestion and mild interstitial edema, while many areas appeared normal. By SEM examination, the control animals had normal appearing lungs (Fig. 1), while those exposed to 100% oxygen for 48 hr presented evidence of generalized thickening of the alveolar septa and prominent congestion of alveolar vessels (Figs. 2 and 3). Lesions observed after 60 hr of exposure to 100% oxygen included interstitial edema and intra-alveolar hemorrhage. After 70 hr of 100% oxygen exposure the pathologic changes were characterized by generalized accumulation of a plasma-like exudate in the alveolar spaces. This exudate contained abundant amounts of fibrin, leukocytes, macrophages, and some erythrocytes (Fig. 4). Also, active proliferation of type II granular pneumocytes (Fig. 5) was observed. In the normal lung these type II pneumocytes are associated with the secretion of surface active phospholipids. Sections of lung in all exposure groups were observed in an atelectatic state.

When comparing the onset and severity of lesions seen in 100%, 85%, and 75% oxygen exposures, we observed a direct correlation in the development of lesions relative to time of exposure and concentration of oxygen. For example, the first evidence of interstitial edema and congestion in the 85% and 75% exposure groups were at 86 hr and 100 hr, respectively. These same changes were observed at 48 hr in the 100% oxygen exposure group. Lungs showing tissue characterized by marked interstitial edema, alveolar exudate containing fibrin, and cellular infiltrates were first observed in the 85% or 75% exposure groups at 92 and 116 hr, respectively. Again, similar lesions were observed at 70 hr in the 100% oxygen exposure groups.

After the initial onset of severe lung lesions in each group, the histopathologic and SEM findings were similar in all extended-time exposure groups.

In summary, the results of this study support our conclusion that the development and severity of pathologic oxygen toxicity lesions are greatly influenced by the concentration of oxygen and the duration of exposure. The SEM proves to be a valuable adjunct tool for investigating lung morphology.

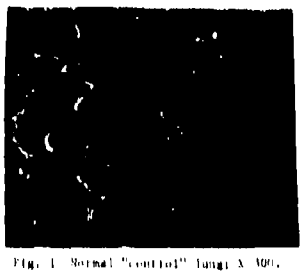

Fig. 1 Normal "control" lung; X 400.

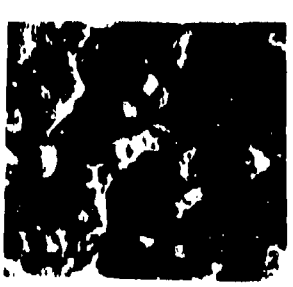

Fig. 2 Alveolar septal thickening after 100% O$_2$ exposure for 48 hr; X 500.

Fig. 3 Alveolar septal vessel congestion after 100% O$_2$ exposure for 48 hr; arrow (RBCs); X 1,000.

Fig. 4 Alveolar (intracellular exudate after 100% O$_2$ exposure for 70 hr. F (fibrin); arrow (RBCs); X 600.

Fig. 5 Alveolar (intracellular exudate after 100% O$_2$ exposure for 70 hr; arrow (Type II granular pneumocytes); X 1,500.

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THE INFLUENCE OF INERT GAS CONCENTRATION ON PULMONARY OXYGEN TOXICITY. M.H. Powell and H.D. Fust. Institut für Flugmedizin, Deutsche Forschungs- und Versuchsanstalt für Luft- und Raumfahrt, Bonn-Bad Godesberg, West Germany.

1. INTRODUCTION

The observation that oxygen at higher than normal pressure has a deliterious effect upon lung tissue dates back to Lavoisier. Two effects are generally noted. The first, or acute oxygen toxicity, occurs when oxygen tension is lower than three bar. Here a neurological component is prominent with convulsions occurring. It was Paul Bert who, in 1878, first showed that the toxic substance responsible for this central nervous system effect was the oxygen in compressed air. The second effect, so-called chronic pulmonary oxygen toxicity, was first described by J. Lorraine Smith in 1899 and is noted following a long exposure when the oxygen pressure is greater than 0.5 bar. It is primarily directed toward the pulmonary tissue with death the ultimate outcome.

The literature contains conflicting evidence concerning the effect of added amounts of inert gas on each of these two types of oxygen toxicity. Added amounts of inert gas appear to exert little influence on C.N.S. oxygen toxicity, which has a very rapid onset, although Burns (1972) did report increased latency to convulsions when helium was added to the oxygen as did Almquist et al. (1969) with oxygen-nitrogen mixtures.

There does exist some experimental evidence in the literature also that increased amounts of inert gas will influence the course of chronic pulmonary oxygen toxicity. Lambertsen (1955) reported the beneficial effects of interruption of oxygen breathing by the substitution of compressed air. The early investigations of Painter (1956) indicated that gross pulmonary damage in guinea pigs was reduced by the presence of inert gas. He postulated that, to a great extent, the chronic toxic effects of oxygen were the result of a locally high oxygen tension in the lungs. Norman and co workers (1971) found that pulmonary damage in rats and mice was reduced when breathing a given oxygen tension with added nitrogen. Protection was not found when systemic oxygen levels were reduced by the addition of carbon monoxide to the oxygen, although anemia and pulmonary denervation were found to be protective by Moon et al. (1976). Bellet and Powell (1972) reported a reduction in pulmonary toxicity in rats when either helium or nitrogen was added to oxygen. No protection of added nitrogen was be so, as recently reported by Bokitka and Rahn (1977), in a treadmill study with mice.

Pulmonary oxygen toxicity in man is generally calculated by the method proposed by Wright (1977), and the result is expressed in "Unit Pulmonary Oxygen Toxicity Doses", or UPTD for short. Basically, one UPTD is equal to one bar of oxygen breathed for one minute. As it is known that the effects of pulmonary oxygen toxicity appear more rapidly and in a disproportionate manner with increased oxygen pressure, the calculation method is pro portionately weighted.

The end-points for a specific number of toxicity doses was expressed as a reduction in the vital capacity of human subjects in addition to such subjective feelings as nausea and substernal burning, two of the most commonly occurring initial events. While the algorithm has appeared to be a useful one, in our opinion it suffers from its inability to account for air pauses commonly made in the final stages of decompression, relative humidity, and gas temperature; also we question its initial premise (Clark and Lambertsen, 1971), that inert gas diluents play a negligible role in the development of pulmonary oxygen toxicity.

In diving procedures developed over the past several years at the Institut für Flugmedizin (Cabarrou et al., 1978), oxygen is employed during the decompression phase with a time-weighted average of 1,9 bar. This results in reductions of decompression times of often more than 50 % over other published tables (Krekeler, Cabarrou, Fust, 1978) with no subjective symptoms of pulmonary oxygen toxicity. Furthermore, since the total decompression time is shortened, the total number of UPTD's is kept comparatively low. By means of the employment of oxygen-enriched gas mixtures, the inert gas is quickly eliminated without the need of the long "oxygen breathing tail" normally found in conventional decompression methods.

In terms of the normally employed UPTD calculation method, this means that most of our oxygen breathing is done with diluted oxygen. For a 150 meter for 30 minute dive, only about 22 % of the toxicity doses are acquired under 100 % oxygen. We therefore wish to determine if there exists a constant effect of the presence of a diluent gas and/or relative humidity on chronic pulmonary oxygen toxicity.

It is the purpose of this study to determine with mice if commonly measured pulmonary and bloodgas parameters are changed when equal oxygen toxicity doses are administered, that is, at a constant time and oxygen partial pressure; the oxygen is administered either in pure form or diluted with inert gas. Additionally, the effect of high and low humidity in the breathing mixture was also studied.

II. MATERIALS AND METHODS

An initial investigation was started to observe the gross effects of pure versus diluted oxygen by means of survival times. For these studies, adult female mice (NMRI strain) with an average weight of 38,6 ± 1,5 grams were used as subjects. They were divided into groups of fifteen each and exposed in a hyperbaric chamber fitted with observation ports; decompression was thus not needed to determine the number of survivors.

Gas was supplied to the chamber from premixed cylinders. Residual air was flushed out quickly so that the end result would be either 100 % oxygen (at 1,75 bar) or 50 % oxygen (1,75 bar)/ 50 % nitrogen (1,75 bar). The chamber was constantly purged with either of these two mixtures, and at the chamber pressure, flow was approximately 2 liters/minute. Carbon dioxide levels were determined with Dräger gas analysis tubes; the chamber equivalent P_{CO_2} was 4,2 ± 1,5 mbar. For the experiments with elevated humidity, the gas was bubbled through air-stones in water; for the low humidity cases, the floor of the chamber was covered with silica gel granules. Relative humidity was determined electronically. The high humidity series ranged from 90 to 95 % while the low humidity series was between 10 and 15 %. All experiments were conducted at temperatures between 21 and 21 °C.

To investigate the sequence of events in the pre-terminal period, blood-gas measurements and gross lung morphological studies were performed. Mice, in groups of 15, were placed in a hyperbaric chamber and exposed for periods of 1 to 20 hours to a P_{O_2} of 1,75 bar. After exposure (with and without nitrogen and at high and low humidity), the subjects were then removed and allowed to come to equilibrium with room air for a minimum of thirty minutes. They were then lightly anesthetized with Nembutal, and blood was collected in a heparinized syringe from a small incision made in the posterior aorta. Repetative measurements were then immediately made for P_{aO_2} and P_{aCO_2} using a blood-gas analyzer. The lungs were also excised and the degree of edema estimated from the lung/ body weight ratio. Gross morphology was also noted.

III. RESULTS

Figure 1 shows the results of survival time in oxygen when the relative humidity is high. A difference in the two curves is easily seen between the 100 % and 50 % oxygen cases.

Figure 2 is again pure and diluted oxygen, but this time with a low relative humidity. In all of the four variations, a minimum of three trials was made, each with 15 mice. The points represent the sum of these trials; a total of 210 mice were used.

A Wilcoxon Rank Sum Test performed on the results shows that the statistical difference between the two curves in Figure 1 is meaningful at the $p = 0,05$ level while that between the curves in Figure 2 is at the $p = 0,01$ level.

At present, our blood-gas measurements are incomplete. Results show that the above measured parameters in mice change with exposure time as also observed by Välimäki (1975) and Schäfer and Citoler (1978).

While the exact cause of death from pulmonary oxygen toxicity has not been proved, it is clearly evident that the physiological changes leading to death are either mitigated or forstalled by the inert gas fraction. This will be discussed.

IV. CONCLUSION

The results found thus far in mice do not allow one to make adjustments in UPTD calculations for manned diving. They do indicate, however, that in a mammalian system, simple calculations of exposure time and oxygen partial pressure are not always sufficient to correctly describe the degree of chronic pulmonary oxygen toxicity. They, furthermore, agree with the results and findings of our manned dive experiments which also indicate a benefical effect of moisture and inert gas.

References will appear in PROCEEDINGS.

Figures 1 and 2 follow.

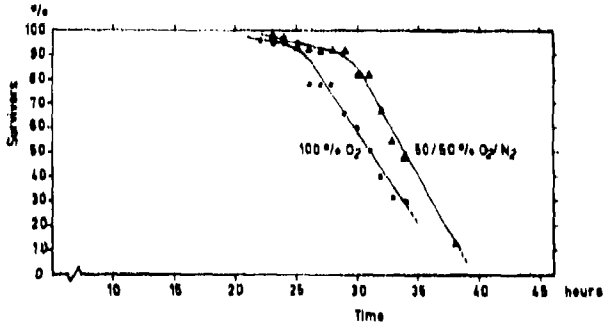

Figure 1. Survival rate of mice in an oxygen pressure of 1,75 bar, and a second case with an equal partial pressure of oxygen in the mixture. The relative humidity = 90 - 95%.

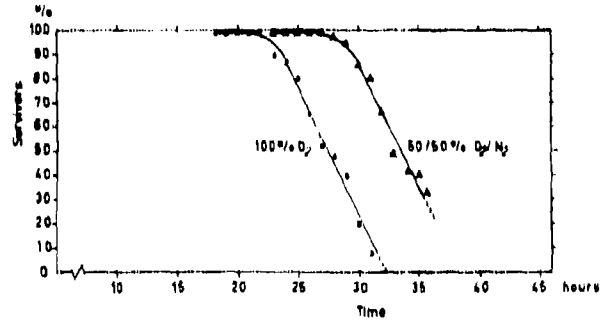

Figure 2. Survival rate of mice in an oxygen pressure of 1,75 bar, and a second case with an equal partial pressure of oxygen in the mixture. The relative humidity = 10 - 15%.

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BRAIN GABA AND cGMP AS INDICES OF METABOLIC DISTURBANCE IN THE CNS DURING ACUTE OXYGEN TOXICITY. M. W. Radomski and W. J. Watson. Defence and Civil Institute of Environmental Medicine, Downsview, Ontario, Canada.

Alterations induced by high pressure oxygen (OHP) in various neurotransmitters (gamma-aminobutyric acid=GABA, norepinephrine, dopamine, serotonin) have been implicated in the mechanism of oxygen toxicity. It is unlikely, however, that these various neurotransmitters act independently in the central nervous system (CNS), but must interact at functional and neurochemical levels to modulate behaviour in a balanced manner. Thus, alteration of one or more neurotransmitters by OHP could produce an imbalance that would be manifested in a convulsion. Of the various neurotransmitters implicated in oxygen toxicity, only changes in GABA metabolism appear to relate to the numerous variables observed in oxygen toxicity (1).

Cyclic GMP (cGMP) which mediates the action of acetylcholine is involved in excitatory responses in the cerebellum. Excitation elevates cGMP and depresses GABA, whereas depression decreases cGMP and elevates GABA. This inverse relationship between cGMP and GABA has been observed in the action of certain chemical convulsants (2), and it has been suggested that cGMP may act as an index of GABA receptor function in the cerebellum (3).

Although a large number of drugs will suppress convulsions induced by OHP, metabolic disturbance may continue to occur in the CNS in the absence of convulsions. Thus, it is important in any evaluation of drug potency to measure in addition to motor activity, some biochemical marker in the brain. This study examined the effects of OHP on the relationship between GABA and cGMP, and using GABA as a biochemical indicator of OHP induced lesions, re-examined several classes of drugs known to affect the occurrence of oxygen convulsions. These drugs included acid-base compounds, hypoglycaemics, antioxidants, di sulfiram, *succinate, and diazepam.*

METHODS

Male Wistar rats (200 to 220g) that had been deprived of food overnight were exposed to 6 ATA of 100% oxygen for 60 min in the convulsion studies. Whole brain GABA and cGMP were measured in non-convulsed animals exposed to 6 ATA oxygen for 20 min.

The 50% convulsion time (CT50) was calculated for each treatment and the anticonvulsant efficacy of each drug was expressed as the Convulsion Reduction Factor (CRF) (ratio of CT50 treated/CT50 untreated). The agents evaluated against oxygen convulsions are listed in Table 2.

RESULTS AND DISCUSSION

GABA and cGMP

The effect of OHP (20 min at 6 ATA) on brain cGMP and GABA levels are given in Table 1. Although the characteristic OHP-induced decrease in GABA was seen, no significant decrease in cGMP levels was found. This was surprising as agents such as isoniazid which produce convulsions when the brain content of GABA is reduced, elevate cGMP levels (3). Furthermore, drugs that either block the synthesis of, or are GABA antagonists, increase the cGMP content in the cerebellar cortex. Our analyses were carried out, however, on the whole brain whereas the cerebellum is the area of highest concentration of cGMP in the brain (4). Use of the whole brain in our studies may have masked any changes in cGMP. It is also possible that OHP may act on GABA-ergic neurons in areas of the CNS which are not related to cGMP. Further, work on specific brain regions is required to resolve this question.

Anticonvulsants

Table 2 shows the effects of various agents on the oxygen-induced changes in brain GABA and on the convulsion reduction factor (CRF). Brain GABA's are shown as the ratio of the oxygen-induced change in brain GABA in the drug treated animals to the change in control animals treated with the solvent (ctrl+d/control). Thus a ratio less than one would indicate that brain GABA dropped less in the treated animal than in the control. Values greater than 1 indicate a greater decrease in the drug-treated animals.

The three hypoglycaemics studied were selected on the basis that tolbutamide increases brain GABA, acetohexamide decreases it, and phenformin has no effect (5). It is evident in Table 2 that tolbutamide protected against oxygen convulsions (CRF 1.91) and modified the decrease in brain GABA (ratio 0.45)); acetohexamide had no protectant effect; and phenformin accelerated convulsions (CRF 0.68). The qualitatively variable effect of these hypoglycaemics on oxygen convulsions suggests that changes in brain GABA are not related to reductions in blood glucose.

Alkalosis with Tris or $NaHCO_3$ significantly delayed convulsions and the decrease in GABA, whereas Diamox precipated oxygen convulsions along with a greater decrease in GABA than in controls (GABA ratio 1.78). The intensification of oxygen poisoning by the inhibition of carbonic anhydrase by Diamox is likely due to an increase in tissue pO_2 evoked by an increase in blood flow by Diamox (1).

With the exception of disulfiram, all of the remaining agents tested (Table 2) increased the CRF concomitant with a decrease in the GABA ratio. A significant inverse linear relationship was found, in fact, between the CRF and the GABA ratio ($r = 0.85$, $P<0.001$).

Table 1 Whole brain GABA and cGMP levels in OHP-exposed rats (20 min, 6 ATA)

Group	GABA (µmoles/g wet wt)	cGMP (pmoles/g wet wt)
Control	1.76 ±0.06	9.82 ±0.14
Oxygen	1.50 ±0.05	9.48 ±0.84
Significance	0.001	n.s

X ± SEM for 9 rats per group

Table 2 Effects of various agents on oxygen convulsions and oxygen-induced changes in brain GABA

Drug	CRF	GABA Ratio* (T/C)
Tolbutamide	1.91	0.45
Acetohexamide	1.15	1.28
Phenformin	0.68	1.07
Diamox	0.19	1.78
Tris	1.44	0.22
$NaHCO_3$	1.47	0.48
Na succinate	1.21	0.66
Glutathione	2.19	0.31
Cysteine + Succinate	1.53	0.49
Cysteine + glutamate	1.42	0.65
Pargyline	1.29	0.71
Propylene glycol	1.80	0.21
Tween-80	1.70	0.21
Disulfiram	1.57	1.21
Diazepam	1.98	

* (T/C) ratio of change in GABA levels in treated animals exposed to OHP to change in control animals exposed to OHP.

Diazepam at two doses of 4 and 8 µmole/kg significantly extended the CRF by 1.50 and 1.88. There is overwhelming evidence that GABA transmission is involved in the action of diazepam in the cerebellum (3) and that diazepam lowers cGMP (4).

In conclusion, strong evidence continues to accumulate that GABA-ergic activity is altered by OHP and is related to the etiology of oxygen seizures.

References will appear in PROCEEDINGS

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PULMONARY PROSTAGLANDIN METABOLISM DURING NORMOBARIC HYPEROXIA. C. L. Lefebvre and M. M. Mathias. Department of Physiology and Biophysics and Department of Food Science and Nutrition, Colorado State University, Fort Collins, Colorado, U.S.A.

INTRODUCTION

Several observations, in our laboratory and others, have led us to investigate the possible role of prostaglandin (PG) metabolism in the etiology of pulmonary oxygen poisoning. We found that hyperoxia decreased PG synthesis by lung homogenates, possibly as a result of decreased availability of two key factors necessary for synthesis--molecular oxygen and lipid precursors. There was a direct relationship between decreased dietary fat content and susceptibility to pulmonary toxicity. Elevating the amount of dietary polyunsaturated fatty acid precursor for PG synthesis improved the PG synthetic potential of lung homogenates. Administration of aspirin, an inhibitor of PG synthesis, markedly exacerbated the symptoms of oxygen poisoning and incidence of mortality.

Additional facts suggest a possible role of PG in pulmonary toxicity. The lungs are the primary organ responsible for clearance and detoxification of PG from the blood. Pulmonary pathology during hyperoxia might impair this vital function. Pulmonary capillary endothelial cells are the most sensitive to oxygen and undergo necrosis early in the onset of toxicity. They are also the primary site of synthesis for PG and the related thromboxanes. Oxygen has been shown to reduce the activity of enzymes containing labile sulphydryl groups. PG dehydrogenase/reductase (PGDH/R), the rate-limiting step of PG degradation and detoxification, is a sulphydryl-containing enzyme which has been shown to be impaired during hyperoxia. Finally, the fact that PG and thromboxanes are currently thought to regulate vascular pressure in peripheral beds prompted the possibility that any imbalance between vasoconstrictor and vasodilator PG during oxygen poisoning might cause, or at least exacerbate, pulmonary complications and edema.

METHODS

Male weanling Sprague-Dawley rats were fed a semi-synthetic diet enriched after that recommended by the average American for three weeks and then exposed to either pure oxygen or air at an absolute pressure of one atmosphere. Ambient temperature, gas flow and light cycle were controlled. After exposure periods of 0, 24, or 48 hours, animals were decapitated and samples of blood and lung taken. Plasma and lung tissue were analyzed by radioimmunoassay for endogenous concentrations of PGI_2, $PGF_{2\alpha}$, $PGE_{2\alpha}$ and 13,14 dihydro-15-keto $PGF_{2\alpha}$ ($DHPGF_{2\alpha}$), the metabolite of $PGF_{2\alpha}$. The activities of PG synthetase (PGS) and PGDH/R, the respective rate-limiting synthetic and degradative enzymes, were also measured in lung homogenates.

RESULTS

Rats exposed to oxygen did not have plasma or lung tissue concentrations of PGI_2, $PGE_{2\alpha}$ or $PGF_{2\alpha}$ which were significantly different from the air-exposed controls at any of the time periods sampled (Table 1). However, plasma concentration of $DHPGF_{2\alpha}$ was significantly ($P<.05$) lower in oxygen exposed animals at 24 and 48 hours than in the controls.

Although PGS activity declined sharply in oxygen exposed rats between 24 and 48 hours, it was not significantly different than control at 48 hours. PGDH/R activity also declined between 24 and 48 hours in the oxygen-exposed rats but was significantly lower than that in the air controls ($P<.01$) at 48 hours (Table 2). The reduced activity of both enzyme probably resulted in the observed decrease of plasma $DHPGF_{2\alpha}$.

DISCUSSION

Normobaric hyperoxia did not change either the pulmonary or circulating levels of at least three pro-inflammation but seemed to impair the lungs' ability to metabolize them. If the rate of degradation was reduced during hyperoxia but endogenous PG concentrations were not, there is the possibility that the rate of PG synthesis was also reduced. These data do not allow us to assess what, if any, effect such a scenario would have on the etiology of pulmonary pathology, but two observations are pertinent.

The first relates to the timing of the observed changes. The rat model tolerates normobaric hyperoxia well for 48 hours. Up to this time, edema and morphological pathology are not in evidence. We have detected changes in the activity of key acid-oxidant enzymes, such as glutathione peroxidase, superoxide dismutase and ribosome-to-PO_4 dehydrogenase by 48 hours, but the significance of these changes is unclear.

Between 48 and 60 hours, edema, pulmonary pathology and mortality are observed. Mortality can be as high as 100% at 72 hours. Thus, one or more events occurring prior to this time may trigger the symptoms of pulmonary toxicity. The precipitous decrease in the activity of PGDH/R between 24 and 48 hours oxygen exposure and the impaired ability to metabolize PG may well be two of the critical events.

Second, the demonstrated vasoactivity of PG and thromboxanes could play a role in edema formation and possibly cell necrosis. PGI_2, a pulmonary vasodilator, is more sensitive to oxygen inhibition than $PGE_{2\alpha}$, a pulmonary vasoconstrictor. Further, hyperoxia decreases the sensitivity of the pulmonary vasculature to PGI_2. It is possible that differential changes in the balance between dilator and constrictor activity such that edema formation is enhanced. We are currently investigating this hypothesis as well as examining the possible role of newly-discovered PGI_2 and thromboxane A_2. The results of these investigations will be discussed.

This work sponsored by ONR contract N00014 76 C 0457 with funds supplied by the Naval Medical Research and Development Command.

TABLE 1. Plasma and lung tissue prostaglandin concentrations ($\bar{X} \pm S.E.M.$).

Exposure	n	Plasma[a] $6\text{-}PGF_{2\alpha}$	Lung[b] $PGF_{2\alpha}$	Lung[b] PGE_1	Lung[b] PGE_2
6 hours					
air	8	NA	7.2 ± 2.6	1.7 ± 0.7	0.55 ± 0.2
oxygen	8	NA	5.8 ± 2.3	1.8 ± 0.9	0.60 ± 0.2
24 hours					
air	4	20.2 ± 1.5	4.7 ± 0.7	1.2 ± 0.3	0.38 ± 0.2
oxygen	8	10.8 ± 3.2^c	5.4 ± 1.9	1.7 ± 0.6	0.64 ± 0.2
48 hours					
air	4	20.6 ± 4.0	4.3 ± 1.5	1.1 ± 1.5	0.51 ± 0.1
oxygen	8	13.1 ± 2.6^c	4.1 ± 0.9	0.97 ± 0.3	0.52 ± 0.2

[a] ng/ml

[b] ng/100 mg dry tissue

[c] $p < .05$ or better

TABLE 2. Lung tissue prostaglandin synthetase and prostaglandin dehydrogenase reductase activities. ($\bar{X} \pm S.E.M.$)

Exposure	n	Synthetase[a]	Dehydrogenase Reductase[a]
6 hours			
air	8	115.5 ± 60.8	198.9 ± 52.2
oxygen	8	124.1 ± 66.7	230.6 ± 71.8
24 hours			
air	4	51.1 ± 17.5	174.5 ± 15.2
oxygen	8	118.8 ± 20.1^b	269.1 ± 134.8
48 hours			
air	4	78.0 ± 23.6	220.5 ± 105.2
oxygen	8	97.0 ± 24.7	29.0 ± 80.5^b

[a] picomoles PG converted/mg protein min^{-1}

[b] $p < .05$ or better

EXERCISE METABOLISM IN HUMANS ON ACUTE EXPOSURE TO A 5.8 BAR NORMOXIC OXYHELIUM ENVIRONMENT. R de G Jamson, R M Grey, M M Wingborough, K S McKenzie and K G M M Alberti. Physiological Laboratory (AMTE), Fort Road, Alverstoke, Gosport, Hampshire, UK, and Southampton University, Hampshire, UK.

Studies on exercise performance are usually carried out under saturation conditions with high partial pressures of helium and a slightly hyperoxic environment (Bradley et al, 1971; Saltzman et al, 1971) and were primarily concerned with physiological variables. This series of experiments was undertaken to investigate the effect that a short exposure to a relatively low pressure of helium might have on exercise metabolism. The subjects were healthy adult males of normal build, all familiar with compression chamber work and the equipment used in this study. The experimental plan called for the subjects to be exposed to three different atmospheres in a random order, the atmospheres being: air - 1.0 bar, normoxic oxyhelium at 1.3 bar and normoxic oxyhelium 5.8 bar. The pressure of the former oxyhelium mixture was chosen as this was the least pressure which would allow the chamber to be sealed. The pressure of the later mixture was chosen since it was calculated that this was the pressure at which the mixture would have the same density as air at 1 bar. A space of 7 days was allowed between each of the subjects' experimental runs to ensure there was no training effect.

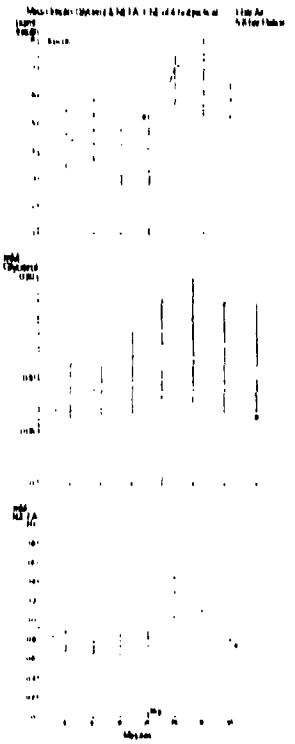
The subjects were fasted overnight (12-14 hours). A cannula was inserted into an antecubital vein. After resting for 5 minutes the first resting sample was taken and 5 minutes later the second. The subjects then exercised for 20 minutes at 60% of their predetermined $\dot{V}O_2$ maximum. Blood samples were taken at 5 minute intervals during exercise. Of the blood 7 ml were transferred to chilled perchloric acid (5% V/V) for analysis of lactate pyruvate, glucose, glycerol, alanine, 3-hydroxybutyrate (3OHB) and acetoacetate (AcAc). Remaining blood was placed in plain glass tubes, centrifuged following decompression, and the plasma stored at -20 °C for later assay of insulin and non esterified fatty acids (NEFA). Ventilatory volume studies were carried out using a dry gasometer and continuous analysis of expired oxygen and carbon dioxide was made from a mixing box by means of a quadruple mass spectrometer. Calibrations were carried out before and after each run. It was found at the end of the experiment that the calibration gases used for the 1.3 bar experiments were unsuitable so these readings were not included in the final results. Throughout the exercise and post exercise period the heart rate was monitored using a Hewlett Packard telemetry system which had previously been tested to 41 bar. The decompression schedule chosen for these experiments was the US Navy's partial pressure table for 190 ft of helium for 60 minutes since the exposure was the equivalent of 18% for 60 minutes. The 4 exposures on this schedule produced fleeting joint pains on surfacing on all dives and on the last dive one of the investigators developed a large urticarial rash over his back and abdomen coupled with a mild ache in one shoulder. Because of these symptoms it was decided not to continue with these exposures for the planned 6 subjects but to limit the numbers to 4.

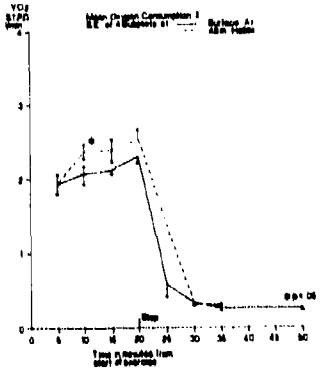
The general pattern of response of the blood metabolites to the exercise was the same at each depth. The lactate and pyruvate levels both rose with exercise; the lactate then stabilising and falling rapidly at the end of exercise while the pyruvate level reached a peak after the end of the exercise and fell more slowly. Alanine concentration rose with exercise as did glycerol. The level of ketone bodies fell while the NEFA level showed a slight fall with a post exercise rise. However, there were some differences between the 5.8 bar exposures and the other two. The statistically significant changes occurred in the blood lactate level which was higher at 5.8 bar, both during recovery and exercise, as was the lactate/pyruvate ratio. The glycerol however was lower at 5.8 bar during and after exercise, being significant at 5 minutes. The NEFA level was highest during the exercise period at 5.8 bar, and the post exercise rise was not so marked. In fact after exercise the levels were lower than at the surface, being significant at 5% minutes.

Perhaps the most interesting finding was the failure of plasma insulin levels to show the characteristic drop with exercise, the difference in levels being statistically significant at the end of exercise. Despite this failure to fall, plasma insulin concentration still showed a post exercise rise to the same level as with all exposures. The relationship between NEFA, glycerol and insulin can be seen in figure 4.

The heart rate rose with exercise but there was no initial any difference between the environments. The $\dot{V}_E$ was not significantly raised in the 5.8 bar environment, compared to air at 1 bar, except at the 15 minute period during the exercise period as can be seen from figure

The respiratory quotient (RQ) was lower at [illegible] at 5.8 bar than at the surface, 0.91 ± 0.01 as against 1.00 ± 0.01. However, an RQ of 0.91 is approximately the level one would expect to ascertain where to under normal conditions (Astrand and Rodahl, 1970). If anything the RQ at the other exposures is a little high.





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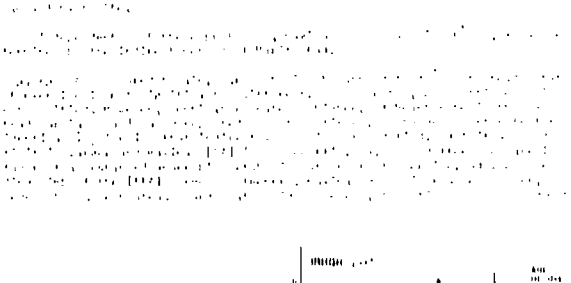

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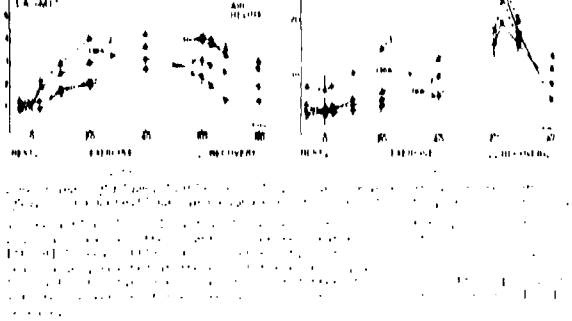
EFFECTS OF EXERCISE AND HYPERBARIC AIR ON VENTILATION AND CENTRAL INSPIRATORY ACTIVITY. C.M. Honger and P. Lind, Department of Environmental Medicine, Karolinska Institutet, S-10401 Stockholm, Sweden.

Several studies in the past have shown that the respiratory responses to CO_2 and muscular exercise may become depressed by acute exposure to raised air pressure. To what extent these effects might be due to increased breathing resistance secondary to increased density of the respired gas, or to a depressant (narcotic) effect of the raised N_2 pressure on the respiratory centers has been a matter of debate. In a recent report from this laboratory (3) it was shown that hyperapnic hyperventilation is reduced by high N_2 pressure despite a concurrent increase of the central inspiratory activity (CIA). It now seemed of interest to investigate whether the relationship between pulmonary ventilation and CIA is changed by hyperbaric air and nitrogen during the hyperpnea of exercise as well.

Methods. Eight, healthy male volunteers were studied. Their age, weight, vital capacity, and $\dot{V}_{O_2max}$ ranged 24-34 yr, 61-82 kg, 4.1-6.1 l, and 46-61 ml·kg^{-1}·min^{-1}, respectively. Each subject performed two tests with progressive-load leg exercise on a cycle ergometer (pedalling rate 60 rpm) placed inside a dry compression chamber. In the first test the subject inhaled O_2 at an ambient pressure of 1.3 ATA, in the other he inhaled air at 6 ATA (same inspired P_{O_2} as in the first test). The ergometer load was increased in steps of 10 W from 0 to 200 W, each step lasting for 1 min, except 0 W which lasted for 2 min. Pulmonary ventilation ($\dot{V}$), tidal volume, respiratory rate, end-tidal P_{CO_2}, and heart rate were recorded continuously, whereas CIA was assessed by determination of the inspiratory occlusion pressure, $P_{0.1}$ (4), at intervals of 25-35 s. To determine the lung volume at which ventilation was carried out, the subjects were requested to make maximal inspirations every second minute during the exercise runs.

Results. The mean values for $\dot{V}$ and $P_{0.1}$ increased progressively with increasing work load, both at 1.3 ATA O_2 (control) and at 6 ATA air (Fig. 1). The rate of increase of $\dot{V}$ was lower than that of $P_{0.1}$ in both conditions. The $\dot{V}$ values at 6 ATA were similar to the control values up to 100 W, but were significantly lower at loads exceeding 100 W. In contrast, the $P_{0.1}$ values were about 20% higher at all loads at 6 ATA than in the control condition. Thus the ventilation per unit occlusion pressure ($\dot{V}/P_{0.1}$) was considerably lower at 6 ATA than at 1.3 ATA, and decreased with increasing load in both conditions. The relationship of $\dot{V}$ to $P_{0.1}$ showed a wide variation between subjects. Mid-expiratory and tidal volumes increased, whereas the expiratory reserve volume (ERV) decreased slightly in both conditions as the work load increased (Fig. 2). End-tidal P_{CO_2} rose with the load, in the control condition from 48 to 51 Torr, and at 6 ATA from 46 to 50 Torr when the load increased from 0 to 200 W. Heart rate rose progressively with increasing load, and was about 5 beats/min lower in the 6 ATA than in the 1.3 ATA experiments ($P<0.05$).



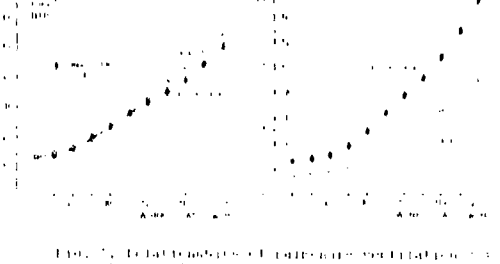


Fig. 2. Relationships of inspiratory ventilation [illegible] end-tidal P_{CO_2}, $P_{0.1}$ [illegible] work load [illegible] at 1.3 ATA O_2 (●—●) and at 6 ATA air (○—○).

References - 1. LAUSBERG-Fabr. Annals Medicus, [illegible] 2. SPEER W. M. and GREENWOOD F.C., [illegible] 3. HONGER C.M. et al. Eur. J. Appl. Physiol. [illegible] 4. MARTIN L. et al. Respiration [illegible] 5. SCHMANN H. Support central CHEMO. [illegible]

This work was supported by C.M.D.N.S. contract nr 77-1260.

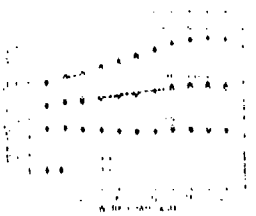


Fig. 2. Relationships of end-inspiratory, mid-inspiratory and end-expiratory lung volumes to work load during progressive-load leg exercise. Symbols as in Fig. 1. Vital capacity = 5.12 ± 0.27 L, BTPS.

Discussion. To the extent that $P_{0.1}$ represents a true index of CIA both during normal and hyperbaric conditions, our observations of higher $P_{0.1}$ values at 6 ATA air than at 1.3 ATA O_2 (Fig. 1D) would indicate that the central inspiratory activity during exercise is enhanced by acute exposure to raised air and nitrogen pressures. However, as discussed in detail in a previous report (3) the $P_{0.1}$-CIA relationship found at normal atmospheric pressure may become altered at raised pressures. Due to the difference in compressibility of the breathing medium, $P_{0.1}$ at a given neural output to the inspiratory muscles will be somewhat higher at raised than at normal atmospheric pressure. In the present 6 ATA experiments the calculated increases of $P_{0.1}$ due to such effects amounted to approximately 0.1 and 0.8 cm H_2O at 0 and 200 W, respectively. Thus only a small fraction of the differences observed in $P_{0.1}$ between 6 and 1.3 ATA can be attributed to the difference in gas compressibility. Also, changes in the breathing pattern at raised pressures may alter the functional residual capacity (FRC) which will affect the $P_{0.1}$-CIA relationship (4). It is probable, however, that the higher $P_{0.1}$ values in the hyperbaric as compared to the control condition were only to a minor degree caused by such FRC-dependent changes in $P_{0.1}$. This follows from the fact that, at any given load, ERV and thus probably also FRC differed but slightly in the two conditions (Fig. 2). It is likely therefore that the relation of $P_{0.1}$ to CIA was approximately the same in the two series of experiments. The higher $P_{0.1}$ at 6 ATA then indicates that during exercise hyperbaric N_2 has no narcotic or depressant effect on the respiratory centers or other neural structures involved in the control of respiration. It may also be concluded that the diminished ventilatory response to exercise at 6 ATA (Fig. 1A) was due mainly to the increased gas density and consequent increase of breathing resistance.

From the above reasoning it follows that the cause and mechanism responsible for the higher $P_{0.1}$ at 6 ATA than at 1.3 ATA must be sought among factors other than pharmacological effects of the high N_2 pressure or differences in FRC and gas compressibility. The raised O_2 pressure can be ruled out as a causative factor, since the same high Po_2 was present in the control condition. That the high pressure per se was the cause is unlikely, since much higher pressures usually must be applied to evoke EEG changes and other signs of CNS affection. End-tidal Pco_2 was higher at 6 ATA than at 1.3 ATA at loads exceeding 100 W, which may explain part of the difference in $P_{0.1}$ in the high-load range. The difference in $P_{0.1}$ in the low-load range, on the other hand, cannot be ascribed to any Pco_2 effect, since end-tidal Pco_2 was of similar magnitude in the two conditions at loads lower than 100 W. It seems likely therefore that the predominant factor responsible for the augmented $P_{0.1}$ response at 6 ATA was the enhanced breathing resistance caused by the raised gas density. This is supported by the observation that the $P_{0.1}$ response to hypercapnia is increased by added inspiratory resistance (2). The present data then support the notion that despite a reduced pulmonary ventilation, the central inspiratory activity is enhanced in hyperbaric air, probably because the increased flow resistance induced by the raised gas density causes a reflex stimulation of the respiratory centers (3).

The observation of wide variation between subjects in the relationship of $\dot{V}$ to $P_{0.1}$ agrees with previous reports (2, 4). That the heart rate was lower at 6 ATA than in the control condition supports the notion that N_2 at high pressure causes a reduction of heart rate, presumably by causing a beta-blockade of the heart (1).

Conclusions. The above results show that the ventilatory response to progressive-load leg exercise is reduced by acute exposure to raised air and nitrogen pressures despite a concomitant increase of the central inspiratory activity. Increased airflow resistance induced by the raised gas density is probably the predominant factor responsible for both the reduction in ventilatory response and the enhancement in CIA response.

References will appear in PROCEEDINGS.

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RESPIRATORY DYSPNEA DURING EXERCISE AT 46 ATA. I. Saltzman, F.M. Cammarota, R. Stolp, H. Saltzman, W. Bell and D. Shelton. U.S. Navy Experimental Diving Unit, Research Facility, Duke University Medical Center, Durham, North Carolina, 27710, USA

It is generally accepted that the capacity for muscular activity at increased ambient pressure during inhalation of a gas mixture denser than air at 1 ATA will be closely related to the maximum voluntary ventilation (MVV) under those conditions (1,2). An additional limitation to work performance while breathing a gas mixture with a density approaching 7 g/l has recently emerged. Inspiratory dyspnea has been reported to be the primary work limiting factor in immersed divers in three independent studies (3,4,5). In each study, the dyspnea appeared not to be chemical in origin.

In this communication we are reporting the results obtained during exercise in a dry chamber at a simulated depth of 460 msw. Inspiratory dyspnea increased in relative proportion to ventilatory levels above 50 l/min limited exercise performance when ventilation exceeded 70 l/min.

Methods. The responses of three experienced subjects to steady-state exercise were studied during the 460 msw Atlantis I trials dive at the Experimental Diving Unit, Duke University, Durham, North Carolina. Exercise was performed while breathing two different gas mixtures, with densities of 7.4 and 10.1 g/l, both containing .5 ATA O_2 at 46.7 ATA. Controls were obtained at 1 ATA breathing either air or 50-50 oxygen-nitrogen as detailed in the following table.

Table 1. Control (1 ATA) and experimental (46.7 ATA) conditions during the Atlantis I dive, 1972.

Pressure (ATA)	Inspired gas	FIO_2 (ATA)	FIN_2 (ATA)	FIHe (ATA)	Density (g/l)
1	air	.21	.79	--	1.11
1	O_2-N_2	.5	.5	--	1.18
46.7	trimix	.5	2.1	43.9	10.1
46.7	heliox	.5	--	46.2	7.4

The subjects were compressed with trimix in 12 hrs and 20 min to a simulated depth of 460 msw. The divers served as subjects and investigators during experimental protocols which were repeated during control measurements in the chamber at 1 ATA. One diver performed 0-1, 15 min MVV measurements and four levels of six-minute periods of work on a bicycle ergometer. Work rates ranged up to $\dot{V}O_2$ max at 1 ATA and from 180 to 900 kpm/min at pressure. The highest work rates at pressure ranged between 65 and 75% of the surface $\dot{V}O_2$ max. 45 to ten minute rest periods were provided between work periods. At pressure the MVV and the physiological responses to exercise were measured while each subject inspired the trimix gas in the morning and while inspiring heliox during a mid afternoon session. The sessions were separated by a lunch and rest period of 2-3 hours.

Humidified gas was supplied to the inspiratory port of the respiratory valve through wide-bore tubing connected to a 200 liter Douglas bag. The gas expired during each minute period was collected in Douglas bags connected to the expiratory side of the breathing valve via large bore tubing and large otopeaks with 45° angles. Volume of gas in the bags was measured in a dry gasometer and exhausted after measurement. The O_2 and CO_2 contents of exhled samples were analyzed by gas-chromatography. Arterial blood samples were collected during the 5th and during the 6th minutes of each exercise period and analyzed for PO_2, PCO_2, and pH.

Heart rates were continuously recorded both at rest and during exercise. Changes in dimensions of the chest cage and abdomen were estimated from two pairs of magnetometers (R. Paterson, Harvard Univ.) affixed to the subject. Oxygen consumption, carbon dioxide production, pulmonary ventilation, tidal volume and respiratory frequency were calculated from the classical equations for the expression of these parameters.

The respiratory circuit resistance during exercise ventilation was reasonably low. Peak mouthpiece pressure swings during the highest work load ventilation averaged 4.5 and 6 cm H_2O in heliox and trimix respectively.

The exercise studies were completed at pressure during the third, fourth and fifth day at 46.7 msw, after the initial alteration in various neurophysiological indices induced by pressure had returned toward surface control values. All subjects demonstrated excellent coordination while completing tasks which required great skills: insertion of radial arterial cannulae, calibration of transducers, etc.

Results. MVV values decreased at pressure as an exponential function of inspired gas density. Density exponents ranged from -.39 to -.46 in our subjects.

O_2 production ($\dot{V}O_2$) in each of the three subjects was greater at any work rate at 46.7 ATA as compared to the same work rate at 1 ATA. There was no significant difference in $\dot{V}O_2$ between trimix or heliox as the inspired gas when work was performed at depth.

Heart rate as a function of work was greater at rest and during exercise for each subject at 460 msw compared to 1 ATA. Heart rates tended to be faster at rest and during exercise when heliox was inspired compared to trimix at 460 msw. For the subject in whom $\dot{V}O_2$ was measured a relative (10%) bradycardia was observed in trimix, but not in heliox, when heart rate was expressed as a function of $\dot{V}O_2$.

Pulmonary ventilation ($\dot{V}_E$) was greater for any work load at 460 msw in two subjects. The increases in $\dot{V}_E$ were, in general, the result of lower respiratory rates and larger tidal volumes than those at 1 ATA. The subject (BS) in whom $\dot{V}_E$ was less at pressure than at the surface exhibited a mild arterial hyperkapnia at the highest work load both during trimix and heliox breathing. (Figure 1).

All subjects experienced some degree of dyspnea during one or more of the work periods at 46.7 ATA. The dyspnea occurred whether the density of the inspired gas mixture was 7.4 g/l (heliox) or 10.1 g/l (trimix). In every case the symptoms were described by the subject as sensations associated with inspiratory insufficiency. The sensations of dyspnea did not correlate with $P_{ET}CO_2$ but were associated with levels of ventilation which represented a sustained utilization of significantly greater fractions of MVV than occurred at 1 ATA. Figure 1 summarizes the relationships between $P_{ET}CO_2$ and $\dot{V}_E$ as a fraction of MVV both at 1 ATA and at 46.7 ATA.

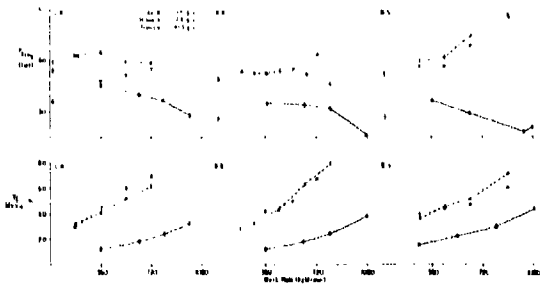

Fig. 1. Resting and 6th min exercise $PaCO_2$ at various work rates for the three experimental subjects. The lower panel presents exercise ventilation, $\dot{V}_E$ (expressed as fraction of the MVV measured in the various conditions) at different work rates. Control results for 1 ATA, air are joined by a continuous line; the 1 ATA O_2-N$_2$ control data were not significantly different from air, and for clarity are not plotted. Experimental points obtained during the 46.7 ATA exposure in heliox and trimix are presented at 460 mmw exercise ventilation represented a much higher fraction of MVV compared to the surface.

The degree of dyspnea appeared to be a function of the ergometric load. One subject (LW) was able to tolerate the discomforting dyspnea at the highest work rate (720 kpm/min) but felt he would have been unable to continue longer than the prescribed 6 minutes. Dyspnea limited work in two subjects, more so in one than in the other. One of the two subjects (BH) was unable to work longer than 5 minutes at his highest work rate (810 kpm/min). The other subject (HB) experienced moderate to severe dyspnea during the fifth and sixth minute of his highest work rate (900 kpm/min) both during trimix and heliox exposures. He was able to complete all six minutes of work in trimix; however, during the sixth minute of exercise while inhaling heliox the dyspnea suddenly became so severe that the subject signaled he could no longer continue the work. The signal was followed by frantic activity including a struggle to remove the mouthpiece and strenuous efforts to breathe. He stated afterwards that if immersed he might have drowned because of the sudden transition from mild to severe dyspnea. The subject's perception was of not getting any gas to inhale. All recorded signals, (EKG, blood pressure monitored directly from the radial artery, inspiratory flow and magnetometer signals) were unchanged prior to and during the inspiratory embarrassment. None of the subjects experienced choking sensations during expiration nor did they become dyspneic during MVV measurements under otherwise similar conditions.

Chest and abdominal diameters as measured by magnetometers consistently reflected changes in tidal volumes. End expiratory diameters did not increase during exercise, even when severe dyspnea was experienced at 46.7 ATA.

Discussion. Analysis of arterial blood gas values demonstrated that arterial hypoxemia was not associated with the sudden onset of dyspnea. PaO_2 remained well in excess of 200 Torr even during the heaviest exercise at 460 mmw. Similarly, pH values varied reciprocally with $PaCO_2$ and at ergometric efforts as high as 900 kpm/min significant metabolic acidosis was not observed. Mild hypercapnia ($PaCO_2$ of 49 Torr) was observed in one subject at the highest level of exercise. ^{6} in heliox and in trimix; hypercapnia was not observed in the other subj[illegible] ing work-limiting dyspnea.

All subjects experienced shortness of breath during transient light physical activity, including talking, eating and climbing an 8 foot ladder to enter a section of the chamber. It was necessary to interrupt these activities to "catch up on breathing", but the subjects felt in control of ventilation. This experience was quite different from the dyspnea which occurred during exercise. For example, one subject stated he felt he was getting further and further behind in his breathing during exercise and this produced a frightening sensation of suffocation.

Unexpectedly dyspnea occurred more frequently and was more clearly work limiting when performing exercise while breathing heliox as compared to observations with trimix. Pulmonary ventilation during the heaviest work loads were also significantly higher in heliox compared to trimix for the two subjects reporting intolerable dyspnea, and in both cases $\dot{V}_E$/MVV exceeded 70%. These data indicate that dyspnea occurred because the metabolic load required a pulmonary ventilation representing a very high fraction of the MVV associated with a given gas density. This percentage in every case was larger than the percentage of the MVV used during $\dot{V}O_2$ max at sea level pressure.

The inspiratory dyspnea of varying degrees observed by these divers during exercise in a dry chamber at 46.7 ATA is similar to that seen in immersed divers at 49.5 ATA by Spaur et al (1) and at 41.4 ATA by Dwyer et al (4). In these deep dives the inspired gas was predominantly helium with a density of approximately 7 g/l. Work limiting dyspnea of an inspiratory nature was seen by Thalmann et al (5) in immersed divers breathing compressed air at 6.8 ATA, with a gas density of 7.7 g/l.

Since inspiratory dyspnea during exercise at depths of 41-50 ATA occurs both in wet and dry divers the cause must reside elsewhere than in the effects of immersion on the cardiorespiratory system. Gas density, the increased helium pressure or hydrostatic pressure, singularly or in combination, may initiate the phenomenon. The occurrence of a similar event in the divers of the study by Thalmann et al (5) at a relatively shallow depth (6.8 ATA) while breathing air would appear to rule out helium and hydrostatic pressure as initiators. An inspired gas density of 7-10 g/l is a common parameter in these diverse studies. The mechanism of action remains elusive. We and other investigators (1,4,5) provide strong evidence that the dyspnea is not associated with a significant CO_2 retention or hypoxemia. The sensation, however, may arise from the perception of a mismatch between respiratory effort usually expended for a given $\dot{V}_E$ breathing air and the effort needed for similar $\dot{V}_E$ while breathing a gas of a higher density. Alternatively, there may be a perception of the expenditure of a higher percentage of one's reserve capabilities (MVV) than is usually required for a given ergometric load. We are not able, at this time, to do more than speculate on the causes of dyspnea.

Supported in part by NIH grant HL07896.

References will appear in PROCEEDINGS.

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CARBON DIOXIDE RETENTION WITH UNDERWATER WORK IN THE OPEN OCEAN. J. Dwyer, J.W. Macdonald, R.W. Stolp, and A.A. Pilmanis. University of Southern California Catalina Marine Science Center, Avalon, California, U.S.A.

Elevation of CO_2 in the arterial blood can result in a variety of manifestations ranging from headache and dizziness to unconsciousness. Underwater, CO_2 retention can lead to work limitations and potentially life-threatening conditions. The primary goal of this study was to determine the alveolar PCO_2 levels in experienced working divers during actual open sea dives. These levels were to be determined at several standardized work loads. The extent of inter- and intra- individual differences was studied. A secondary objective was to determine at what meta olic level (percentage of the maximal O_2 consumption) the alveolar CO_2, and hence the $PaCO_2$, assumes the role of a work limiting factor, i.e. at what % $\dot{V}O_2$ max does CO_2 retention reach a hazardous level. Changes in pulmonary ventilation (VE) and alveolar ventilation (VA) occur underwater and the magnitude of these changes is of critical importance for adequate CO_2 elimination. Thus, studies of ventilation patterns in conjunction with PCO_2 measurements were done to determine their relationship to CO_2 retention. Since arm exercise is of equal, if not more, importance than leg work in underseas operations, and since the physiology of arm and leg work is different, the above objectives were applied to both modes.

Due primarily to data acquisition difficulties studies defining the physiological responses in man during actual ocean diving situations have been few and limited. Moreover, extrapolation of data obtained from hyperbaric chamber and swimming pool experiments is not always valid and does not eliminate the deficiency which currently exists in our understanding of the physiology of man working in the open sea. Methods have been developed at the University of Southern California Catalina Marine Science Center (CMSC) during the past decade for physiological data acquisition on working divers in the open ocean. The combination of mild and predictable weather and sea state conditions, and the proximal physical access ability to clear ocean waters of any depth has permitted the successful utilization of the CMSC data acquisition equipment.

Methods. All land and underwater experiments were conducted at CMSC. Ten experienced male divers served as subjects. Scuba equipment used by the subjects was standard except for the 72 cu. ft. tanks which had the underwater recording and gas sampling systems mounted on them. Three depths were used: 10, 20, and 30 meters. A fifty meter course was marked on the sandy bottom at each depth. These courses are permanent CMSC test sites. All land experiments utilized standard bicycle ergometry and spirometry techniques. Standardization of underwater work rates was done by utilizing a unique leg ergometer and a separate arm ergometer both developed at CMSC (Pilmanis et. al., Ergonomics 20, p. 51, 1977). Underwater data on free swimming divers was obtained with two complimentary pieces of CMSC equipment: (1) the Underwater Data Recorder (Pilmanis et. al. Biotelemetry II, 2nd Int. Symp., Karger, Basel, 1971 p. 411 and (2) the Underwater Gas Sampler (Dwyer, Ergonomics 20, p. 477, 1977). Subjects were exercised through a series of increasing work loads. A steady state in oxygen consumption was reached at each submaximal work rate. The subject was worked at two submaximal work rates and one predicted supramaximal rate. Mixed expired gas samples were analyzed on a Quintron Model 8 gas chromatograph for oxygen (O_2) and carbon dioxide (CO_2) fractions. The methods of Chiodi (Am. Rev. Respir. Dis., 1976, vol. 114, p. 291) were used for CO_2 sensitivity testing. Serial lactate measurements were used to verify that the $\dot{V}O_2$ max attained by the subject was indeed the maximum of his aerobic work capacity.

Results. The data show moderate but consistent CO_2 retention with leg exercise at high work rates on all subjects during ocean diving (Table 1). The characteristic "blowing off" of CO_2 characteristic of land exercise was absent with underwater exercise. The underwater exercise data is a composite of data from experiments at 10, 20, and 30m of sea water.

Table I

% $\dot{V}O_2$ max	$PaCO_2$ (mm Hg)		$\dot{V}_E$ (BTPS)		$\dot{V}_A$ (l/min)	
	Land exercise	Underwater exercise	Land exercise	Underwater exercise	Land exercise	Land exercise
0 to [illegible]	39.2 ± 0.6	41.5 ± 1.1	10.5 ± 1.1	9.3 ± 1.2	10.0 ± 1.1	8.0 ± 1.4
20 to [illegible]	39.6 ± 2.1	46.1 ± 3.0	11.0 ± 1.1	13.0 ± 1.4	10.5 ± 1.2	10.4 ± 1.5
50 to 60	41.1 ± 3.1	51.4 ± 7.9	11.8 ± 1.1	16.8 ± 3.5	11.3 ± 1.3	14.0 ± 3.1
70 to 80	41.4 ± 2.1	60.5 ± 11.2	12.0 ± 1.1	19.6 ± 4.2	11.6 ± 1.2	16.4 ± 3.0
90 to 100	42.0 ± 1.1	76.4 ± 15.8	12.4 ± 1.1	23.4 ± 5.8	11.9 ± 1.2	19.6 ± 4.6

A characteristic hyperventilation was found during the underwater exercise (Table 1). Both $\dot{V}_E$ and $\dot{V}_A$ were significantly reduced at the high work rates underwater. The CO_2 sensitivity curves ($\dot{V}_E$ vs. $PaCO_2$) from land experiments showed great slope variation among the subjects (0.75 to 1.65 l/mm Hg). A relationship was found between the slope and the underwater tendency for CO_2 retention in underwater exercise. The subject with the lowest CO_2 sensitivity (close on land to least sensitive) to CO_2 retained the highest $PaCO_2$ underwater. The most sensitive subject "blew off" more CO_2. Although the inter-individual variation in CO_2 retention is great, the intra-individual variation was small. It is felt that individual predictable CO_2 retention curve. $PaCO_2$ levels with land exercise were significantly ($P < .05$) lower when compared to land leg exercise. However the $PaCO_2$ levels of underwater arm exercise were identical, higher than comparable $\dot{V}O_2$ max underwater leg exercise. The hyperventilation with underwater arm exercise was also significantly greater.

Discussion. CO_2 retention with underwater work occurs at high work loads requiring 6 x $\dot{V}O_2$ max and more. Individual divers attained CO_2 levels as high as 68 mm Hg and complaints of "CO_2 headaches" were frequent above 50 mm Hg. The decreased pulmonary ventilation during underwater work is the apparent cause of the CO_2 retention. There are a number of possible and interrelated explanations for this hypoventilation an CO_2 retention:

1. Inhalation and exhalation resistance of the scuba regulator
2. Restrictions of ventilatory movements by the wet suit and other diving equipment
3. Increased respiratory work with a more dense breathing gas
4. Pulmonary engorgement from body immersion
5. Reduced alveolar CO_2 diffusion in a denser breathing gas
6. Insensitive central chemoreceptors from increasing PO_2

Characteristic individual PCO_2 sensitivity may be especially important in determining the safe levels of underwater work. When viewed in the context of the multiple physiological stresses and unexpected conditions encountered during diving, it is proposed that underwater work limits established by acceptable $PACO_2$ levels may be more important to safe diving practices than previously thought.

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CARDIOPULMONARY FUNCTIONS AND MAXIMAL AEROBIC POWER DURING A 14-DAY SATURATION DIVE AT 31 ATA (SEADRAGON IV). Y. Ohta[1], H. Arita[1], H. Nakagawa[2], S. Tanaka[3], C. Lundgren[4], Y.C. Lin[4], E.H. Smith[5], R. Martin[5], L.J. Patrick[5] and H. Matsuda[3].
[1]Fukui University School of Medicine, Isehara, Japan; [2]Japan Marine Science and Technology Center, Yokosuka, Japan; [3]State University of New York at Buffalo, Buffalo, N.Y., U.S.A.; [4]University of Hawaii, Honolulu, Hawaii, U.S.A.

A simulated saturation dive to an equivalent depth of 300 meters at the Japan Marine Science and Technology Center, Yokosuka, in July-September, 1979, provided us with the opportunity to investigate cardiopulmonary conditions comprehensively. The physiological measurements were conducted on four experienced divers, whose vital statistics (mean ±S.E.) follow: age (yrs), 30±2, height (cm), 171.2±3.1; weight (kg), 64.5±4.2; BSA (m^2), 1.77±0.08. In addition to measurements at the bottom (31 ATA) data were collected at the depths of 100 and 200 meters during both compression and decompression. The composition of the atmosphere in the chamber was maintained at 0.4 ATA oxygen, 0.79 ATA nitrogen and the remainder helium throughout the dive. CO_2 never exceeded 0.004 ATA. Ambient temperature at the bottom was 31° (range, 30.4-31.2)° C and relative humidity about 60%.

Lung volumes and flow rates were determined by conventional spirometry and recordings of maximum expiratory flow volume curves through the use of a low resistance bell spirometer (Pulmorecorder, ANIMA Corp., Japan) fitted with an electrical potentiometer and a differentiator for flow recordings. Free frequency maximum voluntary ventilation (MVV) measurements were obtained by electronic integration of volume of each breath over a 15 second period. Spirometric measurements except for MVV were made in duplicate in each subject and used to obtain mean values. Residual volume (RV) was measured by a rebreathing technique. Regulation of respiration was studied by measuring inspiratory pressure at 100 msec after the beginning of inspiration against a closed inspiratory valve ($P_{0.1}$). The negative pressure developed has been claimed to reflect the central inspiratory activity (CIA). The ventilatory response to elevated P_{ICO_2} was recorded in parallel. With respect to circulatory functions, thoracic impedance (Z0), dZ/dt, heart sounds and ECG were measured at the various depths with the Minnesota Impedance Cardiograph (Model 304, Instrumentation for Medicine, Inc., U.S.A.).

There were no changes in vital capacity (VC). $FEV_{1.0}$ decreased significantly from the predictive value of 84°% (mean 95.0, 1 to 73°±1 at 100 m, to 66±3% (p < 0.05) at 200 m and to 65±1 (p < 0.01) at the depth of 300 m. Predicted MVV values were calculated by Baldwin's formula, and the 3 MVV also showed the well known decrease under pressure: 144±12 predictive, 105±13 at 100 m, 80±11 at 200 m, 76±11 at 300 m and 114±1° predictive the difference from predicted. Peak flows and flows at 25% of vital capacity showed significant decreases under pressure, which were closely related to reciprocals of the square roots of relative gas densities. The maximal expiratory flow at 25% of VC (V_{max}) and the $\dot{V}_{max}$ ratio did not change. It is of interest that $FEV_{1.0}$, MVV and flows at higher than 25% of VC recovered slightly during the stay at the bottom. This may be attributed to the effect of training or acclimation. A consistent increase in maximum inspiratory pressure (p < 0.005) may also indicate a training effect on respiratory muscles, while maximum expiratory pressure showed no increase under pressure. One of the most interesting findings with regard to lung volumes in this dive was a steady increase in expiratory reserve volume (ERV) (p < 0.001, p < 0.01), while VC and RV showed no significant changes. ERV gradually increased during compression and then decreased slightly during the stay at the bottom. The mechanism and meaning of this increase in ERV are uncertain but presumably it is an adaptive response compensating for the increased airway resistance. The air trapping index did not change.

Tidal volume remained essentially unchanged throughout the dive and minute ventilation ($\dot{V}_E$) showed significant decreases at high pressure, 1°±1±0.2 l/min predictive, 10.2±0.15 l/min at 300 m (p < 0.05), 10.68±0.50 l/min at 100 m during decompression (p < 0.001) and 11.38±0.10 l/min predictive (p < 0.001). Although dead space was not recorded it seems reasonable therefore to conclude that there was a tendency for preservation of alveolar ventilation ($\dot{V}_A$) in the face of the decline in $\dot{V}_E$.

As shown in Fig 1, the ventilatory response to CO$_2$ at 300 msec ($P_{0.1}$) significantly depressed to 82.5±3.1% of the 1 atm response (p < 0.01) at a P_{ACO_2} of 50 torr and to 70.1±3.6% (p < 0.001) at a P_{ACO_2} of 60 torr. The $P_{0.1}$ results obtained at 300 msec did not, however, differ from those at 1.0 and 0.5 sec, which may imply that, in the underwater environment, an amount of CIA produced the same volume expansion of the lung under 31 ATA as it did in 1 ATA, or I should be 31 times greater at 31 ATA than at 1 ATA. Two possibilities can be interpreted in two ways. (1) The decrease of an increased $P_{0.1}$ at depth reflects a decrease in CIA or (2) there is a change in the relation between CIA and inspiratory muscle shortening. Should the latter be the case the stands to reason reflect a trimming effect on the relation between CIA and inspiratory muscle shortening rather than between CIA and inspiratory muscle shortening.

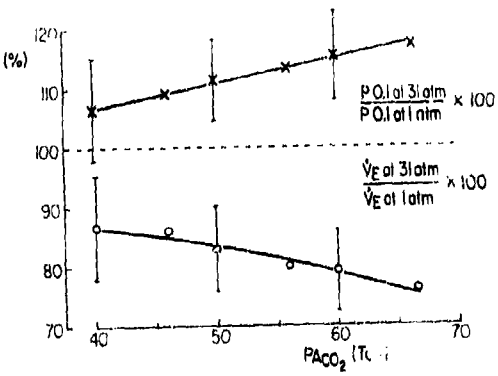

Fig 1 Normalized (to 1.0 atm measurements) mean values ±S.E. of $P_{0.1}$ (top panel) and $\dot{V}_E$ (bottom panel) against P_{ACO_2} in rebreathing experiments in 4 subjects at 31 atm. For further explanations and statistical evaluation see text.

Heart rate during compression decreased slightly from 58±1/min predive to 54±1/min (p < 0.02) at 31 ATA. However, it increased gradually during the stay at the bottom and a marked increase was observed during decompression (68±1/min, p < 0.001). There were no significant changes in blood pressures and in cardiac indexes throughout the dive. Stroke volumes showed a tendency to increase during the compression period, but remained unchanged at the bottom. The specific thoracic impedance (Z0/f) did not change significantly during this dive.

Maximal and submaximal work performance at 31 ATA was evaluated in relation to cardiopulmonary functions. Pedalin, a bicycle ergometer, the maximal tolerable work load during the predive control period was 240±1° watt (air breathing) and maximal O_2-uptake ($\dot{V}_{O_2}$ max) was 3.11±0.18 l/min, and the former was not significantly different from that at the predive period while the $\dot{V}O_2$ max at 2.80±0.1b was significantly lower postdive (p < 0.01) (Fig 2). At 31 ATA the maximal tolerable work load and $\dot{V}_{O_2}$ max decreased to 205±10 watt (p < 0.01) and 2.71±0.10 l/min (p < 0.05), respectively. $\dot{V}_{O_2}$ for a given work load remained essentially unchanged at depth. Decrease in CO output at 31 ATA was significant (p < 0.001) and the gas exchange ratio (R) was not significantly changed.

$\dot{V}_E$ during maximal work decreased more than did maximal work capacity, i.e. from 142.8±7.5 l/min predive to 74.0±3.1 l/min at 31 ATA (p < 0.001). Because V_T/BSA remained unchanged, this suggests a limitation at breathing due to high ge gas density at depth. Ventilatory equivalent to O_2 ($\dot{V}_E/\dot{V}_{O_2}$) increased at work load increased, but at 31 ATA it decreased as work load increased. The $\dot{V}_E/\dot{V}_{O_2}$ at 45.8±4.1 l/min at 1 ATA decreased to 37.5±2.3 at maximal exercise at 31 ATA (p < 0.025). A decrease in heart rate at maximal exercise at 31 ATA was significant at p < 0.01 (from 190±4/min at 1 ATA to 162±2), while the cardiac index remained unchanged. The O_2 pulse (i.e. removal per heart beat) at 31 ATA did not show any significant change from predive values. Thus, maximal work performance at 31 ATA seemed not to be limited in terms of ventilation and O transport.

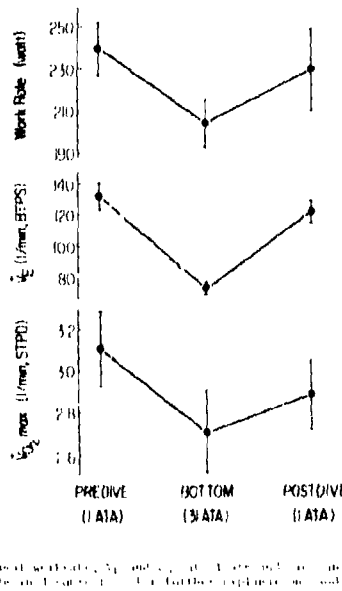

Fig 2 Physical work rate, $\dot{V}_E$ and $\dot{V}_{O_2}$ max at 1 atm pre- and post-dive and at 31 atm at the bottom. For further explanations and statistical evaluation see text.

SPECIES INDEPENDENT MAXIMUM NO-BUBBLE DECOMPRESSION FROM SATURATION DIVE. Y.-C. Lin. Dept. of Physiology, Univ. of Hawaii John A. Burns School of Medicine, Honolulu, Hawaii, U.S.A.

Formulation of an efficient decompression table depends on determination of pressure reduction (ΔP) from a saturation pressure (P_1) to a lower pressure (P_2) and depends on determination of the duration required at P_2 before subsequent pressure reduction can be made. The duration required at each stage of decompression is a function of the rate of inert gas elimination and thus is species dependent. On the other hand, the maximum tolerable ΔP without forming bubbles is determined by the physics of bubble formation and thus may not be species dependent. The objective of this study is to determine and to compare the maximum ΔP allowable from a saturation dive without forming intravascular bubbles in different species. Evaluation of decompression stress by symptomatic observation in animals involves subjective judgment and is relatively imprecise. Since observed symptoms are results of a major decompression stress and are evaluated subjectively in most cases, it is therefore desirable to determine some presymptomatic parameters by means of an objective criterion. This paper is such an attempt, by monitoring the threshold of intravascular bubble formation. The threshold of decompression-induced intravascular bubbles was detected by the ultrasonic Doppler flowmeter.

Male Wistar rats weighing 475 ± 25 g were anesthetized with pentobarbital sodium (40 mg/kg) and surgically prepared by implanting a 2 to 3-mm diameter perivascular Doppler probe (Parks Electronics, Beaverton, OR) on the posterior vena cava caudad to the renal veins. Dogs weighing between 18.0 to 22.0 kg were also surgically prepared in a way similar to the rat by using 16 to 20-mm diameter flowmeter probes, however, the probe was located on the posterior vena cava between the heart and the diaphragm. Two weeks were allowed for recovery following surgery. This chronic preparation was chosen over an external probe to eliminate the necessity of anesthetizing the animal during compression and subsequent decompression. The wire leads from the probe were run subcutaneously to the top of the head between the ears for the rat and on the back of the neck for the dog. The flowmeter probes with frequencies of 8. - 10.0 MHz were tested prior to and following implantation for bubble detecting ability.

Detection of intravascular bubbles was made, using a Parks Electronics Doppler Flowmeter Model 831 with the output signal fed to an audio amplifier, a cassette tape recorder and a pen-writing oscillographic recorder. The bubble can be detected by the distinct Doppler shifted chirping sounds or from recorded traces.

Following a step increase in ambient pressure (P_1) in a 1 liter chamber for the rat for 1 hour, and 6 hours for the dog in a human hyperbaric chamber (Bahn and Clay, Houston, TX), pressure reduction was carried out as rapidly as the system permitted to a predetermined lower pressure (P_2). If there was no indication of bubbles within 1 hour, the decompression was considered bubble-free. For each saturation pressure (P_1), an increasing pressure difference (ΔP) to a lower pressure (P_2) was tried on each exposure on separate experiments until the threshold for bubble detection was found. In the rat, repeat exposures were at least 24 hours but no longer than 3 days after first exposures. For the dog, the repeat compression-decompression exposures were made weekly. Compressed air is used in all experiments.

A total of 54 decompression trials were made on 19 rats. The data shown on Fig. 1 are the greatest ΔP values not producing bubbles and the smallest ΔP values where bubbles were detected during the first pressure exposures. Each point represents one rat for one pressure exposure. Trial points with values greater than the lowest ΔP with bubbles (circles) and less than the highest ΔP without bubbles (squares) have been omitted. The true threshold for Doppler-detected intravascular emboli can be considered to lie between the paired bubble and no bubble lines. Upper dashed lines indicate incidence levels for decompression sickness based on behavioral observation of Berghage et al. (NMR, [illegible], 1976). Data on the repeat pressure exposures for the rat are similarly summarized in Fig. 2. The paired lines without data points indicate the regression lines of the first exposure results as seen in Fig. 1. It is evident that an increased pressure differential is tolerable without forming bubbles upon repeat exposures. It is important to note that no animals represented by the threshold boundary lines exhibited any overt signs or behavioral changes indicating decompression sickness, even if intravascular bubbles were detected.

[illegible] compression-decompression experiments were performed in three [illegible] prepared dogs. Experimental protocols were similar to those for the rat. The first trial began with the average critical pressure for humans (Young, Aviat. Space Environ. Med., [illegible]) in which the ΔP was much smaller than the rat as presented above (Fig. 3, line C). It became apparent that intravascular bubbles do not form for this small ΔP. Subsequent trials were made using the first exposure bubble threshold line of the rat. No bubble was detected in all trials in dogs using the first exposure no bubble schedule for the rat (Fig. 3, line B). However, two out of three exposures in the dogs using the repeat exposure, no bubble line for the rat resulted in intravascular bubbles although no observable symptoms appeared (Fig. 3, line A).

The results of this investigation demonstrate the feasibility of [illegible] implanted Doppler probe in laboratory animals of varying sizes for detection of decompression induced intravascular bubbles. The intravascular bubble threshold indicate a mild, previously undetectable level of decompression sickness in the rat and the dog. Thus, the present method offered a clear and non-subjective criterion for detection of the onset of decompression sickness which [illegible] [illegible] symptomatic. As the threshold of decompression induced intravascular bubble formation, the rat and the dog should be detectable [illegible] decompression [illegible]. This procedure to clearly [illegible] and [illegible] over the behavioral determination of decompression sickness.

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detection of decompression-induced intravascular bubbles. These intravascular bubbles indicate a mild, previously undetectable level of decompression sickness in the rat and in the dog. The findings showed that the maximum tolerable pressure reduction from a saturation diving appears to be not a species dependent phenomenon. We anticipate that this finding will facilitate the extrapolation of decompression schedules among species, since only the consideration of species specific parameter is required, namely, the rate of inert gas elimination.

ACKNOWLEDGEMENT

This investigation was supported by the University of Hawaii Sea Grant College Program under Institutional Grant No. 04-7-158-44129 from NOAA Office of Sea Grant, Department of Commerce. The author gratefully acknowledges the able assistance of [illegible]. Watt, K. Shida, E. Hayashi and B. Respicio in conducting the experiments.

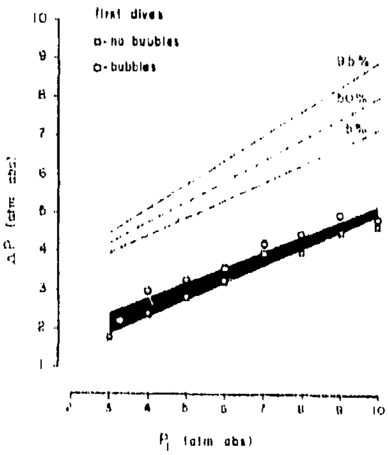
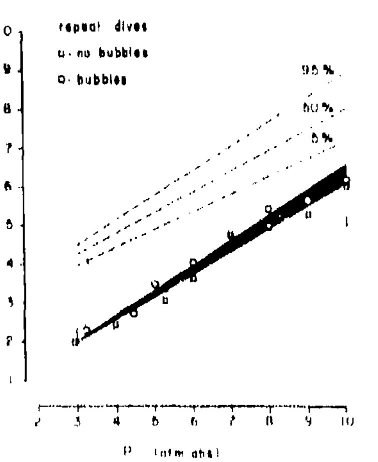


Fig. 1. Doppler-determined decompression sickness thresholds based on the detection of venous gas emboli in the rat during the first pressure exposure. Circles represents the minimum pressure reduction from saturation that produces intravascular bubbles. The squares represent the maximum pressure reduction from saturation that produces no intravascular bubbles. The true decompression-induced bubble threshold can be considered to lie between the bubble and no bubble regression lines. P_1 is the saturation pressure, ΔP the pressure reduction from P_1 to a predetermined lower pressure P_2. Upper dashed lines indicate incidence levels for decompression sickness based on behavioral observation (Berghage, et al., [illegible], 1976).



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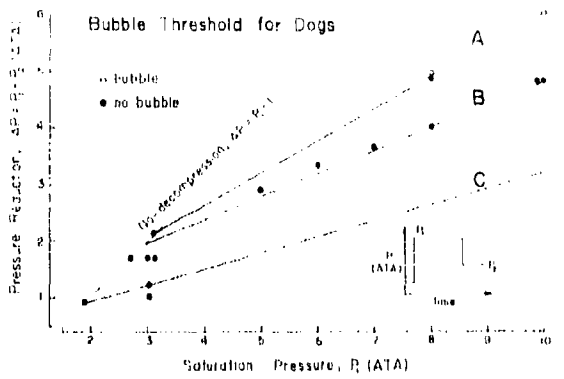

Fig. 1. Doppler-determined decompression sickness threshold based on the detection of venous gas emboli in the dog at weekly exposures. Lines A and B are the no bubble recompression limits for the 1st at the second and first exposures, respectively. Line C is the averaged critical reduction pressure for humans according to Yount (Aviat. Space Environ. Med. Jordd, 1979). Solid circles represent no bubbles and open circles indicate detection of intravascular bubbles during decompression.

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DETERMINATION OF SAFE TISSUE TENSION VALUES DURING THE SURFACE INTERVAL IN SURFACE DECOMPRESSION SCHEDULES FOR HELIUM-OXYGEN DIVES. Peter O. Edel. Sea-Space Research Company, Inc., Harvey, Louisiana, USA.

Although the safe inert supersaturation levels for nitrogen in man during brief surface intervals in surface decompression air dives have previously been determined by empirical tests, no equivalent experiments have been conducted for limiting max tissue tensions following helium-oxygen exposures. Some evidence suggested the possibility of utilization of much higher quantities of inert gas levels in slower tissue half-time compartments than those in current use in military and commercial surface decompression schedules. In tests to develop an emergency surface decompression table following profiles (simulating a total saturation exposure at 47 FSW breathing a 91%N2-9%O2 mixture) for project TEKTITE I, subjects were exposed to surface intervals of 10, 15, and 20 minutes in successive tests following the pressure exposure and prior to re-compression (Edel-1971). The two divers exposed to a 10 minute surface interval and the six divers exposed to a 15 minute surface interval were asymptomatic during the surface interval. Likewise one of the two divers exposed to a 20 minute surface interval was free of symptoms during the surface interval period. However, the other subject experienced serious symptoms of decompression sickness in the 19th minute of the surface interval. It was not, however, the decompression sickness as such, but rather the nature of the symptoms which were unanticipated.

As shown in figure #1, all bodily half-time tissue compartments between 5 and 360 minutes (the latter value thought at that time to represent the slowest tissue half-time compartment in man) were at an approximate state of equilibrium with the nitrogen partial pressure of the breathing medium (68.25 FSW) as indicated by the horizontal line. The intersecting line shown Workman's M values for nitrogen for arrival at sea-level pressure. As shown, tissue compartments with half-times of 5 to 20 minutes do not involve violations of accepted safe criteria for an indefinate period of residence at sea level. Beyond the 20 minute half-time compartment, the degree of inert gas loading in excess of the safe limit increases with tissue half-time. Hence, the slowest tissue half-time compartments have the greatest degree of excess gas beyond the accepted safe limit and would be anticipated to be the most limiting and the likely areas of initial symptoms of decompression sickness. The actual symptoms, however, were not the characteristic "know bends" associated with the slowest tissues but rather of a type associated with much faster tissue half-time compartments. This suggested a response more directly associated with bubble growth than for excess inert gas loading per se. If true, surface decompression schedules, which maintained the tissue tension of the faster half-time compartments within accepted safe criteria at the time wherein the diver is exposed to surface pressure for a brief surface interval, would permit much greater inert gas loading in the slower tissue half-time compartments than is in current usage for such schedules.

A computer was used to construct pressure profiles in which the slowest tissue compartment would control or limit decompression prior to arrival at the final water decompression stop, where, in all schedules, sufficient oxygen was utilized to bring the faster tissue half-time compartments within acceptable limits for a brief surface interval. Using these profiles, experiments were conducted, using a dry test chamber, in which human volunteer subjects were exposed to four hour exposures to 150 FSW breathing helium-oxygen mixtures. At the end of this period the subjects were decompressed in accordance with the computer generated schedule to simulate the water decompression phase. Following this they were brought to surface for a surface interval duration of 5 to 15 minutes. In the initial experiment tissue tensions in the slowest bodily half-time tissue compartment were limited to values currently in use by contemporary methods for arrival at sea-level pressure during the surface interval. The four subjects surfaced after completing the entire profile shown in fig. #2. Following this exposure the subjects were recompressed to 70 FSW. On arrival at this pressure the subjects breathed oxygen for 10 minutes, were then switched to chamber air and brought to 60 FSW. The decompression was completed in accordance with the computer generated schedule in which the subjects ascended to surface in 10 FSW stages breathing intermittent air oxygen mixtures. None of the subjects experienced any signs or symptoms of decompression sickness either during or following this exposure.

In succeeding experiments these levels were elevated in successive stages and the final simulated water decompression stop was accordingly increased to permit surfacing with the increased inert gas loading in the slowest tissue compartment. The points at which the four subjects were brought to surface is indicated by the letters B, C, & D in figure #2. None of the above mentioned schedules resulted in any signs or symptoms of decompression sickness either during the decompression, surface interval, or post dive decompression period.

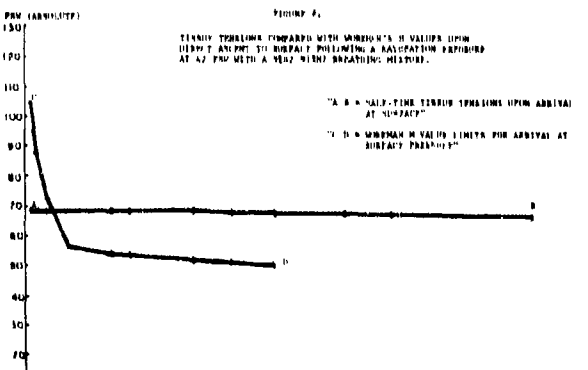
As previously stated, a final period of oxygen breathing, just prior to arrival at surface pressure for the surface interval, is necessary to reduce the faster tissue half-time components to acceptable levels for a brief period of residence at sea-level. Obviously the use of oxygen in the water below 60 FSW would appear to present an unacceptable risk in any practical diving operation. It was thought that one avenue might provide a solution to even larger quantities of inert gas loading prior to surfacing than possible with the schedules utilized up to this point. This involved substitution of a 95%He-5%O2 mixture for the 90%He-10%O2 mixture previously used while on bottom and following the same pressure profile to generate higher tissue tension levels in the slowest bodily tissue half-time compartment. This involved some comparatively small violations of computer assessed safe limits during the water decompression phase. This however would not, according to past experience, often produce problems in "normal" forms of decompression providing such violations were not repeated within the same tissue compartment during the decompression. Accordingly, two subjects were exposed to this profile which resulted in the same tissue tensions upon arrival at surface pressure for the 5 minute surface interval as had resulted in the exposure in which the subjects terminated the water decompression phase at the point marked "C" in figure #2.

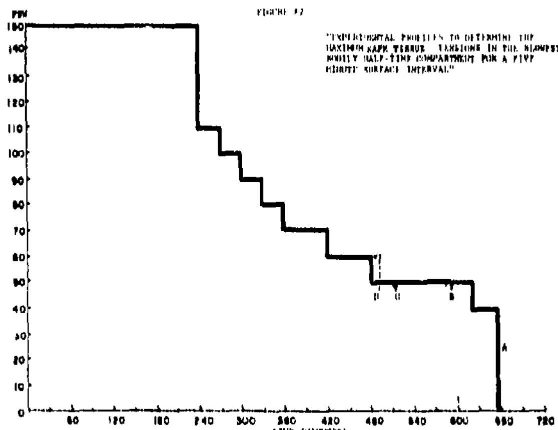
One subject was asymptomatic during the surface interval. The other subject experienced severe pain in both knees after three minutes at surface pressure which was relieved during recompression to 70 FSW. Both subjects were decompressed in accordance with the computer generated profile. No further symptoms or recurrence of symptoms were reported either during the decompression or post dive period.

The ability of the subjects to withstand the much higher tissue tension levels on arrival at surface pressure for surface intervals of 7 to 11 minutes without any evidence of symptoms of decompression sickness, leaves little doubt that the water decompression phase was the primary cause of the decompression sickness in the schedule employing the 95%He-5%O2 breathing mixture at depth. Hence, it would appear that although much higher tissue tensions in the slowest bodily compartment may be achieved by this method, the decompression prior to the surface interval must be handled with great care to avoid the occurrence of decompression sickness during the brief stay at surface pressure. In addition, the experience of the TEKTITE I tests indicate the hazard with regard to elevated tissue tensions in the faster compartments at this point. However, with proper management of these areas, the tests show that much higher levels of inert gas may be tolerated by the body in the slowest tissue half-time compartment without ill effect. Further, the greatly increased levels of inert gas upon arrival at sea-level pressure during the surface interval, strongly indicate that the primary factor in producing decompression sickness in the bubble growth factor as opposed to the degree of excess inert gas loading beyond the accepted safe levels.

As shown, the results indicate that much higher tissue tension levels can be attained in the slowest tissue compartment during a brief surface interval than the levels which are currently utilized in surface decompression procedures. In addition, the final water stop may be 10 to 20 feet below the accepted 40 foot stage allowing significant reductions of in-water decompression time prior to surfacing. In these tests, this has resulted in a reduction of water decompression time, from the initial 423 minutes in schedule "A", to 256 minutes in the final schedule. This provides a reduction of 40 percent water decompression time. Utilizing presently accepted limits with regard to permissible water decompression time or total exposure time for a diver in the water, application of this method can provide for practical increases in exposure time at depth, applied to present exposures within this limit it may be applied to significantly reduce the water decompression time and hence the time required for exposure to a hostile environment.

The research reported here has been supported under the office of Naval Research Contract #N00014-78-C-0147 with funds provided by the Naval Medical Research and Development Command.





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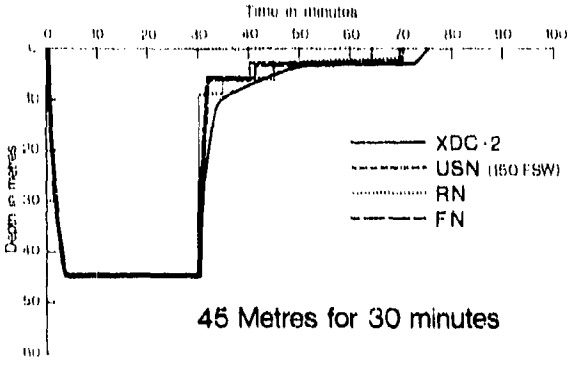
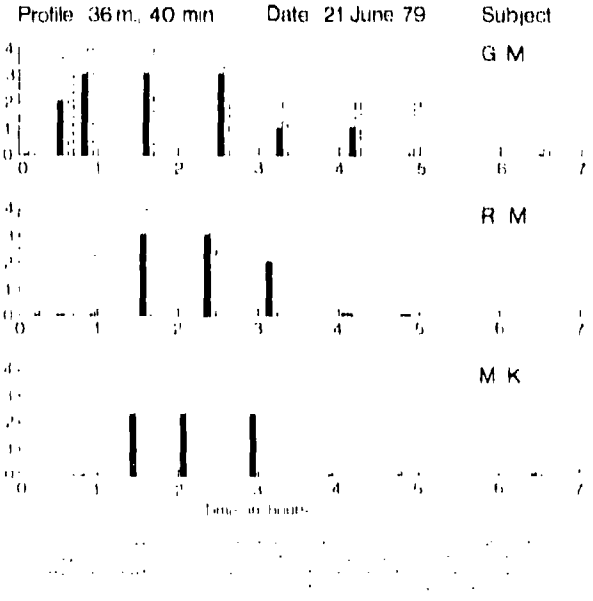
References will appear in PROCEEDINGS.
Table and Figures follow.

TABLE I

Maximum Bubble Grade Detected Over the Precordium, Neck or Head and Scalp - 2 Hour

Depth (msw)	Bottom Time (min)	No. of Man-Dives	No. of Divers with Maximum Bubble Grades					No. of Bends	Bubble Grade for Bends
			0	1	2	3	4		
Phase I									
0	60	12	1	2	5	1	0	0	
	90	12	2	2	2	5	0	1	2
	120	6	0	1	1	3	0	1	2
	240	6	1	0	2	0	0	0	
15	20	6	2	1	1	0	2	0	
	30	12	5	5	1	1	0	0	
	40	12	1	3	2	3	1	1	4
30	15	6	2	1	1	2	0	1	4
	20	12	4	1	3	3	1	3	4
	25	12	1	1	0	5	1	2	4 b
Phase II									
60	30	24		5	5	9	0	0	
75	20	6	5	1	0	1	1	3	
	25	6	4	0	1	0	0	0	
90	10	24	12	3	7	1	0	0	
	15	24	11	8	4	1	0	0	
100	10	24	2	1	7	7	7	0	





MONITORING BUBBLE FORMATION WITH AN INTEGRATING PULSE-ECHO ULTRASONIC METHOD, Daniels, Ian, Davies, John, Paton, W.D.M., and Smith, Eric M., Defence Research Department of Pharmacology, South Parks Road, Oxford, U.K.

Introduction

Bubble formation during or after decompression is generally accepted as the cause of decompression sickness. Historically this view is based on the facts that: (a) elevated inert gas tissue tensions are necessary for the occurrence of symptoms, (b) prompt recompression is therapeutic and (c) bubbles have been frequently observed post mortem. The observation of bubbles in vivo in blood and tissues has become increasingly important in the investigation of decompression sickness. Initially they were observed directly by surgical exposure of a variety of areas (Harvey, 1951; Buckley, Posey, Fever, Miller, Paton & Smith, 1961; Hall, Miller, Paton, Smith & Welch, 1963) but more recently non-invasive, indirect methods have been used. The most successful indirect method is the use of ultrasound and a number of different methods have been developed based either on transmission (Hartley, 1961; Powell, 1962; Walder, 1963) or reflection (Welsby, 1959; Franklin, Schlegel & Rushmer, 1961). The most widely used of these has been the Doppler technique which relies on a shift in frequency of the sound reflected from a moving target (e.g. Willis, Peterson & Karagiannes, 1968; Spencer & Campbell, 1969). Originally, transducers which were surgically implanted onto blood vessels were used but more recently transcutaneous devices have been developed which are completely non-invasive (Willis et al, 1969; Evans & Walder, 1969; Spencer, Simons & Clarke, 1971). Doppler recording has almost exclusively been applied to a "pre-cordial" position with the object of detecting bubbles in the total venous return to the heart (Spencer & Clarke, 1972). However a number of limitations apply to this method. Firstly, the fact that only moving bubbles can be observed has resulted in very poor correlations between the number of bubbles detected and the incidence of decompression sickness after certain types of decompressions, particularly air and saturation dives. Secondly, the localization of the method to the pre-cordial region precludes the examination of the distribution of bubbles to different areas. Finally, there are considerable problems associated with the interpretation of Doppler signals. This has been somewhat improved by the use of advanced signal processing techniques (Belcher, Ahrenberg & Lytle, 1974; Hissam, 1975) although the fundamental limitations of movement and location remain.

A potentially more powerful method of Pulse-Echo Ultrasonic Imaging was suggested by Mackay (1963) and subsequently demonstrated by Ma Lay & Robertson (1971, 1973, 1974). This method is technically more difficult than the Doppler method but it allows all types of bubble, moving and stationary, to be visualised in relation to a cross-section of the tissue area under study. Consequently bubbles can be spatially located with reference to the anatomical features and their temporal distribution with respect to the decompression profile can be plotted. A high resolution pulse echo ultrasonic imaging system has been developed in Oxford (Daniels, Paton & Smith, 1979; Beck, Daniels, Paton & Smith, 1980) and a number of different types of dives have been studied successfully (Daniels, Paton & Smith, 1979; Daniels, Paton & Smith, 1979; Daniels, Paton & Smith, J. Physiol., in press). However, although this technique has been shown to be capable of observing the complex patterns of bubble formation, the procedure of analysing the recorded images is extremely time consuming and prevents any immediate information being available. Such information is likely to be important in managing decompression schedules, in particular therapeutic schedules. To overcome this difficulty a new approach has been studied which we have called the Integrating Pulse-Echo Imaging method (Daniels, 1978).

This technique involves the digitalisation of the echoes which go to make up each image. The sum of the total number of echoes is then displayed in a manner which allows small changes in the number of echoes, due to bubbles, to be observed. This is done after subtracting a fixed quantity representative of the background. A baseline is thus set which is equivalent to the average number of echoes received from the various tissue interfaces. Any bubble-type echoes which are then detected will produce an increase in the sound reflected above this baseline, either transiently if due to their movement through the plane of section or persistently if stationary. A number of experiments have been done to test the equipment's response to such bubbles. These bubbles, 10 to 100 μm in diameter upwards, and that the subject consistently developed a persistent silent bubble envelope over the precordium, were introduced into a pre-cordial vein by passing the injection needle through the skin and veins and then withdrawing it, leaving the injection cannula in the vein. Further small experiments have been done, as expected, after very brief exposure to pure oxygen at 3 bar absolute, the bubbles and noise are displayed on the screen and can be recorded for future analysis if necessary.

Results

A number of preliminary experiments have been completed using the [illegible] in accordance with the existing ethical committee [illegible]. These experiments have all used one method of [illegible] pre-cordial bubbles. Briefly, [illegible] was performed on a [illegible] and then applied to the [illegible]. In all cases the left hand [illegible] was [illegible] and was done the last, and control [illegible] were also [illegible]. Three different [illegible] were used, [illegible] at [illegible] and then [illegible]. Throughout all the experiments the [illegible] was [illegible] and the sound [illegible] the [illegible] was [illegible]. The [illegible] and [illegible] especially, [illegible] were effective [illegible] test. Recompression was applied at [illegible] after the test [illegible] to [illegible] the [illegible] of the [illegible] and [illegible] the [illegible] of the [illegible] and [illegible] the [illegible].

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TABLE 1

	5.5 bar	6.9 bar	8.1 bar
Incidence of decompression sickness	40% (4/10)	50% (14/28)	100% (6/6)
Time for averaged integrator output to rise 3 standard deviations above baseline.	15 min	8 min	6 min
Mean time to first ecg abnormality ± S.E.	24.4 ± 5 min	11.5 ± 1.2 min	6 ± 0.5 min
Mean count number at time of first ecg abnormality ± S.E.	841 ± 341	526 ± 1.5	141 ± 91

Discussion

From Table 1 it can be seen that the separation in time of a significant rise in the integrator output and the onset of decompression sickness, as judged by ecg, increases with decreasing severity of decompression. Furthermore the count is greater at the time of onset of decompression sickness for the less severe dives. These effects appear to be related to the relative proportions of stationary and moving bubles as well as to the total number. That is, after the less severe dives the bubble formation observed is predominantly stationary and in consequence it is observed to build up to a considerable density before serious central embolism occurs and is reflected in the alteration in the ecg. After the more severe decompressions, however, in the first place the total number of bubbles formed in the whole body is expected to be greater and secondly in the peripheral area studied a greater proportion of transient bubbles are observed which are presumed free to move centrally. This then may account for the rapid onset of cardiac arrhythmias due to central embolism at a relatively lower peripheral echo density.

It was found that for recompression to be therapeutic it had to be applied very shortly after the onset of cardiac arrhythmias although even when not therapeutic the echo count in the leg could always be returned to the baseline level by the application of pressure. It was always observed that decompression after a recompression gave rise to bubble formation within an extremely short time after commencing the decompression and frequently with no detectable latent period if the hold at pressure had been for a short time.

As a further check on the sensitivit of the integrating method a frame by frame comparison of the echo count from the integrator and the number of echoes per scan due to bubbles, revealed by a complete analysis of the ultrasonic images, has been done. One such comparison is shown in Fig. 2. It can be seen that the general shape of the two types of trace is the same with only a little sensitivity being lost by the integrator in the initial stages, where the response from only 1 or 2 transient bubbles is masked by the baseline variation.

In view of these encouraging results a new machine is being developed specifically for use with large animal and human subjects. The present system, being an adjunct to the existing high resolution imaging system, is only suitable for use with small animals. The new machine has a lower operating frequency (5 MHz as opposed to 8 MHz) and instead of a single, mechanically scanned transducer, there is a transducer array of 64 elements. Scanning is achieved by electronically switching between these elements at a rate which will give up to 40 frames.s^{-1}. This high scanning frequency allows a further stage of signal processing, whereby a continuous averaging of the count number can be applied, whilst still maintaining an output each second. This will reduce the baseline variability, due to movement, and should also allow the study of moving structures. With this new system we expect to be able to study not only the distribution in time of bubbles and the relative proportions of moving and stationary bubbles but the distribution of bubble formation over a variety of anatomical regions.

Acknowledgements

The work was supported by the Ministry of Defence, under contract No. [illegible]/AMIE 11.

References will appear in PROCEEDINGS.
Figures follow.

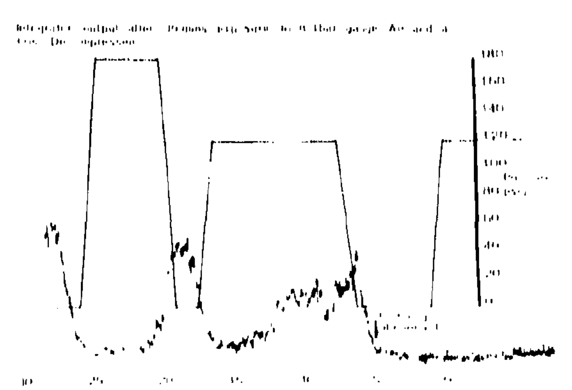

Fig. 1. [illegible]

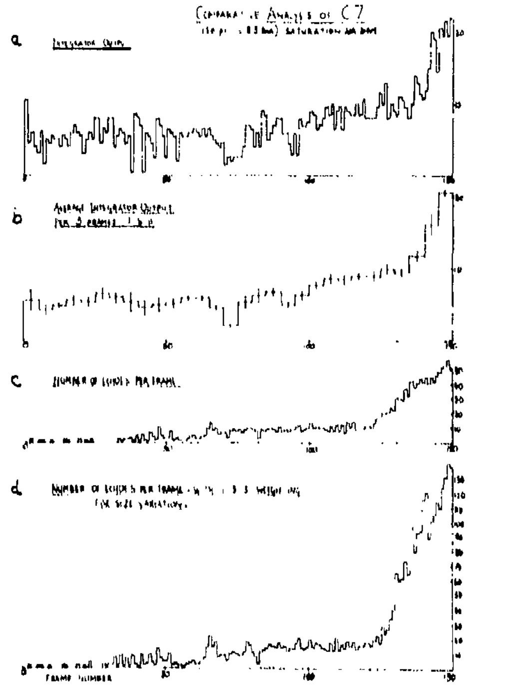

Fig. 2. Comparison of integrator output with number of bubbles per frame identified from a full spatial and temporal analysis of the ultrasound images. In all cases elapsed time after the start of the decompression is shown as frame number (25 frames.min^{-1}). (a) Integrator output, vertical scale in mm above baseline. (b) Integrator output averaged over 5 frame intervals, vertical scale in mm above baseline. (c) Number of echoes identified as due to bubbles from the full analysis of each frame of the ultrasound images. (d) Number of echoes identified as due to bubbles in each frame with weighting factors 1, 3, 5 according to three categories of bubble size, <100 µm diameter, 100-500 µm diameter, >500 µm diameter respectively.

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MIGRATION OF LUNG SURFACTANT TO PULMONARY AIR EMBOLI. B.A. Hills and D.D. Butler, Department of Physiology, University of Texas Medical Branch, Galveston, Texas.

During many decompressions, bubbles can be detected in venous blood (Spencer & Campbell, 1968) and yet they usually remain asymptomatic. This is consistent with other studies which show that the venous system is most tolerant to extra vascular gas and, in dogs at least, has been known to tolerate up to one litre of air (Durant et al., 1947). By comparison, 0.5 ml of air injected into the arterial system has proven fatal (Kent & Bladon, 1942). Hence it is most important to know how effective the lung can be as a bubble filter and what factors can facilitate the release of trapped bubbles into the arterial system where they are so much more dangerous and in evidence physiologically (Butler & Hills, 1979).

Appreciating the great importance of this question several decades ago, Walder (1948) performed a most relevant calculation based upon the Laplace equation in which he estimated that the pulmonary arterial pressure would need to reach at least [illegible] in order to push a bubble through a pulmonary capillary and into the arterial system. However this estimated [illegible] pressure could be appreciably smaller if the trapped bubble were to become coated with any of the surfactants known to be present in the lung. [illegible]

Experimental

Micro-bubbles were produced in a medium consisting of 50% heparinised Ringer's solution plus 50% plasma from the same animal. This was infused into the right ventricle over a period of 5 minutes, the heparin dose amounting to 30,000 units. After a further 60 minutes with ventilation controlled by a Harvard ventilator, the lungs of the sacrificed animal were back-flushed with Ringer's solution via a cannula inserted into the left ventricle through an incision in the left atrial appendage. The main pulmonary arterial trunk was cannulated for collection of the displaced pulmonary capillary blood and flushing fluid as successive 75 ml. aliquots.

12 ml. of each aliquot was placed in a Langmuir trough with ample opportunity for the surface to recruit any surfactant from the hypophase in the trough. This was evidenced by the fifth loop being essentially retraced in subsequent cycles. Thus the form of the fifth loop can be regarded as a physical assay of the presence of substances with surface active properties.

Results

All three samples from each dog showed surface tension/surface area loops which were characteristic of dipalmitoyl lecithin spread on the surface of serum (Barrow & Hills, 1980). Surface tensions were significantly lower than control values. For maximum film compression on the fifth cycle, for example, mean surface tension for six dogs was 31.92 dyne/cm for the first aliquot extracted, 29.25 for the second and 27.17 for the third by comparison with 35.67 dyne/cm for the control sample taken prior to embolisation. Statistical significance exceeds 99%.

Discussion

The significant drop in surface tension with successive back-flushings of the lungs is strongly suggestive of recruitment of surface active substances on to the surface of the bubbles trapped in the pulmonary circulation. The surface tension probably decreases with continued flushing due to the probable contribution to the aliquot of more blood which had been static in embolised capillaries. The shape of the surface tension/surface area loop is so characteristic of those produced when dipalmitoyl lecithin (DPL) is deposited on to the surface of serum (Barrow & Hills, 1980) to make it highly likely that DPL or some similar lung surfactant reaches the blood-bubble interface. There will obviously be some modification in surface properties simply due to deposition of protein at the surface and its well known denaturation by air (Schmidt, 1914). However changes of the same magnitude are not induced when control samples are exposed to air for long periods, so it would seem reasonable to conclude that the effect observed is primarily attributable to surfactants of the type normally associated with the air-alveolar interface.

This conclusion would seem reasonable since, from basic thermodynamic considerations, surfactants would tend to locate at an air-aqueous interface whether it is located within the pulmonary vessels or upon the aqueous hypophase lining the alveoli. These can be less than a micron apart in some places; although permeability of the endothelium to surfactants produced by Type II cells is also a factor and one which is most complex.

The permeability of the capillary wall to proteins leaving the vessel has been studied most extensively, but nothing could be found in the literature concerning the ability of macromolecules such as the phospho-lipids to enter vessels. Permeability would tend to be increased by occlusion. This would deprive the vessel walls of nutrients and alter vascular tone even though ventilation was providing adequate oxygen over the short diffusion distances separating occluded vessels from the nearest alveoli.

Whatever the theoretical limitations, the results offer quite convincing evidence of surfactant entering pulmonary vessels occluded by bubbles. It is difficult to estimate the extent of surfactant recruitment at the bubble surface, but the true reduction in surface tension is likely to be far greater than the 4-8 dyne/cm quoted in the results. This arises from the fact that the surfactant would be greatly diluted by the flushing fluid whose surface tension is in excess of 70 dyne/cm.

Hence the surfactant concentration could be very high in certain bubbles. This would certainly tend to facilitate their release into the arterial system and, moreover, the rate of migration of surfactant molecules to the bubble surface may be the factor needed to explain the delay of 20-30 mins. in the appearance of arterial bubbles after over-loading the venous system with gas either as a bolus (Spencer & Oyama, 1971) or as a shower of micro-bubbles (Butler & Hills, 1979).

If sufficient time has elapsed before such bubbles are recompressed (as may occur in a delayed treatment of a limb "bend"), then the accumulated surfactant could cause a dramatic reduction in surface tension with the decrease in surface area. Hence migration of lung surfactant to trapped pulmonary bubbles could be offered as a possible explanation for arterial embolisation (Gait et al., 1975) and the serious neurologic symptoms which have been known to occur upon recompression (Elliott & Barnard, 1965) - even of a previously asymptomatic diver.

References will appear in PROCEEDINGS.

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PREVENTION OF DECOMPRESSION SICKNESS BY COMBINED CYPROHEPTADINE-AMPHETAMINE TREATMENT. C. Chryssanthou, Louisa Rodriguez and Peter Branden.
Departments of Pathology, Beth Israel Medical Center, New York, N.Y. 10003 and Mount Sinai School of Medicine of the City University of New York, N.Y. 10029

Studies conducted in our laboratories in the last 15 years strongly suggest that smooth muscle stimulating factors are implicated in the pathogenesis of decompression sickness (DS). Compounds which combine activities against histamine, bradykinin and 5-hydroxytryptamine such as dimethothiazine and cyproheptadine [illegible], prevented development of DS in mice, hamsters and dogs when administered prior to compression (Chryssanthou et al., Aerospace Med. 45:279-282, 1974; Fructus et al., Bulletin Medsubhyp, No. 1, pp 87-96, 1976). Since all substances with [illegible] activity produce sedation, the question is raised whether this effect plays a role in the prevention of DS by the above mentioned compounds.

The present communication deals with experiments designed to determine whether cyproheptadine maintains the DS preventive effect when its sedative action is neutralized or counteracted. Elucidation of this question is important not only from a theoretical point of view but also for practical purposes since it would not be advisable to administer drugs which cause drowsiness to divers or compressed air workers.

In the first series of experiments, various dose combinations of amphetamine and cyproheptadine were administered to mice to determine the minimum amount of the former drug which antagonizes the sedative effect of the latter. The degree of sedation was measured in terms of reduction of spontaneous locomotor activity, using the revolving wheel cage method. The experiments were conducted in a dark room with a temperature between [illegible]. Each animal acted as its own control. Baseline (control) levels were established by taking 6 hour counts of activity every other day for six days. [illegible] days were allowed to elapse before [illegible] different treatment.

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INNER EAR INJURIES IN DIVING — DIFFERENTIAL DIAGNOSIS OF INNER EAR DECOMPRESSION SICKNESS AND INNER EAR BAROTRAUMA.

[illegible] C. Farmer, Jr., Duke University Medical Center, Durham, North Carolina, U.S.A.

Persistent inner ear injuries in diving have been noted more frequently over the past decade and have been described in several publications.[1-5] These injuries have been classified as follows: (1) inner ear barotrauma and labyrinthine window rupture, (2) inner ear injuries occurring at stable deep depths, (3) inner ear decompression sickness or air emboli, and (4) sensorineural deafness related to high background noise during diving conditions.

Inner ear injuries occurring at stable deep depths have been documented on only one occasion and occurred shortly after the beginning of breathing a different inert gas by mask while the chamber remained at a stable pressure with the original helium-oxygen atmosphere.[6] Injuries related to high background noise have been suggested.[7] The exact frequency of these injuries is not known.

Most divers who suffer inner ear injuries related to diving have inner ear barotrauma and possible labyrinthine window rupture or inner ear decompression sickness. In some instances the differential diagnosis of inner ear barotrauma and inner ear decompression sickness is difficult. Some of the related dives are air exposures close to the no decompression limits. Divers occasionally do not know when during the dive the symptoms began. Also, signs of middle ear barotrauma suggesting the possibility of inner ear barotrauma or signs suggesting decompression sickness may not be present. An accurate diagnosis is important, for the likely mechanisms of such injuries strongly suggest that recompression would be an inappropriate treatment for inner ear barotrauma and possible labyrinthine window rupture, and unnecessary delay in recompression therapy for those cases of inner ear injuries resulting from decompression sickness can increase the likelihood of permanent deficits.[2] Therefore, the following review of current treatment recommendations and the differential diagnosis of these two types of inner ear injuries in diving is presented.

Inner Ear Barotrauma

Inner ear injuries occurring after relatively shallow diving or with symptoms onset during the compression phase in deeper diving have been termed inner ear barotrauma. Such injuries were first documented and named by Freeman and Edmonds in 1972[?] and have been related to labyrinthine window ruptures.[?] Implosive and explosive mechanisms for such ruptures have been postulated. The explosive mechanism suggests that with inadequate middle ear clearing during descent, intracochlear pressure as well as ambient pressure increases relative to middle ear pressure. With straining or a Valsalva maneuver a further increase in this pressure differential occurs with rupture of the round window or oval window membrane into the middle ear and a subsequent perilymph fistula. The implosive mechanism suggests that with a sudden Valsalva maneuver which does result in middle ear ventilation, the rapid increase in middle ear pressure results in rupture of the round window or oval window into the intracochlear space. Simmons has suggested that such pressure changes may also result in membrane breaks within the inner ear.[8] Which of these mechanisms is more often the cause of inner ear injuries during the compression phase of diving as well as the overall frequency of such injuries has not yet been established.

Treatment principles for inner ear barotrauma include the following:

1. The best treatment is prevention. This includes the avoidance of descent or compression when middle ear pressure equilibration problems exist and the avoidance of forceful Valsalva maneuvers at depth. Recompression therapy should be avoided since such therapy exposes the diver to conditions similar to those which resulted in the injury.
2. A complete otologic evaluation by a knowledgeable physician should be [illegible].
3. Until such an evaluation can be obtained and appropriate treatment [illegible] [illegible] should be avoided. This includes the avoidance of coughing, straining or [illegible]. Bed rest with head elevation is indicated.
4. Drugs [illegible] increase inner ear blood flow are generally not effective and may result [illegible] [illegible] shunting of the [illegible] circulation [illegible]. Anticoagulants should be avoided because of possible hemorrhage from traumatized tissues.
5. The need for immediate exploratory tympanotomy is controversial. Some [illegible] have advocated [illegible] exploration [illegible] [illegible]. Others have suggested reserving surgery for those who do not improve after [illegible] of bed rest with head elevation. Singleton et al.[?] [illegible] labyrinthine window fistula [illegible] [illegible] [illegible]

[illegible]

[illegible]

1. Vertigo, nausea, vomiting, tinnitus, and/or hearing loss beginning during or shortly after the decompression phase of dives in which decompression sickness is possible should be considered forms of decompression sickness and recompressed promptly. Divers who experience such symptoms during or shortly after a switch to an air environment during decompression from a deep helium-oxygen exposure should be switched back to the presymptom helium-oxygen atmosphere and recompressed promptly.
2. The optimum treatment depth or depth of recompression has still not been established. Theoretically, the optimum recompression depth is the lesser of the depth of relief or the bottom depth. However, labyrinthine trauma resulting from inner ear bubble formation may result in hemorrhage or structural deformities and prompt relief will not be seen even though an adequate depth of recompression to drive bubbles back into solution is achieved. Also, returning to the bottom depth in some situations may be hazardous or impractical. Therefore, we arbitrarily suggest that the optimum treatment depth in these situations should be at least three atmospheres deeper than the depth of symptom onset. Whether or not a lesser treatment depth will suffice has not been established.
3. Drugs which supposedly increase intracranial and inner ear blood flow are generally not effective and may result in shunting of blood to the periphery. Also, if hemorrhage has occurred, anticoagulants may result in additional intracochlear bleeding. Thus, these agents are considered potentially harmful and not recommended. Diazepam, 5-10 mg given intramuscularly has been noted to result in significant relief of vertigo, nausea and vomiting during otologic decompression sickness. This agent can suppress the nystagmus accompanying such injuries and thus mask a sign of optimum treatment. However, in many cases the symptoms are so severe that relief is desired. Fluid replacement is also indicated.

Differential Diagnosis Of Inner Ear Barotrauma And Inner Ear Decompression Sickness

The most important factors in accurately differentiating inner ear barotrauma and inner ear decompression sickness include the knowledge that such injuries can occur in diving, familiarity with the likely pathophysiological mechanisms, and the obtaining of an accurate history and physical examination. Other factors to be considered in this differential diagnosis include the following:

1. The time of symptom onset is important. Divers who indicate that their symptoms started during compression are certainly more likely to be suffering from inner ear barotrauma, whereas, divers whose symptoms start during or shortly after decompression are more likely to be suffering from inner ear decompression sickness.
2. Knowledge of the dive profile is important. Symptoms of inner ear dysfunction associated with shallow dives which do not approach the no decompression limits and in which decompression sickness is unlikely should not be suspected of resulting from decompression sickness. Inner ear barotrauma is certainly more likely in these instances. Also, dives associated with rapid descents are more likely to result in inadequate middle ear pressure equilibration during compression, especially in inexperienced divers, and subsequent inner ear barotrauma.
3. The presence or absence of associated symptoms should be noted. Divers experiencing ear pain or blockage or fullness during compression are more likely to develop inner ear barotrauma. Freeman notes that the common factor in his series of labyrinthine window ruptures in divers is difficulty in clearing the ears when descending.[?] However, divers who note symptoms suggestive of decompression sickness involving other organs or tissues should certainly be suspected of having their inner ear symptoms secondary to bubble formation of the inner ear structures and/or vasculature.
4. The presence or absence of associated physical findings are important. Divers who exhibit physical findings compatible with middle ear barotrauma, such as [illegible], hemorrhages, or ruptured ear drums should certainly be suspected of having a possible labyrinthine window rupture as an explanation of their inner ear symptoms. Divers who exhibit other types of decompression sickness, such as [illegible] neurological deficits, should be suspected of having their inner ear symptoms on the basis of inner ear decompression sickness.

An accurate differential diagnosis can be difficult in those cases in which inner ear symptoms are first noted after or [illegible] decompression dives [illegible] to the no decompression limits or deeper dives in which apparently adequate decompression schedules were followed and in which the divers cannot recall the time of symptom onset. [illegible] [illegible] associated signs and symptoms should be considered. Divers who have associated symptoms and/or signs of middle ear barotrauma should be suspected of having inner ear barotrauma and possible labyrinthine window rupture. For divers without these signs and/or symptoms, additional factors to be considered would be the presence or absence of other types of symptoms suggestive of decompression sickness or air emboli. If these are absent and if none of the above factors are helpful in arriving at a diagnosis, the overall type of diving should be considered. [illegible] associated with air diving in which proper decompression procedures were followed are more likely to represent inner ear barotrauma [illegible] decompression sickness [illegible] more common with deeper [illegible]. Fortunately, [illegible] adequate determination of the time of symptom onset, dive profile, and the presence or absence of other types of symptoms [illegible] accurate diagnosis [illegible]

All [illegible] inner ear injury during diving should [illegible] complete [illegible] otologic evaluation after treatment. [illegible] [illegible] symptom relief has occurred. Individuals who exhibit permanent inner ear [illegible] [illegible] diving [illegible] [illegible] to result in [illegible] permanent [illegible]

[illegible]

[illegible]

MECHANISMS OF AURAL BAROTRAUMA.
J. Miller, A. Axelsson, D. McPherson, and W. Potter. Department of Otolaryngology, University of Washington, Seattle, Washington, U.S.A.

Middle ear (M.E.) changes with barotrauma include development of effusions, vascular hemorrhage, tympanic membrane perforations and disarticulation of the ossicles (Fields, 1958; Goodhill, 1971; Edmonds & Thomas, 1972; Goodhill et al, 1973; Beasly, 1974; Compere, 1974). Suggested inner ear changes have included membraneous labyrinth distortion and rupture, round window perforation, and hemorrhage (Vail, 1929; Simmons, 1968; Farmer, 1977; Freeman & Edmonds, 1972; Harker et al, 1974). The majority of these suggested and observed changes derive from observations in man. On the basis of animal studies, a number of them, including inner ear changes, have been confirmed to some extent (McCormick et al, 1973; Lamkin et al, 1974). For the most part these animal investigations have been performed under conditions of whole-body hyperbarotrauma or local static M.E. pressure changes. We have initiated studies to evaluate the effects of phasic M.E. pressure changes. The program is aimed at evaluating the structural and physiological mechanism underlying phasic barotrauma. The studies to be reported will concern the chronic effects on middle and inner ear structures of phasic pressure change. We will also describe acute changes in middle and inner ear structures and associated changes in M.E. transmission and inner ear electrophysiological response immediately following phasic M.E. pressure change.

Methods

A total of 94 guinea pigs were used in this investigation. In each animal one ear was randomly designated "control" the other "experimental". Following the exposure of the bulla of both ears, the experimental ear was exposed to a positive or negative phasic pressure change, of rapid rise/fall times and 1-2 seconds in duration, ranging in magnitude from 1,000 to 6,000 mm H_2O. Following pressure exposure in 20 animals, the incisions were closed and the subjects treated with antibiotics. These animals were sacrificed following three week survival for anatomical study. Seventy-four animals were used in acute studies of immediate structural (56) and physiological (18) changes.

Histological study included evaluation of drum and M.E. structures with the aid of the operating microscope, followed by light and phase-contrast microscopic study of inner ear sensory neuro-epithelium and vasculature. Histological preparation of inner ear structures was based upon soft surface preparation procedures (Axelsson et al, 1974, 1975) in 52 animals and serial paramodiolar sectioning of collodin embedded material in 22 animals. Surface prepared tissue was stained with osmic acid. Serially sectioned material was stained with H & E. Physiological study included evaluation of phasic M.E. pressure induced changes in absolute and relative M.E. input impedance and a measure of cochlear impedance derived from cochlear microphonic activity (after Møller, 1965) for frequencies from 200 to 13,000 Hz. (See McPherson et al, 1976).

RESULTS

Anatomical Observations: Primary M.E. structural changes in both acute and chronic animals included distortion and perforation of the tympanic membrane and effusions and hemorrhage. These changes were correlated with direction and magnitude of applied pressure. Tympanic membrane perforations and serous and serosanguinous effusions were most frequent with high negative pressures. Hemorrhagic fluid and perivascular hemorrhages were most common with high negative pressures in acute animals and more frequently observed with high positive pressure in chronic animals. These findings were influenced by the presence of infection in approximately 50% of the chronic material. Hemorrhage was observed in 52% of the acute and 5?% of the chronic experimental ears. In the absence of infection hemorrhage occurrence in chronic ears was 20%.

Round window perforations were observed in over 50% of the chronic animals exposed to high (6000 mm H_2O) pressure of either direction. Each of these ears exhibited M.E. infection. Round window perforations were not observed in any acute animal nor in control infected ears of chronic animals.

No instance of endolymphatic hemorrhage was observed. In acute material 9% exhibited experimentally induced perilymphatic hemorrhage, 86% were related to negative pressure. In chronic animals, after 3 weeks survival, perilymphatic hemorrhage was observed in 58% of the material--78% of the ears were exposed to positive pressure and 40% was observed in ears exposed to negative pressure. This relationship of perilymphatic hemorrhage in chronic ears to positive pressure is reduced in the absence of infection.

Hydrops, sagging and rupture of the vestibular membrane was occasionally observed in paramodiolar serially sectioned material. They did not appear to be related to M.E. pressure direction or magnitude and were observed also in control ears.

Physiological Observations: Phasic pressure change exhibited a variable effect on both M.E. input impedance and cochlear impedance. Both increases and decreases in impedance were observed for specific frequencies in each measure. In general M.E. input impedance changed little for low frequency stimuli (for frequencies below the resonant frequency of the M.E.). Greater changes were observed for high frequency stimuli, with positive pressure exhibiting a somewhat greater influence than negative pressure. Typically, the change was to decrease input impedance to the M.E. Equivalent cochlear impedance was decreased across frequency. The influence of phasic pressure was greater on equivalent cochlear impedance than on M.E. impedance. Also distortion products in the cochlear response were increased following the phasic pressure exposure.

Discussion

Observations in both acute and chronic guinea pigs of phasic M.E. pressure change are consistent with observations in man. [illegible]

Functional changes are consistent with the varied middle and inner ear pathology observed. They indicate that ossicular changes are produced by the phasic pressure change. (Our anatomical methods in the middle ear were not sensitive to small changes in the ossicles and their articulation of potential functional significance.) The data are consistent with a conductive loss due to partial disarticulation (decoupling of the cochlea) and a cochlear loss due to mechanical changes, perhaps induced, by inner ear fluid changes (hemorrhage).

References will appear in PROCEEDINGS.

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WATER-BORNE MICROBIAL PATHOGENS AND DIVING ENVIRONMENTS

O.P. Daily, S.W. Joseph, J.D. Gillmore, R.J. Seidler, D.A. Allen, and R.R. Colwell

Naval Medical Research Institute, Bethesda, Maryland, USA; Department of Microbiology, Oregon State University, Corvallis, Oregon, USA; Department of Microbiology, University of Maryland, College Park, Maryland, USA

Public health specialists have long recognized health risks, such as cholera, shigellosis and typhoid fever, associated with contaminated drinking water and, more recently, have become concerned about exposure to polluted waters via water related recreation and/or occupation [illegible]. Global documentation of increasingly higher levels of pollution in coastal waters has prompted various agencies, such as the United Nations, National Oceanic and Atmospheric Administration (NOAA), Environmental Protection Agency (EPA) and the U.S. Navy, to expand efforts designed to assess and control public health problems associated with polluted waters.

Since a significant number of commercial and military diving operations are conducted in heavily polluted waters, notably major harbor areas, there is a potential for increased health hazards because of exposure to water borne microbial pathogens. In the past decade, the presence of a number of water borne pathogens in polluted waters has been established [illegible]. Documented infections caused by members of the genus Vibrio, especially V. parahaemolyticus and V. alginolyticus, lactose positive vibrios and non-01 vibrios, Campylobacter, Aeromonas, Legionella, Pseudomonas and Yersinia spp., in addition to previously established pathogens, including Escherichia coli and Klebsiella, Salmonella and Shigella spp., have been reported to occur following contact with polluted waters from various locations throughout the world [illegible].

We are currently studying water borne pathogens isolated from locations where diving operations are ongoing: the Anacostia River, Washington, D.C., site of the U.S. Naval School, Diving and Salvage, and the New York Bight, site of NOAA diving operations.

Our first encounter with a water-borne infection resulted when a U.S. Navy diver suffered a leg puncture wound while conducting night SCUBA operations in the Anacostia River. The diver developed an infection subsequently shown to have been caused by two species of Aeromonas, A. hydrophila and A. sobria. The result of this incident and the bacteriological follow up have been reported [illegible].

[illegible]

were taken with saline wetted sterile swabs, placed in 2 ml of sterile saline, immediately transported to the laboratory, inoculated onto an appropriate medium, incubated for 24 hours at 37°C and Aeromonas spp. were enumerated and isolated for further study. Figure 1 shows the results of two such surveys. The first survey, conducted in August, 1978, when the Aeromonas counts were 300/ml and the water temperature was 28°C, indicated that approximately 90% of the divers' ears and masks were contaminated after exposure to the polluted water. Approximately 7% colonization in noses and 27% in throats was recorded. The second survey, conducted in October, 1978, when the Aeromonas counts in the water were 50/ml and the temperature was 13°C, showed generally lower colonization rates, except for the ear samples, which remained at about 90%.

The results of these experiments indicated it was, indeed, possible for divers to become colonized with Aeromonas spp., even when the numbers of Aeromonas in the water were as low as 50/ml. The total number of Aeromonas and coliforms isolated from each positive swab averaged 130. Exposure to polluted water for a time as brief as 30 minutes significantly altered the composition of the skin microflora, to an extent resembling that of the diving environment. While it is not known what number of Aeromonas are required to establish a wound infection or a gastrointestinal disorder, a short period of exposure may facilitate pathogenesis, i.e. attachment of microorganisms to the skin surface, as a first step in the disease process.

Conclusions. To date results of our studies of water-borne pathogens indicate that polluted waters, i.e. water containing fecal coliforms in numbers greater than 1 per 100 ml or total coliforms in numbers greater than 200 per 100 ml, contain potentially pathogenic species in significant numbers (Table 1). Several of these organisms produce cytotoxins and/or enterotoxins. Exposure to these organisms has been shown, in reports from our laboratories and elsewhere, to be capable of causing wound infections and/or gastrointestinal disease. Thus, it can be concluded that presence of these organisms poses an additional health risk associated with diving operations in polluted waters.

Diving medical officers should recognize the potential health problems presented by water-borne pathogens and provide clinical documentation by keeping accurate records involving any infectious disease occurrences within the diving community. Without proper documentation it is difficult to determine the magnitude of such risks. In addition, because some of the water-borne microorganisms are only now becoming recognized as true pathogens, it is imperative that diving medical officers or attending physicians involved in treatment of infectious disease problems of divers, become familiar with the unusual, as well as familiar pathogens present in polluted waters.

Acknowledgments. This work was performed under Naval Medical Research and Development Command Work Unit number ZF51524009.0057 and National Oceanic and Atmospheric Administration contract 01-8-M01-2027.

The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

References will appear in PROCEEDINGS.
Table and Figure follows.

Table 1

Microbial Parameters and Potential Pathogens Isolated from Polluted Waters

Isolates	Concentrations per 100 ml water: Anacostia River		New York Bight	
Total Viable Count	5 x 10[6]	2 x 10[7]	100	76,000
Total Coliforms	4,000	20,000	[illegible]	[illegible]
Total Fecal Coliforms	100	6,000	0	[illegible]
Total Anaerobic Count	[illegible]	10,000	[illegible]	30,000
Aeromonas spp.	1,000	50,000	0	[illegible]
Bacteroides spp.	[illegible]			
Clostridium spp.				
Enterobacter spp.				
Escherichia coli				
Klebsiella spp.				
Salmonella spp.				
Vibrio cholerae				[illegible]
Vibrio parahaemolyticus				
Vibrio [illegible]	[illegible]			

[illegible]

[illegible]

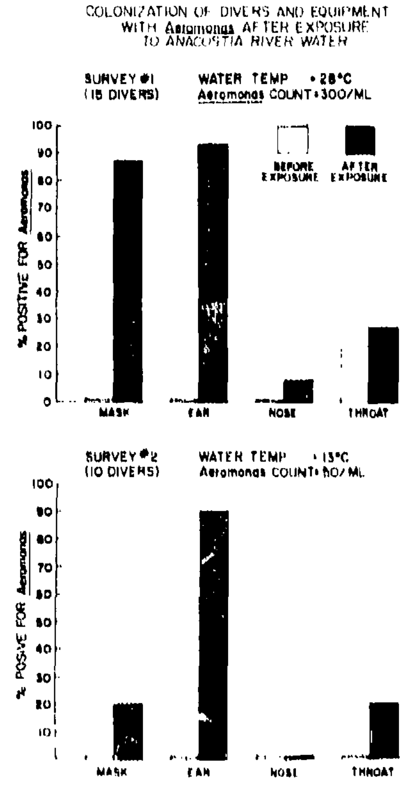

Figure 1. Colonization of divers and equipment with Aeromonas after exposure to Anacostia River water.

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MANAGEMENT OF HEALTH HAZARDS ASSOCIATED WITH THE SALVAGE OF TOXIC CHEMICALS USING A SATURATION DIVING TECHNIQUE. A. Marroni [illegible], T. Gelbring [illegible] and D. Zannini [illegible].

[illegible] Head, Industrial Medicine Dept., [illegible] Milan
[illegible] Medical Officer, The Associated Octel Company Ltd., London
[illegible] Director, Hyperbaric Medicine [illegible], Institute of Occupational Medicine, University of Genova

On [illegible] July [illegible] a collision occurred between the M.V. CAVTAT and the steamship LADY RITA. The M.V. CAVTAT was badly damaged amidships, and sank in [illegible] metres of water. The M.V. CAVTAT was carrying a general cargo but of special interest were [illegible] drums of antiknock compound.
The drums contained tetramethyl lead (TML) and tetraethyl lead (TEL) or a mixture of the two compounds.
This paper deals with the problems of salvage of toxic compounds from a depth of [illegible] metres and the various safety and medical recommendations which were made to ensure that the operation could be successfully completed.

TOXIC EFFECT OF LEAD ALKYL COMPOUNDS

[illegible]

DIVING PROCEDURE

Saturation diving techniques were adopted for the task. The 15 divers employed on this task spent 1235 hours working on the Gattat wreck and performed a total of 247 no-decompression saturation excursion dives of an average duration of 5 hours each.

Lead alkyl HV is normally considered to be 100 mg Pb_3 of gas for an exposure of 8 working hours as saturation divers work and live in a confined habitat 24 hours a day, the HV should be, in this case, 2.5 mg Pb_3 of gas. This quantity would correspond to the evaporation of a lead alkyl droplet of a radius less than 0.01 millimetres. Considering this and the total lack of experience about toxic effects of lead alkyl under increased hydrostatic pressure conditions, and the extreme difficulty to exclude the possibility that such a small quantity of lead alkyl compound was not taken into the habitat, we considered the limit of 20 mg Pb_3 an emergency threshold and aimed for the total absence of lead alkyl vapour in the habitat atmosphere. Therefore, the divers were all submitted to a step by step decontamination procedure which assumed the diver, the bell and the transfer lock were contaminated until the contrary was proven by testing.

To prevent skin absorption the diver was provided with a PVC suit and gloves. In addition to his normal hot water diving suit, when he returned to the diving bell he removed the PVC suit and gloves and the flippers which were left in a basket outside the bell, or abandoned.

The umbilical was designed to be relatively buoyant, to prevent contamination on the sea bed. Should contamination have occurred the umbilical was cleaned before the diver entered the bell. After entering the bell after a dive both the diver and the tender breathed through a mask, in order to prevent possible exposure to lead alkyl vapour in the bell. The diving bell was fitted with an activated charcoal filter, to remove lead alkyl vapours. After the bell reached the surface and was connected to the transfer lock, its outlet valve was opened to ensure a light but constant gas flow from the transfer lock to the bell, which would prevent the passage of lead vapour from the bell to the transfer lock. The divers then stripped and transferred to the interlock, where they showered whilst continuing the "mask on" system. Only when the analytical results of the gas were shown to be within normal limits did the diver transfer to the living chamber.

In case of persistent contamination of the bell and the transfer lock, the bell was detached, depressurised and thoroughly cleaned while the activated charcoal filtering system was shifted to the transfer locks. If this procedure proved insufficient, the divers were isobarically transferred to the living chamber, "mask on" order was given to all occupants of the DDC, the transfer lock depressurised, cleaned and repressurised; all the divers were then transferred to the interlock and the procedure repeated for the living chamber.

BIOLOGICAL MONITORING OF THE DIVERS

At the onset of the operation each of the divers had a complete physical examination and a full blood count. During the working period urinary lead levels were checked weekly. At the end of the diver's tour of duty he had a further medical examination and estimations for blood lead and urinary lead. Normal levels of urinary lead in subjects with no previous occupational exposure varies between 0 to 50 mg lead/litre. Cases of symptoms following exposure to tetraethyl lead were reported for levels below 200 mg lead/litre of urine, and it was decided for this salvage operation to regard 120 mg lead/litre of urine as an "alert" level for immediate further investigation on safety procedures and biological monitoring.

RESULTS

During the period from March to 7 to April 1975 medical surveillance was carried out on 15 divers who were working on the recovery of drums from the M.V. CASTAL. As can be seen from the lead in urine results, the vast majority of these were within the prescribed "alert" level of 120 mg lead/litre of urine.

The last saturation period, number ..., had the highest lead in urine levels. The divers employed at the time were very experienced and were working hard to finish the job. The mean level may be distorted by two high figures from two men, one of 260 mg and one of 280 mg. It was found that the umbilical being used by these two divers was contaminated by compound and a "Drag test" gave a lead in air level of 14 mg/m³. Because of this high level it was considered possible that compound may have penetrated through the walls of the umbilical to contaminate the gas mixture supplying the divers.

The umbilical is routed into the bell and contamination to this degree would result in high lead levels in the bell.

The described procedure and the mask on system used in the bell and the interlock were designed to provide a closed system of respiration for the divers in a potentially contaminated environment of the bell and the interlock. The lead in urine results show that this concept was successful and on the occasions when the lead in urine levels showed a rise in absorption of lead alkyls this was due to a failure in the safety system. The most common failure was due to non-wearing of masks either in the bell or the interlock, but contamination of the umbilical were also an important factor. In the last saturation period combination of these two factors that is contamination of the umbilical causing lead alkyl vapour to contribute to the breathing system and in addition contamination of the outside of the umbilical causing raised lead in air levels in the bell caused fairly significant levels of lead in urine in the divers. These raised levels only occurred for a few days and none of the divers had any untoward symptoms. The results show that by applying stringent safety controls, including the step by step decontamination procedure described above, backed up by adequate biological monitoring, salvage of such potentially toxic chemicals, such as tetraethyl lead and tetramethyl lead can be carried out safely, despite the use of saturation diving technique which allows for normal variations of microclimatic conditions, with only fairly modification of a routine saturation diving procedure.

References will appear in PROCEEDINGS, Tables follow.

Period 1 to 1975 to 25.4.1975

Saturation Periods	23
Saturation Dives	50
Saturation Manhours	14,628
Bottom Time Hours	1,235
Saturation excursion Dives	247
Average duration of excursion	5 hours

Results and number of lead in air controls performed during operations

mg/m³ Pb	PPH	INTERLOCK
0	40	102
41	50	12
81	120	4
121	160	1
161	200	1
201	300	2
301	400	1
	1,000	

Saturation Period No.		Date	Mean Urine Lead mg/l	Range
1	4 divers	1 4.75 – 22 4.75	40	1 –
2	4 divers	24.4.75 – 6.5.75	–	– –
3	4 divers	12.5.75 – 27.5.75	60	1 –
4	4 divers	29.5.75 – 12.6.75		1 – 6
5	4 divers	16.6.75 – 1.7.75	50	10 – 1
6	4 divers	3.7.75 – 21	50	20 – 110
	4 divers	4.7.75 – 18.7.75	30	11 – 61
7	4 divers	21 7.75 – 1 8.75	40	10 – 1
8	4 divers	2.8.75 – 20.8.75	40	1 – 1
10	4 divers	22.8.75 – 10.9.75	50	6 – 1
11	4 divers	11.9.75 – 24.9.75	1	1 –
12	4 divers	26.9.75 – 14.10.75	1	1 – 1
13	2 divers	15.10.75 – 6.11.75	1	1 – 1
14	2 divers	15.11.75 – 27.11.75	1	1 – 1
15	2 divers	1.12.75 – 1.12.75	1	1 –
16	2 divers	1.12.75 – 1.12.75	1	1 –
17	2 divers	1.12.75 – 1.12.75	1	1 –
18	2 divers	1.12.75 – 1.12.75	1	1 –
19	2 divers	1.12.75 – 1.12.75	1	1 –
20	4 divers	1.12.75 – 1.12.75	1	1 – 1
21	4 divers	1.12.75 – 1.12.75	11	1 – 1
22	4 divers	1.12.75 – 1.12.75	11	1 – 2
23	4 divers	1.12.75 – 1.12.75	1	1 – 1

Blood Lead mg/100 ml	Range
Mean at start	1
Mean at finish	11 – 1
Mean overall	1 – 1

THE PRESENT STATUS OF BONE NECROSIS RESEARCH D.N. Walder, University of Newcastle upon Tyne, NE1 4LP, UK.

At present the M.R.C. Decompression Sickness Central Registry in Newcastle upon Tyne has the radiographs of the bones of about 4000 professional divers. Of these persons 8 are known to have juxta-articular (A) lesions with broken joint surfaces (the most serious outcome of bone necrosis). A further 41 have unbroken juxta-articular (A) lesions which are potentially disabling and 126 have (A) lesions which are not. So far, these have considered to be of any significance to the health or efficiency of the subject concerned.

In addition we have noted in our records that 30 divers have suspected juxta-articular lesions and 66 divers have unconfirmed head, neck and shaft lesions. From experience we know that some of these suspected lesions will become definite in a year or so.

In terms of an overall problem bone necrosis in divers does not appear to be overwhelming. However, because the management of the condition is so difficult it does seem to be important to try to understand why it occurs and what the natural history is so that we can either stop it occurring or select the optimum time for treatment.

Ultimately the aim must be to avoid the condition altogether. Most decompression tables in use at present are unreasonably inflexible, at least by those who use them, to be satisfactory. This I suggest means that they will be safe for most (say, for example, 90%) but not all, of the population at risk. It now transpires that bone necrosis can occur in the absence of obvious attacks of decompression sickness although a history of such attacks does increase the likelihood of bone necrosis occurring. It therefore now appears that there may be some additional factor independent of the decompression which makes a diver susceptible to bone necrosis.

Research into bone necrosis following hyperbaric exposure has been proceeding simultaneously along several avenues and although at first sight they may appear to be unrelated, they are in fact all directed towards the development of an integrated picture of the total problem.

1. Epidemiology

First I would like to express my thanks to all those radiologists in every part of the world who have followed the M.R.C. Decompression Sickness Panel's system of radiological skeletal survey and classification of bone lesions and who have thereby enabled us to build up a clear idea about the overall problem that would otherwise not be possible.

In addition to obtaining some idea about the prevalence of bone necrosis in North Sea divers it has also been possible to study the influence of (1) the type of diving carried out (Gas pressure, Depth of exposure, Time of exposure), (2) Decompression Sickness, and (3) some personal factors such as alcohol on the prevalence of the condition.

A question currently being asked is whether or not the appearance of a B lesion is an indication that the individual is more likely to develop an A lesion if he continues to dive than a normal person. This has proved difficult to answer. The crux of the problem lies in finding men with comparable hyperbaric experiences. At the Newcastle Central Registry we have recently and at long last found a way in which this difficulty can be solved and the relevant figures will be presented.

2. Animal Models

Bone necrosis research has proceeded slowly over the years because it has been difficult to find a satisfactory animal model. Many laboratory animals can be shown to develop intravascular evidence of bone necrosis when examined after severe decompressions but under realistic conditions of depth and duration of exposure only the mini-pig develops macroscopic lesions similar to those seen in man.

An interesting alternative model system which we have used successfully has been the animal shown in rabbits of pebble cobalt by glass spheres. After these particles had been injected into the arterial circulation, the rabbits developed both shaft and juxta-articular lesions which appear to be identical with those seen in man after hyperbaric exposures.

3. The development of diagnostic techniques

One of the practical problems in dealing with divers is to isolate any bone lesion which may appear to the casual incident. As diagnosis by radiographs cannot be immediate, and usually requires a period of at least three months between the initiating insult and the development of changes sufficient to be seen on a radiograph, efforts have been made to seek more sensitive but none the less practical indicators. So far the most encouraging and conveniently detectable sign of incipient bone damage appears to be a rise in the serum ferritin level. This is of course a non localizing sign and when positive has to be followed up with a bone seeking radioisotope scan in order to identify the site of the abnormality.

This is a very sensitive technique and one of the dangers with any such method is that it may be too sensitive and detect lesions which will in any case heal spontaneously. It is, therefore, important to gain more experience in this area before concluding that all positive serum ferritin results will necessarily end up as definite bone lesions.

4. A predisposing factor

Bone lesions occur mainly in a less well known sites in the skeleton. As there is no absolute relationship between being treated for decompression sickness and subsequently developing bone necrosis, this raises the question as to whether there is some additional predisposing factor which has so far not been considered. Recently we have been studying in animals the circulation of radiolabelled fats from bone marrow before, during and after decompression from simulated dives. As will be reported elsewhere at this meeting it does look as though there are times when the marrow circulation is embarrassed and would be more than usually vulnerable to a small additional episode. Should the cause for this diminution in circulation rate be one which could be controlled it might be possible to minimize the damage to bone marrow and bone during decompression.

5. Treatment

Unfortunately by the time most A lesions are seen the joint surface has broken and is irreparable. Essentially the choice lies between arthroplasty and the replacement of the damaged joint by a prosthesis. Whilst one or other of these options may be perfectly acceptable for patients at the end of an active life neither is desirable in young and otherwise fit men.

Post mortem studies of juxta-articular lesions have provided evidence that almost always the body makes a considerable effort to repair the damaged area of bone but rarely succeeds completely. In most cases the healing process comes to a halt just short of the articular surface to leave a sequel of dead bone at this epiphyseal point. It transpires that the explanation for this failure to repair totally lies in the fact that the repair process involves the deposition of new bone on the dead trabeculae. These eventually become so thick that where they lie close together the spaces between them are occluded and this results in obstruction to the forward progress of new capillaries and hence a blood supply to the tissue beyond. The repair process stops. Now that this mechanism is appreciated possible ways in which the difficulty could be overcome are clear and can be tested.

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ARTICULAR BONE AND CARTILAGE COLLAGEN METABOLISM IN EXPERIMENTALLY INDUCED DYSBARIC OSTEONECROSIS. Diane Brickley Parsons and Mark E. Bradley. The George Washington University, Washington, D.C., U.S.A. and Naval Medical Research Institute, Bethesda, Md., U.S.A.

Dysbaric osteonecrosis is a debilitating, chronic disease found in those individuals exposed to changes in ambient pressures. Despite interest, concern and study during the last half century, the etiology and pathogenesis of dysbaric osteonecrosis remain in enigma.

The main functions of the skeletal tissues, bone and cartilage are to provide the body with mechanical support and motion. Mineral formation and resorption, as well as the quality and quantity of the structural protein collagen, play crucial and critical roles in carrying out these functions and in doing so efficiently. In recent years it has become increasingly apparent that collagen exhibits an extensive chemical heterogeneity. At least some of this molecular polymorphism undoubtedly reflects the biological adaptation of both molecules for special tissue requirements. Thus, for example, cartilage contains a type of collagen that is genetically distinct from that of bone; yet the collagens of bone and cartilage both contain intermolecular crosslinks that display similar distinctive features. Such unique chemical components equip the tissue with the special properties that are important to its physiological functions. Thus, it seems reasonable to assume that the skeletal deterioration observed in Dysbaric Osteonecrosis may turn out to be linked to abnormalities in collagen metabolism.

Over the past two years, our laboratory has been extensively involved in studying collagen synthesis, maturation and degradation of bone and cartilage in the early, intermediate and latent stages of induced dysbaric osteonecrosis. Our studies using the experimental mouse model of thyroxine/hormone revealed a number of striking changes in the composition of bone and cartilage at the molecular level.

Four groups of male, genetically-obese, hyperglycemic mice were subjected to 25 psig air pressure in a pressure chamber for 3 hours. The compression was either rapid, 25 psig in 60 seconds or staged, the staged compression involved steps at 10 psig (6 min), 15 psig (30 min), 45 psig (10 min), and 90 psig (60 min). All decompression was staged with steps at 45, 40, 30, 20, and 10 psig for 5, 25, 15, 25, and 120 min respectively. Two groups of mice were subjected to either rapid or staged compression 3 times per week, 2 other groups were exposed 2 times per week. A fifth group of mice was not subjected to dysbaric exposure and served as age and site matched controls.

Bone from the epiphyses of the proximal tibia, distal femur, proximal tibia and humerus, the mid shafts of the femur, tibia and humerus and cartilage from the femoral and humeral head and the knee joint were analyzed chemically for: 1. dry fat extraction, 2. total amino acid analysis, 3. cortical profile, 4. amino acid specific content, 5. collagen specific, and 6. specific content.

Samples from a total of 400 mice were analyzed biochemically to determine the accumulation and crosslinking of collagen of bone and cartilage as a function of the development of osteonecrosis. Table 1 shows the incidence of osteonecrosis induced by exposure to dysbaric conditions as diagnosed by abnormal collagen composition. These data clearly demonstrate that with daily exposure to rapid compression, the incidence of abnormal alterations in collagen metabolism is higher and the latent period shorter than with 3 exposures per week or with staged compression.

Table II shows a striking, temporal correlation between the rate of compression and the content of hydroxylysine in the collagens analysed. Amino acid analyses of bone collagen from the epiphyses of the proximal tibia and femur revealed a marked increase in the hydroxylysine content as a function of daily exposure to rapid compression. Increased hydroxylysine content in the epiphyses of the distal femur and proximal humerus demonstrated that these areas were the least affected by exposure to either rapid or staged compression, with hydroxylysine values only slightly higher than the age matched controls. The hydroxylysine content of the collagen of the mid shafts of all experimental bones was identical to the control suggesting the absence of collagen abnormality in these areas.

These data strongly support that the bone cells, in response to injury by repeated dysbaric exposure, synthesize an abnormally hydroxylated collagen similar to that repair type I collagen synthesized in the healing of bone fracture in adult bone in those mice exposed to either rapid or staged compression for 3 times per week also showed an increasing hydroxylysine content (Table not shown). The time factor required to detect these changes was greater than 3 months using rapid compression and greater than 12 months using staged compression.

Further evidence for the synthesis of a repair collagen is demonstrated in the reducible crosslink profile. In all control mice, a decrease in the amount of reducible crosslinks and precursor aldehydes as a function of age was observed. However in all experimental mice, particularly those exposed to rapid compression, increasing amounts of a hydroxylysine derived addition and precursor aldehydes were consistently observed. Moreover, ion exchange chromatography on selected samples for detection of collagen type showed the loss of both normal and elevated levels of hydroxylysine to be compared solely to a type I bone collagen. No type II cartilage collagen was detected.

The ratio of type I to type II collagen in selectively pooled samples of femoral head and knee articular cartilage increased dramatically with time in those mice exposed daily to rapid compression. Approximately, 50% of the

Table I

Influence of the Rate of Compression and the Frequency and Duration of Hyperbaric Exposure on the Incidence of Abnormal Collagen in Mice.

Exposure Duration (months)	Rapid Compression		Staged Compression	
	7/week	1/week	7/week	3/week
3	60% (12/20)	15% (7/20)	- (0/20)	- (0/20)
6	70% (14/20)	40% (8/20)	- (0/20)	- (0/20)
9	85% (17/20)	55% (11/20)	15% (3/20)	5% (1/19)
12	90% (18/20)	60% (11/19)	21% (4/19)	10% (2/20)
15	93% (14/15)	80% (12/15)	12% (6/19)	11% (2/18)
18	100% (16/16)	81% (13/16)	11% (5/15)	20% (4/20)

Table II

Alterations in the Hydroxylysine Content of Bone Collagen as a Function of Daily Exposure to Hyperbaric Conditions (Values expressed as residues of hydroxylysine /collagen chain)

Rapid Compression

Exposure Duration (months)	Epiphysis				Mid-Shaft	
	Px Tibia	Px Femur	Ds Femur	Px Humerus	Tibia	Humerus
0	6.5	6.4	6.4	6.2	5.2	5.2
3	8.4	6.9	6.5	6.2	5.2	5.2
6	10.6	8.4	7.6	6.2	5.2	5.1
9	12.7	10.1	8.1	7.7	5.1	5.1
12	14.4	12.6	8.5	7.7	5.1	5.2
15	14.7	11.1	7.7	6.1	5.2	5.1
18	14.6	11.4	7.5	6.4	5.1	5.2

Staged Compression

0	6.5	6.4	6.4	6.2	5.2	5.2
3	6.5	6.6	6.4	6.2	5.2	5.1
6	6.6	6.4	6.1	6.2	5.1	5.1
9	7.1	6.4	6.8	6.3	5.2	5.1
12	8.9	7.8	6.9	6.4	5.1	5.2
15	9.2	8.1	7.2	6.8	5.2	5.1
18	9.1	8.6	7.6	6.9	5.1	5.2
Control (all ages)	6.5	6.4	6.4	6.2	5.2	5.2

cartilage was type I after exposure for 1 months. At 6 months, the type I collagen increased to 10%, at 9 months 41%, at 12 months 90% and at 15 and 18 months over 75% of the collagen was type I. Age matched controls consistently gave values of 15-20% type I collagen. Identical cartilage samples taken from mice exposed to staged compression exhibited only a slightly increased molecular perisomphism. Samples of humeral articular cartilage from mice exposed to either rapid or staged compression at all time intervals showed no increase in poly morphism over the age matched controls.

Electrophoresis of the collagen cyanogen bromide peptides of concentric slices of femoral knee cartilage from mice exposed to rapid compression for 3, 6, and 9 months showed that the type II collagen was predominant at the surface and the type I collagen in the deeper layers. However, samples taken after 12 and 15 months exposure times revealed a shift in the distribution of collagen with the type I collagen being detected closer to the surface. In 6/10 mice exposed to rapid compression for 18 months, marked concave defects were observed in the articular surfaces. Electrophoresis of concentric slices of these samples showed that type I collagen was predominantly at the surface and the type II collagen in the deeper layers. This finding was supported by increased hydroxylysine glycoside and hexosamine content of the tissue with depth. Amino acid analyses of surface slices from a subchondral sclerotic lesion revealed that the type I collagen was hydroxylated with the residue/chain value calculated at 12.4 and 13.6 residues/chain, respectively.

The present study demonstrates that rapid changes in collagen synthesis, crosslinking and distribution of genetic type occur in the short, precise and reversible bone and cartilage lesions induced by repeated hyperbaric conditions. Although hydrostatic stress was considered to be one of the experimental determinants in this study, the fact that staged and the frequency of exposure greatly influenced the appearance and progression of abnormal collagen metabolism in cartilage and bone, suggests that the abnormal collagen metabolism is a direct consequence of the hyperbaric exposure rather than a secondary phenomenon. Moreover, it is clear that a repair, hyperhydroxylated type I collagen is synthesized in response to dysbaric conditions. However unlike repair collagen that is synthesized and then resorbed after bone fracture healing, the collagen synthesized in response to dysbaric exposure fails to be contained at the bone cartilage junction and continues to invade the overlying articular cartilage.

It was apparent that in the early stages, collagen metabolism in the articular cartilage remained viable and functioned normally despite the development of osteonecrosis in the epiphyseal bone. Thus, the findings of this study strongly suggest that the initial response to connective tissue injury by dysbaric exposure is the synthesis of a repair tissue containing hyperhydroxylated type I collagen. In addition to resorbing and destroying the epiphyseal bone, the invasion of this repair tissue into the cartilage contributes to the development of osteoarthritis and ultimate destruction of the adjacent joint. Although extrapolation of the findings of this small-animal study must be undertaken cautiously, the synthesis and fate of an abnormal repair tissue may have potentially serious implications for human connective tissue function in individuals subjected to dysbaric exposure.

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A DETAILED HISTOLOGICAL AND RADIOLOGICAL CONTROLLED STUDY OF SELECTED BONES FROM DIVERS. G. R. Weatherley, W. M. Park, M. Haddaway and L. Calder. The Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, Salop, U.K. and The London Hospital, London, U.K.

Observations have been made which suggest that the full extent of bone necrosis occuring in divers may be much underestimated. The histological examination of autopsy material from divers has clearly shown that even in typical lesions of bone necrosis there is cellular death beyond the boundary of the lesion defined by radiology. (McCallum et al 1966; Weatherley et al 1977 a). The absence of radiological changes is also in itself no proof that bone necrosis is not present. Experimental work in animals has shown that extensive areas of necrosis may be present in both the marrow and cortex of bones in the absence of detectable radiological changes (Weatherley et al 1977 b). Recent studies in divers on the diagnostic value of scintigraphy for dysbaric osteonecrosis suggest similar findings. In one study post dive scanning changes were noted in 6 out of 14 divers although only 1 man subsequently developed definite radiographic changes. (Harrison et al 1977), whilst such observations indicate that in any one diver the extent of bone necrosis may be greater than that indicated by radiology they also suggest that more divers may be affected than is at present accepted. Such observations also illustrate the considerable limitations of routine radiology as a method of detecting dysbaric osteonecrosis.

Information that would help to define the full extent and incidence of dysbaric osteonecrosis at a histological level would be of value for several reasons. As already suggested it is possible that the insult which can result in dysbaric osteonecrosis in man may at other times result in permanent and detectable histological changes in the bone in the absence of radiological changes. Moreover, even when dysbaric osteonecrosis is present on radiological examination there may be no obvious or easily detected damage to the other tissues of the body and furthermore there may be no history of decompression sickness. Under such circumstances a detailed examination of the skeleton may offer the only guide that the diving procedures followed by that man have not been entirely innocuous. In the case of exposure to saturation diving this is of even greater importance as the long term safety of this now accepted technique has yet to be confirmed. A detailed histological examination of whole bones has perhaps been made even more urgent by recent reports which suggest a higher incidence of bone necrosis in saturation divers. The detailed histological examination of whole bones from divers also offers the opportunity to establish the accuracy of a radiological assessment. If bones are available in which typical lesions of osteonecrosis are present such a comparative histological and radiological study may also provide further information on such problems as the apparent failure of lesions to repair completely. Cognizant of the limitations of conventional radiology as a diagnostic method, recent research has considered the possible role of biochemical methods of early detection (Edgar & Calder 1977; Weatherley et al 1977 c). The possible success of such methods is governed to a large extent by the amount of bone involved. The histological examination of bones from divers may, therefore, elucidate the likelihood of such methods being useful.

Materials and Methods

As the humerus and the femur are bones that may be affected by dysbaric osteonecrosis a number of these bones have been obtained at autopsy examination for this analysis. Where possible each man's diving history has been obtained noting such particulars as subjective exposure and decompression sickness together with any relevant medical details. An identical sequential analysis has been carried out on all these bones. This has begun with basic measurement followed by standard antero-posterior and lateral radiographs. Following this each bone has been bisected in the coronal plane and the cut surfaces examined macroscopically and then photographed. One hemisection of each bone has then been divided longitudinally into 2 equal blocks and 1 complete 2 mm. transverse section cut at the 4 sites of division of the bone. A 2 mm. longitudinal section has then been cut from each block to give a total of 4 sections for each bone. Each of these 4 sections has then been subjected to microfocal radiography prior to preparation for histological examination.

To obtain an objective analysis in this study which is at present in progress the radiological and histological findings are to be reported independently and without reference to each other. However, in order to obtain an accurate correlation of the radiological and histological findings the outline of each section on the microfocal radiograph has been traced and delivered with the specimens to the pathologist so that the location of any pathological changes may be precisely recorded, when both the radiological and histological reports are completed the combined findings are to be reviewed together and considered in relation to the diving and medical histories. As controls for this study femora and humeri obtained at autopsy from men of comparable age and physique who are not divers are also to be submitted to the same sequential analysis. Whilst there is some evidence that loss of osteocytes from interstitial bone lamellae may occur with age and ischaemic disease (Bermann & Schmerwitz 1965; Gatto 1970, this is not reported in the bones of otherwise healthy young men. As a result of this detailed controlled study it is hoped that we may be in a better position to provide answers to the questions outlined in the introduction.

THE EFFICACY OF SPINAL ANESTHESIA AT HIGH PRESSURE, M. L. Strandness, H. Bellhorn, and R. Levy,
Naval Medical Research Institute, 8500, Bethesda, Maryland 20014

Pressure antagonizes general anesthesia induced with inhaled anesthetic gases or with intravenous agents which are widely varied in nature. Antagonism is manifested by increased requirements or shortened duration of effect, or both. Similarly, pressure also antagonizes the nerve conduction block caused by some local or general anesthetics (Brindly and Cohen 1977; Roth, Smith, and Paton 1976). It is therefore important to determine how much pressure may influence the dose requirement and the duration of the block if spinal anesthesia is to remain a viable alternative to general anesthesia under pressure.

MATERIALS AND METHODS

Male guinea pigs (300-500 g) were employed in this study. Spinal anesthesia was induced with tetracaine hydrochloride crystals dissolved in 5% dextrose in lactated Ringer's solution. Under clinical conditions, to make the anesthetic solution heavier than cerebrospinal fluid, one uses 5% dextrose in water as the vehicle for the active ingredients. Instead, we used 5% dextrose in lactated Ringer's solution because we feared that solutions with electrolyte contents too different from that of the plasma or cerebrospinal fluid might influence ionic transfer across the nerve membrane, especially during hyperbaria, and therefore change the recovery times. To assure the same quality of the anesthetic, we prepared from the same parent solution aliquots of 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, and 0.625 mg/ml in equal volumes. To each 4 ml of the solution, we added 0.2 ml of 1:1000 epinephrine. The concentration of the anesthetic solution was not known at the time of use. Each concentration was studied in groups of four guinea pigs at a time, either at 1 ATA of air (surface control) or at 12 ATA helium with 0.15 O_2.

Lumbar puncture was performed under general anesthesia (halothane-air) at the first or second lumbar vertebral interspaces. A 27-ga 3/4 G needle was introduced into the spinal canal at 1 cm depth where 0.1 ml of the anesthetic solution was injected. We made no attempt to obtain cerebrospinal fluid. The onset of the spinal block was instantaneous, manifested by loss of abdominal muscle tone and loss of urinary sphincter tone. Within minutes, the animals emerged from the general anesthesia. Only those with bilateral motor blocks were included in the study.

To evaluate recovery of muscle function, we placed blocked animals in an electrically driven drum that rotated at 4-5 rpm when activated. The drum, divided into sections, was located inside a 180 L hyperbaric chamber. Evaluations were carried out at 5 to 10 min intervals. The recovery was considered complete when three criteria were satisfied: (a) motor function--ability to sustain posture for at least one rotation, (b) strength--ability to lift and support caudal half of the body off the floor of the rotating drum, and (c) proprioception--ability of the hind extremities to follow the rotation by taking alternate steps, as in normal gait.

The groups that were studied under pressure were compressed at 4 ATA per min as previously described (Strandness, Bitondomo, Tobey, and Bailey 1979). When all of the animals had recovered function, or after 3 h at 12 ATA, they were subjected to euthanasia by rapid decompression.

Statistical handling of the data required natural log transformation of the variables (duration and dose) to make the distribution more homoscedastic. Comparison of the duration was by regression analysis; the slope of the intercept was determined by t-test.

RESULTS

A total of 114 observations were made. The mean duration of spinal block in each dose (concentration) and condition are presented in Table 1. Increasing the dose of anesthetic agent consistently produced longer-duration spinal block. The effect of pressure was not significant. Up to 12 ATA, duration of spinal anesthesia induced with the same doses were close to or identical with those on the surface. Figure 1 is a graphic presentation of the effect of doubling the concentration of the solution on the duration of spinal block.

Table 1
Duration of spinal block

Concentration (mg/ml)	Mean duration (min) ± SE	
	Surface Control	12 ATA
0.625	61.1 ± 4.5	57.8 ± 5.1
1.25	76.8 ± 5.2	72.5 ± 11.4
2.5	92.5 ± 5.8	89.5 ± 5.5
5.0	118.0 ± 8.4	125.2 ± 6.2

SE = standard error.

DISCUSSION

The anesthesia induced in the experimental animals was patterned as closely as possible to clinical practice. Recovery was surprisingly complete for most of the guinea pigs except for four that showed residual nerve damage (poor to total sphincter control), either from trauma during the lumbar puncture or infection.

Our findings show that spinal anesthesia is a practical alternative to general anesthesia under hyperbaric conditions. There is neither an increase in requirements nor a change in the duration of the block. We suspected that spinal and other techniques of conduction anesthesia that bathe the nerves in the anesthetic solution would be effective under pressure. We reasoned that the concentrations of the drug employed for any of these techniques are much larger than those required to cause nerve block. The concentrations employed should mask whatever increased requirement high pressure might induce.

The findings reported here are not necessarily at variance with those of Brindly et al., and Roth et al. because the experimental methods are different. Those investigators used isolated nerve preparations, whereas we used intact animals with more complicated pharmacodynamics. The end point in their studies was a return of electrical conduction toward baseline to show antagonism whereas, our criteria may have been too strict to show similar grades of pressure antagonism. Neural conductivity may have recovered long before muscular strength and proprioception recovered, because these functions require a more refined modulation of nerve impulse through a system of feedback mechanisms.

Based on our data, we conclude that spinal anesthesia with tetracaine, at dosages practical for clinical use, should be safe at depths of 12 ATA and could be safely used for any brief procedure that are usually performed under spinal anesthesia at surface conditions.

ACKNOWLEDGMENTS

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References will appear in PROCEEDINGS.
Figure follows.

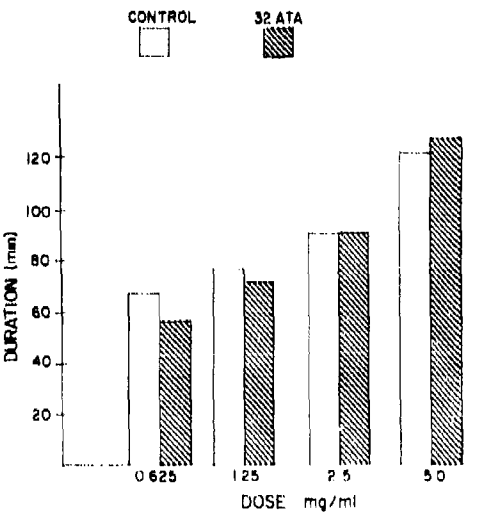

Figure 1. The effect of doubling the dose (concentration) of the drug on the duration of spinal block.

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