

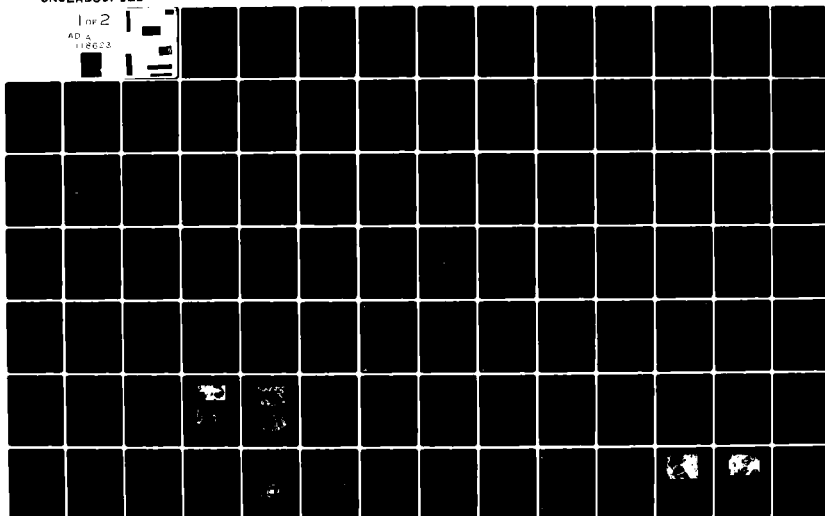
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STUDIES ON THE MECHANISM AND PREVENTION OF DECOMPRESSION SICKNESS--ETC(U)
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STUDIES ON THE MECHANISM AND
PREVENTION OF DECOMPRESSION SICKNESS
FINAL TECHNICAL REPORT
ONR CONTRACT NO. N00014-75-C-0312
C. CHRYSANTHOU, M.D.

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FINAL TECHNICAL REPORT

STUDIES ON THE MECHANISM AND PREVENTION
OF DECOMPRESSION SICKNESS

BY

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PREFACE

This is a Final Technical Report on our investigations on the pathogenesis and prevention of dysbaric disorders conducted under ONR Contract #N00014-75-C-0312 from June 1, 1968 to February 28, 1982.

The studies focused on three main areas: Decompression Sickness, Dysbaric Osteonecrosis and Dysbaric Alteration of the Blood-Brain Barrier. On decompression sickness, attempts were made to elucidate pathogenetic mechanisms. Several hypotheses were advanced and novel pathogenetic concepts were proposed. On the basis of new theoretical considerations we were able to develop means for the prevention or amelioration of the disease. Regarding dysbaric osteonecrosis, we developed an animal model which permitted studies on the etiology predisposition and pathogenesis of the disorder. The most recent investigations covered by this report involve dysbaric modification of blood-tissue barriers, an observation which was made in our Laboratories few years ago.

The report includes an overview (summary) of the investigations conducted in these areas with references to pertinent publications. In lieu of detailed description of the work done, reprints of representative articles have been attached. We hope that this material might be of help to those interested in the fields of dysbaric disorders.

I am grateful to the Office of Naval Research, U.S. Navy, for the support and cooperation that made these investigations possible. My appreciation and thanks is also extended to my collaborators, Drs. F. Teichner and G. Goldstein and to G. Molenje, S. Marrin, O. Yalis, J. Rice and E. McManus, for their technical and secretarial assistance.



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July 12, 1982

INTRODUCTION

Technological advances in the last 30 years made it possible for man to dive deeper into the seas and climb higher into the atmosphere and the space beyond. The expanding industry of off shore drilling, the popularity of scuba diving and other accelerated commercial, military, scientific and recreational activities are responsible for more people getting farther away from the surface of our planet more often and for longer periods.

Exposure to compressed air environments and underwater conditions is associated with potential hazards including dysbaric disorders. The most common serious disorder resulting from exposure to pressure changes is decompression sickness which may develop following decompression from higher to lower or sea level pressures (divers, caisson workers) or from atmospheric to sub-atmospheric pressures (aviators, astronauts). In addition to the immediate and overt manifestations of dysbaric disorders, pressure changes can cause potentially serious conditions which exhibit no obvious clinical signs (e.g., blood brain barrier alterations) or develop after long latent periods (osteonecrosis). These silent or latent disorders are not necessarily associated with decompression sickness and can develop in the absence of the disease. Because of these considerations, physiologic problems related to the effects of pressure changes have assumed greater importance and renewed attention has been focused on the etiology, mechanism, prevention and treatment of dysbaric disorders. No significant progress in the prevention and treatment of these conditions is expected as long as their pathogenesis remains obscure. Under ONR support, we conducted for 14 years intensive biomedical investigations on the mechanism and prevention of dysbaric disorders. Our work was presented in many national and international meetings and resulted in 38 publications.

Introduction (continued)

Among our contributions in the field of dysbarism is the theory that development of dysbaric disorders depends not only on the direct mechanical effects of gas bubbles but also on complex secondary changes triggered by intravascular or tissue bubbles which do not necessarily produce clinical manifestations ("silent" bubbles). Smooth muscle stimulating substances, e.g., bradykinin, histamine, serotonin, prostaglandins and SMAF (a new factor reported from our laboratory), are thought to be implicated in the pathogenesis of decompression sickness and in other dysbaric disorders. On the basis of this theory we were able to develop means for the amelioration and even prevention of decompression sickness in mice. The effectiveness of our prophylactic treatment was subsequently confirmed by other investigators working with dogs and hamsters. SMAF which has been isolated in various tissues of several species, including humans, appears to play a role not only in dysbaric disorders but also in other pathologic conditions such as shock.

Another contribution made by our studies is the observation that dysbaric exposure can cause alteration of the blood-brain barrier. We also proposed the possible involvement of blood-bubble interface activity in the mechanism of dysbaric phenomena and provided data suggesting activation of humoral factors by gas bubbles.

The spectrum of our research on dysbaric disorders also includes significant work on dysbaric osteonecrosis. Our laboratories developed an animal model suitable for clinical, histological and biochemical studies on this bone lesion. This model which has recently been included in the handbook, "Animal Models of Human Disease" of the Armed Forces Institute of Pathology, has successfully been used by us and other investigators in research on the etiology, pathogenesis, predisposing factors and possible prevention of dysbaric osteonecrosis.

The following overview of the studies conducted from 1968 to 1982 and the charts that summarize proposed pathogenetic mechanisms present a synopsis of our ONR supported investigations. Details of this work can be found in the attached reprints of related publications.

OVERVIEW

DECOMPRESSION SICKNESS

Decompression sickness (DS) in caisson workers, divers, or aviators, although it differs in the circumstances of development and progression of the process, involves the same fundamental mechanism and thus exhibits many similarities in its manifestation. Despite uncertainties regarding the origin, site, and mode of action, gas bubbles are generally accepted as the basic initiating factor in the production of the disorder.

Gas bubbles are formed first in tissues and the venous circulation, appearing later in the arteries. The formation and growth of gas bubbles in tissues and blood have several direct, as well as indirect, potentially detrimental effects. They may obstruct blood flow and results in ischemia and infarction. Expanding bubbles in muscles and tendons may cause pain by distorting and deforming nerve endings. Ischemia and release or activation of humoral agents may also contribute to the production of pain. Gas bubbles arising in the vessels and lipid-rich tissues of the brain and spinal cord or air embolization of the central nervous system could be responsible for neurologic manifestations, and embolization to the lung could contribute to the respiratory signs of the disease.

The bubble theory, however, is not all-inclusive and leaves an appreciable deficit in our understanding of various phenomena and problems in dysbaric disorders. Signs of DS may develop without evidence of circulatory obstruction by gas bubbles, and gas bubbles can exist without manifestation of the disease (the so-called "silent" bubbles). Furthermore, gas bubbles, or at least their direct effects, cannot explain certain complications.

It seems plausible that gas bubbles only initiate a complex and self-propagating disease process, the development and seriousness of which depend more on the involvement of biohumoral and other factors than on the gas bubble itself.⁽¹⁶⁾

Note: The numbers on the superscript refer to the list of publications.

Overview (continued)

Fat emboli produced by decompression injury to bone marrow and adipose tissue or resulting from a gas-induced disruption of lipoprotein linkages in the blood, have been implicated in the pathogenesis of the syndrome. Alterations of serum lipids produced by exposure to compression-decompression or by bubbling air have been reported by our laboratory.⁽²⁵⁾ Clumping of red blood cells was considered a secondary complicating factor as early as 1938. Disseminated intravascular coagulation associated with a fall in the circulating platelet count may also play an important pathogenetic role.

Many of the previously mentioned complicating factors may be the result of surface activity of the bubbles. Intravascular gas bubbles may act as foreign surfaces to cause denaturation of plasma proteins, clumping of red blood cells, platelet aggregation and adhesion, coalescence of plasma lipids, and activation of the Hageman factor, which in turn could result in activation of the coagulation mechanism, of the kinin system, and of other humoral agents.^(3,15,16,19) Gas-induced osmosis resulting in changes of water concentration in certain tissues has also been recently considered as a factor in DS and dysbaric osteonecrosis.

Our studies on dysbaric disorders led us to theorize that smooth muscle-stimulating substances (vasoactive agents) are implicated in the pathogenesis of DS. (See charts 1,2 and 3). This concept is based on the following observations which were made in our laboratories in the last two decades.

- a) Several of the histologic changes seen in animals injected with bradykinin resemble the pathologic alterations observed in DS. (Aerospace Med. 35:741-746, 1964)
- b) Administration of bradykinin to animals subjected to compression-decompression intensifies pathologic alterations and increases mortality in DS. (Aerospace Med. 35: 741-746, 1964)

Overview (continued)

- c) Compounds which combine activities against bradykinin, histamine and 5-hydroxytryptamine when administered prior to compression prevent or decrease the intensity of pathologic changes and significantly reduce mortality in DS. (^{4,18,31,33} and Aerospace Med. 35:741, 1964) Dimethothiazine (migristene), 2-(phenylpiperazinylmethyl)-cyclohexanone (PPCH) and cyproheptadine are most effective in preventing DS. (^{18,31})
- d) The activity of a new humoral Smooth Muscle Acting Factor (SMAF)* increases in DS. (³)
- e) SMAF administration after compression and just prior to altitude decompression increases susceptibility of thin mice to DS. (⁴)
- f) PPCH counteracts this effect of SMAF and prevents development of DS. (⁴)
- g) PPCH blocks the in vitro potentiating effect of SMAF on bradykinin. (³⁹)
- h) Preliminary experiments suggest that SMAF increases and PPCH decreases (³⁹) blood coagulability.
- i) Preliminary experiments also suggest that SMAF induces platelet aggregation while dimethothiazine (migristene), which prevents DS, acts against platelet aggregation, as a "platelet protector". (³⁸)

*SMAF which was isolated in our laboratory originally from mouse lung was subsequently found in several species (rabbit, rat, mouse, dog, human) and in a variety of organs. Currently, it is being prepared from human placenta. SMAF is a polypeptide with physical, chemical and pharmacological properties distinct from those of similar substances. The most striking activity of SMAF is its ability to increase responsiveness of smooth muscle to stimulants. In addition to its involvement in DS, SMAF has also been implicated in hemorrhagic and endotoxic shock. (¹⁷)

Overview (continued)

j) Blood or plasma subjected to compression-decompression or bubbled with air exhibits an increase in smooth muscle stimulating activity and in SMAF levels.⁽¹⁵⁻¹⁹⁾ This increase is not entirely dependent upon formed elements of the blood. The activity is not attributable to histamine, acetylcholine or 5-hydroxytryptamine. Polypeptides may account for at least part of the smooth muscle stimulating activity.⁽¹⁵⁾

The above observations clearly indicate that administration of smooth muscle stimulating substances increases susceptibility or aggravates DS while inhibitors or antagonists of these substances prevent or ameliorate the disease. Our experiments also show that extracts from animals or from blood subjected to compression-decompression exhibit an increase in smooth muscle stimulating activity.

These findings led to a new pathogenetic concept. According to our theory, smooth muscle stimulating factors released or activated in DS by a variety of possible triggering mechanisms (e.g., gas-blood interphase phenomena, Hageman factor, enzymes released from injured or anoxic cells, etc.) (see chart 2) induce tissue responses that could contribute to the production of the syndrome (e.g., circulatory changes favoring nucleation and growth of gas bubbles; respiratory changes including bronchoconstriction and perivascular edema which could interfere with elimination of nitrogen and also cause respiratory distress; production of pain; aggregation of platelets; increased vascular permeability contributing to hemoconcentration and to hypovolemic shock) (see chart 3).

These pathogenetic considerations provided the basis for a novel pharmacologic approach to the prevention or amelioration of DS. The DS-preventing effect of dimethothiazine (a drug approved for certain clinical applications in Europe and Canada) reported from our laboratories has attracted the interest of several investigators. Experimental work done in other laboratories indicates that dimethothiazine protects dogs against decompression sickness, (Bull. Medsubhyp.

No. 12, p. 87, 1975) thus, confirming our original observations.

The drugs which prevent DS also cause a varying degree of drowsiness probably because of their antihistaminic effect. This action is undesirable and also raises the question as to whether central depression caused by these compounds plays a role in their prophylactic effect.

Preliminary studies suggest that cyproheptadine, one of the drugs that prevents or ameliorates DS, retains its protective effect when its sedative action is neutralized or counteracted.⁽³³⁾ Further investigations, however, are required to establish the effectiveness of this treatment.

DYSBARIC OSTEONECROSIS

Dysbaric osteonecrosis (DO) is a potentially crippling disease which has recently been recognized as a major hazard in individuals subjected to large changes in ambient pressure. The incidence of the lesion is alarmingly high ranging from 4% in Royal Navy divers to 60% in Japanese diving fishermen. The disorder has also disabled pilots who have been exposed to hypobaric conditions and men subjected to simulated high altitudes.

It is important to remember that DO is a latent lesion, not necessarily associated with decompression sickness and can develop after many years in subjects who never experienced any dysbaric manifestations.

The etiology and pathogenesis of the disease are still obscure. Lack of a suitable animal model is one of the reasons for slow progress in this field. We developed an experimental osteonecrotic lesion with close similarities to the human disease and several other advantages. Our animal model which has been included in the Fascicles, "Animal Models of Human Disease" of the Armed Forces Institute of Pathology⁽³⁶⁾ has been successfully used by us and other investigators^(8,10,21,26,27,34) and Proc. 7th Symp. Underwater Physiol., p. 837, 1981)

for studies on the incidence, pathogenesis and predisposing factors of DO. A total of 2,500 bones of animals subjected to dysbaric conditions were histologically examined in our laboratories. Our investigations yielded data which led to the following conclusions:

- a) Dybaric osteonecrosis can be experimentally produced in mice, particularly in obese strains
- b) There is a latent period of several months before the lesion is manifested
- c) In obese mice, the incidence is greater and the latent period shorter than in thin siblings
- c) With multiple exposures, the incidence is higher and the latent period shorter than with single exposure
- d) With stage compression, the incidence is lower than with rapid compression
- f) Dysbaric osteonecrosis in mice appears to be independent of decompression sickness
- g) The pathogenesis of dysbaric osteonecrosis may involve several initiating and contributing factors that act in concert or in sequence. (See chart 4.)

DYSBARIC MODIFICATION OF THE "BLOOD-BRAIN AND BLOOD-LUNG BARRIERS"

The presence of a blood-brain barrier has long been established. A blood-lung barrier has been proposed to explain failure of certain circulating substances to penetrate pulmonary tissue (Proc. XVI Int'l. Congr. Zoology 2:87, 1963). In 1976 we reported that dysbaric exposure appears to "break" these barriers.⁽²³⁾ Intravenously injected dyes (e.g., trypan blue) were found in significantly higher concentrations in lung and brain tissue of animals which were subjected to dysbaric conditions than in corresponding controls.⁽²⁴⁾ The extent of dye permeation into the tissues was evaluated by gross and microscopic examination and by spectrophotometric determination of dye concentration in tissue extracts. Subsequently, it was shown that dysbaric exposure also modifies brain permeability

to antibiotics.⁽³⁰⁾

These findings which have been confirmed by other investigators (Neurology 66:238, 1979) have both, theoretical and practical implications. Dysbaric alteration of blood-organ barriers and, particularly, of the blood-brain barrier is relevant and important in dysbaric medicine for several reasons including the possible implication of such alterations in the pathogenesis of decompression sickness. Another consideration which is noteworthy, is that increased permeability of the blood-brain barrier under dysbaric conditions may be important regarding pharmacotherapy of persons subjected to such conditions. Alteration of the barrier in divers and compressed air workers may allow certain drugs to enter the brain in larger amounts and produce toxic or undesirable effects. Finally, modification of the blood-brain barrier by dysbaric exposure may suggest new methods for therapeutic or diagnostic administration of compounds that, under normal conditions, do not penetrate the barrier or do so to a very limited extent.

The mechanism of dysbaric blood-brain barrier modification is still obscure. Among various pathogenetic considerations, it was hypothesized that cerebral vessel permeability may increase by the action of chemical agents released or activated by gas bubbles.⁽²⁴⁾ We have shown in this regard, that SMAF, which increases vascular permeability and is activated by dysbaric exposure in vivo⁽³⁾ as well as by gas bubbles in vitro,⁽¹⁵⁾ alters the blood-brain barrier when injected intracarotidly into rabbits.⁽³⁵⁾ We also explored reversibility of dysbaric alterations of the blood-brain barrier. Preliminary studies yielded data suggesting that the barrier is restored in 24 hrs after dysbaric exposure.⁽³⁷⁾

PROPOSED PATHOGENETIC MECHANISMS OF DECOMPRESSION SICKNESS

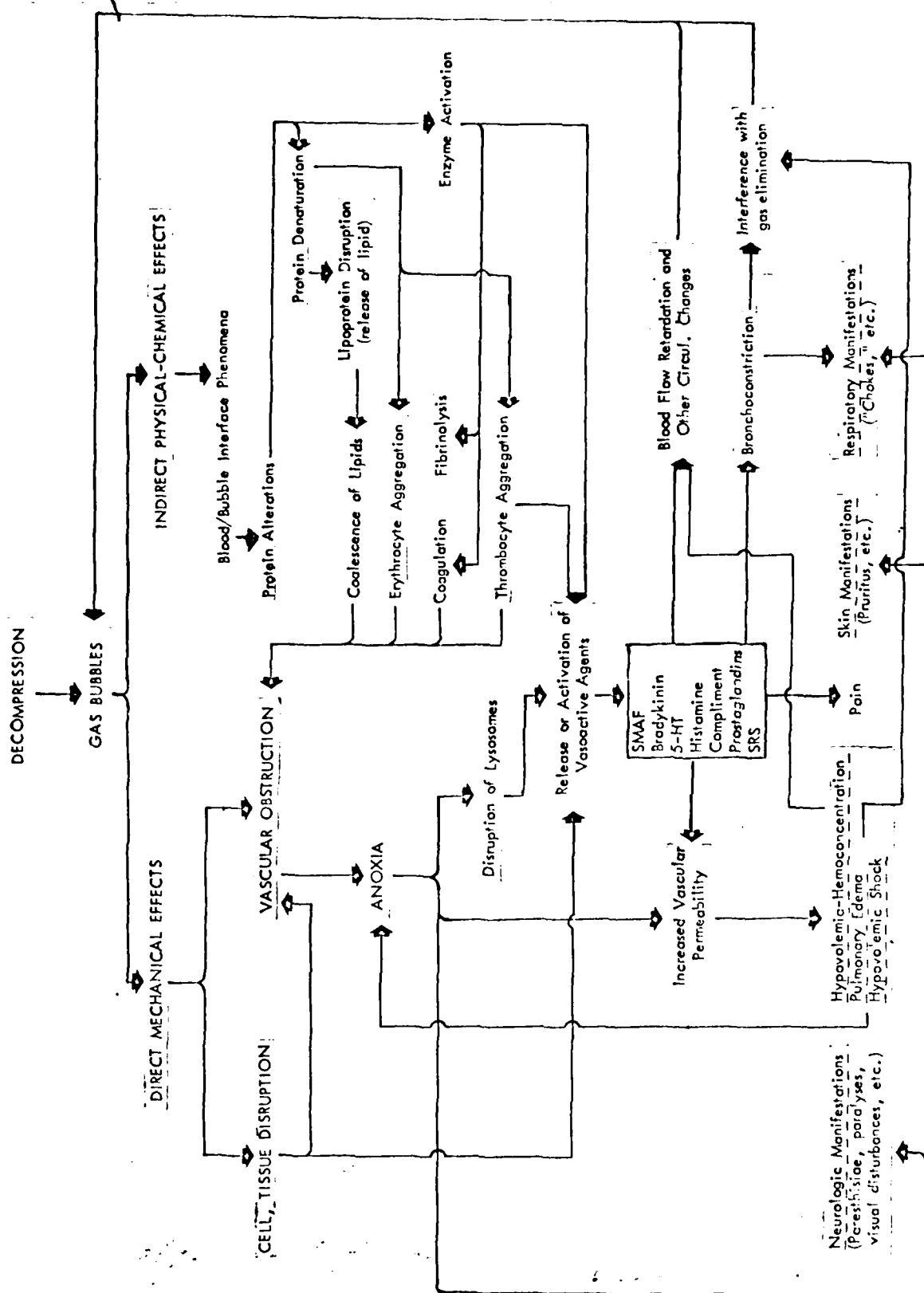


Chart 1

MECHANISM OF RELEASE OR ACTIVATION OF VASOACTIVE AGENTS IN DECOMPRESSION SICKNESS

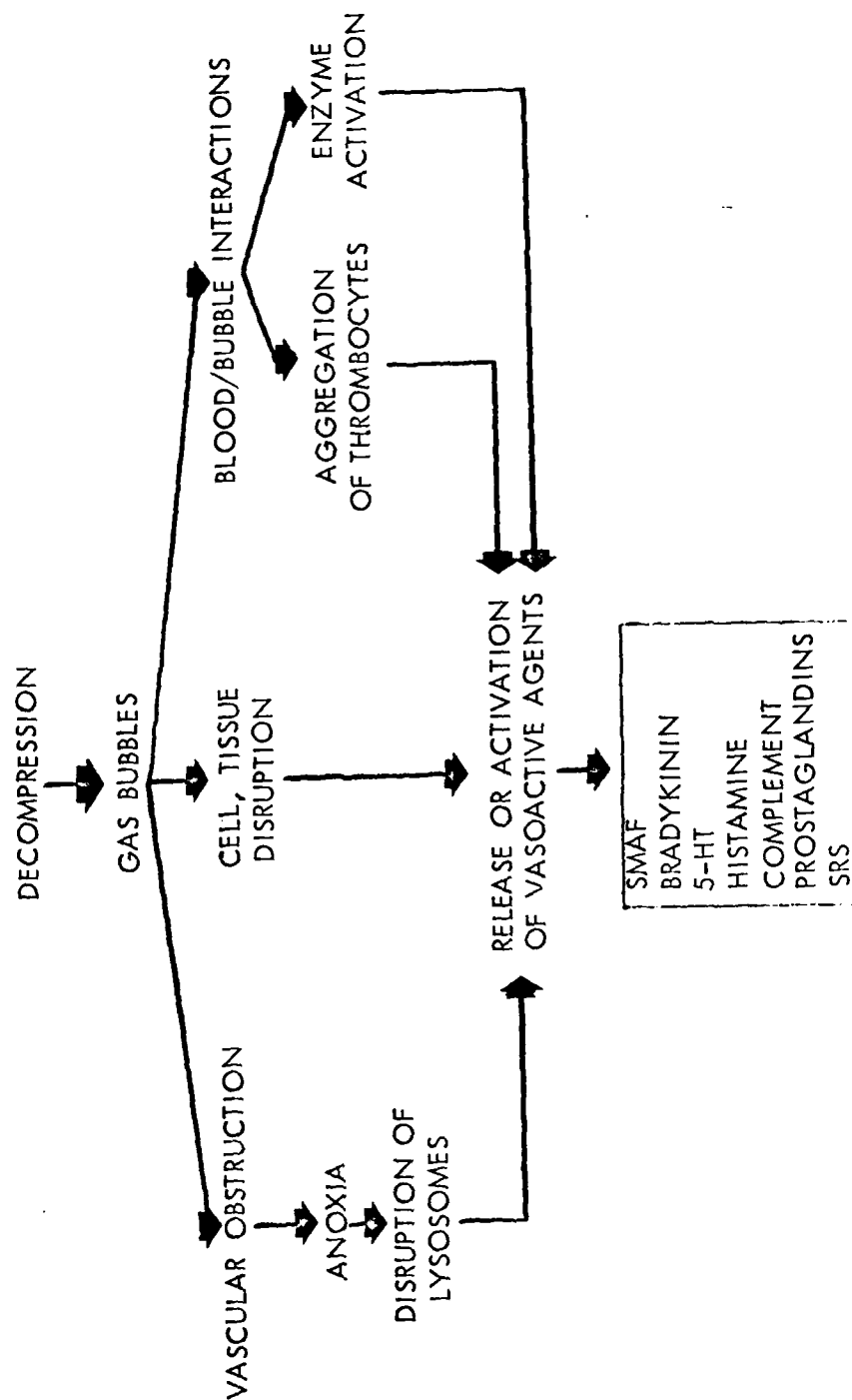


Chart 2

IMPLICATION OF VASOACTIVE AGENTS IN THE PATHOGENESIS OF DECOMPRESSION SICKNESS

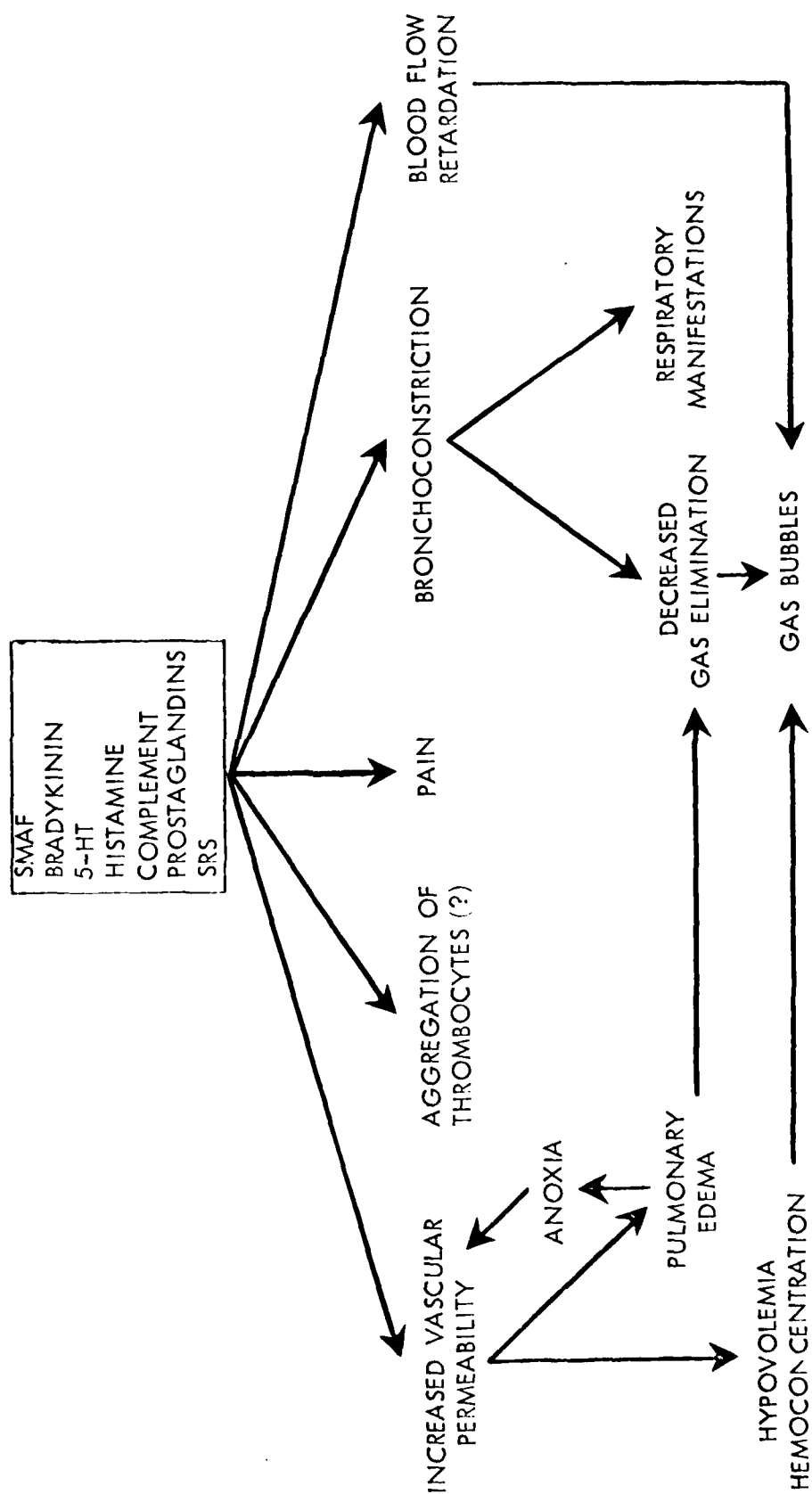


Chart 3

PROPOSED PATHOGENIC MECHANISMS IN DYSBARIC OSTEO NECROSIS

COMPRESSION — DECOMPRESSION

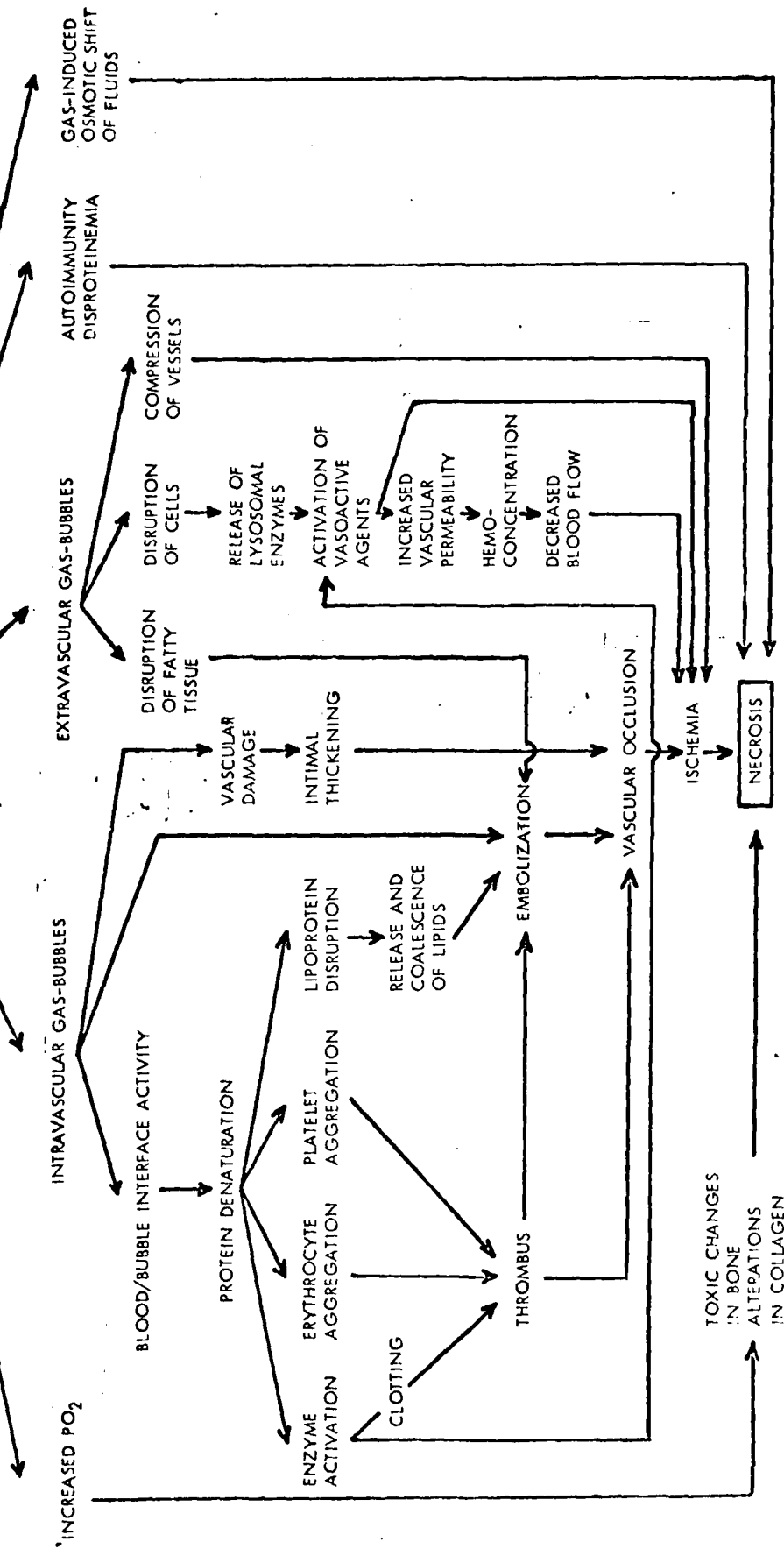


Chart 4

PUBLICATIONS AND REPORTS WHICH STEMMED FROM THE O.N.R. SUPPORTED STUDIES IN
THE PERIOD JUNE 1968 - MARCH 1982

1. Studies on the Chemical Nature of a Smooth Muscle Acting Factor (SMAF)
Extracted from Rabbit Lung. Chryssanthou, C., Goldstein, G., Teichner, F.
Antopol, W.; Fed. Proc. 28:799, 1969
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Extracted from Rabbit Lung. Chryssanthou, C., Goldstein, G., Teichner, F.
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F., Goldstein, G., Kalberer, J., Jr. and Antopol, W.; Aerospace Medicine 41:
864-867, 1971.
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W.; Aerospace Medicine 42:864-867, 1971
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Teichner, F., Goldstein, G. and Antopol, W.; Proceedings of the International
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6. Production and Prevention of Decompression Sickness in "Non-Susceptible"
Animals. Chryssanthou, C., Teichner, F. and Antopol, W.; Aerospace Medical
Association Meeting Preprints, pp. 111-112, 1971
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Chryssanthou, C.; Abstracts of BuMed-ONR sponsored Navywide Workshop in
High Pressure Biomedical Research, Published by the Naval Submarine Medical
Research Laboratories, p. 42, 1971

8. Experimental Production of Aseptic Bone Necrosis in Mice. Chryssanthou, C. and Antopol, W.; Aerospace Medical Association Meeting Preprints, pp. 255-256. 1972
9. Mechanism and Prevention of Decompression Sickness. Chryssanthou, C.; Seminar, Naval Submarine Medical Center, Groton, Conn., Feb. 3, 1972
10. Animal Model for Aseptic Bone Necrosis. Chryssanthou, C; Seminar, Naval Submarine Medical Center, Groton, Conn., Feb. 3, 1972
11. Decompression Sickness and a Smooth Muscle Acting Factor (SMAF). Chryssanthou, C.; Proceedings of the Stress Study Group Meeting, Philadelphia, Pa., pp. 109-129, 1972
12. Newer Concepts on the Mechanism and Prevention of Decompression Sickness. Chryssanthou, C., Teichner, F., Goldstein, G. and Antopol, W.; Abstracts of Papers, XXth International Congress of Aviation and Space Medicine, p. 66, 1972
13. Prevention of Decompression Sickness by Dimethothiazine. Chryssanthou, C., XXIst International Congress of Aviation and Space Medicine Preprints, pp. 54-55, 1973
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15. Generation of SMAF Activity in Blood by Gas Bubbles. Chryssanthou, C., Waksman, M. and Koutsoyiannis, M.; Undersea Biomedical Research 1:A9, 1974
16. Pathogenesis and Treatment of Decompression Sickness. Chryssanthou, C.; New York State Journ. of Med. 74:808-812, 1974

17. The Possible implication of a Humoral Smooth Muscle Acting Factor (SMAF) on Shock. Chryssanthou, C.; The Mount Sinai Journal of Medicine 41:260-266, 1974.
18. Studies on Dysbarism V. Prevention of Decompression Sickness in Mice by Dimethothiazine. Chryssanthou, C., Teichner, F. and Koutsoyiannis, M.; Aerospace Medicine 45:279-282, 1974
19. Humoral Factors in the Pathogenesis of Decompression Sickness. Chryssanthou, C.; In:Blood-Bubble Interaction in Decompression Sickness. K.N. Ackles (Ed.) DCIEM Conference Proceedings No. 73-CP-960, pp. 165-170, 1974
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28. Prophylaxis Against Decompression Sickness by Cyproheptadine. Chryssanthou, C., Teichner, F. and Graber, B.; Undersea Biomedical Research 5:27, 1978
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31. Amelioration of Decompression Sickness in Mice by Pretreatment with Cyproheptadine. Chryssanthou, C., Teichner, F. and Graber, B. Undersea Biom. Res. 7:321-329, 1980.
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34. Animal Model of Human Disease, Dysbaric Osteonecrosis, Dysbaric Osteonecrosis in Mice. Chryssanthou, C.; Amer. J. of Path. 103:334-36, 1981
35. Modification of the Blood-Brain Barrier by Smooth Muscle Acting Factor (SMAF) Chryssanthou, C., Kersh, R., and Margiotta, M.; Undersea Biomed. Res. 8:31-32, 1981
36. Dysbaric Osteonecrosis, Model No. 227. Chryssanthou, C.; In Handbook: Animal Models of Human Disease; Fasc. 10; Edited by C.C. Capen, D.B. Hackel, T.C. Jones and G. Migaki. Registry of Comparative Pathology, Armed Forces Institute of Pathology, Washington, D.C., 1981, 3p.
37. Reversibility of Dysbaric Alteration of the Blood-Brain Barrier. Chryssanthou, C., Fuhrer R., and Higgins, D.; Undersea Biomed. Res. 9:30, 1982
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39. Unpublished data.

DETAILED REPORTS

DECOMPRESSION SICKNESS:
PATHOGENESIS AND PREVENTION

Studies on Dysbarism: III. A Smooth Muscle-acting Factor (SMAF) in Mouse Lungs and Its Increase in Decompression Sickness

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Studies on Dysbarism: III. A Smooth Muscle-acting Factor (SMAF) in Mouse Lungs and Its Increase in Decompression Sickness

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A smooth muscle-acting factor (SMAF) was derived from mouse lungs. The procedure for extraction and partial purification is described. SMAF was shown to: (1) elicit contraction of smooth muscle, (2) potentiate smooth muscle contractions produced by bradykinin, acetylcholine, 5-hydroxytryptamine and histamine and (3) increase vascular permeability. The activity of SMAF was significantly higher in lung tissue from animals subjected to compression-decompression than in lung tissue of equal weight from controls. SMAF is probably a polypeptide or a mixture of polypeptides. Physicochemical and pharmacological properties differentiate SMAF from other polypeptides with similar actions. The possible role and significance of SMAF in the pathogenesis of decompression sickness is discussed.

IT WAS PREVIOUSLY REPORTED that bradykinin and possibly other humoral smooth muscle stimulating substances may be implicated in the pathogenesis of decompression sickness.¹ This hypothesis was supported by the following observations: (1) Several of the histologic changes seen in decompression sickness are similar to those produced by bradykinin, (2) bradykinin intensifies the pathologic alterations and increases mortality in decompression sickness, (3) bradykinin antagonists and certain anti-inflammatory compounds ameliorate or prevent development of the disease as evidenced by the striking reduction in mortality and absence or decrease in severity of the pathologic changes.

Seeking more direct evidence, the possibility that smooth muscle stimulating or sensitizing substances are

released or activated in decompression sickness was explored. The present communication concerns a smooth muscle-acting factor (SMAF), extracted from mouse lungs, the activity of which increases in decompression sickness.²

MATERIAL AND METHODS

Production of Decompression Sickness: Hereditary obese hyperglycemic mice which are susceptible to decompression sickness¹ were employed. These animals, weighing 38-65 grams, were obtained from Jackson Memorial Laboratories, Bar Harbor, Maine. They were housed in metal cages in animal rooms with controlled temperature ($71 \pm 2^\circ\text{F}$) and relative humidity (50%) and were fed Purina Laboratory Chow and water *ad libitum*.

Decompression sickness was produced in these animals by a method previously described.⁴

Preparation of SMAF: In each experiment, lung extracts were prepared from two groups, one consisting of five mice subjected to compression-decompression, the other of five controls. A total of 14 experiments were performed. In the extraction procedure, precautions were taken to avoid or minimize the possibility of *in vitro* activation of smooth muscle stimulating substances, and to protect active substances against enzymatic degradation. As soon as the animals were sacrificed (in the experimental group the animals that did not succumb were sacrificed 20 minutes after decompression), the lungs were rapidly excised, pooled according to group, weighed, and immediately placed in 1N hydrochloric acid (300 mg of lung tissue/ml) in a boiling water bath for ten minutes. During this heating period, the lungs were minced and homogenized by means of a glass tissue grinder with teflon pestle or by sonication. Following heating, the homogenates were rapidly cooled

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in an ice bath, soybean trypsin inhibitor (200 mcg/ml homogenate) was added, and the pH was raised to 7.6. After centrifugation to remove coarse particulate matter the supernatants were dialyzed against 30 volumes of deionized water for 24 hours. The dialysates were concentrated under reduced pressure in a boiling water bath, and seven volumes of absolute ethanol were added. The precipitate formed was removed by centrifugation and discarded. The supernatant was placed in a boiling water bath and evaporated to dryness under reduced pressure. The residue obtained was extracted with 90% ethanol; the extract was centrifuged and the supernatant mixed with four volumes of ethyl ether. The precipitate formed was desalted on Sephadex G-10 and then fractionated on Sephadex G-25. When the absorption at 270 nm was plotted against the Sephadex G-25 fraction number, the resulting curve showed two distinct peaks. The first peak was completely separated from the second by repeated passage through the column. The eluates constituting the first peak were collected, lyophilized and used for bioassays and other tests. This material will be referred to as SMAF, an acronym derived from "smooth muscle-acting factor."

Bioassays: The bioassays for detecting and estimating smooth muscle stimulating and/or sensitizing activities of SMAF were carried out on isolated guinea pig ileum and hen rectal cecum suspended in a 5 ml bath of con-

tinuously oxygenated (95% O₂ and 5% CO₂) Tyrode's solution at 34°C and on rat uterus and duodenum suspended in a 5 ml bath of continuously oxygenated DeJalon's solution at 25°C and 34°C respectively. Contractions were recorded on a Grass polygraph. In order to detect and estimate smooth muscle stimulating activity, SMAF from animals subjected to compression-decompression and from controls were introduced into the bath alone and the responses elicited by corresponding doses (derived from equal amounts of wet lung tissue) were compared to each other and to those produced by known amounts of bradykinin. When smooth muscle sensitizing activity to bradykinin, 5-hydroxytryptamine, acetylcholine and histamine was to be assessed, SMAF was introduced into the bath and after 2-3 minutes, without washing, followed by the addition of one of the above agents. Whenever SMAF potentiated the effect of any of the smooth muscle stimulating substances, the potentiation test was repeated and "bracketed" by responses to the smooth muscle stimulating substance alone. In some bioassays SMAF was preceded by introduction into the bath of chymotrypsin in order to determine whether this agent sensitizes the muscle to SMAF. According to Edery's findings,^{6,7} which were confirmed in our laboratories, chymotrypsin sensitizes guinea pig ileum to various kinins, but not to substance P, eledoisin, and angiotensin. All bioassays were repeated two or three times on the same muscle preparation and at least once on another muscle preparation.

Permeability Studies: The effect of SMAF on vascular permeability was studied by utilizing the "blueing" of the rabbit's skin method. Pontamine blue (37 mg/kg) was injected intravenously in rabbits. Fifteen minutes later SMAF from decompressed and control animals was intradermally injected in the depilated abdominal skin at different sites. One hour after the intradermal injections the intensity and diameter of the resulting "blueing" was recorded. In some experiments, a mixture of SMAF and bradykinin or SMAF and histamine was injected in order to explore the possibility that SMAF may potentiate the effect of these agents on vascular permeability. The volume of each intradermal injection was 0.1 ml.

The following substances were used in these investigations: soybean trypsin inhibitor (5x cryst.) (Mann Res. Lab., Inc.), synthetic bradykinin (Sandoz), serotonin creatinine sulfate (Mann Res. Lab., Inc.), histamine dihydrochloride (Fisher Scientific Co.), acetylcholine bro-

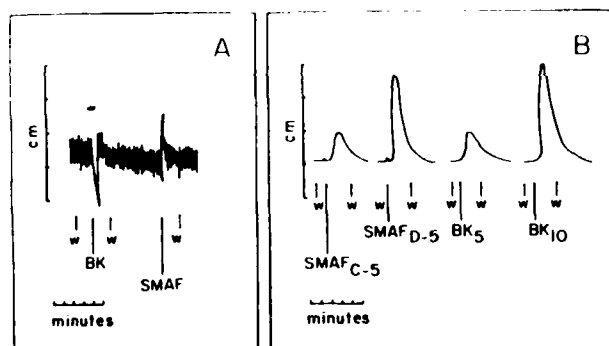


Fig. 1. A. Contrasting effects of SMAF and of bradykinin on rat duodenum. SMAF (5 mg wet lung tissue/ml bath), BK (bradykinin 20 ng/ml bath), w (washing). B. Comparison of smooth muscle stimulating activity of SMAF from controls and from decompressed animals on rat uterus. SMAF_{C-5} (controls, 5 mg wet lung tissue/ml bath), SMAF_{D-5} (decompressed 5 mg wet lung tissue/ml bath), BK₅ and BK₁₀ (bradykinin 5 ng and 10 ng/ml bath), w (washing).

TABLE I. COMPARISON* OF ACTIVITY OF SMAF FROM DECOMPRESSED ANIMALS AND CONTROLS

Effect on Smooth Muscle	Total No. Experiments**	Greater Activity In Decompressed (No. Experiments)	Greater Activity In Controls (No. Experiments)	Equal Activity (No. Experiments)	Statistical Significance
Stimulating	13	11	1	1	P<0.001
Sensitizing***	14	9	2	3	P<0.05

*SMAF preparations from decompressed animals and controls were compared in corresponding doses. (Derived from equal amounts of wet lung tissue).

**In each experiment two SMAF preparations were made. One from the pooled lung tissue of 5 animals subjected to compression-decompression, the other from the pooled lung tissue of 5 controls.

***Increase of the smooth muscle responsiveness to bradykinin.

mide (Matheson Coleman & Bell), crystalline chymotrypsin (Miles Chemical Co.), carboxypeptidase B DFP (Worthington), pyribenzamine hydrochloride (Ciba), SQ 10,643 (anti-serotonin compound, Squibb), atropine sulfate (Burroughs Wellcome & Co.), pontamine sky blue 6BX (DuPont).

RESULTS

Effects on Isolated Organs: Most SMAF preparations, both from controls and from animals subjected to compression-decompression, elicited slow responses of guinea pig ileum and rat uterus resembling those produced by bradykinin. SMAF also produced contractions of rat duodenum and hen rectal cecum in contrast to bradykinin, which caused relaxation or had no effect (Figure 1A). When SMAF from animals subjected to compression-decompression was compared to that from the control group in corresponding doses, the former produced an appreciably greater response in most of the experiments (Table I). Figure 1B shows that SMAF from control animals in a dose corresponding to 5 mg of wet lung tissue/ml of bath elicited a contraction of the rat uterus equal to that produced by 5 ng of bradykinin/ml of bath. A corresponding dose of SMAF from animals subjected to compression-decompression produced approximately a twofold greater response, corresponding to 10 ng bradykinin/ml of bath.

In addition to its smooth muscle stimulating activity, SMAF increased the sensitivity of smooth muscle to bradykinin, histamine, acetylcholine and 5-hydroxytryptamine. The potentiation of bradykinin was of greater magnitude and more constant than that of the other agents. Figure 2 shows that SMAF extracted from compressed-decompressed animals corresponding to 2.5 mg of wet lung tissue/ml of bath increased the response produced by 5 ng of bradykinin/ml of bath to that corresponding to 10 ng of bradykinin. In most experiments, SMAF from animals subjected to compression-decom-

pression exhibited a greater potentiation of bradykinin than did corresponding doses of SMAF from control animals (Table I) (Figure 2). A dose response relationship was observed between the amount of SMAF and

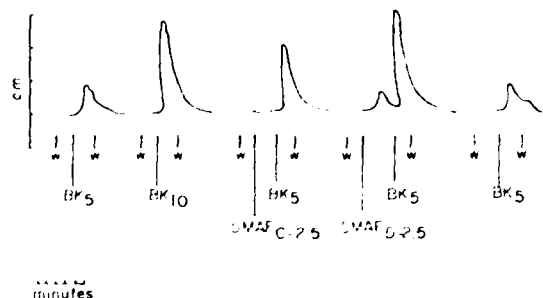


Fig. 2. Comparison of the bradykinin-potentiating activity of SMAF from controls and from decompressed animals on rat uterus. BK₅ and BK₁₀ (bradykinin 5 ng and 10 ng/ml bath), SMAF_{C-2.5} (controls 2.5 mg wet lung tissue/ml bath), SMAF_{D-2.5} (decompressed 2.5 mg wet lung tissue/ml bath), w (washing).

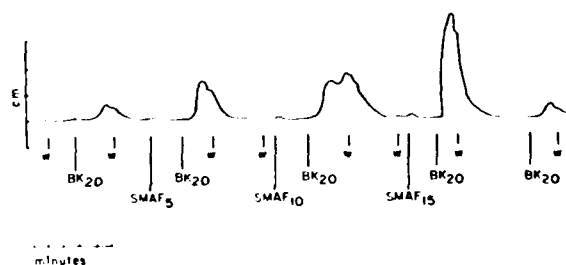


Fig. 3. Dose response relationship of the bradykinin-potentiating activity of SMAF on rat uterus. BK₂₀ (bradykinin 20 ng/ml bath), SMAF₅, SMAF₁₀ and SMAF₁₅ (5 mg, 10 mg, and 15 mg wet lung tissue/ml bath), w (washing).

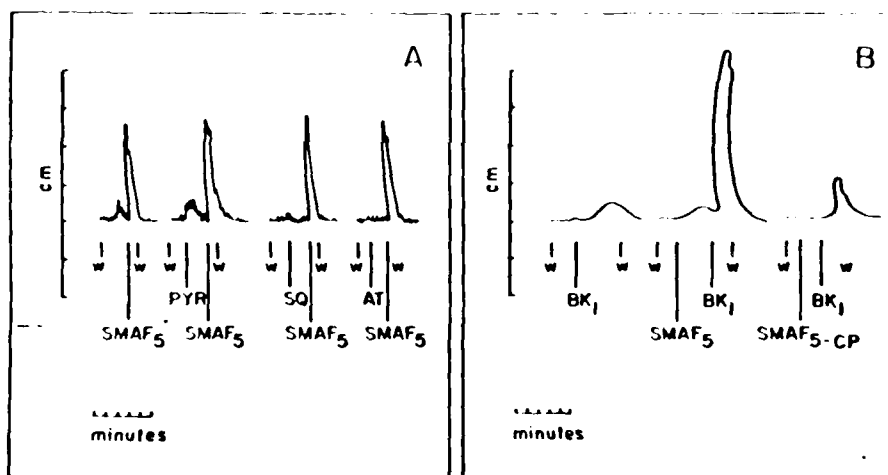


Fig. 4. A. Effect of antihistaminics, antiserotonin compound and atropine on the smooth muscle stimulating activity of SMAF on guinea pig ileum. SMAF₅ (5 mg wet lung tissue/ml bath), PYR (pyribenzamine 0.2 mcg/ml bath), SQ (SQ 10643 0.2 mcg/ml bath), AT (atropine 0.2 mcg/ml bath), w (washing).

B. Effect of carboxypeptidase B on the smooth muscle stimulating and sensitizing activity of SMAF on rat uterus. BK₁ (bradykinin 1 ng/ml bath), SMAF₅ (5 mg wet lung tissue/ml bath), SMAF_{5-CP} (Same as SMAF₅, but incubated with carboxypeptidase B), w (washing).

the degree of potentiation (Figure 3). SMAF in doses which, by themselves, did not elicit contraction increased the sensitivity of smooth muscle so that it responded even to subthreshold doses of bradykinin. The muscle stimulating activity as well as the potentiating effect of SMAF were not inhibited by antihistaminics, atropine, or antiserotonin compounds (Figure 4A), but were abolished or markedly decreased when SMAF was incubated with carboxypeptidase B (1 mcg/50 mg wet lung tissue) prior to its introduction into the organ bath (Figure 4B). The degree of SMAF inactivation by carboxypeptidase B was directly related to the incubation period.

When SMAF was tested following addition of chymo-

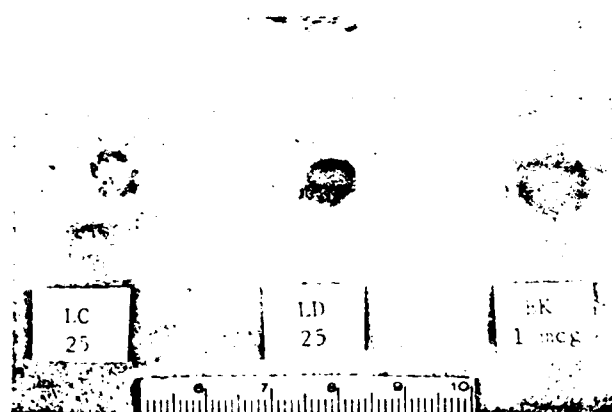


Fig. 5. Comparison of the effect of SMAF from controls and from decompressed animals on vascular permeability ("blueing" of rabbit's skin), I.C. (SMAF from controls, 25 mg wet lung tissue), I.D. (SMAF from decompressed animals, 25 mg wet lung tissue), BK (bradykinin 1 mcg).

trypsin to the bath (0.2 mg/ml of bath), its smooth muscle stimulating activity was markedly potentiated. Chymotrypsin sensitized the rat uterus to SMAF to a greater degree than it did to bradykinin, since, following chymotrypsin, subthreshold doses of SMAF elicited responses greater than those produced by above threshold doses of bradykinin.

Effects on Permeability: SMAF both from animals subjected to compression-decompression and from controls increased vascular permeability, as evidenced by the production of "blueing" in the rabbit's skin. SMAF from compressed-decompressed animals produced "blueing" over a larger area and of greater intensity than did corresponding doses of SMAF from control animals (Figure 5). The effect of bradykinin and histamine on vascular permeability was increased when these substances were mixed with SMAF. It was not possible, however, to determine whether this was a potentiating or an additive effect.

Physicochemical Properties: Considering the extraction and purification procedures of SMAF, it can be stated that the active agent(s) is heat stable (100°C), dialyzable, water soluble, ethanol soluble, and ethyl ether insoluble. The fact that it is dialyzable and can be inactivated by incubation with carboxypeptidase B suggests that the active factor(s) is a polypeptide or a mixture of polypeptides of relatively small molecular weight.

Differentiation from Other Similar Polypeptides: SMAF shares many of its physicochemical characteristics and biological effects with a number of known polypeptides, but it cannot be identified as any single one of them. Table II presents some of the differences between SMAF and some other biologically active poly-

TABLE II. DIFFERENCES BETWEEN SMAF AND SOME BIOLOGICALLY ACTIVE POLYPEPTIDES

	SMAF	Brady- kinin	Subs. P	Angio- tensin	Vaso- pressin	Oxy- tocin	Peptide B*	BPE**	Kuta- pressin***
Ethanol (Abs.) soluble	Yes	Slightly							
Inactivated by carboxypeptidase B	Yes	No					No		
Contraction of rat uterus	Yes							No	
Contraction of guinea pig ileum	Yes					Relax- ation		No	
Contraction of rat duodenum	Yes	Relax- ation			No	No			
Contraction of hen rectal cecum	Yes	Relax- ation			Relax- ation	Relax- ation			
Inhibited by atropine (guinea pig ileum)	No			Partially					
Potentiated by chymotrypsin	Yes	No	No						
Potentiates bradykinin	Yes	No							
Potentiates 5-HT	Yes								No
Potentiates histamine	Yes							No	No
Potentiates acetylcholine	Yes							No	
Increases capillary permeability	Yes			Not in rabbits	No	No		High doses only	

*A bradykinin potentiating peptide released from fibrinogen by thrombin (see ref. 11)

**A bradykinin potentiating factor obtained from snake venom (see ref. 9)

***A bradykinin potentiating liver extract (see ref. 14)

peptides and agents which have been reported to potentiate bradykinin.^{2,11,14}

DISCUSSION

The data presented indicate that SMAF prepared by the methods described is capable of eliciting smooth muscle contractions, of enhancing the responses of smooth muscle to bradykinin, 5-hydroxytryptamine, acetylcholine and histamine, and of increasing vascular permeability. The increase in the magnitude of contraction elicited by smooth muscle stimulating substances following addition of SMAF to the organ bath is interpreted as a potentiation of their action. This conclusion is based on the fact that the increase in the magnitude of contraction was in excess of that expected if it were due merely to an additive effect.

The exact chemical composition of SMAF has not been determined. Its dialyzability and its inactivation by proteolytic enzymes suggest a polypeptide nature. Further purification and characterization of SMAF is in progress. The possibility that the smooth muscle stimulating activity of SMAF is due to the presence of histamine, 5-hydroxytryptamine or acetylcholine can be ruled out on at least three counts: the method of SMAF preparation, its enzymatic inactivation by carboxypeptidase B, and the fact that antihistaminics, atropine, and antiserotonin compounds did not inhibit smooth muscle responses elicited by SMAF. The enzymatic inactivation of SMAF also excludes the possibility that its smooth muscle sensitizing activity might be due to thiol compounds (Glutathione, cysteine) which have been reported to potentiate the response of guinea pig ileum to bradykinin.¹⁰

SMAF resembles several smooth muscle stimulating polypeptides, particularly bradykinin, with which it shares many physicochemical characteristics and pharmacologic effects. In view of the above and considering the implication that SMAF is a new factor of polypeptide nature, its differentiation from similar biologically active polypeptides is necessary. Table II summarizes some of the differences between SMAF and other similar smooth muscle stimulating or sensitizing agents.

The fact that the activity of SMAF extracted from animals subjected to compression-decompression is greater than that of SMAF extracted from equal amounts of lung tissue from control animals suggests that SMAF may be released or activated in decompression sickness. This further supports the original hypothesis that smooth muscle stimulating substances are involved in decompression sickness.⁴ The presence of SMAF in lung extracts of obese-hyperglycemic mice is not unique, since it was also found in thin mice² as well as in rabbits.³

The role of SMAF in the pathogenesis of decompression sickness may be due to its direct effects on smooth muscle and on vascular permeability as well as to its potentiation of the effects of humoral factors with smooth muscle stimulating activity. Direct effects on smooth muscle could cause bronchoconstriction and circulatory changes which may influence decompression

sickness in a way similar to that postulated for bradykinin.⁴ The increased vascular permeability produced by SMAF could cause extravasation of plasma and subsequently hypovolemic shock which has been observed as a complication of decompression sickness and has been considered a major factor in the death of animals after decompression.⁵ The smooth muscle "sensitizing" effect of SMAF may have even greater importance. In view of the possible implication of smooth muscle stimulating substances in decompression sickness, agents which can modify tissue responsiveness to them may play critical roles. Minimal concentrations or even sub-threshold levels of smooth muscle stimulants may produce significant tissue reactions when tissues are sensitized by SMAF. Certain investigators have considered the possibility of involvement of some smooth muscle stimulating substances in decompression sickness in rats but failed to demonstrate significant increase in their concentration in tissues following rapid decompression.¹¹ In the light of our findings, failure to show an increase in the level of these substances in decompression sickness does not necessarily rule out their involvement in the disease since they could elicit stronger reactions without an increase in their concentration if the responsiveness of the muscle is enhanced by sensitizing factors.

The mechanism by which SMAF is released or activated in decompression sickness is obscure. Activation of biologically active substances has been reported in shock^{12,13} and following tissue injuries by mechanical pressure, burns, and freezing.⁴ It is not impossible that among other triggering mechanisms, expanding gas bubbles, causing circulatory impairment, anoxia and mechanical injury of tissues may initiate reactions resulting in release or activation of SMAF. Speculations regarding the mechanism of SMAF activation or release, however, seem to be premature.

The possible significance of SMAF may extend beyond the pathogenesis of decompression sickness. SMAF or similar humoral agents may play important roles by modifying smooth muscle responsiveness to physiologic or abnormal stimuli.

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GENERATION OF SMAF ACTIVITY IN BLOOD BY GAS BUBBLES.

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The nature and properties of a hitherto unidentified humoral Smooth Muscle-Acting Factor (SMAF) and its possible implication in the pathogenesis of decompression sickness (DS) has been previously reported. (Aerospace Med., 41: 43-48, 1970 and 42:864-867, 1971.) Among various conceivable mechanisms, the possibility that SMAF is released or activated in DS in the course of chain reactions initiated by blood-bubble interphase activity was explored. Samples of blood, cell-free plasma and plasma containing leukocytes and platelets were subjected to bubble generating compression-decompression, or to direct bubbling with air. SMAF was extracted from these samples and from corresponding controls (not bubbled) and its level determined both by bioassays and by measurement of the optical density (at 270 nm) of the isolated material. The level of SMAF was higher in bubbled samples than in corresponding controls. The observation that bubbling of cell-free plasma resulted in an increase of SMAF levels suggests that generation of SMAF activity is not dependent upon formed elements of the blood. There are indications that the Hageman factor may participate in SMAF releasing or activating reactions triggered by gas bubbles. (Supported by the Office of Naval Research, Department of the Navy, Contract # N00014-68-A-0393.)

Studies on Dysbarism: IV. Production and Prevention of Decompression Sickness in "Non-Susceptible" Animals

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CHRYSSANTHOU, C., F. TEICHNER and W. ANTOPOL. *Studies on dysbarism IV. Production and prevention of decompression sickness in "non-susceptible" animals.* *Aerospace Med.* 42(8): 864-867. 1971.

Thin mice subjected to 90 psi absolute air pressure for 5 hours and then decompressed to sea level within one minute do not develop decompression sickness. A relatively small incidence of the syndrome is observed when the animals, after a short surface interval, are further decompressed to a simulated altitude of 26,000 ft. SMAF (a previously reported smooth muscle-acting factor) markedly increases the incidence if administered prior to exposure to altitude. When, however, 2-(4-phenyl-1-piperazinylmethyl) cyclohexanone (a compound which blocks bradykinin and histamine effects) is given before exposure to 90 psi none of the animals develops the disease. The findings support the postulated implication of humoral smooth muscle-stimulating factors in the pathogenesis of decompression sickness, thus providing a new pharmacological approach for the prevention or amelioration of the syndrome.

THE PRESENT investigation deals with the production and prevention of decompression sickness in animals which, under the experimental methods that have hitherto been employed in our laboratories, were not susceptible to this disease. The following observations provided the basis for these studies:

1. Exposure to 90 psi absolute air pressure for six hours followed by decompression to sea level within one minute produces decompression sickness in obese but not in thin mice.^{1,2,3}

This investigation was supported by the Office of Naval Research, Department of the Navy, under Contract #N00014-68-A-0393 (NR 101-735), the Saul Singer Foundation, and the Lenore Weinstein Fund.

2. Bradykinin and possibly other smooth muscle-stimulating substances are considered to play a role in the mechanism of decompression sickness since compounds with antibradykinin and antihistaminic activity, such as 2-(4-phenyl-1-piperazinylmethyl) cyclohexanone (PPCH) prevent or ameliorate the disease, while bradykinin intensifies pathologic alterations and increases mortality.^{6,7}

3. SMAF, a smooth muscle acting factor extracted from lung capable of increasing vascular permeability and responsiveness of smooth muscle to bradykinin and other stimulants, has been implicated in the pathogenesis of the disease, since its activity increases in animals subjected to compression decompression.^{6,8}

In view of these observations, the possibility that administration of SMAF could render thin mice susceptible to decompression sickness was entertained. The ability of PPCH to counteract the effect of SMAF and prevent development of the syndrome was also explored.

MATERIALS AND METHODS

The animals employed in these investigations were thin siblings (18-27 gms) of the hereditary obese hyperglycemic mice from Jackson Memorial Laboratories, Bar Harbor, Maine. They were housed in metal cages in a room with controlled temperature (71 ± 2 F) and relative humidity (50%) and were fed Wayne Lab-Blox and water *ad libitum*.

The substances employed were 2-(4-phenyl-1-piperazinylmethyl) cyclohexanone HCL (PPCH) (Miles-Ames) solution containing 30 mg/ml and SMAF solution containing 2 mg/ml. The latter was prepared with the previously described Sephadex G-25 fraction of the lung extract⁷ and corresponded to 2 gm of wet lung tissue/ml of solution.

A series of four experiments were conducted utilizing a total of 110 mice which were divided into the following groups:

- Exp. #1: Group A (10 mice) and Group B (10 mice)
- Exp. #2: Group A (10 mice) and Group B (10 mice)
- Exp. #3: Group A (10 mice), Group B (10 mice), Group C (10 mice) and Group D (10 mice)
- Exp. #4: Group A (10 mice), Group B (10 mice) and Group C (10 mice)

In all of these experiments Groups A, B and C were placed in the previously described pressure chamber,⁶ subjected to 90 psi absolute air pressure for 5 hours, decompressed to sea level within one minute (usually 30 seconds) and 1 minutes later further decompressed to a simulated altitude of 26-28,000 ft. (270-247 mm Hg) for 40 minutes. Groups A received no other treatment and served as controls; Groups B and C were intraperitoneally injected with 0.1 ml of SMAF solution immediately following decompression to sea level (before exposure to altitude); Groups C received 3 mg of PPCH subcutaneously immediately before exposure to 90 psi; Group D received 0.2 ml of SMAF intraperitoneally and was kept at sea level (SMAF control).

The various groups of each individual experiment were placed in the pressure chamber in separate cages and exposed simultaneously to the identical pressure conditions. All animals were observed through the port-holes of the chamber for clinical manifestations during exposure to altitude.

RESULTS

Decompression from 90 psi absolute air pressure to sea level did not produce clinical manifestations in any of the groups. Soon after exposure to altitude, however, the majority of the animals of Groups A and B exhibited chokes, hiccup-like spells and convulsions. Some of them ran about erratically, others fell on their sides gasping and twitching, and several ceased to move and appeared dead. Fatalities were confirmed and recorded only after removal of the cages from the chamber.

The animals of Groups C did not develop the symptoms observed in Groups A and B.

Table I presents mortalities in the various groups. It can be seen that treatment with SMAF increased mortality from 12.5% (Group A controls) to 45% (Group B).

Treatment with PPCH, on the other hand, counteracted the effect of SMAF and lowered mortality from 45% (Group B) to 0% (Group C). Statistical evaluation of these results shows significance at high levels of confidence (Table I).

Autopsies on the animals which died in Groups A

TABLE I. EFFECT OF SMAF AND PPCH ON MORTALITY IN DECOMPRESSION SICKNESS

Experiment #	Compression Decompression (Group A)	% Mortality		
		Compression SMAF Decompression (Group B)	PPCH Compression SMAF Decompression (Group C)	SMAF (Group D)
1	20 (2/10)*	50 (5/10)		
2	10 (1/10)	60 (6/10)		
3	10 (1/10)	20 (2/10)	0 (0/10)	0 (0/10)
4	10 (1/10)	50 (5/10)	0 (0/10)	
Total	12.5 (5/40)	45 (18/40)*	0 (0/20) ^b	0 (0/10) ^c

*Number of dead animals/total number of animals in group.

*Significantly differs from mortality in group A ($P < 0.01$).

^bSignificantly differs from mortality in group B ($P < 0.001$).

^cSignificantly differs from mortality in group B ($P < 0.05$).



Fig. 1. Gas bubbles in spleen from mouse treated with SMAF immediately before exposure to simulated altitude. (Stained with hematoxylin and eosin. Original magnification 25 $\times$.)

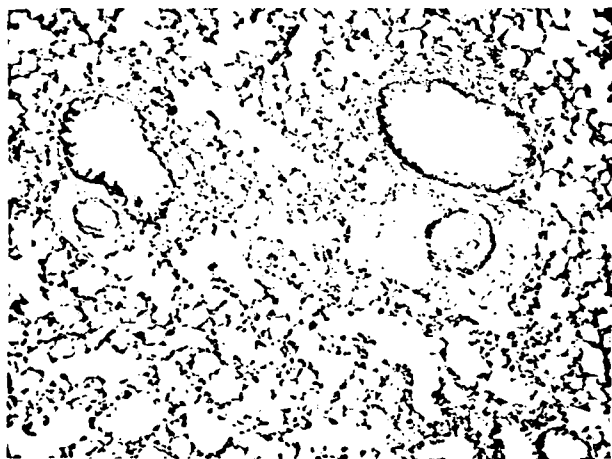


Fig. 2. Striking perivascular space in lung of mouse treated with SMAF immediately before exposure to simulated altitude. (Stained with hematoxylin and eosin. Original magnification 32 $\times$.)



Fig. 3. Lung from mouse treated with SMAF immediately before exposure to simulated altitude. Several round spaces resembling gas bubbles are present in the lumen of the artery at the bottom. Smaller spaces can be seen in the lumen of the artery in the upper right. (Stained with hematoxylin and eosin. Original magnification 25 $\times$.)

and B, performed immediately after their removal from the chamber, revealed distended abdomen, inflated stomach and intestines and gas bubbles in the inferior vena cava and other veins. On microscopic examination, several of these animals showed gas bubbles in the spleen (Figure 1) and marked congestion with increase in the perivascular space in the lung (Figure 2). These changes were similar to those previously observed in obese mice² which are susceptible to decompression sickness. In addition, well circumscribed round spaces resembling gas bubbles were seen in pulmonary arteries (Figure 3). No autopsy was performed on animals of Group C since they all survived and could not be sacrificed so that mortality rates could be determined. However, in experiments preliminary to this study, mice subjected to the same conditions as Group C were sacrificed at intervals of 15, 30 and 60 minutes following exposure to altitude. None of these animals displayed the pathologic alterations described above.

DISCUSSION

Thin mice exposed to 90 psi absolute air pressure for six hours and then decompressed to sea level within one minute do not develop decompression sickness.² This is consistent with the generally accepted fact that small animals are less susceptible to this disease than larger animals and man. The short circulation time of the small animals might be one of the factors responsible for this difference. Obese mice, on the other hand, subjected to the same experimental conditions do develop compression sickness.^{2,3,6} The greater susceptibility of the obese mouse is considered to be due to the relatively large amount of adipose tissue and the high solubility of nitrogen in fat which results in high PN_2 in this tissue and in high supersaturation ratios at decompression.

The present investigation indicates that a small percentage of the "non-susceptible" thin mice develop decompression sickness when decompression from 90 psi to sea level is followed, after a four minute interval, by further decompression to a simulated altitude of 26,000 feet. This is in accord with the reported occurrence of decompression sickness in human subjects who flew at high altitudes following scuba diving or were exposed to similar hyperbaric-hypobaric conditions with short surface intervals.^{10,11}

The findings presented demonstrate that SMAF markedly increases the incidence of decompression sickness in thin mice and that PPCH significantly counteracts this effect, as evidenced by the clinical and autopsy findings and by the respective mortality rates. The clinical manifestations and high mortality observed in animals which were subjected to hyperbaric-hypobaric conditions and received SMAF were not due to SMAF *per se*, since control mice injected with even double the dose of SMAF but kept at sea level (not exposed to compression-decompression) did not exhibit any abnormal signs and had a mortality of zero.

In view of the postulated implication of smooth muscle-stimulating agents in the pathogenesis of decom-

pression sickness,⁴ it is possible that SMAF increases the susceptibility of thin mice by its direct action on smooth muscle and/or by its ability to increase responsiveness of smooth muscle organs to bradykinin and other stimulants.⁷ In either case, possible bronchoconstriction interfering with elimination of nitrogen, circulatory changes favoring bubble formation and increased vascular permeability may be factors contributing to the development of the syndrome. The role of these factors in the pathogenesis of decompression sickness was discussed in earlier communications.⁶

The ability of PPCH to prevent decompression sickness was striking. The animals which were pretreated with this compound, even though they received SMAF and were exposed to the identical pressure conditions as the other groups, did not develop any clinical symptoms or pathologic alterations and all survived.

PPCH has also been shown to prevent or at least ameliorate decompression sickness in obese mice.⁵ It was postulated that this protective effect was due, at least in part, to antagonism of humoral factors considered to play a role in the pathogenesis of the syndrome, although at that time no direct evidence for such antagonism was available.

Recent investigations in our laboratories, however, have shown that PPCH, which is an anti-inflammatory compound, blocks histamine and bradykinin effects on intact animals and isolated organs.¹² The anti-histaminic activity of PPCH was subsequently corroborated.⁹

It is very difficult, from the data presented, to determine the mechanism of the protective effect of PPCH. It is possible that PPCH blocks the direct effects of SMAF and/or inhibits smooth muscle-stimulants, such as bradykinin, to which SMAF increases the responsiveness of smooth muscle-organs.

The results of this investigation as well as the previously reported findings strongly support the postulated implication of humoral smooth muscle-stimulating factors in the pathogenesis of decompression sickness since: (1) bradykinin-treated mice (under normal atmospheric pressure) develop histologic changes similar to some of those observed in decompression sickness;^{3,5} (2) Bradykinin administration increases mortality and intensifies the pathologic changes produced in the disease;⁵ (3) antagonists of bradykinin and of other smooth muscle-stimulating agents prevent or at least ameliorate the syndrome;⁵ (4) SMAF, a humoral smooth muscle-stimulating factor, increases susceptibility of thin mice

to the disease and PPCH, a compound blocking the action of smooth muscle stimulants, counteracts this effect.

The ability of SMAF to increase the incidence of decompression sickness in thin mice may provide a method of experimental production of the syndrome in relatively less susceptible animals or with smaller partial pressure gradient (lower supersaturation ratios).

The striking protective effect of PPCH observed in these studies strengthens the possibility that this new pharmacologic approach may provide the means for prevention or amelioration of decompression sickness.

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The Possible Implication of a Humoral Smooth Muscle-Acting Factor (SMAF) in Shock*

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Disparity between the circulating volume of blood and the volume capacity of the vascular system is the major factor underlying the reduction of blood flow and the resulting decreased perfusion and anoxia which sustain and further increase the circulatory deficiency in shock. Hypovolemia is a frequent but not a necessary condition for the development of the disparity between volume and capacity. Alterations in the physiologic state of hemodynamics other than or in addition to reduction of blood volume are significant pathogenetic factors. Marked increase in the vascular bed produced by dilation of the resistance and/or of the capacitance vessels could result in severe disruption of peripheral vascular homeostasis with subsequent circulatory collapse. Changes in vascular tone that could lead to the development of shock are primarily induced by humoral agents which are the main mediators governing the state of microcirculation.

A broad spectrum of humoral vasoactive factors has been implicated in the circulatory changes both of the early compensatory as well as the subsequent decompensatory phase of shock. Catecholamines, vasoexcitator material (VEM), vasodepressor material (VDM) (10, 19), myocardial depressant factor (MDF) (14), plasma kinins (3, 21), bacterial factors, prostaglandins (15) and other humoral agents (1, 2, 13) have been proposed as possible mediators of circulatory changes in shock. In view of the implication of vasoactive substances in the mechanism of shock it is apparent that humoral agents capable of modifying the responsiveness of vascular smooth muscle to vasoactive substances may play important roles in the pathogenesis of the disorder.

The present communication is intended as a preliminary report regarding the possible implication of such an agent in the pathogenesis of hemorrhagic and endotoxin shock. This agent, probably a polypeptide, is referred to as SMAF, the acronym derived from Smooth Muscle-Acting Factor. Originally found in mouse lung (6), SMAF, has been subsequently isolated from extracts of various organs (lung, kidney, placenta and blood) in several animal species (mouse, rabbit, rat, dog, human). The method for extraction and purification as well as the physical, chemical, biological and pharmacological properties of SMAF have

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been previously reported (8, 9). The properties of SMAF distinguish it from similar known substances including bradykinin, substance P, edotoisin, prostaglandin, factor BPF (11), Kutapressin (20), peptide B (12), and the polypeptides described by Said (16, 17).

The most striking action of SMAF, which is relevant to the subject of this report, is its ability to increase responsiveness of smooth muscle-organs (including blood vessels) to vasoactive substances (8, 9). SMAF also increases vascular permeability and elicits slow, weak contractions of smooth muscle (8, 9).

The possible role of SMAF in pathologic conditions has already been suggested in studies on the pathogenesis of decompression sickness (4, 7, 9).

This report deals with the observed increase of the level of SMAF in the blood of animals subjected to endotoxin and hemorrhagic shock.

Methods

Endotoxin Shock: Thirteen female albino rabbits weighing 2-3 kg were anesthetized with intravenous administration of sodium pentobarbital (30 mg/kg body weight as an initial dose, with additional intravenous doses if and when needed to maintain anesthesia). Mean carotid blood pressure was continuously monitored by means of a Statham pressure transducer coupled to a Grass polygraph. Heart rates were calculated from ECG or blood pressure records. Endotoxin shock was produced by an intravenous injection of 1 mg/kg *E. coli* or *Proteus vulgaris* endotoxin. Arterial blood samples (10 ml) were obtained from all animals before (control sample) and after endotoxin administration. The latter samples were collected when the mean carotid blood pressure fell below 40 mm Hg, which usually occurred 1-6 hours after endotoxin administration. In some animals additional blood samples were collected at earlier stages of the hypotensive response before blood pressure reached the 40 mm Hg level.

Hemorrhagic Shock: Three female albino rabbits were anesthetized and the mean carotid blood pressure and heart rate monitored as described above. A cannula, connected to a plastic constant-pressure calibrated reservoir, was inserted in the carotid artery. The reservoir, tubing, etc., were all siliconized. Heparin (1500 U/kg) was intravenously administered prior to bleeding. The animal was bled into the reservoir in which the pressure was set to remain constant at 40-45 mm Hg by bubbling 95% O₂ + 5% CO₂ through the blood into the reservoir. After a variable interval following initiation of bleeding, blood flow was spontaneously reversed from the reservoir into the animal. When 40% of the shed blood was spontaneously reinfused, hemorrhagic hypotension was terminated by gradually raising the pressure in the reservoir to permit the remaining shed blood to be infused back into the animal. The purpose of gradual reinfusion was to avoid excessive rise in the central venous pressure. Preliminary experiments have shown that with the method described above, shock progresses to a terminal stage when the animal's mean arterial blood pressure declines below 60 mm Hg. Arterial blood samples (10 ml) were obtained before or at the time of initiation of bleeding (control sample) and during the hypotensive period which follows the reinfusion of blood.

SMAF Level Determination: The blood samples as soon as they were drawn, were placed in 1N hydrochloric acid (0.3 ml of blood, 1 ml acid) in a boiling water bath for ten minutes. SMAF was extracted and isolated by the previously reported methods for extraction of SMAF from tissues (9). The level of SMAF in the various samples was determined by measuring the optical density at 270 nm of the material extracted from 1 ml of blood and dissolved in 1 ml of water. Since the purity of the extracted material and consequently the validity of the optical density as an index of SMAF concentration can be questioned, the activity of SMAF in various samples was also determined by bioassays on isolated organs. In view of the observed correlation between SMAF activity and optical density, the latter was used as an expression of SMAF levels in the various blood samples.

Results

Endotoxin Shock: The initial immediate hypotensive response to the intravenous injection of endotoxin reported in dogs and other animals, which is followed by a transient recovery, was not observed in the rabbits used in these experiments. Following a varying interval after endotoxin administration the animals exhibited a rise in the heart rate and a gradual and progressive fall in the mean arterial blood pressure, which eventually led to the death of the animals. Table I shows SMAF levels expressed in terms of optical density of extracted

TABLE I
Increase of SMAF in Endotoxin Shock

Rabbit *	Before Endotoxin		After Endotoxin		% Change in SMAF OD/ml blood
	B.P. mm Hg	SMAF OD/ml blood	B.P. mm Hg	SMAF OD/ml blood	
17110	96-101	0.050	20-30	0.245	+390
17111	92-96	0.020	30	0.146	+600
17112	100-112	0.081	0-40	0.140	+67
17113	108-112	0.051	20-30	0.110	+104
17095	120	0.026	30	0.048	+85
17096	120	0.015	50*	0.052*	+246
17111	100	0.070	†	†	†
17118	100-112	0.093	†	†	†
17119	92-108	0.081	30	0.069	-14
17120	92-101	0.069	28-30	0.131	+89
17121	100-108	0.081	44‡	0.106‡	+30
17128	82-108	0.090	20-30	0.076	-16
17129	110-130	0.120	30-40	0.143	+19

* The animal died before blood pressure reached 40 mm Hg and therefore there was no sample drawn at that stage. The sample drawn at an earlier stage, however, revealed a marked increase in SMAF level.

† The animal died before blood pressure reached 40 mm Hg. No samples were drawn at earlier stages.

‡ The animal revealed an increase in SMAF at an earlier stage when blood pressure had dropped to 44 mm Hg.

SMAF in blood of the control blood sample (pre-endotoxin) and the sample drawn when blood pressure declined below 40 mm Hg. It can be seen that in 7 of 13 animals SMAF concentrations were appreciably increased when blood pressure fell below 40 mm Hg level. In two animals the increase in SMAF was manifested at an earlier stage and in another two animals the level of SMAF slightly decreased. The remaining two animals died before blood pressure reached 40 mm Hg and no samples were obtained at earlier stages. Excluding the two latter animals, the results indicate that SMAF levels increased in endotoxin shock in 9 out of 11 animals. Statistically evaluated, these results are significant at the $P = 0.01$ level of confidence (t test for paired observations). In those animals from which several blood samples were drawn during the post-endotoxin hypotensive period the level of SMAF showed a progressive increase.

Hemorrhagic Shock: Figure 1 is a representative chart showing the changes in the mean arterial blood pressure in relation to the hemorrhage and reinfusion. The figure also shows the level of SMAF (expressed in optical density of the extracted material in blood) in the blood samples drawn at the time of initiation of hemorrhage (sample #1, control) and during the initial (sample #2)

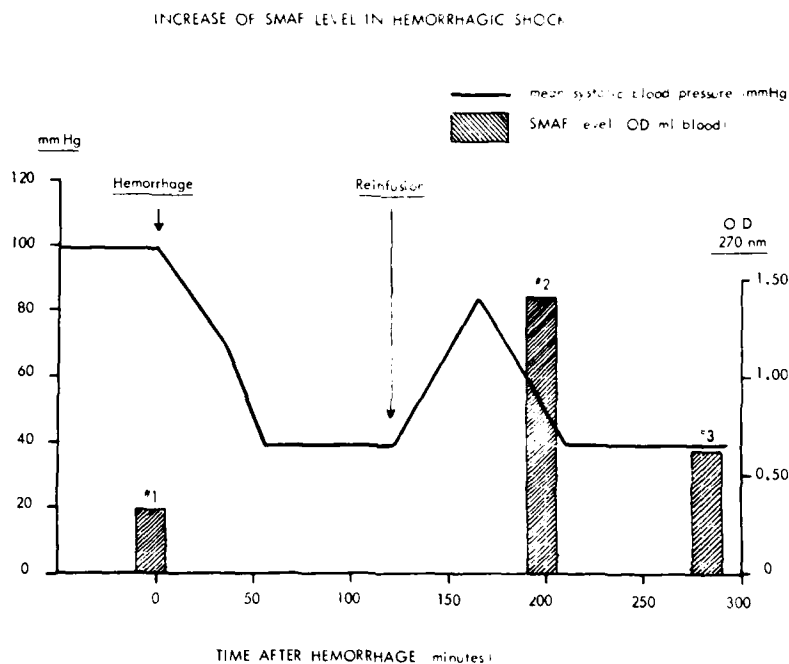


FIG. 1. Continuous mean arterial blood pressure recording with superimposed levels of SMAF in arterial blood samples drawn just prior to bleeding (#1), and in the early (#2) and late (#3) post-reinfusion hypotensive period. At the first arrow (Hemorrhage) the reservoir was opened and bleeding initiated. At the second arrow (Reinfusion) the shed blood was reinfused from the reservoir back into the animal. Note the increase in SMAF during the post-reinfusion hypotensive period.

and late stage (sample #3) of post-reinfusion hypotensive period. It can be seen that the level of SMAF in sample #2, drawn when blood pressure had declined to 50 mm Hg, shows a fourfold increase as compared to the control sample #1. In sample #3, drawn later in the terminal stage of shock and shortly before the animal succumbed, the concentration of SMAF, although it shows a relative decrease, is still above control level. In the remaining animals the results were similar, indicating an increase of SMAF level in the early post-reinfusion period with a relative decrease in the later terminal stage.

Discussion

The scope of the present investigation was confined to exploration of the possible release or activation of SMAF in experimental shock. The results of these preliminary experiments indicate that blood SMAF levels increase in the course of endotoxin and hemorrhagic shock. In the former type of shock the increase in SMAF appears to be progressive and in inverse relationship to the level of blood pressure. Regarding the hemorrhagic shock the available data suggest that SMAF levels increase in the early phase of the post-reinfusion hypotensive period with a relative decrease in the terminal stage.

Although SMAF has not yet been chemically identified, it exhibits chemical, physical and pharmacological properties (8, 9) which distinguish it from all known similar substances which act on smooth muscle. SMAF, probably a low molecular weight polypeptide, is capable of increasing responsiveness of smooth muscle-organs to stimulants. This property of SMAF may be of great significance regarding its possible role in physiologic functions and pathologic conditions. It can be postulated that the function of smooth muscle organs is regulated not only by neurogenic stimuli and myotropic substances which directly cause contraction or relaxation of smooth muscle cells but also by factors, like SMAF, which modify the capacity of these cells to respond to stimuli. SMAF has already been implicated in the pathogenesis of *decompression sickness* (4, 7, 9). On the basis of the present preliminary investigation, the question of possible involvement of SMAF in the mechanism of shock can only be a matter for speculation. The possibility that SMAF, by increasing the reactivity of vascular muscle, may augment the effect of vasomotor stimuli in shock and thus contribute to the deterioration of the peripheral circulation is an attractive and provocative hypothesis. It is of interest in this respect that although depletion of kininogen was observed in several types of shock (18) and release of kinin during anaphylaxis was found in dogs (3), little correlation was seen between the severity of the shock and the amount of kinin formed (3). It is conceivable on the basis of the hypothesis discussed earlier that small increases in bradykinin levels and even "normal" concentrations of this vasodilator agent may induce significant vascular changes if the smooth muscle of the vessels has been sensitized by factors like SMAF with a resulting increase in responsiveness to bradykinin. In addition to the potentiation of the action of vasoactive agents, SMAF, which also increases capillary permeability (9), could cause extravasa-

tion of fluid and thus further increase the disparity between volume and capacity of the vascular system.

The stimulus and the mechanism whereby SMAF is released or activated in shock is at present unknown. Several possibilities merit consideration. It is conceivable that liberation or activation of SMAF is the end point or an intermediate link in chain reactions triggered by physiological and biochemical alterations in shock, such as decreased tissue perfusion and anoxia with disruption of lysosomes and release of proteolytic enzymes. Liberation or activation of intra- and/or extracellular enzymes with formation of chemical mediators from precursor substrates has already been reported in shock (10). It is also possible that SMAF may be released from tissues following ischemic injury, or activated by mechanisms similar to those which trigger the coagulation or kinin systems.

Summary

The level of a humoral Smooth Muscle-Acting Factor (SMAF) increases in the blood of animals subjected to hemorrhagic and endotoxin shock. The possible involvement of SMAF in the pathogenesis of shock and proposed mechanisms regarding its release or activation are discussed.

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**Studies on Dysbarism: V. Prevention
of Decompression Sickness in Mice
by Dimethothiazine**

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Studies on Dysbarism: V. Prevention of Decompression Sickness in Mice by Dimethothiazine

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CHRYSSANTHOU, C., F. TEICHNER, and M. KOUTSOYIANNIS. *Studies on dysbarism: V. -Prevention of decompression sickness in mice by dimethothiazine.* Aerospace Med. 45 (3):279-282, 1974.

Dimethothiazine, a compound with activities against smooth muscle stimulating agents, exhibits protective effects against decompression sickness in obese mice which are susceptible to the disease. In groups receiving dimethothiazine prior to compression, mortality is significantly reduced and clinical manifestations and pathologic changes are less frequent and less pronounced than in corresponding control groups subjected to identical pressure conditions. The results of this report are in accord with the previously proposed pathogenetic concept which implicates humoral smooth muscle stimulating factors in the mechanism of decompression sickness.

EXPERIMENTAL DATA indicating that humoral smooth muscle stimulating agents are implicated in the pathogenesis of decompression sickness have been presented in previous communications (3,4,5,7). Among the findings which support this concept is the observation that administration of smooth muscle stimulants, such as bradykinin and SMAF (Smooth Muscle-Acting Factor) (7), aggravates decompression sickness in mice and increases their susceptibility to the disease, while inhibitors or antagonists of smooth muscle stimulating agents prevent or ameliorate the syndrome (3,4).

The present investigation deals with the possible decompression sickness-preventing effect of dimethothiazine (dimethylsulfamido-3 [dimethylamino-2 propyl]-10 phenothiazine). Dimethothiazine is a phenothiazine derivative which combines activities against bradykinin, histamine and serotonin (8), and therefore exploration of its effectiveness against decompression sickness could provide a further testing of the above-mentioned concept.

MATERIALS AND METHODS

A total of 200 hereditary obese hyperglycemic mice,

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weighing 40 to 80 grams, obtained from Jackson Memorial Laboratories, Bar Harbor, Me, were used in a series of nine experiments. These mice were selected for the present studies because of their susceptibility to decompression sickness (1). They were housed in metal cages under controlled temperature $22 \pm 1^\circ\text{C}$ and relative humidity (50%) conditions, and fed Wayne Lab-Blox and water *ad libitum*. In each experiment, a control and an experimental group, consisting of mice of the same sex and of corresponding weights, were placed in a pressure chamber and subjected to compression-decompression simultaneously to ensure exposure of the two groups to identical conditions. In all experiments the animals were subjected to $6,327 \text{ g/cm}^2$ absolute air pressure for 6 hours and then decompressed to sea level within 1 minute (usually 30 seconds). Immediately prior to compression, the control groups in all experiments received a subcutaneous injection of normal saline while the experimental groups were subcutaneously injected with dimethothiazine (Migristène, Rhône-Poulenc-Specia). In six experiments the dose of dimethothiazine was 40 mg/kg and in the remaining three experiments the dose employed was 4, 10 and 20 mg/kg respectively. Following decompression, the animals were observed for at least 1 hour for clinical manifestations. When an animal died, the survival period from the time of decompression was recorded and an autopsy was immediately performed. Sections of various organs were taken for microscopic examination. Autopsies were also performed on animals sacrificed at intervals of 15, 30 and 60 minutes after decompression.

RESULTS

Under the conditions of these experiments, the clinical manifestations of decompression sickness included scratching, reduced locomotion, chokes, and convulsions. Almost all control animals exhibited these signs and the majority of them succumbed in less than 1 hour following decompression, their death being preceded by twitching and severe respiratory distress with gasping and hiccup-like spells. At autopsy, in almost all control ani-

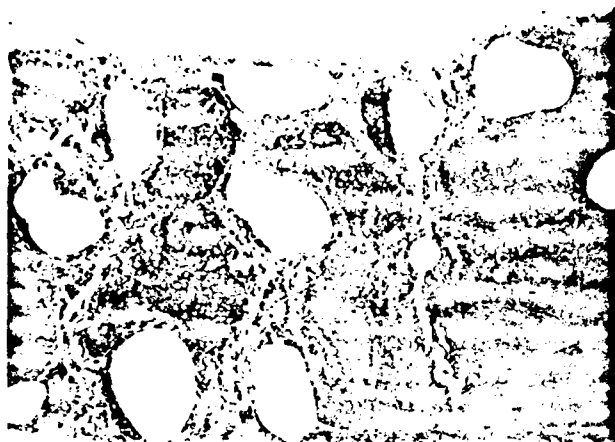


Fig. 1A



Fig. 1B

Fig. 1. (A) Numerous gas bubbles of varying size in the spleen of a control compressed-decompressed mouse. (B) Absence of gas bubbles in the spleen of a mouse treated with 40 mg/kg dimethothiazine prior to compression. (Stained with hematoxylin and eosin. Original magnification $\times 7.5$.)

TABLE I. EFFECT OF DIMETHOTHIAZINE (40 mg/kg) ON MORTALITY OF OBESE MICE IN DECOMPRESSION SICKNESS.

Exp. No.	% Mortality	
	Control	Dimethothiazine
992	100 (8/8)*	25 (2/8)
1/27/72	63 (5/8)	25 (2/8)
1002	50 (6/10)	10 (1/10)
1003	20 (2/10)	0 (0/10)
1004	56 (5/9)	50 (5/10)
1005	70 (7/10)	0 (0/10)
TAL	60 (33/55)	17 (10/56)†

* Number of dead animals/total number of animals in group.

† Significantly differs from mortality in control groups ($p < 0.001$ by chi-square test using Yates correction).

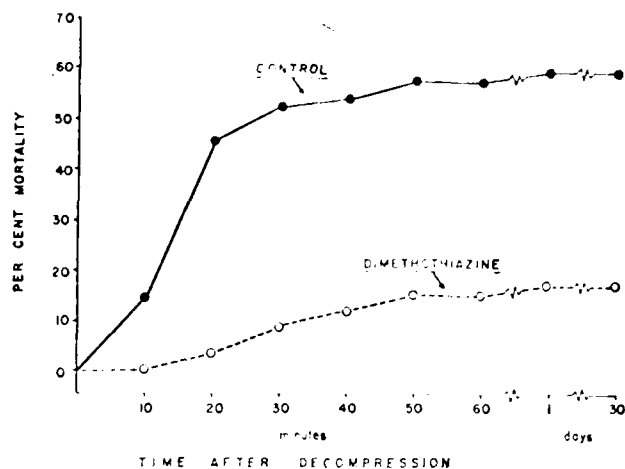


Fig. 2. Mortality curves of mice subjected to compression-decompression. Upper curve: percent mortality of 55 animals treated with normal saline prior to compression. Lower curve: percent mortality of 56 animals treated with 40 mg/kg dimethothiazine prior to compression.

imals that died and in most of those sacrificed, the abdomen appeared enlarged, due to gaseous distension of the stomach and intestines. Grossly visible gas bubbles were seen in the subcutaneous and intra-abdominal fat, in the spleen and in several blood vessels, being most conspicuous in the inferior vena cava and the right chambers of the heart. Microscopic examination revealed minute bubbles as well as large accumulations of gas in several organs and tissues, including the spleen (Fig. 1A), adrenals, bone marrow, adipose tissue, lung and, less frequently, liver and heart. The lung, in addition to intravascular bubbles, revealed perivascular edema. Hyperemia was observed in various organs, being most pronounced in the bone marrow. "Rouleaux formation" was seen in the spleen of an occasional animal.

The 40 mg/kg dose of dimethothiazine, in six separate experiments, exhibited a profound protective effect against decompression sickness, as indicated by the prolongation of the survival time, the significant decrease in the overall mortality, and by the amelioration or absence of clinical manifestations and pathologic alterations. Fig. 2 shows that 44.5% of the control animals had succumbed within 20 minutes after decompression, while only 3.6% of mice treated with dimethothiazine died by the end of this period. Dimethothiazine reduced overall mortality from 60% to 17% (Table I), an effect which statistically is significant at high levels of confidence ($p < 0.001$). These percentages represent a 72% reduction in the overall mortality. The majority of the mice treated with 40 mg/kg dimethothiazine did not exhibit convulsions or signs of respiratory distress. Several animals showed varying degrees of drowsiness. In most of the animals which succumbed and in all of those sacrificed, there was no gaseous distension of the gastrointestinal tract, the tissues revealed minimal or no bubble formation (Fig. 1B) and the perivascular edema in the

lung was absent or less pronounced than in corresponding controls.

The low doses (4, 10, and 20 mg/kg) of dimethothiazine had no appreciable effect on the clinical manifestations, pathologic alterations or mortality.

DISCUSSION

The incidence and severity of decompression sickness in animals subjected to hyperbaric-hypobaric conditions is influenced by a variety of factors, both intrinsic and extrinsic. Among the former, the degree of obesity is an important variable influencing susceptibility (1,2). Among the latter, the composition of the breathed gas mixtures, the level and duration of compression and the rate of decompression are factors of obvious significance.

In the present investigation, the possible influence of differences in these factors was eliminated by the fact that the relatively small size of the animals employed permitted the placement of control and experimental groups in the same chamber at the same time, for a simultaneous exposure to identical environmental conditions. In addition, control and corresponding experimental groups consisted of animals of the same sex, and of approximately the same age and weight. Furthermore, the relatively large number of animals used afforded randomization of the influence of other independent factors, with a resulting increase in the statistical significance of the observed drug effect.

The observation that, in animals treated with 40 mg/kg dimethothiazine prior to compression, the clinical manifestations and morphologic changes seen in decompression sickness were absent or less pronounced and mortality was significantly reduced, indicates that this compound exerts a considerable protective effect against decompression sickness in mice.

The 4, 10, and 20 mg/kg doses of dimethothiazine were ineffective in protecting mice against decompression sickness, although doses even smaller than those have been reported to exhibit activities against smooth muscle stimulating agents in rats and guinea pigs (8). It should be borne in mind, however, that dimethothiazine in our experiments was administered to mice 6 hours prior to decompression. During that interval it is possible that excretion, biotransformation and other processes might have resulted in a concentration of the compound in the blood and/or tissues below effective levels. It should also be noted that the experiments with various doses of the compound were conducted for purposes of orientation and the number of animals which received each of the smaller doses is not sufficient to provide statistical significance of the respective results. Considering the above, it is clear that the results presented in this report should not imply that 40 mg/kg is necessarily the minimal effective dose of dimethothiazine.

Previous communications presented data strongly suggesting that humoral smooth muscle stimulating agents are implicated in the pathogenesis of decompression sickness. Among them, the following observations are of particular interest: 1. Administration of bradykinin intensifies pathologic alterations and increases mortality in decompression sickness (3). 2. SMAF (a humoral fac-

tor which augments responsiveness of smooth muscle organs to stimulants and increases vascular permeability) is released or activated in decompression sickness (6,7). 3. Administration of SMAF increases susceptibility of thin mice to decompression sickness (4). 4. Compounds with activities against smooth muscle stimulating agents prevent or ameliorate the disease (3,4).

In view of the above, and considering the fact that all agents which, in our investigations, offered protection against decompression sickness exhibit activities against smooth muscle stimulants, it is reasonable to attribute the decompression sickness preventing effect of dimethothiazine to its activity against bradykinin, histamine and serotonin. Thus the results of this investigation provide additional support to the postulated concept regarding the pathogenesis of decompression sickness. According to this concept, humoral smooth muscle stimulating agents, released or activated in decompression sickness (by a mechanism involving gas-blood interphase or by other processes), may induce several tissue responses that could contribute to the production of the syndrome. For example, bradykinin, histamine, SMAF and other humoral agents, by causing bronchoconstriction, could interfere with the elimination of nitrogen and also contribute to the respiratory distress. These agents could also induce circulatory changes favoring nucleation and/or growth of gas bubbles. Furthermore, they increase vascular permeability which, in turn, could be responsible for the perivascular edema in the lungs observed in our experiments and for the hypovolemic shock that often complicates decompression sickness. Thus, the absence of perivascular edema in the lungs of animals treated with dimethothiazine could be attributed, at least in part, to a blocking of agents which increase vascular permeability. Also, inhibition of bronchoconstrictor agents, by improving ventilation and consequently nitrogen elimination, could have contributed to the lack or diminution of nitrogen bubble accumulation which was observed in the dimethothiazine-treated mice.

The soporific effect of dimethothiazine, observed on several animals, does not fit the above concept but, nevertheless, merits consideration and further exploration. It seems, however, unlikely that the sedative effect alone was responsible for the protective action of dimethothiazine, particularly in view of the observation that in hamsters, chloralose exhibited appreciably less protection against decompression sickness as compared to PPCH* although the sedative effect of chloralose was greater than that of PPCH (9). The effectiveness of dimethothiazine against decompression sickness, which was demonstrated in the present study, warrants further investigations including potential experimentation on human decompression sickness. Dimethothiazine, in contrast to the decompression sickness-preventing com-

*PPCH (2-[4-phenyl-1-piperazinylmethyl]cyclohexanone HCl) (Miles-Ames) is a compound with antibradykinin and antihistaminic activities which was previously reported to prevent or ameliorate decompression sickness (3,4).

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pounds previously studied (3), is already in clinical use against other disorders in several countries, including France, England, and Canada, and has been well tolerated with minor or no side effects.

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**REVUE
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NEWER CONCEPTS ON THE MECHANISM AND PREVENTION OF DECOMPRESSION SICKNESS

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Our work on the mechanism and prevention of decompression sickness (DS) led us to theorize that smooth muscle stimulating substances are implicated in the pathogenesis of the disease. This concept is based on the following observations: 1. Bradykinin intensifies pathologic alterations and increases mortality in DS.¹ 2. Compounds such as 2 (4-phenyl-1-piperazinylmethyl) cyclohexanone (PPCH), 1, 2 and dimethothiazine (Migristene)³ with activities against smooth muscle stimulating substances (e.g. bradykinin, histamine, 5-hydroxytryptamine) ameliorate and even prevent DS. 3. The activity of a new humoral Smooth Muscle Acting Factor (SMAF) increases in DS.^{4,5} 4. SMAF potentiates bradykinin and other smooth muscle stimulants, causes bronchoconstriction and increases vascular permeability.^{4,5,6} 5. SMAF increases susceptibility to DS.²

DS was produced in these mice by subjecting the animals to 90 psi (absolute) air pressure for 6 hours and then decompressing them within 1 minute to sea level. The various groups of control drug-treated animals were all simultaneously exposed to the identical pressure conditions. Soon after decompression, the control animals exhibited scratching, respiratory distress with choking, hiccough convulsions, and most of them expired within 30 minutes following decompression. Gross and microscopic examination revealed gaseous distension of the gastrointestinal tract, accumulation of gas in the adipose tissue, presence of gas bubbles in the bone marrow, spleen and adrenals, vena cava, right side of the heart and in pulmonary and other vessels. Among other findings, there was an increase in the perivascular space in the lung (edema?), bronchoconstriction and pronounced hyperemia and hemorrhagic foci in the bone marrow.

Administration of bradykinin immediately after decompression exaggerated many of these pathologic alterations and increased mortality. In contrast, in animals treated with PPCH or dimethothiazine prior to compression, clinical manifestations were ameliorated, pathologic

changes were minimal or absent and mortality was significantly reduced as the following table 1 indicates.

TABLE 1
EFFECT OF ANTI-INFLAMMATORY DRUGS ON MORTALITY OF ORISE MICE IN DECOMPRESSION SICKNESS

DRUG	% MORTALITY	STATISTICAL SIGNIFICANCE
CONTROL	78 (31/40)	
PPCH* (60 mg/kg)	43 (12/23)	0.001 < P < 0.01
PPCH (120 mg/kg)	19 (3/16)	P < 0.001
CONTROL	78 (14/18)	
PPBP** (60 mg/kg)	44 (8/18)	0.05 < P < 0.1
CONTROL	70 (23/33)	
MIGRISTENE*** (40 mg/kg)	21 (7/33)	P < 0.001

* PPCH 2 - (4 - phenyl - 1 - piperazinylmethyl) cyclohexanone (Miles - Armes, USA).

** PPBP 1 - (N - methyl - piperidyl - 4) - 3 - phenyl - 4 benzyl - pyrazolone - 5 (Sandoz, USA).

*** MIGRISTENE dimethothiazine (Specia, France).

The DS-preventing effect of PPCH has recently been confirmed by other investigators⁷ in experiments with rats and hamsters.

Seeking additional support for the hypothesis that smooth muscle stimulating substances play a role in the mechanism of decompression sickness, the possibility that bradykinin is activated in animals exposed to hyperbaric-hypobaric conditions was explored. Lungs from control and decompressed mice were extracted but we found no evidence of bradykinin activation. We did come across, however, a new smooth muscle stimulating factor in the lung extract, the activity of which increases in decompression sickness. This previously unidentified factor was called S.M.A.F., which is the acronym derived from Smooth Muscle Acting Factor. SMAF is probably a polypeptide which elicits slow weak contractions of

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smooth muscle, causes bronchoconstriction, increases vascular permeability, exhibits hypotensive effects and increases responsiveness of smooth muscle to bradykinin and other stimulants.^{5,6} SMAF was subsequently found also in blood, kidney and other organs of several species including humans.

The increase of SMAF activity in DS strengthens the theory that smooth muscle stimulating substances are implicated in the pathogenesis of the disease. According to this theory, bradykinin, SMAF and other smooth muscle stimulating substances released or activated in DS induce several tissue responses which contribute to the production of the syndrome. For example, bradykinin and SMAF may cause bronchoconstriction, which could interfere with the elimination of nitrogen. This could also contribute to the respiratory distress observed in decompressed animals. These agents could also induce circulatory changes favoring nucleation and growth of gas bubbles. Furthermore, they may increase vascular permeability which could contribute to the production of hypovolemic shock that often complicates DS.

Exposure to the pressure conditions described previously produces decompression sickness in obese but not in thin mice.⁸ Even when decompression from 90 psi to sea level is followed by exposure to simulated altitude only a small percentage of thin mice developed DS.

In view of these observations, the possibility that administration of SMAF could render thin mice susceptible to DS and that PPCH could counteract this effect of SMAF was entertained.

Thin mice were subjected to 90 psi air pressure for 5 hours, decompressed to sea level and after a short surface interval further decompressed to a simulated altitude of 26-28,000 ft. Clinical manifestations of DS and pathologic alterations were observed in a few animals and mortality was only 12.5%. When, however, SMAF was administered before exposure to altitude most of the animals developed symptoms and pathologic changes as seen in the susceptible obese mice and mortality reached 54%. Treatment of the animals with PPCH before compression counteracted the effect of SMAF and prevented DS. All of the animals survived, and none exhibited any pathologic alterations. Considering the postulated implication of smooth muscle stimulating factors in the mechanism of DS it is hypothesized that SMAF increases the susceptibility of thin mice to DS by its direct action on smooth muscle and/or by its ability to increase responsiveness of smooth muscle organs to bradykinin and other stimulants.

The striking DS-preventing effect of PPCH and dimethothiazine is considered to be primarily due to the ability of these compounds to block the action of smooth muscle stimulating substances. Other mechanisms however, cannot be ruled out. The findings of these investigations are consistent with the postulated involvement of humoral factors in the mechanism of DS. This new pathogenic consideration provides the basis for a novel pharmacological approach for the prevention or amelioration of the disease.

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RESUME

CONCEPTS RECENTS SUR LE MECANISME ET LA PREVENTION DE LA MALADIE DE DECOMPRESSION.

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Les substances stimulantes du muscle lisse (Bradykinine, Histamine, 5-Hydroxytryptamine, etc.) paraissent jouer un rôle dans la pathogénie de la maladie de décompression. Cette hypothèse est basée sur les observations ci-après: 1) La Bradykinine intensifie les altérations pathologiques et augmente la mortalité par maladie de décompression chez des souris obèses exposées pendant 6 heures à une pression absolue de 90 psi puis décompressées en une minute à la pression du niveau de la mer. 2) Les composés à activité antibradykinine et antihistamine (tels que le 2,4-phényl piperaziny methyl - cyclohexanone) préviennent ou atténuent la maladie de décompression chez la souris, le hamster et le rat. 3) Les expériences suggèrent que la diméthothiazine (Migristène) qui combine des actions antihistamine, antibradykinine et anti 5-Hydroxytryptamine, prévient aussi la maladie de décompression chez la souris. 4) Un nouveau facteur d'activation du muscle lisse capable d'augmenter la perméabilité vasculaire et la réactivité du muscle à la bradykinine et aux autres stimulants, a été récemment trouvé dans le sang et les tissus animaux et humains. 5) L'activité de ce facteur augmente lors de la maladie de décompression. 6) Une décompression à partir de 90 psi jusqu'au niveau de la mer provoque la maladie de décompression chez les souris obèses mais non chez les souris minces. Même lorsque la décompression au niveau de la mer est suivie par une exposition à l'altitude, un petit pourcentage de souris minces, seulement, présentent une maladie de décompression. Cependant, lorsque le facteur d'activation du muscle lisse est administré à ce dernier groupe, l'incidence de la maladie augmente. 7) Le 2,4-P.P. cyclohexanone contrebalance les effets du facteur d'activation et prévient la maladie de décompression.

Les facteurs d'activation du muscle lisse libérés ou activés au cours de la maladie de décompression peuvent induire des réponses tissulaires qui pourraient contribuer au développement du syndrome. Ceci conduit à de nouvelles conceptions pathogéniques et fournit la base pour une approche pharmacologique originale de la prévention et de l'amélioration de la maladie.

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Pathogenesis and Treatment of Decompression Sickness

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SYMPOSIUM

Underwater
Physiology

**Pathogenesis and
Treatment of
Decompression
Sickness***

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Recent technologic advances have enabled man to descend deeper into the earth's hydrospace and to climb higher into the atmosphere and the space beyond. Scientific curiosity, socioeconomic needs, newer developments in air travel, and the increasing popularity of scuba diving are some of the reasons responsible for more people getting farther away from the surface of our planet more often. But as man leaves his natural environment on the surface of the earth he exposes himself to potentially dangerous alien conditions, including changes in atmospheric pressure. Such changes have ill effects on the human body which, in extreme situations, may be fatal.

Because of our accelerated aerospace and underwater activities, physiologic problems related to the effects of pressure changes have assumed greater importance, and renewed attention is being focused on the etiology, mechanism, prevention, and treatment of the pathologic conditions which may be caused by such pressure changes. One of these pathologic conditions is dysbarism. Dysbarism is a syndrome, exclusive of hypoxia, resulting from the existence of a pressure differential between the total ambient barometric pressure and the total pressures of dissolved and free gases

within the body tissues, fluids, and cavities. The term hyperbarism applies to the condition developing when the ambient gas pressure exceeds that of the gas within the body. Conversely, hypobarism applies to the condition resulting from an excess of the gas pressure within the body over the ambient gas pressure. Often the term dysbarism is used to refer to the latter condition, the pathogenesis and treatment of which is the subject of this report. This condition is also known by several other names which have created some confusion and controversy regarding the proper terminology. The most popular, but maybe the least appropriate, term is "the bends." Although this term denotes only one of the symptoms, namely pain in the joints, it is often used to characterize the overall syndrome. Other terms implying causes, mechanism, or circumstances of development of the disorder include aeroembolism, caisson disease, divers' disease, aviators' disease, and decompression sickness. The term decompression sickness, being more inclusive and having a broader use, will be employed in this presentation. It will refer to the syndrome which results from a reduction in barometric pressure and which is characterized by a variety of symptoms including joint pains (bends), cough, chest pain and dyspnea (chokes), vertigo, skin disturbances such as rash and pruritus (itch), and central nervous system symptoms such as visual disturbances, aphasias, paralyzes, and so forth. Shock and long-delayed effects, such as aseptic bone necrosis, may complicate the syndrome.

Historic aspects

The signs of decompression sickness were first noted in 1659 by Boyle¹ in animals subjected to reduced atmospheric pressure. He observed convulsions preceding the death of the animal and postulated that air-bubble formation in the blood and tissues of the body contributed to the premortal agony. Speculating on the effects of intravascular bubbles, he says: "... and so by choking up some passages, and vitiating the figure of others, disturb and or hinder the due circulation of the Blood. Not to mention the pains that such distensions (of blood vessels) may cause in some Nerves, and membranous parts..."

Following Boyle's observations and prophetic remarks, the subject remained almost untouched

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until the nineteenth century when Pol and Watelle² gave the first account of "compressed air illness" (caisson disease) and came to the remarkable conclusion that the danger was proportional to the degree and period of compression and especially to the rapidity of decompression. They also indicated that if symptoms of the disease occurred, the treatment should be recompression followed by even slower decompression. A more systematic study was published in 1878 by Bert³ who indicated that the cause of the symptoms of caisson disease was the liberation of nitrogen bubbles and demonstrated that if animals were placed in an oxygen rich atmosphere for some time before being rapidly decompressed, no bubbles were formed.

The first report of air bubbles in animals exposed to altitude (62,000 feet) was given by Hoppe-Seyler in 1857,⁴ and Henderson,⁵ sixty years later, considered the possibility of decompression sickness in flying personnel, but he commented that airplanes at that time were not getting high enough and fast enough for the disorder to develop. Bends at simulated altitudes were reported by Jongbloed in 1930,⁶ and a year later by Barcroft *et al.*⁷ Several other investigators, including Heller, Mager, and von Schrötter,⁸ who made the first and maybe most detailed study on divers' bends, Boycott and Damant,⁹ and Hill¹⁰ made essential contributions to our knowledge of decompression sickness. Finally, the work of Armstrong,¹¹ and later that of Behnke,¹² provided a basis for many more recent developments in underwater and aerospace physiology which have improved our understanding of decompression sickness.

Etiology and pathogenesis

Disorders associated with changes in barometric pressure are better understood when one considers certain simple laws of physics. Dalton's law states that the total pressure of a mixture of gases is the sum of the partial pressures of each gas. Henry's law states that the amount of gas dissolved in a liquid is proportional to the partial pressure of that gas. Accordingly, at sea level a certain amount of nitrogen is dissolved in the blood and tissues of the body, that amount corresponding to the partial pressure of nitrogen at sea level. Solubility of nitrogen, or of any other substitution gas, in various tissues is another factor which influences the amount and site of gas concentration. When the ambient air pressure increases the partial pressure of nitrogen also increases, and thus more gas will be dissolved in the fluids and tissues, and a new equilibrium will be established, providing that exposure to the hyperbaric environment has been given sufficient time.

When one is exposed to an environment of lower barometric pressure, the nitrogen already dis-

solved in the blood and tissues will be in disequilibrium with the environment. This will occur when one returns to sea level from a higher pressure, as in the case of an ascending diver, or when one leaves the surface of the earth and exposes oneself to the reduced pressure of high altitudes and, of course, to the lack of pressure in space. In both situations the gas dissolved in blood and tissues of the body, having a partial pressure higher than that in the environment, tends to leave the liquid phase. If the rate of the reduction of the ambient pressure is slow enough to permit gradual elimination of the "excess" nitrogen through the lungs, in all probability no ill effects will be observed. But if the fall in barometric pressure is rapid, the blood and tissues will become supersaturated with nitrogen which will tend to "precipitate" as bubbles.

The ΔP (differential pressure) or tendency for a gas to leave the liquid phase is given in the equation introduced by Harvey in which $\Delta P = t - P$, where t is the total tension of the dissolved gas(es) in the medium, and P is the ambient pressure consisting of the total pressure of gases in the environment plus the hydrostatic pressure of the blood or tissue. It is apparent that when the P decreases, as during exposure to an environment of lower barometric pressure, the ΔP , that is the tendency of gas to come out of solution, increases.

It is not difficult to appreciate the fact that decompression sickness of caisson workers, divers, or aviators, although differing in the circumstances of its development and progression of the process, involves the same fundamental mechanism and thus exhibits many similarities in its manifestation. Despite uncertainties regarding the origin, site, and mode of action, gas bubbles are generally accepted as the basic initiating factor in the production of the disorder.

The gas bubbles are formed first in tissues and the venous circulation, appearing later in the arteries. The formation and growth of gas bubbles in tissues and blood have several direct, as well as indirect, potentially detrimental effects. They may obstruct blood flow and result in ischemia and infarction. Expanding bubbles in muscles and tendons may cause pain by distorting and deforming nerve endings.¹³ Ischemia and release or activation of humoral agents may also contribute to the production of pain. Gas bubbles arising in the vessels and lipid-rich tissues of the brain and spinal cord or air embolization of the central nervous system could be responsible for neurologic manifestations, and embolization to the lung could contribute to the respiratory signs of the disease.

The bubble theory, however, is not all-inclusive and leaves an appreciable deficit in our understanding of various phenomena and problems in decompression sickness. Signs of the sickness may develop without evidence of circulatory obstruction by gas bubbles, and gas bubbles can exist

without manifestation of the disease (the so called "silent" bubbles). Furthermore, gas bubbles, or at least their direct effects, cannot explain certain complications.

It seems plausible that gas bubbles only initiate a complex and self-propagating disease process, the development and seriousness of which depend more on the involvement of biohumoral and other factors than on the gas bubble itself.

Fat emboli produced by decompression injury to bone marrow and adipose tissue or resulting from a gas-induced disruption of lipoprotein linkages in the blood, have been implicated in the pathogenesis of the syndrome.¹⁴⁻¹⁶ Clumping of red blood cells was considered a secondary complicating factor as early as 1938.¹⁷ Disseminated intravascular coagulation associated with a fall in the circulating platelet count may also play an important pathogenetic role.¹⁸

Many of the previously mentioned complicating factors may be the result of surface activity of the bubbles.¹⁹ Intravascular gas bubbles may act as foreign surfaces to cause denaturation of plasma proteins, clumping of red blood cells, platelet adhesion and aggregation, coalescence of plasma lipids, and activation of the Hageman factor, which in turn could result in activation of the coagulation mechanism, of the kinin system, and of other humoral agents.^{15,19-23} Gas-induced osmosis resulting in changes of water concentration in certain tissues has also been recently considered as a factor in decompression sickness and in aseptic bone necrosis produced by exposure to dysbaric conditions.²⁴

Prevention and treatment

Susceptibility to decompression sickness and severity of the pathologic changes depend on many predisposing factors, including species, age, sex, amount of adipose tissue (obesity), and prior exposure to dysbaric conditions. For example, diving preceding exposure to altitude predisposes one to decompression sickness, and obesity increases susceptibility to the disease. Preoxygenation for several hours at ground level by removing nitrogen (denitrogenation) reduces the potential of bubble formation and serves to protect against the disease. Heparin, with its anticoagulant and lipid-clearing effect, has been used for both prophylaxis and treatment of decompression sickness.^{20,25,26} Plasma expanders, such as dextran, have been employed in the treatment of the disease, particularly to counteract the hemoconcentration and hypovolemic shock that often complicate the syndrome.^{14,27} Dextran, in addition to compensating for the plasma deficit, may also act by means of its anticoagulant effect and its inhibition of platelet adhesiveness.

Several other pharmacologic agents have been used for the prevention and treatment of decom-

pression sickness. Some of them appeared promising; others generated skepticism. Many did not prove commensurate with the expectations and were abandoned. Research is in progress on new pharmacologic approaches. However, until more light is shed on the obscure pathogenesis of decompression sickness and the effectiveness of new treatments is established, conventional prophylactic and therapeutic measures will still prevail. These include gradual safe decompression and breathing of oxygen or oxygen and inert gas mixtures as a prophylactic procedure and immediate oxygen or air recompression followed by gradual decompression as a therapeutic method. Because these procedures are neither foolproof nor their application always possible, the need for alternative or supplementary treatments is evident.

Studies conducted in our laboratories

Our work on the mechanism and prevention of decompression sickness led us to theorize that smooth muscle-stimulating substances are implicated in the pathogenesis of the disease. This concept is based on the following observations: (1) bradykinin intensifies pathologic alterations and increases the mortality rate in decompression sickness;²⁸ (2) compounds such as PPCH (2-(4-phenyl-1-piperazinylmethyl)cyclohexanone)^{28,29} and dimethothiazine (Migristene)³⁰ with activities against smooth muscle-stimulating substances (for example, bradykinin, histamine, 5-hydroxytryptamine) ameliorate and even prevent decompression sickness; (3) the activity of a new humoral SMAF (smooth muscle acting factor) increases in decompression sickness;²² (4) SMAF potentiates bradykinin and other smooth muscle stimulants, causes bronchoconstriction, and increases vascular permeability;^{23,31} and (5) SMAF increases susceptibility to decompression sickness.²⁹

Decompression sickness was produced in obese mice by subjecting the animals to 90 psi (absolute) air pressure for six hours and then decompressing them within one minute to sea level. The various groups of control and drug-treated animals were all simultaneously exposed to identical pressure conditions. Soon after decompression the control animals exhibited scratching, respiratory distress with choking, hiccup, convulsions, and most of them expired within thirty minutes following decompression. Gross and microscopic examination revealed gaseous distention of the gastrointestinal tract, accumulation of gas in the adipose tissue, and presence of gas bubbles in the bone marrow, spleen, adrenals, vena cava, right side of the heart, and in pulmonary and other vessels. Among other findings there was an increase in the perivascular space in the lung (edema?), bronchoconstriction, and pronounced hyperemia and hemorrhagic foci in the bone marrow.

Administration of bradykinin immediately after

TABLE 1. Effect of "anti-inflammatory" drugs on mortality rate of obese mice in decompression sickness

Drug	Mortality Rate (Per Cent)	Statistical Significance
Control	78 (31, 40)	...
PPCH (60 mg. per kilogram)	43 (12, 28)	$0.001 < P < 0.01$
PPCH (120 mg. per kilogram)	19 (3, 16)	$P < 0.001$
Control	70 (23, 33)	...
dimethothiazine	21 (7, 33)	$P < 0.001$

decompression exaggerated many of these pathologic alterations and increased the number of deaths. In contrast in animals treated with PPCH or dimethothiazine prior to compression clinical manifestations were ameliorated, pathologic changes were minimal or absent, and the mortality rate was significantly reduced (Table I). The decompression sickness-preventing effect of PPCH has recently been confirmed by other investigators in experiments with rats and hamsters.³²

Seeking additional support for the hypothesis that smooth muscle-stimulating substances play a role in the mechanism of decompression sickness, we explored the possibility that bradykinin is activated in animals exposed to compression-decompression. We extracted tissues from control and decompressed mice, but we found no evidence of bradykinin activation. We did, however, come across a new smooth muscle-stimulating factor in the lung extract, the activity of which increases in decompression sickness. This previously unidentified factor was called SMAF, which is the acronym derived from smooth muscle acting factor. SMAF is probably a polypeptide which elicits slow weak contractions of smooth muscle, causes bronchoconstriction, increases vascular permeability, and potentiates bradykinin and other smooth-muscle stimulants.^{22,31} SMAF was subsequently also found in blood, kidney, and other organs of several species including human beings. The physical, chemical, biologic, and pharmacologic properties of SMAF distinguish it from similar substances, such as bradykinin, substance P, prostaglandin, and various other factors which either stimulate smooth muscle or increase its responsiveness to stimulants.

The increase of SMAF activity in decompression sickness strengthens the theory that smooth muscle-stimulating substances are involved in the pathogenesis of the disease. According to this theory, bradykinin, SMAF, and other smooth muscle stimulating substances released or activated in decompression sickness induce several tissue responses which contribute to the production of the syndrome. For example, bradykinin and SMAF, by causing bronchoconstriction, could interfere with the elimination of nitrogen. This could also contribute to the respiratory distress

observed in decompressed animals. These agents could also induce circulatory changes favoring nucleation and growth of gas bubbles. Furthermore, they may increase vascular permeability which could contribute to the production of hemoconcentration and hypovolemic shock that often complicates decompression sickness. The mechanism by which SMAF, bradykinin, and/or other smooth muscle stimulating agents are activated or released is at present a matter of speculation. Gas bubbles may cause cellular injury or alteration resulting in the release of such agents or of their activators. Or, surface activity of bubbles may activate plasma components such as the Hageman factor which in turn may, directly or indirectly, cause release or activation of humoral agents.

Exposure to the pressure conditions described previously produces decompression sickness in obese but not in thin mice.³³ Even when decompression from 90 psi to sea level is followed by exposure to simulated altitude only a small percentage of thin mice developed decompression sickness.²⁹

In view of these observations, the possibility that administration of SMAF could render thin mice susceptible to decompression sickness and that PPCH could counteract this effect of SMAF was entertained.

Thin mice were subjected to 90 psi air pressure for five hours, decompressed to sea level, and after a short surface interval further decompressed to a simulated altitude of 26,000 to 28,000 feet. Clinical manifestations of decompression sickness and pathologic alterations were observed in a few animals, and the mortality rate was only 12.5 per cent. When, however, SMAF was administered before exposure to altitude most of the animals developed symptoms and pathologic changes as seen in the susceptible obese mice, and the mortality rate reached 45 per cent. Treatment of the animals with PPCH before compression counteracted the effect of SMAF and prevented decompression sickness. All of the animals survived, and none exhibited any pathologic alterations.²⁹ Considering the postulated implication of smooth muscle-stimulating factors in the mechanism of decompression sickness it is hypothesized that SMAF increases the susceptibility of thin mice to the sickness by its direct action on smooth muscle and/or by its ability to increase responsiveness of smooth muscle organs to bradykinin and other stimulants.

The striking decompression sickness-preventing effect of PPCH and dimethothiazine is considered to be primarily due to the ability of these compounds to block the action of smooth muscle stimulating substances. Other mechanisms, however, cannot be ruled out. The findings of these investigations are consistent with the postulate that humoral factors are involved in the mechanism of decompression sickness. This pathogenetic concept

provides the basis for a new pharmacologic approach to the prevention or amelioration of the disease.

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BLOOD-BUBBLE INTERACTION IN DECOMPRESSION SICKNESS

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Humoral Factors in the Pathogenesis of Decompression Sickness (DS)*

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INTRODUCTION

The role of gas bubbles as an initiating factor in the pathogenesis of decompression sickness (DS) is generally accepted. However, the nature of several bubble-induced pathologic alterations and the mechanism of their development are still obscure. Direct effects of gas bubbles, such as vascular obstruction, although they explain some of the components of the syndrome, leave an appreciable deficit in our understanding of several pathophysiologic phenomena observed in the disease. Signs of DS may develop without evidence of circulatory obstruction by gas bubbles, and gas bubbles can be present without manifestation of DS, the so-called "silent" bubbles.

DS may be a complex self-propagating disease process in which gas bubbles, in addition to their direct effects, may initiate chain reactions involving biohumoral and other factors which could be responsible for several pathologic changes occurring in this malady. In fact, the nature and severity of structural and functional alterations in DS may depend more upon the effects of such secondary factors than upon the gas bubble itself.

Clumping of red blood cells was considered a secondary complication as early as 1938 (1). Hypovolemia and hemoconcentration (2), fat emboli resulting from decompression injury to bone marrow and adipose tissue or from gas-induced disruption of blood lipoproteins (3, 4, 5), and gas-induced osmosis resulting in changes of water concentration in certain tissues (6), have been implicated in DS. Recently, disseminated intravascular clotting associated with a fall in the circulating thrombocyte count was emphasized as an important pathogenetic factor (7). Many of these contributing or complicating factors may come into play as a result of surface activity of the bubbles (8). Intravascular gas bubbles may act as foreign surfaces to cause denaturation of plasma proteins, clumping of red blood cells, platelet adhesion and aggregation, coalescence of plasma lipids and activation of the Hageman factor and other enzymes which in turn could trigger the coagulation mechanism and activate kinins and other biohumoral agents (4, 8-12).

Our work in DS in the last ten years provided data strongly suggesting that smooth muscle stimulating substances may play important roles in the pathogenesis of this disease (12-19).

The effect of smooth muscle stimulants and of their antagonists on DS

The first observations that drew our attention to the possibility that smooth muscle stimulating agents may be involved in the mechanism of DS, was the similarity of certain pathologic alterations produced by bradykinin to some of those seen in animals subjected to compression-decompression. We were thus led to explore the possible influence of smooth muscle stimulants and of their antagonists on DS.

DS was produced in genetically obese mice, which are susceptible to the disease, by subjecting the animals to 90 psi (absolute) air pressure for 6 hours and then decompressing them within 1 minute to sea level. The various groups of control and drug-treated animals were all simultaneously exposed to identical pressure conditions. Soon after decompression, the control animals exhibited scratching, respiratory distress with choking, hiccough-like spells, convulsions, and most of them expired within 30 minutes following decompression. Gross and microscopic examination revealed gaseous distension of the gastrointestinal tract, accumulation of gas in the adipose tissue, and presence of gas bubbles in the bone marrow, spleen, adrenals, vena cava, right side of the heart and pulmonary vessels. Among other findings, there was an increase in the perivascular space in the lung (edema?), bronchoconstriction and pronounced hyperemia and hemorrhagic foci in the bone marrow.

Administration of bradykinin immediately after decompression exaggerated many of the above-mentioned pathologic alterations and increased mortality (14). In contrast, in animals pretreated with compounds which combine activities against bradykinin, histamine and 5-hydroxytryptamine, clinical manifestations of DS were ameliorated, pathologic alterations were minimal or absent, and mortality was significantly reduced (14, 16, 19). The agents used included 2-- (4 phenyl 1 piperazinylmethyl) cyclohexanone (PPCH), diomethothiazine (Migristene), aminopyrine, and 1 (N-methyl piperidyl 4) 3 phenyl 4 benzyl pyrazolone S. The following table presents the influence of the two most effective compounds on mortality in DS.

EFFECT OF ANTAGONISTS OF SMOOTH MUSCLE STIMULANTS OF OBESE MICE IN DECOMPRESSION SICKNESS

Drug	% Mortality	Statistical Significance
Control	78 (31/40)	
PPCH (60 mg/kg)	43 (12/28)	0.001 <P < 0.01
PPCH (120 mg/kg)	19 (3/16)	P < 0.001
Control	70 (23/33)	
Migristene	21 (7/33)	P < 0.001

The DS-preventing effect of PPCH has recently been confirmed by other investigators (20) in experiments with rats and hamsters.

The observations described above provided reasonable support to the hypothesis that bradykinin and possibly other smooth muscle stimulating humoral agents play a role in the mechanism of DS. Seeking additional evidence, we explored the possibility that bradykinin is activated in animals exposed to compression-decompression. We extracted tissues from control and decompressed mice, but we found no evidence of bradykinin activation. We did, however, encounter a new smooth muscle stimulating factor in the lung extract, the activity of which increases in decompression sickness (12). This previously unidentified factor was called SMAF, which is the acronym derived from Smooth Muscle Acting Factor.

SMAF and its possible implication in the pathogenesis of DS

SMAF is probably a polypeptide which elicits slow, weak contractions of smooth muscle, causes bronchoconstriction, increase vascular permeability and potentiates bradykinin and other smooth muscle stimulants. SMAF, which was originally extracted from mouse lung, was also found in blood, kidney, placenta and other organs of several species, including humans. The method of SMAF extraction and partial purification, as well as its physical and chemical properties and biological activities, were dealt with in previous communications (12, 15, 17). Recent attempts to further purify SMAF by Sephadex G 25 chromatography in 6 M urea enabled isolation of at least 3 different subfractions. One of these subfractions, which exhibited the greatest pharmacologic activity, was further fractionated by means of ion-exchange chromatography (DEAE-cellulose) using a buffer concentration gradient at pH 8. The elution curve revealed several peaks which are being analyzed and pharmacologically evaluated.

The observed increase of SMAF activity in the lungs of decompressed mice (12) strengthens the hypothesis that smooth muscle stimulating substances are involved in the pathogenesis of the disease. According to this theory, SMAF and possibly bradykinin and other biohumoral agents released or activated in DS, induce several tissue responses which could contribute to the production of the syndrome. For example, bradykinin and SMAF, by causing bronchoconstriction, could interfere with the elimination of nitrogen and also contribute to the respiratory distress. These agents could also induce circulatory changes favoring nucleation and growth of gas bubbles. Furthermore, they increase vascular permeability which could lead to hemoconcentration and hypovolemic shock which often complicate DS. In addition, bradykinin and other smooth muscle stimulants could be responsible at least in part for the pain which is one of the most common symptoms in DS.

Consideration of SMAF as a pathogenetic factor in DS would be on a safer basis if it could be demonstrated that administration of this agent aggravates or increases susceptibility to the disease and that compounds which block the effects of SMAF prevent or ameliorate the syndrome.

Exposure to the pressure conditions described previously produces decompression sickness in obese mice but not in their thin siblings. In fact, we have observed a significant correlation between degree of obesity and mortality in DS (13). Even when decompression from 90 psi to sea level is followed by exposure to simulated altitude, only a small percentage of thin mice developed DS (16).

In view of these observations, the "non-susceptible" thin mouse seemed a convenient modality to explore the possible DS-enhancing effects of SMAF and at the same time investigate if PPCH could counteract such effects.

Thin mice were subjected to 90 psi air pressure for 5 hours, decompressed to sea level and after a short surface interval further decompressed to a simulated altitude of 26-28,000 ft. Clinical manifestations of DS and pathologic alterations were observed in a few animals and mortality was only 12.5%. When, however, SMAF was administered before exposure to simulated altitude, most of the animals developed symptoms and pathologic changes as seen in the susceptible obese mice and mortality reached 45%. Treatment of the animals with PPCH before compression counteracted the effect of SMAF and prevented DS. All of the PPCH-treated animals survived, and non exhibited any pathologic alterations (16). Considering the postulated implication of smooth muscle stimulating factors in the mechanism of DS, it is hypothesized that SMAF increased the susceptibility of thin mice to DS by its direct action on smooth muscle and/or by its ability to increase responsiveness of smooth muscle organs to bradykinin and other stimulants.

Accordingly the striking DS-preventing effect of PPCH could be due to inhibition of the direct effects of SMAF and/or to the antagonism of bradykinin and of other smooth muscle stimulants to which SMAF increases the responsiveness of smooth muscle organs.

The mechanism of release or activation of smooth muscle stimulants in DS.

The mechanism involved in the release or activation of smooth muscle stimulating substances in DS is obscure, but several possibilities merit consideration.

Biohumoral agents may be released or activated as a result of direct tissue injury by expanding gas bubbles. Or, gas bubbles may trigger an indirect mechanism involving circulatory impairment, decreased tissue perfusion, anoxia with disruption of lysosomes and release of enzymes (21, 22) that could activate humoral agents.

Recent data regarding gas-liquid interphase phenomena provide additional background for speculation. It has been reported that the blood-gas interphase may interact with plasma proteins and with formed elements of the blood, resulting in adhesion and aggregation of thrombocytes and possibly activation of enzyme systems (4, 8-11).

In view of these hypotheses, it is not inconceivable that smooth muscle stimulating agents may be liberated from thrombocytes and/or activated in the course of chain reactions initiated by the surface activity of gas bubbles. Such reactions could involve, for example, the kinin system triggered by the activation of the Hageman factor, or the complement system with formation of prostaglandins and mast cell degranulating factors. These and other known or still undiscovered mechanisms may be implicated in the process of generation of smooth muscle stimulating activity in DS.

To explore the possibility that gas-blood interphase may cause release or activation of smooth muscle stimulating agents, the following pilot experiments were conducted in vitro: Rabbit blood was either subjected to compression-decompression in a pressure chamber or in a syringe, or was bubbled with nitrogen. Some blood samples contained anticoagulant, others did not. In both cases, strict precautions were taken to ensure that the blood came in contact only with siliconized plastic surfaces. When anticoagulant was used (heparin, EDTA or citrate), cell-free and thrombocyte containing plasma was prepared and aliquots of each preparation were either compressed and decompressed in the chamber or bubbled with nitrogen. Extracts of these blood and plasma samples and of corresponding controls were tested for smooth muscle stimulating activity on isolated rat uterus.

The results of these experiments, which are still in progress, will be fully reported in future communications. Preliminary data, however, indicate that both compression-decompression and nitrogen bubbling increase the smooth muscle stimulating activity of blood. In cell-free plasma, however, the increase in activity was appreciably less pronounced, suggesting that formed elements of the blood probably are implicated in the mechanism whereby gas bubbles generate smooth muscle stimulating activity.

The nature of the smooth muscle stimulating agent (s) involved has not yet been determined. Partial reduction in the activity of the extracts following incubation with carboxypeptidase B, suggests, however, the possibility that at least part of the activity is due to substances with peptide moieties.

CONCLUSIONS

The results of our investigations on the pathogenesis of DS indicate:

1. Certain pathologic alterations observed in DS exhibit similarities to some of the histologic changes seen in animals treated with bradykinin.
2. Bradykinin increased mortality and intensifies some of the pathologic changes produced in DS.
3. The activity of SMAF, a new humoral smooth muscle stimulating agent, increases in DS.
4. Obese mice are more susceptible to DS than their thin siblings.

5. SMAF increases the susceptibility of thin mice to DS.
6. Compounds with activities against bradykinin, histamine and serotonin prevent or ameliorate DS in obese mice and counteract the increased susceptibility induced in thin mice by SMAF.
7. Preliminary observations suggest that in vitro compression-decompression or nitrogen bubbling of blood increase its smooth muscle stimulating activity.

The above findings are consistent with the hypothesis that smooth muscle stimulating agents are implicated in the mechanism of DS. This pathogenetic concept and the striking protective effect of compounds which antagonize smooth muscle stimulants provide the basis for a new pharmacologic approach to the prevention or amelioration of the disease.

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GAS INDUCED ALTERATIONS OF SERUM LIPIDS

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INTRODUCTION

The pathogenesis of decompression sickness is still obscure. Increasing evidence suggests that gas bubbles, in addition to their direct effects, may trigger a sequence of blood and tissue alterations which could contribute to the development of the disease.(1)

Lipid embolization is one of the proposed pathogenetic factors which recently attracted considerable attention.(2-4) The fact that fat embolization is not an uncommon finding in victims of decompression sickness, particularly in high altitude decompression, supports the possible role of lipid emboli in the mechanism of the disease. The ameliorating effect of heparin on decompression sickness is also consistent with this hypothesis if one considers the lipid-clearing action of the drug.

Most of the early communications on fat embolization dealt with the liver, bone marrow and adipose tissue as potential sources of lipid emboli. Newer concepts implicate circulating plasma lipids in decompression-induced fat embolization. Suggested mechanisms include gas-induced disruption of serum lipoproteins with subsequent aggregation of the released lipid, coalescence of chylomicrons and incorporation of coalesced lipids into aggregates of formed elements of the blood.(2-5)

The present communication is a preliminary report on lipid particle formation resulting from serum-bubble interface activity in vitro.

METHOD

Serum-bubble interface: Pooled human sera were distributed into control and experimental plastic test tubes which were placed into a water bath at 37°C. Polyethylene tubing connected to a compressed air manifold was immersed in the serum in each test tube. The experimental sera were bubbled for 60 minutes at a flow rate of 5-10 ml of air/minute. No air was passed through the control sera.

Filtration of samples: Following bubbling the experimental samples were pooled into one container and the controls (non-bubbled) into another. 10 ml aliquots from each pool were then passed through microporous

filters of varying pore size. The filters used were Uni-Pore polycarbonate membranes (Bio-Rad) of pore size 0.1-8 microns, and millipore membrane filters (Falcon) or cellulosic discs (Amicon Microporous filters) of 0.45 micron pore size. Filtration was conducted by gravity or under reduced pressure with a differential pressure on the two sides of the filters not exceeding 25 mm Hg.

Staining of filters: After the filters were dried they were stained for lipids with Oil Red O d.e, Nile Blue sulfate or phosphomolybdic acid. Filters sprayed with the latter reagent were heated to intensify the reaction. The staining of experimental and control filters was comparatively evaluated by gross inspection and microscopic examination.

Lipid concentration determination: Triglycerides, cholesterol and total lipids were determined in bubbled and non-bubbled sera before and after filtration by appropriate chemical methods.

RESULTS

Bubbled sera contained a larger amount of unfilterable lipid material than corresponding controls (non-bubbled sera). This was suggested by the more extensive and intense staining of membranes used to filter bubbled sera than of those used for controls (Fig. 1). The difference in the amount of unfilterable lipid between bubbled and non-bubbled sera was more pronounced when 0.45 micron-pore membranes were used. Nevertheless experiments with sequential filtration through membranes of decreasing pore size indicated that bubbling of serum results in the formation of lipid particles between 5-8 microns and even larger than 8 microns as suggested by the retention of lipid-positive particles on polycarbonate membranes of 8 micron pore size. Figure 2-A is a microscopic picture (100x) of lipid particles stained with phosphomolybdic acid on an 8 micron-pore membrane following filtration of bubbled sera. In Figure 2-B no particles are seen on an identical membrane after filtration of non-bubbled (control) sera. This finding was supported by the results of lipid determinations. In several cases total lipids and triglycerides in bubbled sera were reduced to a greater extent than in control sera after filtration through an 8 micron-pore membrane.

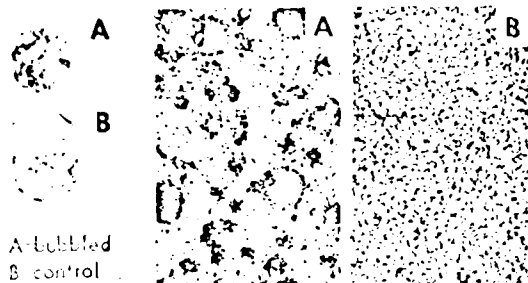


FIGURE 1.

FIGURE 2.

DISCUSSION

The results of these preliminary investigations show that membranes used for the filtration of bubbled sera stained (with dyes for lipids) to a greater extent than those used for non-bubbled sera (controls). Furthermore the decrease of lipid concentration in the filtrates of bubbled sera was greater than that in the controls. These observations suggest that bubbling of sera resulted in the formation of lipid particles which were unfiltrable through the membranes used.

The instability and alterations of plasma lipids may be caused by gas bubbles. Blood-bubble interface activity could denature lipoproteins with liberation of the lipid moiety and could also induce coalescence and adhesion of lipid particles.

The size of the particles is important when one considers their potential role as embolic material. An attempt was made to approximate their size by using membranes of varying pore size. Filtration was carried out with a differential pressure on the two sides of the membranes of less than 25 mm Hg. This precaution was necessary to avoid deformation of lipid particles and forced passage through filter pores under pressure condition substantially different than those anticipated in *in vivo* situations. The results with various pore size filters suggest that bubbling of serum causes formation of particles even larger than 8 microns but the majority of the material retained by the filters consists of smaller particles. It is reasonable to assume that lipid particles larger than 8 microns are potentially capable of causing embolization. However, particles even smaller than 8 microns may contribute to the formation of embolic material by being incorporated into platelet thrombi (5) or other aggregated elements of the blood. It is also possible that small lipid particles may coalesce *in vivo* to form larger emboli.

Gassed and lipid embolization may have relevance not only in the pathogenesis of decompression sickness but also in cardiopulmonary bypass and other similar procedures utilizing membrane oxygenators. It is of interest in this respect that gassing and disruption of lipoproteins resulted in fat embolization during extracorporeal kidney perfusion and that embolization was prevented by prior denaturation and removal of plasma lipids (5,7).

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Amelioration of decompression sickness in mice by pretreatment with cyproheptadine

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Chryssanthou C, Rubin L, Graber B. Amelioration of decompression sickness in mice by pretreatment with cyproheptadine. Undersea Biomed Res 1980; 7(4):321-329. — Substances that stimulate smooth muscle have been previously implicated in the pathogenesis of decompression sickness. This concept was strongly supported by the demonstration that compounds that combine activities against histamine, bradykinin, and serotonin prevent or ameliorate decompression sickness. This communication deals with the prophylactic effect of cyproheptadine (*Periactin*), a drug exhibiting such pharmacologic properties. More than 500 obese mice were used. Experimental groups, subcutaneously injected with cyproheptadine (0.5–40 mg/kg) prior to compression, and corresponding control animals were simultaneously subjected to 75 psig air pressure for 6 h and then rapidly decompressed. Most control animals exhibited signs of decompression sickness (chokes, scratching, twitching, convulsions, paralysis) and died. Gross and histologic examination revealed gas bubbles in vessels and tissues, perivascular edema, and other changes. In cyproheptadine-treated animals the incidence and severity of clinical manifestations and pathologic alterations were reduced, and mortality was markedly decreased. Statistically significant results were obtained with doses of 2.5–10.0 mg/kg. The 5-mg/kg dose lowered mortality by 45.9%. These results support the proposed pathogenetic concept and suggest a potential preventive treatment for human subjects.

decompression sickness	antihistaminic
mice	antibradikinin
prevention	pharmacology
prophylaxis	smooth muscle stimulating substances
treatment	pathogenesis
drugs	Periactin
cyproheptadine	

Previous work provided data indicating that smooth muscle stimulating factors are implicated in the pathogenesis of decompression sickness (1–5). Consistent with this concept is the observed decrease in morbidity and mortality in the disease when compounds that combine activities against histamine, bradykinin, and 5-hydroxytryptamine are administered prior to compression (1–4). The prevention of decompression sickness by such compounds, originally shown in studies conducted in our laboratories on mice, was subsequently confirmed by other investigators working with hamsters and dogs (6, 7).

Even though this pharmacologic approach to the prevention or amelioration of decompression sickness was successful on several animal species, no human studies have been initiated to date. One of the reasons for the reluctance to undertake investigations on human subjects is that none of the drugs used to prevent decompression sickness in animals are FDA-approved for any human use, and seeking FDA approval is a complicated and time-consuming process.

We therefore explored the possibility of ameliorating decompression sickness by use of cyproheptadine, which also combines activities against smooth muscle stimulants, but unlike the previously used compounds, it has FDA approval for use in human beings.

MATERIALS AND METHODS

Approximately 500 male hereditary obese mice (C57BL/6J-ob obtained from Jackson Memorial Laboratories, Bar Harbor, ME), which are susceptible to decompression sickness, were used. The animals were housed in metal cages kept in rooms with controlled temperature ($72^{\circ}\text{C} \pm 2^{\circ}\text{C}$) and relative humidity (50%). They were fed Wayne Lab-Blox and water ad libitum. After a 2–3 week stabilization period they were divided into control and experimental groups of corresponding weights. In each experiment (run) one control group and 2 or 3 experimental groups, each consisting of 6–8 mice, were placed in a hyperbaric chamber (Bethlehem Corporation Model 1836 HP) and subjected to compression-decompression simultaneously to ensure exposure of control and experimental groups to identical dysbaric conditions. A total of 17 experiments were conducted. All animals in all experiments were subjected to 75 psig air pressure for 6 h and then decompressed to sea level within 1 min (usually 30 s). Previous experiments have shown that with this simulated dive profile approximately 60%–80% of the animals develop decompression sickness (1, 4). Immediately prior to compression the experimental groups received a subcutaneous injection of cyproheptadine HCl (Periactin, Merck Sharp & Dohme), whereas the control animals were injected with an equal volume of the vehicle (normal saline). The dose of cyproheptadine ranged from 0.5 to 40 mg/kg body wt. In a few experiments cyproheptadine (2.5–20.0 mg/kg) was administered immediately after decompression.

Upon decompression to sea level, the animals were taken out of the chamber and observed for at least 1 h for signs of decompression sickness (gasping, chokes, hiccough-like spells, scratching, twitch, paralysis, convulsions). Animals that succumbed were autopsied immediately, and representative tissues were removed for microscopic examination. Survival time after decompression was recorded. Autopsies and histologic examination were also performed on a few animals killed at intervals after decompression. These animals were not included in the mortality statistics. The effects of cyproheptadine on mortality were statistically evaluated by the chi-square test.

RESULTS

As in preceding studies, control animals exhibited previously described signs of decompression sickness (1, 4), consisting mainly of severe respiratory distress, twitching, and convulsions, and most of them died within 1 h after decompression. Gross and microscopic examination of mice that succumbed to decompression sickness revealed the usually observed enlargement of the abdomen caused by gaseous distention of the gastrointestinal tract (Fig. 1), pronounced hyperemia of the bone marrow, perivascular edema in the lung (Fig. 2A), and the presence of gas bubbles in various tissues and organs. Intravascular gas bubbles were present



Fig. 1. Gaseous distention of stomach and intestines in animal that succumbed to decompression sickness.



Fig. 2. A: lung from control animal that died 45 min after decompression. Note several intravascular gas bubbles (small arrows) and perivascular edema (big arrow). B: corresponding tissue from cyproheptadine treated animal killed 60 min after decompression. Gas bubbles and perivascular edema are absent. Hematoxylin and eosin stain, orig. magnif. $\times 25$.

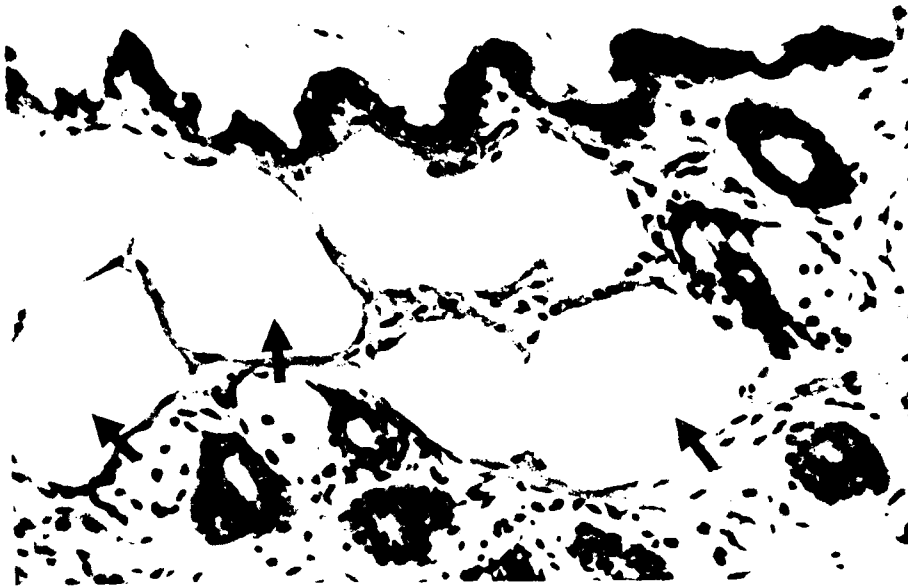


Fig. 3. Skin from animal following exposure to compression-decompression. Note presence of gas bubbles (arrows) in the dermis. Hematoxylin and eosin stain; orig. magnif. $\times 50$.

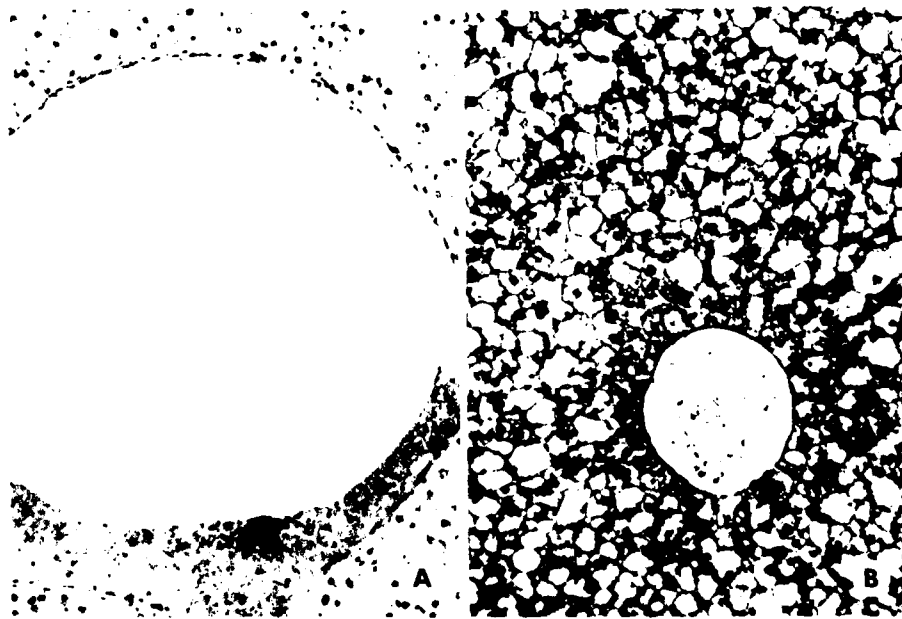


Fig. 4. A: liver from control animal subjected to compression-decompression showing large gas bubbles in markedly distended central vein. B: corresponding tissue from animal treated with cyproheptadine prior to compression. Central vein is free of gas bubbles. Hematoxylin and eosin stain; orig. magnif. $\times 20$.

in many vessels but were predominantly observed in the inferior vena cava and in pulmonary vessels (Fig. 2A). The spleen, adrenals, adipose tissue, skin (Fig. 3), and bone marrow, and to a lesser extent the liver (Fig. 4A), kidney, pancreas, and heart, showed minute bubbles or large accumulations of gas. Although in many instances the gas bubbles were intravascular, it was not always possible to determine whether the accumulation of gas was in vessels or in tissue spaces.

In the experimental groups, particularly those injected prior to compression with 2.5, 5.0, and 10.0 mg/kg cyproheptadine, the frequency and severity of clinical manifestations and pathologic changes were markedly reduced, survival time was prolonged, and mortality was significantly decreased. Microscopic examination revealed that gas bubbles were present in larger numbers and had a wider distribution in controls than in cyproheptadine-treated animals. In the control group 57% of the autopsied mice exhibited gas bubbles in more than two organs; this was observed in only 15% of the experimental animals. The frequency of intravascular gas bubbles in the lung and liver of control animals was 57% and 43%, respectively. In cyproheptadine-treated animals the corresponding frequencies were 20% and 5%, respectively. Figures 2A and 4A show representative pathologic alterations in tissues of control animals (subjected to dysbaric conditions, without drug pretreatment). Corresponding tissues from animals pretreated with cyproheptadine in Figs. 2B and 4B show absence of such alterations. Figure 5 compares mortality of control and cyproheptadine-treated (5.0 mg/kg) animals. It can be seen that 10 min after decompression, 20% of the control animals died, whereas only 8% succumbed in the treated group. Similarly, 30 min after decompression, 55% of the control animals but only 25% of the cyproheptadine-treated animals died.

Table 1 shows the effect of cyproheptadine on mortality at the seven dose levels used. The dose of 2.5 and 5.0 mg/kg reduced mortality by about 45%. The results with the doses at 2.5–10.0 mg/kg are statistically significant at high levels of confidence, as Table 1 indicates.

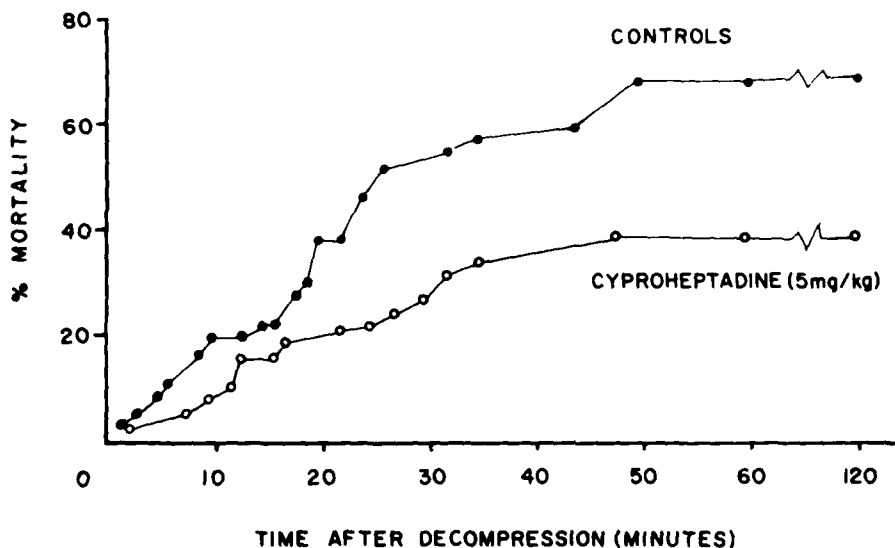


Fig. 5. Mortality in 37 control mice and 38 cyproheptadine-treated mice subjected simultaneously to identical dysbaric conditions.

TABLE 1
EFFECT OF PRECOMPRESSION ADMINISTRATION OF CYPROHEPTADINE ON MORTALITY OF
MICE IN DECOMPRESSION SICKNESS

Dose, mg/kg	Mortality, %			Statistical Significance
	Control Animals	Experimental Animals	Reduction in Mortality, %	
0.5	75.0 (6/8)*	75.0 (6/8)	0	NS**
1.0	78.3 (36/46)	65.2 (30/46)	16.7	NS
2.5	71.4 (15/21)	39.3 (11/28)	45.0	$P=0.05$
5.0	73.0 (27/37)	39.5 (15/38)	45.9	$0.001 < P < 0.01$
10.0	75.7 (53/70)	55.0 (38/69)	27.3	$0.01 < P < 0.02$
20.0	69.7 (23/33)	60.6 (20/33)	13.0	NS
40.0	37.5 (3/8)	100.0 (8/8)	—	NS

*(Number of dead animals/total number of animals in group); **NS = not significant.

The reduction in mortality was not as pronounced with the 1.0- and 20.0-mg/kg doses. The overall mortality in the control groups was 73% (163/223), which is the predicted rate for these animals with the dive profile used.

Cyproheptadine administered immediately after decompression did not influence clinical manifestations and produced only insignificant changes in mortality (Table 2). In these experiments the overall mortality of control animals was 56.8%, which is somewhat lower than that in the preceding experiments. This could be because the animals used in the experiments that are listed in Table 2 were of a lower weight and thus less susceptible to decompression sickness (8).

DISCUSSION

The results of this investigation indicate that cyproheptadine, when administered prior to compression, protects mice against decompression sickness. This prophylactic effect was evidenced by the observed reduction in the frequency and severity of clinical manifestations and pathohistologic alterations and by the significant decrease in mortality. Optimal effect was

TABLE 2
EFFECT OF POST-DECOMPRESSION ADMINISTRATION OF CYPROHEPTADINE ON MORTALITY OF
MICE IN DECOMPRESSION SICKNESS

Dose, mg/kg	Mortality, %			Statistical Significance
	Control Animals	Experimental Animals		
20.0	56.3 (9/16)*	68.8 (11/16)		NS**
5.0	57.1 (12/21)	66.7 (14/21)		NS
2.5	57.1 (4/7)	85.7 (6/7)		NS

*(Number of dead animals/total number of animals in group); **NS = not significant.

obtained with the intermediate doses of 2.5, 5.0, and 10.0 mg/kg. The ineffectiveness of the high doses (20.0 and 40.0 mg/kg) could result from toxic effects of the drug at such high dose levels. On the other hand, failure of the low doses (0.5 and 1.0 mg/kg) to decrease morbidity and mortality may be due to insufficient concentrations of the drug in the blood and tissues, even though these low doses are slightly higher than the therapeutic dose of cyproheptadine when used as an antiallergic drug. This may be because the cyproheptadine was administered several hours prior to decompression, which in all probability is the critical phase during which the drug can exert its protective effect; obviously, during this prolonged period, the concentration of the drug in the blood and tissues may be reduced by excretion or biotransformation, or both.

Administration of cyproheptadine immediately after decompression did not ameliorate decompression sickness. The ineffectiveness of the post-decompression administration of the drug may be due to irreversible changes triggered by gas bubbles before the drug is absorbed in concentrations sufficient to prevent or interrupt bubble-induced chain reactions.

In view of the above considerations, one can hypothesize that protection against decompression sickness may be achieved with smaller doses if the drug is administered shortly before decompression.

This possibility was not tested in the present investigation because of mechanical limitations that prevented administration of injections while the animals were being compressed.

All the compounds that in our hands were found effective against decompression sickness exhibit activities against histamine, bradykinin, and serotonin (1, 3, 4). These compounds include 1-(*N*-methyl-4-piperidyl)-3-phenyl-4-benzyl-5-pyrazolone (PPBP), 2-(4-phenyl-1-piperazyl methyl)-cyclohexanone HCl (PPCH), and dimethothiazine (Migristene). The fact that cyproheptadine, which was also shown to ameliorate decompression sickness, has antihistaminic, antiserotonin, and antibradykinin properties (9) provides additional support to our concept that agents that stimulate smooth muscle are implicated in the pathogenesis of the disease. Such agents could be released or activated in decompression sickness by several mechanisms, including mechanical disruption of tissues by expanding gas bubbles, gas-blood interphase activity, or vascular obstruction causing hypoxic damage to cells with subsequent release of lysosomal enzymes (2, 5, 10-13). The release or activation of smooth muscle stimulants could produce circulatory changes favoring further formation and growth of gas bubbles. They could also cause increased vascular permeability with subsequent edema, hemoconcentration, and hypovolemia. Furthermore, they could cause pain and produce changes that contribute to respiratory distress.

Considering the above and the fact that antagonism of histamine, serotonin, and bradykinin is the common denominator of all compounds that were shown to prevent decompression sickness, it is tempting to attribute the ameliorating effect of cyproheptadine to antagonism of smooth muscle stimulants.

The described pharmacologic approach suggests a potential prophylactic treatment for human decompression sickness. One drawback of this treatment is the drowsiness that may be produced by cyproheptadine. This undesirable side effect, however, could conceivably be alleviated by combining cyproheptadine with a psychomotor stimulant. It has recently been shown that amphetamine, which antagonizes the depression produced by antihistaminics (14, 15), counteracts the sedative action of cyproheptadine in mice without neutralizing its protective effect on decompression sickness. (16)

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Chryssanthou C, Rubin L, Graber B. L'amélioration de la maladie de décompression chez les souris par le pré-traitement avec la cyproheptadine. *Undersea Biomed Res* 1980; 7(4):321-329.— Les substances qui stimulent les muscles lisses ont été impliqués préalablement dans la pathogenèse de la maladie de décompression. Ce concept a été fortement soutenu de la démonstration que les composés qui combinent les activités contre l'histamine, la bradykinine, et la sérotonine empêchent ou améliorent la maladie de décompression. Cette communication regarde l'effet prophylactique de la cyproheptadine (Periactin), une drogue qui montre telles qualités pharmacologiques. Plus de 500 souris obèses ont été utilisés. Les groupes expérimentaux ont été injectées en manière sous-cutanée avec la cyproheptadine (0.5-40 mg/kg) avant la compression, et des souris de vérification ont subi simultanément une pression de l'air de 75 psig pour 6 h et puis décompressés rapidement. La plupart des souris de vérification ont montré des indications de la maladie de décompression (des engorgements, des grattements, des contractions nerveuses, des convulsions, de la paralysie) et sont morts. L'examen gros et histologique a montré des bulles gazeuses dans les vaisseaux sanguins et les tissus, un oedème périvasculaire, et des autres changements. Chez les animaux traités avec de la cyproheptadine, l'occurrence et la sévérité des manifestations cliniques et des altérations pathologiques ont été réduits, et la mortalité a été diminuée d'une façon marquée. Du point de vue de la statistique, des résultats significatifs ont été obtenus avec les doses de 2.5-10.0 mg/kg. La dose de 5 mg/kg ont abassé la mortalité à 45.9%. Ces résultats soutiennent le concept pathogénique proposé et suggèrent un traitement préventif chez les sujets humains.

maladie de décompression
souris
prévention
prophylaxie
traitement
drogues
cyproheptadine

antihistaminique
antibradykinine
pharmacologie
substances qui stimulent les muscles lisses
pathogenèse
Periactin

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UNDERWATER PHYSIOLOGY VII

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AMELIORATION OF DECOMPRESSION SICKNESS BY COMBINED AMPHETAMINE-CYPROHEPTADINE TREATMENT

C. Chryssanthou, L. Rodriguez, and P. Branden

Previous 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 (DCS).

The following are some of our observations that lend support to this concept:

1) Several pathological alterations in DCS are similar to those seen in bradykinin-treated animals (1).

2) Smooth muscle stimulants, such as bradykinin and smooth muscle acting factor (SMAF), increase susceptibility of animals to DCS (1,2).

3) SMAF is released or activated in DCS (3,4).

4) Decompression or nitrogen bubbling of blood in vitro generates smooth muscle stimulating activity (4,5).

5) Smooth muscle stimulating substances could account for several clinical manifestations and pathologic alterations in DCS (6,7).

6) Compounds which combine activities against histamine, bradykinin, and 5-hydroxytryptamine prevent or at least ameliorate DCS in mice (1,7,8). The latter observation was confirmed by other investigators working with hamsters and dogs (9,10). Dimethothiazine and cyproheptadine are among the compounds which exhibit an appreciable DCS preventing effect as evidenced by a significant reduction in morbidity and mortality (7,8).

The fact that all compounds in our studies prevented DCS-produced sedation, probably because of their antihistaminic activity, raises the question of this central depressant action playing a role in the prophylactic effect of these compounds.

The present communication deals with experiments designed to determine whether drugs that ameliorate DCS retain their protective effect when their sedative action is neutralized or counteracted. Elucidation of this question is important not only from a theoretical point of view but also for practical reasons because it would not be advisable to administer to divers or compressed air workers DCS-preventing drugs that cause drowsiness.

Two series of experiments were conducted. The first series determined the minimum amount of amphetamine required to antagonize the sedative effect of dimethothiazine and cyproheptadine at doses which ameliorate DCS in mice. The second series of experiments assessed the effectiveness of optimum amphetamine-cyproheptadine combinations in the amelioration of DCS.

MATERIAL

Animals. C57BL/6J mice (Jackson Memorial Laboratories, Bar Harbor, ME), weighing 22-38 g, were used in the first experimental series and their obese littermates (C57BL/6J-ob), weighing 40-78 g, were used in the second series. The reason for using both thin and obese mice is that thin animals are better suited than obese for experiments on the effects of drugs on locomotor activity (obese are too inactive for such studies). Obese mice, on the other hand, are preferable for studies on DCS because of their greater susceptibility to the disease as compared to their thin littermates (11). Only male animals were used as subjects because their activity is not influenced by cyclic changes as in females.

Drugs. The compounds used in these investigations were: dimethothiazine (Migristene, Rhône-Poulenc-Special), cyproheptadine (Periactin, Merck Sharp & Dohme), pseudoephedrine HCL (Chromalloy Pharmaceuticals), and *dl*-amphetamine sulfate (Amend). All solutions for injection were made in sterile normal saline.

Revolving wheel cage. A revolving drum, 18 cm in diameter and 11.5 cm in width, were used. Friction was minimized by ball bearings and thus locomotion of the animals by even a fraction of an inch caused partial rotation of the drum. The revolving drum measured only lateral movement; vertical movement (standing on hind legs and jumping) or leaning and turning could not be measured. A mechanical counter connected to the revolving drum responded to every one-half rotation in either direction and counted once every two such one-half rotations.

Hyperbaric chamber. A Model 1836HP Bethlehem Corporation chamber with controlled temperature and humidity was used. The chamber was pressurized with air (dry air cylinders, Matheson Company, Inc.).

METHODS

The animals were kept in metal cages in rooms with controlled temperature ($22 \pm 2^\circ\text{C}$) and relative humidity (50%) for a 2-3 week stabilization period.

They were fed Wayne Lab-Blox and water ad libitum.

The degree of sedation produced by dimethothiazine and cyproheptadine and the only antagonistic effect of pseudoephedrine and amphetamine were measured in thin mice in the first experimental series. The central depressant or stimulant effect of the drugs on the animals was expressed in terms of changes in spontaneous locomotor activity measured by the revolving wheel cage.

The effectiveness of the optimum cyproheptadine-antagonist combination in preventing DCS was tested in obese mice in the second series of experiments.

Measurement of spontaneous locomotor activity. These measurements, which were assumed to reflect drug induced sedation or stimulation of the animals, were made in a dark quiet room so that visual and auditory stimuli were eliminated. Temperature ranged between 22° and 26°C. Variations of temperature within 5°C have a negligible effect on locomotor activity (12). The previously described revolving wheel cage was used to record locomotion. Because of minimal friction between the wheel and the horizontal axis, the animals did not use extra effort to rotate the wheel and were thus not discouraged from walking or running. No food or water was available to the animals during the recording period. They were given Lab-Blox and water ad libitum before and after testing.

Each animal served as its own control because of great variations in locomotor activity even among littermates of the same sex under the same experimental conditions (12). Activity was recorded every 15 min during a 4-h run, which was conducted once a day every other day for a total of 5 runs. The test substances were injected subcutaneously following the first 2 h of activity in the wheel cage. Normal saline was administered to all animals in the first three runs to establish base-line (control) levels of activity. The same animals received dimethothiazine (40 mg/kg) or cyproheptadine (5 mg/kg) in the 4th run. Administration of the same drugs in the same dose to the same animals was repeated in the 5th run 15 min after a subcutaneous pseudoephedrine or amphetamine (1-10 mg/kg) injection. Activity was expressed as mean rotations per hour (RPH). The results were statistically evaluated by the Student's *t*-test for paired observations.

Testing effectiveness of drug combinations on DCS. Decompression sickness was produced in obese mice by exposure to 6.12 ATA (90 psi) absolute air pressure for 6 h followed by decompression to sea level within 1 min (usually 30 s). This simulated dive profile has been previously shown to produce DCS in obese mice with approximately 60-80% mortality rate. A total of 64 mice were used in 4 experiments. The animals were divided into one control and one experimental group of corresponding weights. In each experiment eight animals from each group were placed in the chamber at the same time and subjected to the above dive profile simultaneously to ensure exposure of control and experimental animals to identical dysbaric conditions. No food or water were available to the animals during dysbaric exposure. Immediately before compression, the experimental animals received a subcutaneous injection of the optimum amphetamine-cyproheptadine combination. Normal saline was

administered to the control mice in a volume equal to that of the drug injections. The effect of amphetamine alone on DCS was tested on 16 additional animals in 2 preliminary experiments.

Following decompression, the animals were taken out of the chamber and observed for at least 60 min for clinical manifestations of DCS. Animals that succumbed were immediately autopsied and representative tissues were removed for histologic examination. Postdecompression survival times were recorded. Autopsies and histologic examinations were also performed on a few animals which were subjected to euthanasia at various intervals after decompression. These animals were not included in the mortality statistics. The results were statistically evaluated by the chi square test with Yates correction.

RESULTS

First Experimental Series: Effect of Drugs on Spontaneous Locomotion

Both cyproheptadine and dimethothiazine caused a dramatic reduction in spontaneous locomotor activity. Within 15 min following drug administration locomotion began to decrease precipitously and 1 h after injection most animals exhibited minimal or no activity (Fig. 1). In one group of 19 animals locomotor activity of 211 RPH during the control run (following saline administration) was reduced to 47.5 RPH after an injection of 5 mg/kg cyproheptadine (Table I). Similarly, dimethothiazine administered to another group of 12 animals lowered activity from a control level of 257 RPH to only 35 RPH (Table II).

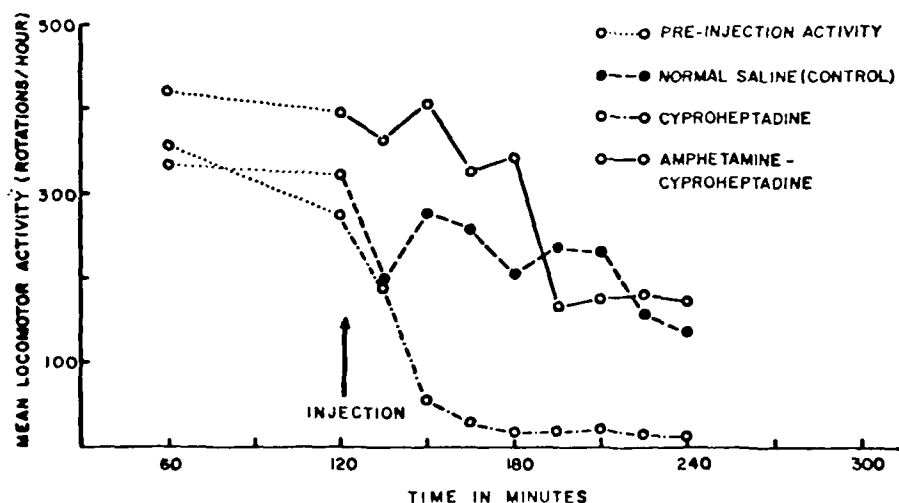


Fig. 1. Time-course of spontaneous locomotor activity of mice treated with cyproheptadine alone and in combination with amphetamine.

TABLE I
Effect of Cyproheptadine and Amphetamine-Cyproheptadine on Spontaneous Locomotor Activity of Mice

Animal No.	Rotations per hour		
	Control (Normal Saline)	Cyproheptadine (5 mg/kg)	Amphetamine (7.5 mg/kg) plus Cyproheptadine (5 mg/kg)*
1	319.5	62.0	301
2	380.5	40.5	342
3	38	14.5	20.5
4	263.5	47.5	123.5
5	155.0	56.5	400.0
6	394.0	44.0	221.5
7	105.0	92.0	408.0
8	52.5	21.5	140.0
9	2.5	5.5	232.0
10	287.5	82.5	329.5
11	112.5	56.5	226.0
12	452.0	96.5	402.5
13	31.5	26.5	175.5
14	83.5	23.5	179.0
15	502.5	72.5	330.0
16	70.5	102.0	251.0
17	408.5	35.0	424.0
18	172.5	14.5	335.5
19	185.5	9.0	219.5
Mean $\pm$ SE	211.4 $\pm$ 36	47.5 $\pm$ 7	266 $\pm$ 25

* Cyproheptadine was administered 15 min after the amphetamine injection.

TABLE II
Effect of Dimethothiazine and Amphetamine-Dimethothiazine on Spontaneous Locomotor Activity of Mice

Animal No.	Rotations per hour		
	Control (Normal Saline)	Dimethothiazine (40 mg/kg)	Amphetamine (7.5 mg/kg) plus Dimethothiazine (40 mg/kg)*
1	488.5	49.5	305.5
2	357	98	666
3	427.8	13	122
4	91.5	24.5	314
5	133.1	26.5	46.5
6	475.5	28.5	144.5
7	135.5	30	185.5
8	96.5	49	132.5
9	353.8	35.5	21.5
10	180.5	23.5	200.5
11	196	38	130.5
12	121	16.5	156
Mean $\pm$ SE	257.28 $\pm$ 41	35.07 $\pm$ 6	198.42 $\pm$ 45

*Dimethothiazine was administered 15 min after the amphetamine injection.

Pseudoephedrine (10 mg/kg) administered to 10 animals 15 min before or after a cyproheptadine (5 mg/kg) or dimethothiazine (40 mg/kg) injection did not influence the depressant effect of the latter compounds on locomotor activity.

Amphetamine, on the other hand, significantly antagonized the sedative action of both cyproheptadine and dimethothiazine (Fig. 2). The time-course of the depressant action of cyproheptadine and the antagonistic effect of amphetamine is illustrated in Fig. 1. Table I shows that 5 mg/kg cyproheptadine, when administered alone, lowered mean locomotor activity from a control level of 211 RPH to 47.5 RPH and did not depress locomotion when preceded by an injection of 7.5 mg/kg amphetamine as evidenced by a mean locomotor activity of 266 RPH. In fact, all animals exhibited a significantly greater locomotor activity when amphetamine preceded cyproheptadine administration. Likewise, Table II indicates that the sedative effect of 40 mg/kg dimethothiazine reflected in the reduction of locomotion from 257 to 35 RPH, was counteracted by 7.5 mg/kg amphetamine, which increased mean locomotor activity to 198 RPH when administered 15 min before dimethothiazine. These results are statistically significant at high levels of confidence ($P < 0.001$). Lower doses of amphetamine did not sufficiently antagonize the sedative effect of cyproheptadine or dimethothiazine; higher doses produced stimulation resulting in locomotor activity much higher than base line levels.

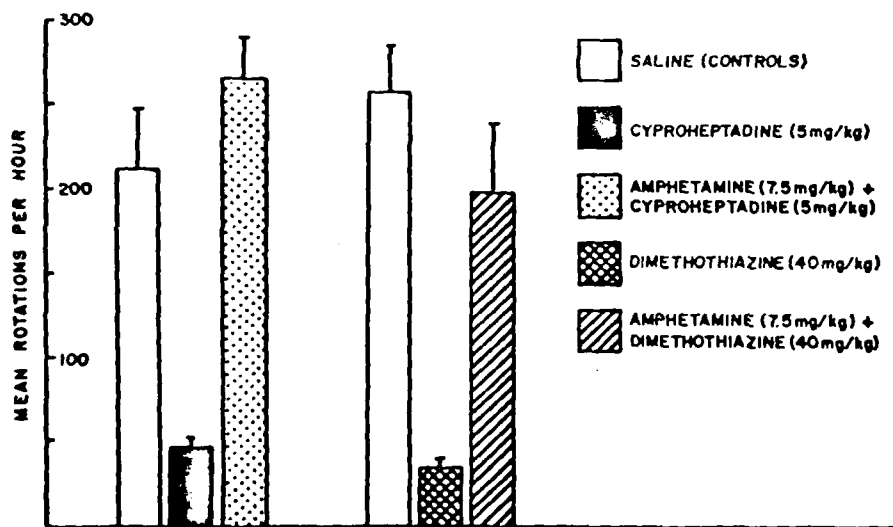


Fig. 2. Amphetamine antagonism of the inhibitory effect of cyproheptadine and dimethothiazine on spontaneous locomotor activity in mice. Height of columns represents mean revolutions per hour of all animals in the group during the entire test period. Vertical lines represent SEM.

Second Experimental Series: Effect of Amphetamine-Cyproheptadine on DCS

Animals that developed DCS, as a result of their exposure to the previously described dive profile, began to exhibit signs of the disease soon after decompression. Clinical manifestations of DCS included scratching (possibly because of the formation of subcutaneous gas bubbles), reduced locomotion, chokes, and convulsions. Almost all control animals exhibited these signs and the majority of them succumbed in less than 1 h following decompression; their death was preceded by twitching and severe respiratory distress with gasping and hiccough-like spells.

In the groups that received the combined amphetamine-cyproheptadine treatment before compression, a smaller number of animals manifested signs of DCS than in corresponding control groups. Table III shows that in all experiments fewer animals died in the groups treated with amphetamine-cyproheptadine than in controls. The overall mortality decreased from 83.8% to 51.7%, a reduction of 38.3%, which is statistically significant ($0.02 < P < 0.01$).

Autopsies of animals that succumbed to DCS revealed the previously described gross alterations (1,11). Most striking were the abdominal enlargement caused by gaseous distention of the gastrointestinal tract and the presence of grossly visible gas bubbles in the vena cava, the subcutaneous and intra-abdominal adipose tissue, and, in some cases, in the adrenals and spleen.

Histologic examination showed perivascular edema in the lungs, severe congestion of the bone marrow, rouleaux formation, and the presence of gas bubbles in various tissues and organs including the lung, spleen, adrenal

TABLE III
Effect of the Combined Amphetamine-Cyproheptadine
Treatment on Mortality of Obese Mice in Decompression
Sickness

Experiment No.	Mortality %	
	Controls	Amphetamine (7.5 mg/kg) plus Cyproheptadine (5 mg/kg)*
1168	87.5(7/8)†	62.5(5/8)
1171	100(8/8)	62.5(5/8)
1172	62.5(5/8)	25.0(2/8)
1177	85.7(6/7)	37.5(3/5)
Total	26/31(83.8%)	15/29(51.7%)

* Cyproheptadine was administered 15 min after the amphetamine injection.
† Number of dead animals/total number of animals in group.

(Fig. 3A), skin, bone, adipose tissue, and less frequently, liver, pancreas, and heart (Fig. 4A). It was not always easy to determine whether the gas bubbles were intravascular or in tissue spaces. Sometimes serial sections revealed that bubbles which on coarse examination appeared extravascular, were continuous with the lumen of a blood vessel. Not infrequently, widely separated nuclei of flattened endothelial cells were observed around such bubbles. Gas accumulations were particularly numerous in the spleen and adrenals giving these organs a spongy appearance (Fig. 3A).

The above histologic alterations were more frequent and more severe in control animals than in those treated with amphetamine-cyproheptadine. Surviving animals that were subjected to euthanasia at intervals after decompression revealed minimal or no changes (Fig. 3B and 4B).

Amphetamine administered alone did not reduce morbidity or mortality in DCS. In fact, in one group treated with amphetamine alone, mortality was higher than in corresponding controls. The number of animals in this group, however, was not sufficient to allow statistical evaluation of this effect.

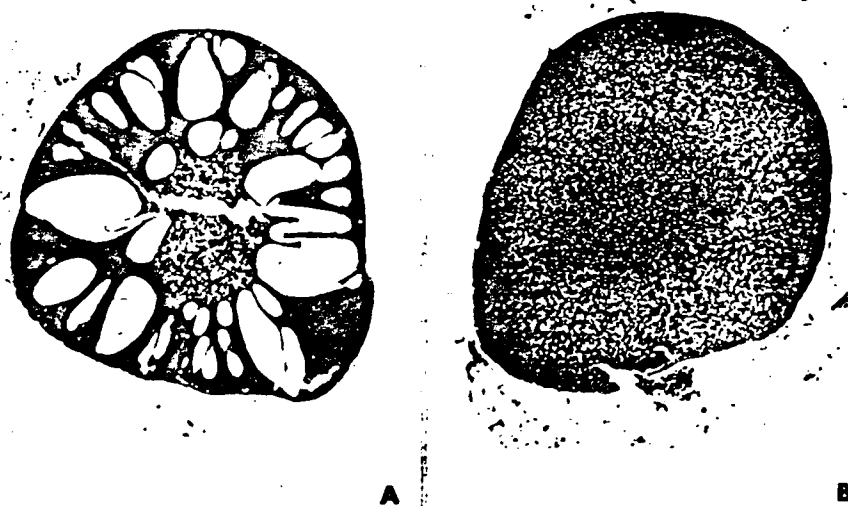


Fig. 3. A. Adrenal from control animal (subjected to compression-decompression without drug treatment), which died 40 min after decompression. The organ has a sponge-like appearance because of numerous gas bubbles of varying size located predominantly in the cortex. B. Corresponding adrenal from animal treated with amphetamine-cyproheptadine, which was subjected to euthanasia 60 min after decompression. Both the cortex and the medulla are free of gas bubbles. (Stained with hematoxylin and eosin. Original magnification 7.8 X.)

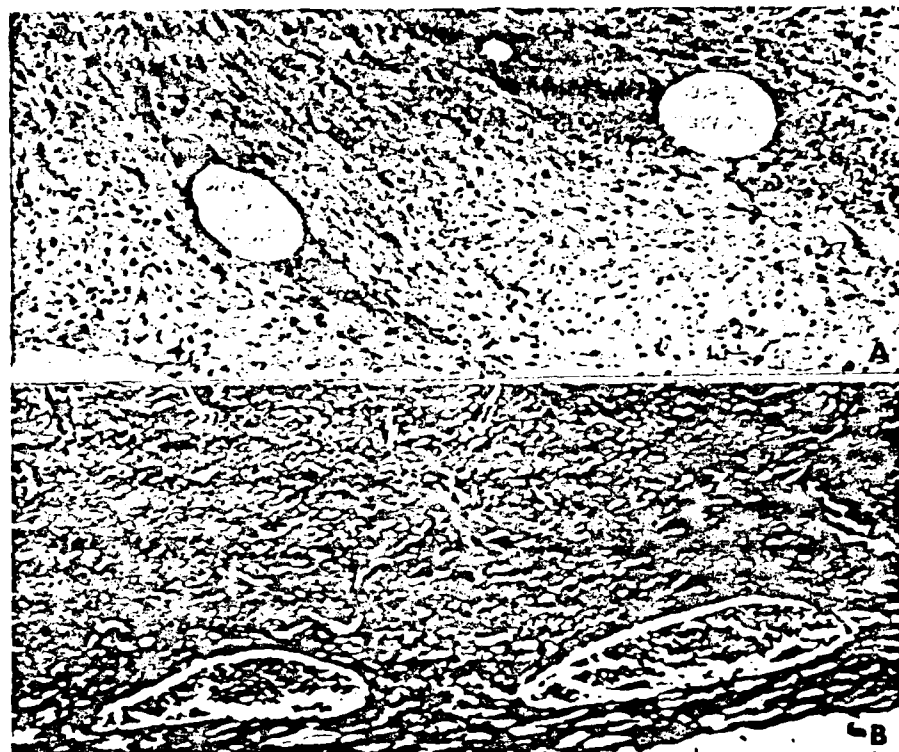


Fig. 4. A. Myocardium from a control animal (subjected to compression-decompression without drug treatment) showing intravascular gas bubbles. B. Corresponding tissue from animal that was treated with amphetamine-cyproheptadine before compression. No gas bubbles are present. (Stained with hematoxylin and eosin. Original magnification 25 X.)

DISCUSSION

The first series of experiments demonstrated a central depressant effect of cyproheptadine and dimethothiazine as well as antagonism of this effect by amphetamine. Evaluation of the depressant or stimulant action of the drugs was based on measurements of spontaneous locomotor activity, which is easily quantitated and expressible in simple units (13). This method obviously does not measure all the complex behavioral changes produced by compounds with sedative or excitatory effects. It does, however, provide a consistent measure of an important parameter of psychomotor activity, and it was therefore assumed to reflect with a reasonable degree of reliability the central depressant or stimulation action of the drugs. Measurements of locomotor activity have

already been used in many studies of behavioral changes induced by a variety of drugs (14,15).

It is evident from Figs. 1 and 2 and from Tables I and II that 7.5 mg/kg amphetamine sulfate counteracted the depressant effect of 5 mg/kg cyproheptadine and 40 mg/kg dimethothiazine. The doses of the latter drugs were those which were previously shown to ameliorate DCS (7,8). Consequently, the combination amphetamine-cyproheptadine in the above doses were used to test the effectiveness of cyproheptadine in ameliorative DCS when its sedative action is counteracted.

The results indicate that the combined amphetamine-cyproheptadine treatment decreased the incidence and severity of clinical manifestation and histologic alterations and reduced mortality by 38.3%, as seen in Table III. This decrease in mortality does not significantly differ from the 44.4% reduction obtained when the same dose of cyproheptadine was administered alone in previous studies (16). Because the pharmacologic effects of dimethothiazine are similar to those of cyproheptadine, it is reasonable to expect that dimethothiazine will also retain the DCS-ameliorating effect when its sedative action is counteracted. This, however, will remain a speculative extrapolation until further experimentation explores the effect of the combined amphetamine-dimethothiazine administration on DCS.

The observations of these investigations leads to the conclusion that the DCS-ameliorating effect of cyproheptadine, and conceivably of dimethothiazine, is independent of the sedative action of the compounds. The observation that sedation is not responsible for the protection against DCS is consistent with the reported failure of hypnotics (chloralose) to prevent DCS (10).

As mentioned earlier, demonstration of the ability of cyproheptadine to decrease morbidity and mortality in DCS when its central depressant effect is counteracted is of theoretical as well as practical importance. From a theoretical point of view, the results of the present investigation suggests that the DCS-ameliorating effect of cyproheptadine is related to its activity against bradykinin, histamine, and serotonin rather than to the central depressant action of the compound. This in turn supports the previously proposed implication of smooth muscle stimulating substances in the pathogenesis of DCS (1-7). The practical aspect of the study is related to the obvious problems with the use of DCS-ameliorating drugs that produce sedation and drowsiness. Such effects could impair performance of divers and compressed air workers and, in extreme situations, jeopardize their missions and even their safety. Elimination of the undesirable sedative effect of cyproheptadine and dimethothiazine by the use of psychomotor stimulants, such as amphetamine, alleviates these difficulties and provides an attractive and promising pharmacologic approach for the amelioration of DCS in humans.

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DYSBARIC OSTEONECROSIS:
EXPERIMENTAL MODES, PATHOGENESIS,
PREDISPOSING FACTORS

**ANIMAL MODEL OF HUMAN DISEASE
DYSBARIC OSTEONECROSIS
DYSBARIC OSTEONECROSIS IN MICE**

C. CHRYSSANTHOU

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ANIMAL MODEL OF HUMAN DISEASE

Dysbaric Osteonecrosis

Dysbaric Osteonecrosis in Mice

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DYSBARIC OSTEONECROSIS, a potentially disabling disorder, has recently been recognized as a major hazard in divers and compressed-air workers. The incidence of the disease ranges from 4% in Royal Navy Divers¹ to 50–60% in Japanese diving fishermen.^{2–3} The etiology and pathogenesis of dysbaric osteonecrosis are still obscure.⁴ An animal model could be very helpful in improving our understanding of causative and predisposing factors and in the development of preventive, diagnostic, and therapeutic procedures. The hereditary obese hyperglycemic mouse develops dysbarism-induced bone necrosis that closely parallels human dysbaric osteonecrosis.

Biologic Features

Hereditarily obese hyperglycemic C57BL/6J mice (Jackson Memorial Laboratories, Bar Harbor, Maine) consistently develop dysbaric osteonecrosis when subjected to 75 psig (6327 g/sq cm) air pressure for 2–3 hours followed by stage decompression with stops at 50, 40, 30, 20, and 10 psig for 5, 25, 35, 75, and 120 minutes, respectively.⁵ With this simulated dive profile, the majority of the animals (about 93%) do not exhibit clinical signs of decompression sickness.

Histologic evidence of osteonecrosis appears after a latent period of at least 2 months following dysbaric exposure. The necrotic lesion involves the spongy tissue of a part or of the entire epiphysis. In early stages the osteocytes in epiphyseal trabeculas exhibit pyknosis and karyorrhexis, and the marrow cells show indistinct cellular boundaries and loss of nuclear staining. In more advanced lesions the lacunas in the necrotic trabeculas are devoid of osteocytes, and the intertrabecular marrow spaces contain amorphous masses of

granular debris (Figure 1) and sometimes fragments of necrotic bone. Several microracks (fissures) are seen between lamellas, usually extending to the surface of the trabeculas (Figure 1), occasionally resulting in tissue fragmentation. In some cases necrotic epiphyseal trabeculas appear fractured, and occasionally collapse of the articular surface is observed. In other cases there is erosion of the articular cartilage and of the subjacent bone of the epiphysis with formation of concave defects, sometimes associated with epiphyseal collapse.⁵ At later stages fibrovascular tissue invades intertrabecular spaces and replaces necrotic marrow (Figure 2). Sometimes vascular connective tissue showing evidence of osteoclastic activity can be seen surrounding partially resorbed bone fragments.⁵ Appositional new bone formation is observed in only few cases (Figure 2).

Comparison With Human Disease

Etiology

Dysbaric osteonecrosis in mice, like the human disease, is induced by exposure to dysbaric conditions that do not necessarily produce clinical manifestations of decompression sickness.

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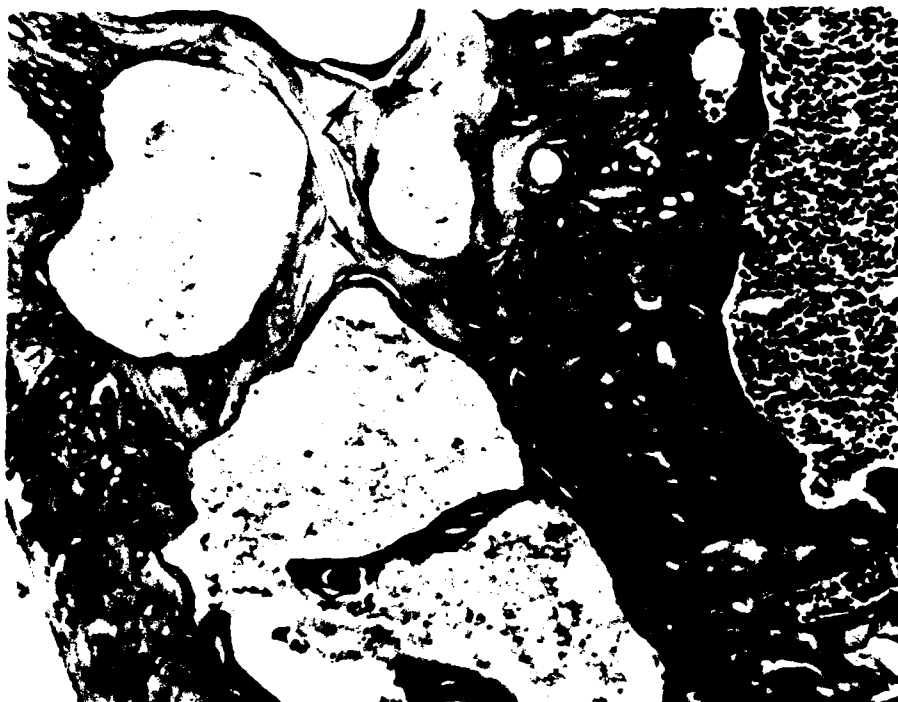


Figure 1—Epiphysis of the proximal end of the tibia of a mouse 5 months after multiple dysbaric exposures. Note necrotic trabeculas with microcracks (arrows) and lacunae devoid of osteocytes and the intervening marrow spaces containing granular debris. Normal bone marrow can be seen in the right portion of the microphotograph. (H&E, $\times 25$)

Latent Period

In both species the necrotic lesion appears after a latent period of at least 2 months; in mice this period can be 9 months or longer, and in man it can reach several years.

Distribution

The lesion involves predominantly the epiphysis of long bones in both the mouse and man; diaphyseal lesions, which occur in man, have not been observed in mice. The distal end of the femur and proximal end of the tibia is a frequent site in both species. The head of the femur and humerus on the other hand is commonly involved in man, but only rarely in mice. These differences in distribution could be due to circulatory peculiarities and differences in postural characteristics of the two species.

Histopathology

The histologic changes observed in the mouse model closely resemble those seen in the human disease. The similarity is evident both in the development of the necrotic lesion as well as in the repair process.

Radiology

Osteonecrotic lesions in humans can be diagnosed by X-rays. No attempts were made to detect lesions in mice radiologically because of the minute size of the structures involved.

Incidence

The magnitude of pressure, frequency of exposure and rate of decompression affect the incidence in both man and mice.^{4,6,7} Obesity predisposes mice to the disease, and there are indications that it may have similar effects in man.^{4,6,8,9} The rate of compression influences the incidence of osteonecrosis in mice.^{4,6} The effect of this factor in man has not been explored.

Usefulness of the Model

The mouse model is suitable for studies on the etiology, pathogenesis, and prevention of dysbaric osteonecrosis because, as in the human disease, the lesion is induced by dysbarism and not caused by artificially produced ischemia, as in some other models.^{10,11} Furthermore, unlike other animal models, a large number of mice can be subjected to compression/decompression.

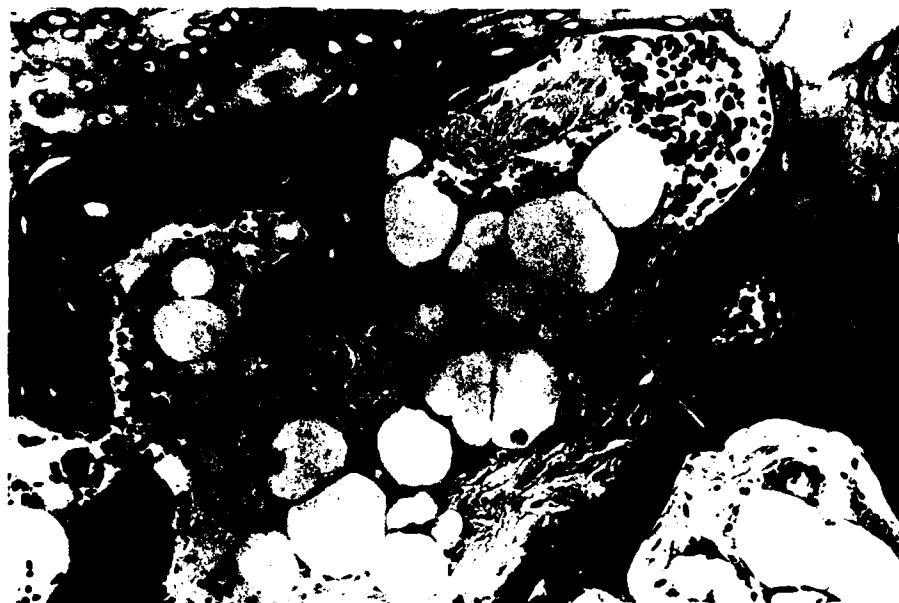


Figure 2—Epiphyseal lesion in the proximal end of the tibia 10 months after hyperbaric exposure. Necrotic trabeculae and subchondral bone with empty lacunae are evident. New appositional bone with lacunae containing osteocytes is sharply demarcated from the adjacent necrotic tissue (*left*). The vascular connective tissue which has replaced necrotic marrow contains hematopoietic and adipose tissue elements. (H&E, $\times 64$)

sion simultaneously in the same chamber to ensure exposure of various groups to identical environmental conditions. This facilitates controlled experiments on predisposing factors (eg, obesity), preventive treatment, and other comparative studies. The large number of animals that can be used also permits statistical analysis of the data. The mouse model has been used for studies on the influence of various factors on the incidence and latency of dysbaric osteonecrosis^{5,6} and on bone and cartilage collagen metabolism at various stages of the lesion and under various conditions.¹²

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Dysbaric Osteonecrosis

Etiological and Pathogenetic Concepts

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Dysbaric Osteonecrosis

Etiological and Pathogenetic Concepts

CHRYSSANTHOS P. CHRYSSANTHOU, M.D.*

The expanding industry of off shore oil drilling, the popularity of scuba diving, the continuing underwater and space exploration and many other commercial, scientific and military activities are responsible for an increasing exposure of man to abnormal pressures. More people than ever before are now more often subjected to dysbaric conditions, working longer in compressed air environments, diving deeper into the seas and flying higher into the atmosphere.

Exposure to abnormal pressures is associated with potential hazards including dysbaric disorders. One such disorder which may develop in individuals subjected to changes in ambient pressure is dysbaric osteonecrosis. This potentially disabling disease is alarmingly widespread in divers and compressed air workers. The incidence of dysbaric osteonecrosis varies depending on the conditions of hyperbaric exposure. In some groups of Japanese diving fishermen the incidence reaches 50–60%.^{20, 36}

The relatively high incidence of dysbaric osteonecrosis, our inability to prevent its

development, and the latency of the lesion which precludes early diagnosis have created great concern.

No significant progress in the prevention, diagnosis and management of dysbaric osteonecrosis can be expected as long as the etiology and pathogenesis of this condition remain obscure. Our knowledge of the role of various etiologic and predisposing factors and the sequence of events leading to dysbaric bone necrosis is extremely limited despite a plethora of theories and speculations. Most of the hypotheses on the etiology and pathogenetic mechanisms lack support by convincing scientific data and are at best controversial. This uncertainty is in part due to the absence of a satisfactory experimental model for testing the validity and significance of the various postulates.

Experimental aseptic bone necrosis was produced in dogs by subjecting them to compression–decompression;⁴³ in rabbits by dysbaric exposure,²⁵ artificial emboli (glass beads)⁵¹ and lipid embolism;²⁶ in sheep by severing specific vessels;²¹ and in miniature swine and mice by exposure to compression–decompression.^{10, 44}

There are serious doubts, however, that the lesions produced in experimental animals are identical to those observed in man. Furthermore, it is questionable that the disorder in these animal models has the same etiology

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and pathogenesis as in humans. Nevertheless, this is the only information available from animal studies and although extrapolations should be made cautiously, certain experimental observations in conjunction with epidemiological data could form the basis for working hypotheses.

There are many fundamental questions challenging researchers. Is dysbaric osteonecrosis associated with decompression sickness? What is the relative role of the magnitude of pressure, duration and frequency of exposure, rate of compression and decompression and nature of inhaled gases? Is the lesion ischemic necrosis? If so, is ischemia caused by vascular changes, thrombosis or embolization? If embolization is implicated, what type of emboli are responsible (gas bubbles, thromboemboli or lipid material)? Is it one, or multiple pathologic alterations which lead to the development of the lesion? Is the gas bubble the single initiator of these alterations? Are there other etiologic and pathogenetic factors related to compression and or decompression but independent of gas bubbles? This communication will attempt to analyze certain etiological and pathogenetic concepts and present relevant experimental data obtained by the author.

CORRELATION OF DYSBARIC OSTEONECROSIS WITH DECOMPRESSION SICKNESS

The association of dysbaric osteonecrosis with decompression sickness is still a controversial subject. The question is not moot. It is relevant to the etiology and pathogenesis of dysbaric osteonecrosis and may have significant practical applications in the prevention of the lesion.

Statistical data do not support the thesis that bone necrosis is associated with a history of decompression sickness. Surveys on U.S. Navy divers, and on West Coast tunneling projects and data collected by the United Kingdom registry of compressed air workers

failed to reveal any significant correlation.^{1,2,4} Many victims of decompression sickness do not develop osteonecrosis, and bone lesions are detected in divers and compressed air workers who had no symptoms of the disease. In fact only 15.8% of the U.S. Navy divers with osteonecrosis reported decompression sickness.¹² Furthermore, no correlation was found between the number of decompression sickness episodes and incidence of bone lesions.¹⁰

The results from our experimental studies are consistent with the above observations. By selecting appropriate dive profiles we were able to produce osteonecrotic lesions in animals which did not exhibit signs of decompression sickness at any time.¹⁰

It has been suggested that lack of correlation between decompression sickness and osteonecrosis may be due to the fact that individuals with bone lesions may have failed to report a prior "hit" because of reluctance to enter a recompression chamber or for other reasons. The argument certainly does not apply to experimental animals, and it is questionable whether it represents a significant factor in human statistics.

The assumption that dysbaric osteonecrosis is independent from decompression sickness does not necessarily imply that the 2 conditions have entirely different etiologies and pathogenetic mechanisms. It is generally accepted that gas bubbles initiate the chain of reactions which are responsible for decompression sickness. Gas bubbles could also initiate changes leading to bone necrosis. Depending on certain conditions gas bubbles could trigger either mechanism or both. In light of this theory, the frequent occurrence of dysbaric osteonecrosis without preceding history of decompression sickness and the seemingly conflicting reports of increased incidence of bone lesions in individuals who suffered decompression sickness are not necessarily contradictory.

"Safe" decompression tables are considered adequate as long as they prevent develop-

ment of decompression sickness. It is conceivable, however, that safe decompression, while keeping gas-tension levels below those required to produce acute manifestations of decompression sickness, may permit supersaturation of the long half-time fatty bone marrow with subsequent bubble formation. In addition, it is known that gas bubbles may be present even after routine asymptomatic decompression.⁴ These asymptomatic gas bubbles are termed "silent." They may be "silent" in terms of decompression sickness but not in terms of dysbaric osteonecrosis. Showers of silent embolic bubbles during repeated decompressions have been implicated in the pathogenesis of bone lesions.²²

Harvey²² has stated that "... the low tolerance of bone for inert-gas supersaturation, may precipitate development of lesions (osteonecrotic) when present-day decompression tables are followed." If this is so, decompression tables may require recalculation in consideration of the longer half-time tissues and of the questionable "innocence" of silent bubbles. In view of the above, it is apparent that gas bubbles may represent a common denominator in the etiologies of decompression sickness and dysbaric osteonecrosis.

Although the possibility cannot be ruled out that development of the 2 conditions may follow entirely different pathways, it seems more likely that dysbaric bone lesions and decompression sickness share some of the pathogenetic alterations and initiating factors.

CERTAIN FACTORS INFLUENCING THE INCIDENCE OF DYSBARIC OSTEONECROSIS

MAGNITUDE OF PRESSURE

The correlation between the degree of pressure and the incidence and severity of bone lesions has been shown both in animal and human studies. McCallum reported that

osteonecrosis in caisson workers was directly related to the degree of pressure and the number of exposures,³⁵ and Kawashima observed a relationship between bone lesions and diving depth in a study of 268 cases of osteonecrosis in Japanese shell divers.²⁹ These observations are in accord with experimental data which indicate that, under controlled conditions, the severity of bone and joint changes in rabbits is correlated to the degree of pressure.²⁵

NUMBER OF EXPOSURES

There are several reports indicating that the incidence of dysbaric osteonecrosis is correlated with the number of dysbaric exposures. These communications include the findings of the Decompression Sickness Panel of the Medical Research Council (United Kingdom), studies on Japanese divers²⁹ and caisson workers,³⁵ and data from animal investigations.^{10, 25}

Results obtained in our laboratories show that repeated exposures of mice to dysbaric conditions not only significantly increases the incidence of bone lesions but also shortens the latent period. With multiple exposures the incidence of the lesion within 3 months after the initial exposure was 25%. During the same period following single exposure, however, no bone abnormalities were detected.¹⁰

Multiple exposures may have cumulative effects. Harvey considers repeated showers of silent bubbles more important in the development of bone lesions than a single insult.²² It is possible that with a single episode the affected area of the bone is too small to be detected or the healing is so perfect that restituted necrotic tissues reveal no evidence of prior damage. Subsequent infarctions, on the other hand, may either result in coalescence of minute lesions or cause further circulatory impairment and thus prevent healing. These possibilities were considered by Jones in reference to single and

multiple episodes of fat embolization.²⁷ It is also conceivable that gas micronuclei remaining after the initial exposure may precipitate bubble formation in subsequent exposures at lower levels of gas supersaturation.

COMPRESSION AND DECOMPRESSION SCHEDULES

In assessing the effect of different decompression profiles on the incidence of dysbaric osteonecrosis, several factors have to be taken into account, including the magnitude of pressure, duration of exposure (work schedules) and type of decompression (oxygen or air inhalation). Although no specific studies were reported where different decompression profiles were compared, with all other variables being the same, it seems that the rate of decompression does influence the incidence of bone lesions. This is evident, for example, when one compares the high incidence of osteonecrosis in compressed air workers in the U.S. before 1963, when inadequate decompression tables were used, with the dramatic reduction in the incidence of the disorder after the Washington State tables with extended decompression time were adopted.^{16,32}

We recently reported what we believe to be the first study on the influence of the rate of compression on the development of bone lesions.¹⁰ This work revealed that the rate of compression appreciably affects the incidence of bone lesions under otherwise identical experimental conditions. In a total of 57 mice subjected to rapid compression 27 developed osteonecrosis (47.3%) while in 34 animals subjected to stage compression only 8 had bone lesions (23.5%). It is premature to speculate on the mechanism of this effect based on a single observation. Nevertheless, it is tempting to consider the possible role of gas-induced osmosis, which may be influenced by the rate of compression and has been implicated in the pathogenesis of osteonecrosis.²³ Assessment of the possible role of

the rate of compression in dysbaric phenomena is important and requires further investigations.

OBESITY

Our experimental studies revealed a striking difference in susceptibility to dysbaric osteonecrosis between obese and thin mice.¹⁰ Under identical experimental conditions the incidence of dysbaric bone necrosis in animals with an average weight of 24, 54 and 78 g was 5.8, 26.3 and 38.6% respectively. Obesity not only increased the incidence of osteonecrosis but also shortened the latent period.

The increased susceptibility of obese mice to dysbaric osteonecrosis may be related to the higher content of fat in their bone marrow and the high solubility of nitrogen in fat. Exchange of nitrogen in the fatty bone marrow proceeds slowly and decompression could cause great supersaturation of the gas in this tissue with the potential of generating gas bubbles over relatively long periods of time. Such extravascular gas bubbles released from fatty elements of the bone could, within the rigid confines of the osseous tissue, exert sufficient pressure to compress blood vessels and cause ischemic changes. Initially extravascular gas bubbles would affect veins which are more susceptible to compression, thus resulting in stasis. The observed congestion and hemorrhagic foci in the bone marrow of animals which died within 48 hours post-decompression¹⁰ are consistent with this hypothesis. Another factor to be considered is that obese mice have fatty livers. It has been suggested that the fatty liver is capable of spontaneously releasing embolic fat globules into the circulation.³⁷

Obesity and hyperlipidemia have been considered predisposing factors in nondysbaric aseptic bone necrosis.⁵³ Conti advises that because of an apparent relationship between hyperlipidemia and osteonecrosis, no individual with such a condition should be per-

TABLE 1. Dysbaric Osteonecrosis

<i>Etiological and Pathogenetic Factors</i>		
<i>Ischemic Changes</i>		<i>Non-Ischemic Changes</i>
<i>Embolie</i>	<i>Non-Embolie</i>	
Gas bubbles	Extravascular bubbles (compressing vessel)	Autoimmunity and dysproteinemia
Lipid material	Intravascular bubbles (obstructing lumen)	Elevated PO_2 (toxic changes in bone) (alterations in collagen)
Thromboemboli (fibrin, platelets, etc.)	Intimal thickening (narrowing of lumen)	Gas-induced osmotic shift of fluids
	Thrombosis	
	Vasoactive substances	

mitted to dive.¹⁶ Caution regarding exposure of obese individuals to dysbaric conditions may also be advisable until more information from clinical observations becomes available.

ETIOLOGIC AND PATHOGENETIC FACTORS

In order to somewhat simplify our review of the numerous and diverse etiologic and pathogenetic concepts that have been advanced in reference to the development of dysbaric osteonecrosis we arbitrarily listed the most important of the various implicated alterations under the headings of ischemic (embolic and nonembolic) and nonischemic changes (Table 1). Furthermore we attempted to present a comprehensive sequence of events that could lead to bone necrosis by charting the suggested alterations in distinct pathways and indicating interactions and overlapping chain reactions (Fig. 1).

ISCHEMIC CHANGES

Most authors agree that dysbaric osteonecrosis represents an ischemic lesion. The controversy begins when the mechanism of ischemia is considered. Is it embolization or nonembolic changes? What is the nature and origin of the embolic material?

Embolization. Intravascular gas bubbles, lipid particles, and thrombotic material can all conceivably produce embolization. Bone necrosis has been produced experimentally by embolic obstruction of blood vessels using lipid particles, glass beads and other artificial emboli.^{17, 27, 28}

Many investigators consider embolization by gas bubbles a major factor in dysbaric osteonecrosis. It is an established fact that gas bubbles are present in the vascular system despite the use of standard decompression profiles. Furthermore intravascular bubbles have been detected even after asymptomatic decompression (silent bubbles).² The presence of gas bubbles has been detected by Doppler ultrasonic monitors and other devices.^{34, 47} Intravascular gas bubbles have also been observed by light and electron microscopy of various organs and tissues including bone.⁴⁹ Some authors question whether round empty spaces always represent gas bubbles. To a certain extent their doubts are justified even though there are several criteria which help identify gas bubbles (negative fat stains, deformity and evidence of pressure in structures surrounding the empty space, etc.).

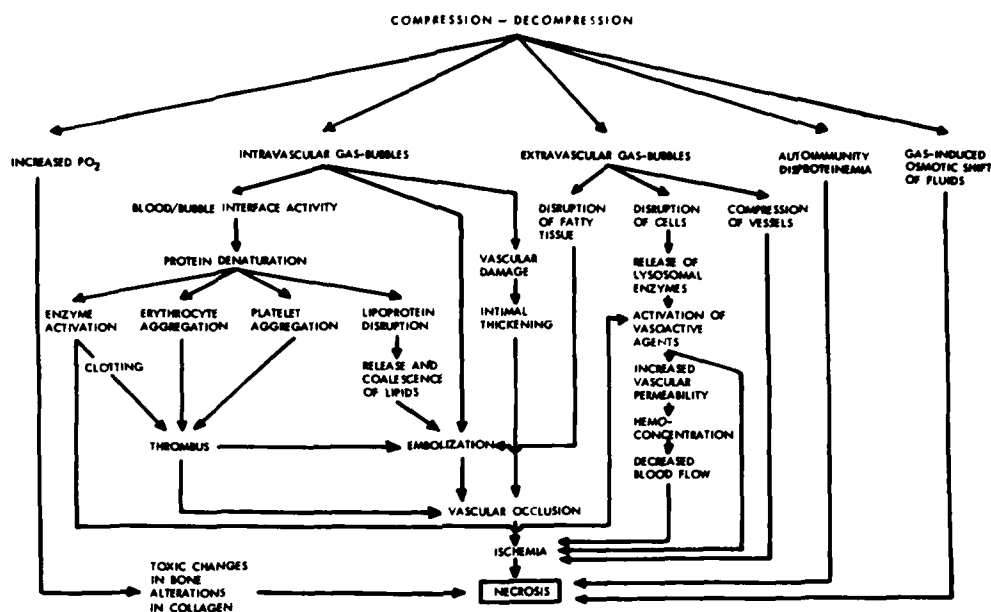


FIG. 1. Proposed etiologic factors and suggested pathogenetic mechanisms in dysbaric osteonecrosis.

In general it seems that there is little doubt that intravascular gas bubbles are formed not only in cases of inadequate decompression but also following standard "safe" procedures. What is questionable, however, is not so much the presence of gas bubbles in blood vessels but rather the ability of those bubbles to cause sufficient ischemia to produce bone lesions. Arterial air embolism in the hind legs of dogs and exposure of animals to dysbaric conditions that generated intravascular gas bubbles in bones failed to produce osteonecrosis.^{15, 21, 28} It has been suggested that vascular obstruction by nitrogen gas bubbles is only temporary, until gaseous emboli either pass through the capillary bed or are dissolved in the blood.²⁷ This, of course, depends, among other factors, on the size of the bubble and the relative nitrogen saturation of tissue and blood. It was mentioned earlier that fatty marrow is a "slow" tissue which can remain supersaturated for long

periods of time following decompression. This could sustain obstructing gas bubbles for sufficient time to produce ischemic changes.

Whether or not intravascular gas bubbles will produce detectable ischemic changes is determined by several factors: type of tissue, duration of obstruction, size of the affected area, vascularization of the affected area, extent of repair, *etc.* It is reasonable to expect that fatty marrow with its long half-time and inadequate collateral circulation would favor production of detectable ischemic changes by gas bubbles particularly if sustained or repeated insults increase the size of the affected area and interfere with healing processes.

Inadequate vascularization of certain areas in bones may be an important predisposing factor. End-artries which favor embolic occlusion¹⁹ are present under open epiphyseal plates and articular cartilages, which

probably explains the frequent occurrence of metaphyseal and subchondral bone necrosis.²⁷ Poor arterial supply of the femoral head in rabbits has been cited as the reason for the ease by which ischemic lesions were produced by injection of embolic particles.¹⁷

Vascular obstruction by fat emboli is another controversial question. Some authors consider fat embolization an important etiologic factor;²⁻²⁷ others have voiced the opposite view.¹³ Jones, *et al.*, succeeded in producing intraosseous fat embolization²⁸ and osteonecrosis in the femoral heads of rabbits by a single intusion of lipid material into the distal aorta.²⁷ If indeed fat emboli are involved in the pathogenesis of dysbaric osteonecrosis, we are still left with the unsettled question of their origin. Figure 1 indicates 2 sources of embolic fat particles: fatty tissue disrupted by expanding gas bubbles and lipid derived from lipoproteins. The presence of gas bubbles in the fatty bone marrow and in adipose tissue following rapid decompression has been demonstrated by several investigators.^{11, 29-31} Since adipose tissue and bone marrow are "slow" tissues, supersaturation of dissolved nitrogen can occur even at gas-tension levels which produce no symptoms of decompression sickness. It is conceivable, therefore, that even under conditions of "safe" decompression, gas bubbles may be generated in these tissues. These bubbles could cause disruption of fat cells and introduction of potentially embolic lipid material into the circulation. Detection of marrow fragments in pulmonary vessels tends to support this hypothesis.²⁷ Additional support derives from the observation that obese animals with excessive amounts of adipose tissue and fatty marrow are significantly more susceptible to dysbaric osteonecrosis than their thin littermates.¹⁰ Pauley and Cockett suggested that expanding gas bubbles may cause liver injury resulting in alteration of the stability of lipids with subsequent extrusion of unstable fats and for-

mation of emboli.³² Embolic lipid particles can also be formed by coalescence and adhesion of plasma lipids to the blood-gas interface.³³ Another possible sequence of events involves denaturation of plasma lipoproteins by blood-bubble interface activity, release of the lipid moiety, coalescence of the liberated lipid and formation of embolic particles (Fig. 1). This concept of lipid emboli formation following gas-induced disruption of lipoprotein linkages is supported by interesting observations related to extracorporeal kidney perfusion. Such a procedure utilizing a pulsatile pump and membrane oxygenator resulted in injury of the isolated kidney from lipid embolization. However, preliminary denaturation of plasma protein to remove lipid, prevented embolic injury to the kidney.³⁴

In vitro experiments currently conducted in our laboratories further support the hypothesis of bubble-induced lipid alterations. In these experiments, pooled human plasma or serum is bubbled with air at a rate of 5-10 ml minute for 30-60 minutes at 37°. A control sample from the same pooled material is subjected to identical conditions except for the introduction of air bubbles into the sample. The experimental and control samples are then passed through microporous filters of varying pore size. After filtration is completed the filters are dried and stained with reagents for lipids. In addition chemical determinations of various lipids are performed in the samples before and after filtration. Preliminary experiments suggest that bubbling of serum with air induces the formation of lipid particles which are retained by a 1.0 μ m and, to a lesser extent, by a 5.0 μ m, pore size filter.¹¹

Ischemic bone changes due to embolization by thrombotic material is another possibility that merits consideration. Figure 1 presents some of the pathways that may lead to thromboembolism. There are several changes in dysbaric disorders which can trigger the

coagulation mechanism or provide conditions favoring thrombus production. The initiating event is again the gas bubble. The blood-bubble interface may interact with proteins, lipids and formed elements of the blood resulting in adhesion and aggregation of thrombocytes, clumping of erythrocytes and coalescence and adhesion of plasma lipids.^{9, 39} Clumping of red blood cells was considered a secondary complicating factor in decompression sickness as early as 1938.¹⁸ The reported denaturation of proteins as a result of gas-liquid interface activity³³ led to investigations suggesting that gas bubbles activate the Hageman factor,^{9, 14} thus triggering the coagulation mechanism. Disseminated intravascular coagulation associated with a fall in the circulating thrombocyte count has been thought to play an important role in decompression sickness.⁴⁰ In addition to the above alterations, decompression-induced hemoconcentration and sluggishness of the blood (Fig. 1) could also favor coagulation. Once fibrin and platelet thrombi are formed, it is not an unusual complication for this thrombotic material to detach and form emboli.

Most of the above mentioned hematologic alterations are bubble-induced. Therefore, they could also be implicated in the pathogenesis of dysbaric osteonecrosis even in the absence of a history of decompression sickness, since asymptomatic (silent) bubbles can also initiate such blood changes.⁴⁵ Failure, however, to prevent development of bone lesions by anticoagulants and platelet function inhibitors^{45, 50} casts some doubts on thromboembolism as a pathogenetic factor in osteonecrosis.

Nonembolic ischemic changes. The various nonembolic alterations that could cause ischemia are listed in Table 1. Extravascular gas bubbles released during decompression from the supersaturated fatty elements of the bone marrow could compress blood vessels and impair tissue perfusion. As mentioned

earlier, it is conceivable that within the rigid confines of the osseous tissue gas bubbles may build up sufficient pressure to reduce blood flow in intraosseous vessels or even cause complete obstruction. The presence of thrombotic or embolic material, as well as pre-existing stenotic intimal changes, could, in combination with extravascular pressure, produce more severe vascular occlusion.

Obstruction of intraosseous blood vessels by autochthonous gas bubbles and thrombi needs no further elaboration since specific conditions regarding bubble formation in bones and mechanisms of decompression-induced clotting and platelet aggregation have already been dealt with.

Ischemic changes can also be caused by stenotic vascular changes. Light and electron microscopic studies on long bones in miniature swines revealed that intravascular gas bubbles cause arterial injury. Intimal thickening and myointimal cell proliferation follows such injury with consequent narrowing of the vessel lumen.^{48, 49} These changes predispose to thrombosis which could aggravate the circulatory impairment.

Since it has been reported that vasoactive substances are released or activated as a result of gas-blood interactions,^{9, 12, 14} the possibility of vasomotor changes playing at least a contributory role in ischemic changes should be borne in mind. Supporting this view is the observation that intraarterial administration of vasodilator substances causes a redistribution of blood flow in the limb by increasing the flow through the muscles at the expense of that through the bone marrow.⁴² This decrease of blood flow could also intensify the effects of extravascular compression or of vascular obstruction by thrombotic and embolic material.

NONISCHEMIC CHANGES

All of the previously discussed hypotheses concern ischemic changes, with the gas bubble as the protagonist causing directly or in-

directly circulatory impairment. Alternatively, there is, however, the possibility that factors other than, or in addition to, ischemia and gas bubble related events may play significant pathogenetic roles. In this regard a number of interesting concepts deserve attention.

Gas-induced osmotic shift of fluids. It has been suggested that gas-concentration gradients resulting from rapid pressure changes can produce osmotic changes and fluid shifts that could contribute to the production of bone lesions.^{2,3} Consequently, the rate of compression becomes a factor that may influence the development of bone changes. Supporting this hypothesis is our previously mentioned finding that, with rapid compression, the incidence of dysbaric osteonecrosis is higher than with stage compression.¹⁰ In this connection it has also been reported that the severity and frequency of hyperbaric arthralgia are reduced in divers subjected to slow compression rates; gas-induced osmosis was implicated in this phenomenon as well.⁸

Increased PO_2 . Toxic effects from increased oxygen tension in hyperbaric environments have also been implicated in the pathogenesis of bone lesions. Long exposures of divers and compressed air workers to hyperoxia may cause collagen modification as it has been observed in *in vitro* experiments.¹⁰ Such modification of bone collagen, including loss of its ability to tolerate twisting and extension, could result in osteonecrosis.

Autoimmunity. Another speculation on the pathogenesis of dysbaric osteonecrosis implicates altered immunity and dysproteinemia. This hypothesis of an autoimmune process is based on the latency of bone lesions and on the observation of rouleaux formation, amyloidosis and long delayed membranous glomerulitis in some animals with dysbaric osteonecrosis.³

FACTORS AFFECTING THE SUSCEPTIBILITY OF BONE TO DYSBARIC NECROSIS

The various pathogenetic mechanisms of dysbaric osteonecrosis which have been reviewed implicate many reactions which are systemic. One wonders then, why is bone the only site that manifests the effects of widespread alterations? Why does only bone suffer from hemostatic and rheologic changes induced by compression-decompression? How can we explain selective infarction of bone when gas bubbles and thrombi could be formed in any vessel, and lipid particles and other embolic material, once they pass into the systemic circulation, could obstruct vessels in virtually any organ.

"SLOW" TISSUE

To begin with, not all of the changes which may contribute to bone lesions are systemic. It was mentioned earlier that gas exchange in the fatty marrow proceeds at different rates than in other tissues and that decompression could cause great supersaturation of this "slow" tissue. Because of this and of the low tolerance of bone for inert gas supersaturation, injury can be afflicted upon this tissue by decompression profiles that do not affect other tissues and organs. Inert gas supersaturation in bone may persist for long periods of time, resulting in generation and growth of gas bubbles capable of compressing or occluding intraosseous blood vessels.

RIGID TISSUE

It is questionable that extravascular bubbles in elastic and compressible tissues can build sufficient pressure to cause circulatory impairment. In the rigid compartments of the bone, however, evolving extravascular gas bubbles might compress intraosseous vessels to a degree that could cause obstruction.

POOR VASCULARIZATION

Certain segments of the long bones may be more vulnerable to ischemic changes because of relatively poor vascularization. In previous paragraphs, it was mentioned that the arteries supplying subchondral and metaphyseal areas are thought to be terminal. Such end-arteries favor production of embolic injury.

DECREASED BLOOD FLOW

Redistribution of blood flow in the extremities in favor of muscles, as a result of the action of vasoactive substances, may produce a critical reduction in bone perfusion, particularly in the presence of other circulatory impairments. It has been suggested that vasoactive substances are released or activated as a result of blood-bubble interactions or through other mechanisms.^{9, 11, 12, 14}

URANIUM 238 AND MICRONUCLEI

Since gas bubbles appear to be directly or indirectly involved in the pathophysiologic alterations that lead to bone necrosis, conditions which favor bubble formation in the bones could play a primary role in the peculiar bone susceptibility. We have already suggested that the long half-time of the fatty marrow is such a condition. Another intriguing hypothesis^{5,2} deals with the role of uranium 238, an isotope present in the body. Formation of gas bubbles requires micronuclei. The energy decay of U_{238} may result in the formation of micronuclei which could cause precipitation of gas bubbles. The interesting aspect of this hypothesis is that this isotope appears to be concentrated principally at the ends of the long bones which are the usual sites of dysbaric osteonecrosis.

CONCLUSIONS

Dysbaric osteonecrosis appears to be independent of decompression sickness but the

2 conditions may share etiologic and pathogenetic factors. The incidence of bone necrosis is influenced by the magnitude of pressure, number of dysbaric exposures, decompression profile, and possibly by the rate of compression. Obesity is also suggested as a predisposing factor.

The etiology and pathogenesis of dysbaric osteonecrosis are still unclear. Most authors agree that the lesion is ischemic. The gas bubble plays a primary role causing ischemia either by direct vascular obstruction or by initiating a sequence of events that lead to circulatory impairment. These events that can also be triggered by asymptomatic ("silent") bubbles include thrombosis, platelet aggregation, embolization by bubbles, lipid particles and other embolic material, rheologic changes, redistribution of blood flow and injury of vessels with consequent arterial narrowing. It is conceivable that several of these factors act in concert or in sequence. Nonischemic mechanisms, such as gas-induced osmotic shift of fluids, hyperoxic injury, etc., also deserve attention though they are highly speculative.

The peculiar susceptibility of the bone to necrotic lesions may be related to a number of conditions. The fatty bone marrow is a "slow" tissue which may remain supersaturated and cause formation of injurious gas bubbles, following decompression profiles that leave other tissues unaffected. Gas bubbles have more devastating effects in the rigid confines of the bone than in soft tissues. Uranium 238, which is concentrated in bones, may be responsible for the formation of micronuclei which promote formation of gas bubbles. End-arteries in certain parts of the bone may contribute to the vulnerability of the tissue to the effects of vascular obstruction.

There is little doubt that most of the above statements represent only tentative

conclusions. Many of the etiologic and pathogenetic concepts presented are speculative and controversial. There are several promising possibilities which are inadequately explored and until further research yields conclusive evidence, the causes and pathogenesis of dysbaric osteonecrosis will remain a challenging enigma.

SUMMARY

Dysbaric osteonecrosis appears to be independent of decompression sickness. The 2 conditions, however, may share etiologic and pathogenetic factors. The incidence of osteonecrosis is influenced by the number of hyperbaric exposures, extent of pressure, decompression profile and possibly by the rate of compression and degree of obesity. Though etiology and pathogenesis are unclear, osteonecrosis is probably due to ischemia, with gas bubbles causing direct or indirect circulatory impairment. *In vitro* experiments, as well as human and animal studies, suggest multiple pathogenetic mechanisms: intraosseous vessel compression by extravascular bubbles; vessel obstruction by bubbles, fibrin thrombi, platelet aggregates, clumped erythrocytes or coalesced lipids; and narrowing of arterial lumina by bubble-induced myointimal thickening. Obstructing materials, whether autochthonous or embolic, may result from blood-bubble interface reactions. Rheologic changes and blood flow redistribution could play contributing roles. It seems likely that multiple pathogenetic factors act in concert or sequentially. Proposed nonischemic changes, such as hyperoxic injury, gas-induced osmosis, or autoimmunity, lack sufficient supporting evidence. The peculiar vulnerability of bone may be related to gas supersaturation of the fatty marrow; sensitivity to extravascular gas pressure because of tissue rigidity; poor vascularization; and the presence of uranium 238 which promotes nucleation and subsequent gas bubble formation.

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Dysbaric osteonecrosis in mice

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Chryssanthou, C. P. 1976. Dysbaric osteonecrosis in mice. *Undersea Biomed. Res.* 3(2): 67-83. The histopathology of dysbaric osteonecrosis and the influence of the number of exposures, compression rate, and obesity on the incidence and latency of the lesion were studied in 438 mice (2505 bones were examined). The animals were subjected to 75 psig air pressure for 2-6 hours (single or multiple exposures). Compression was rapid or stage. Decompression was *salt*. Osteonecrosis developed in the epiphysis of the tibia and/or femur in 34.1% of obese and in 5.8% of thin animals after a latent period of 2 to at least 12 months. It was concluded that: 1. dysbaric osteonecrosis appears to be independent of decompression sickness; 2. in obese mice the incidence is higher and the latent period shorter; 3. multiple exposures result in higher incidence and earlier lesions than single exposure; 4. the incidence is lower with stage than with rapid compression; 5. the pathogenesis of osteonecrosis may involve several factors (circulatory impairment by extravascular or intravascular bubbles, emboli, thrombi, vasoactive substances, gas-induced osmosis, autoimmunity) acting in concert or in sequence.

dysbarism	ischemia	obesity
bone	osmosis	compression rate
bubbles	fat embolism	repetitive diving
histopathology	susceptibility	pathogenesis
	aseptic bone necrosis	

Dysbaric osteonecrosis has recently been recognized as a major hazard in individuals subjected to large changes in ambient pressure. The latest statistics leave little doubt that this potentially disabling disorder is alarmingly widespread in divers and compressed-air workers. The incidence of the disease determined in relatively extensive surveys ranges from 4% in Royal Navy divers (Elliott and Harrison 1970) to 50-60% in Japanese diving fishermen (Kawashima, Torisu, Hayashi, and Kamo 1973; Ohta and Matsunaga 1974). This wide variation in the incidence can be attributed to the difference in the conditions of dysbaric exposure. Certain factors such as degree of pressure, duration of exposure, rate of decompression, and frequency of exposure are known to influence the incidence of the lesion. The effect of other factors, including rate of compression and obesity, remains to be assessed. In addition it remains to be determined whether the latent bone lesions are associated with acute manifestations of decompression sickness. Such uncertainties in our understanding of dysbaric osteonecrosis reflect our ignorance of the etiology and pathogenesis of the disorder.

An animal model for dysbaric osteonecrosis could provide some answers to these important questions. It would enable one to evaluate the influence of various factors on the incidence, severity, and latency of the lesion under controlled experimental conditions. Furthermore, an animal model could be used for studies on the etiology and pathogenesis as well as on the prevention and treatment of the disease.

Dysbaric osteonecrosis has been experimentally produced in several animal species including mice (Antopol, Kalberer, Kooperstein, and Chryssanthou 1964), rabbits (Horvath and Vizkelety 1973), and miniature swine (Smith and Stegall 1974). The mouse as a model for such studies permits large scale experiments to be conducted to provide statistical validation. In addition, large numbers of animals may be subjected simultaneously to compression/decompression in the same chamber, thus ensuring exposure to identical environmental conditions.

This report deals with studies on the histopathology of dysbaric osteonecrosis in mice and on the influence of obesity, number of exposures, and rate of compression on the incidence and latency of the lesion.

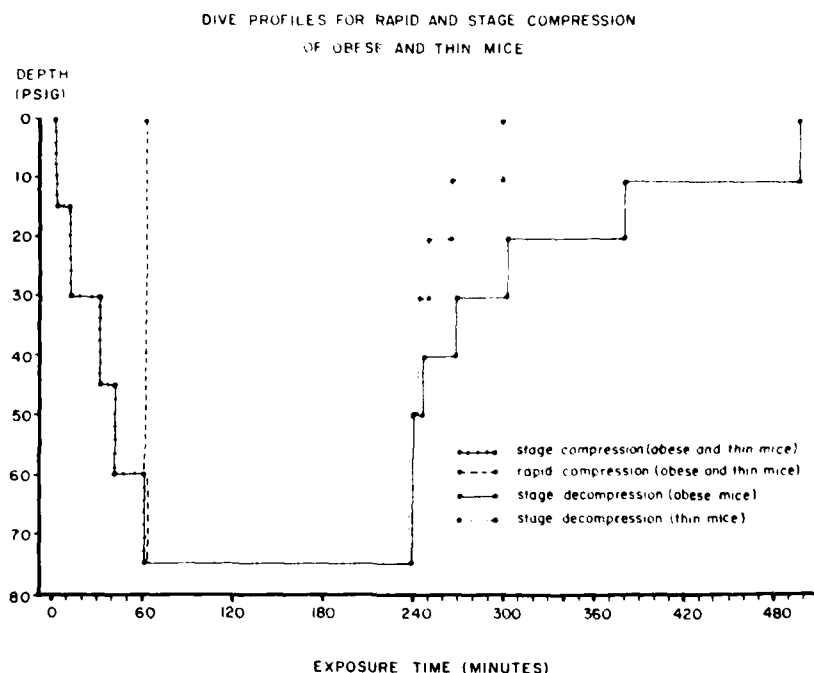


Fig. 1. Compression/decompression schedules for thin and obese mice.

MATERIAL AND METHODS

A total of 580 male hereditarily obese hyperglycemic mice and their thin siblings were used. They were obtained from Jackson Memorial Laboratories, Bar Harbor, Maine. There were two weight ranges of obese animals: 38-60 gm (average 54 gm) and 61-90 gm (average 78 gm). Thin mice weighed 18-35 gm (average 24 gm). All animals were housed in metal cages in animal rooms with controlled temperature ($72 \pm 2^\circ \text{F}$) and relative humidity (50%) and were fed Wayne Lab-Blox and water ad libitum. They were kept under these conditions for a stabilization period of at least 3 weeks before they were used.

The animals in each weight range were randomly divided into an experimental group (subjected to dysbaric conditions) and a control group (kept at ambient pressure). Prior to the initiation of the experiments both control and experimental animals were numbered and their corresponding weights recorded. The experimental animals were subjected to 75 psig (6.12 ATA) air pressure for 2-6 hours in a hyperbaric chamber (Bethlehem Corporation Model 1836 HP) with controlled temperature ($72 \pm 2^\circ \text{F}$) and relative humidity (50%). Bottom time for thin mice was longer (4-6 hours) than for obese mice (2-3 hours). Figure 1 shows the dive profiles employed. Compression for both obese and thin mice was either rapid (to 40 psig in 30 s, to 75 psig in 60 s) or stage. Stage compression involved stops at 15, 30, 45, and 60 psig for 10, 20, 10, and 20 min respectively. Decompression was always stage with stops at 50, 30, 20, and 10 psig for 2, 5, 15, and 30 min respectively for thin mice and at 50, 40, 30, 20, and 10 psig for 5, 25, 35, 75, and 120 min respectively for obese mice. The animals were subjected to these conditions once (single exposure) or 3-8 times (multiple exposures) at weekly intervals. Upon reaching surface the animals were immediately removed from the chamber and observed for signs of decompression sickness (e.g. chokes, scratching, twitch) for at least 1 hour.

The animals died or were sacrificed at intervals up to 17 mo after initiation of dysbaric exposure. The bones of the extremities and, in some animals, the sternum were removed and fixed in 10% neutral buffered formalin for at least 3 days. Prior to fixation the soft tissue surrounding the bone was carefully trimmed off. Following fixation the bones were decalcified (Omega Decal solution) for a minimum of 24 hours and stored in 80% alcohol until processing by autotechnicon. The latter process consisted of sequentially treating the specimen for 1 hour with each 10%, 95%, 95%, 95%, 95%, and 100% alcohol, chloroform, paraffin (paraplast), and paraffin. The specimens were then embedded, cut (5-6 micron section), and stained with hematoxylin-eosin.

Bones of animals that died within 24 hours were examined to observe acute histologic changes but were not included in the statistics of dysbaric osteonecrosis. Also excluded from the studies were animals that were autolyzed or cannibalized after death as well as those in which processing of tissue was unsatisfactory. Because of these eliminations the statistical data of this report are based on 438 animals and a total of 2505 examined bones (Table 1).

In the statistical analysis of the results, the chi square test with Yates correction was used for comparison of distribution frequencies and the Pearson's correlation coefficient for testing dependence of two variables.

RESULTS

With the dive profiles used in these studies, thin mice did not exhibit apparent clinical manifestations of decompression sickness except for an occasional animal. In obese mice signs of decompression sickness were observed in about 7% of the animals. Mice which developed decompression sickness usually died within 24 hours after decompression.

TABLE I
Number of bones histological examined

	Compressed/Decompressed		Controls		Total
	Obese	Thin	Obese	Thin	
Femur	316	378	72	110	876
Tibia	316	378	72	110	876
Humerus	191	237	4	34	466
Sternum	51	156	36	44	287
TOTAL	874	1149	184	298	2505

HISTOLOGIC FINDINGS

Histologic examination of the bones of animals that died within 48 hours after decompression revealed pronounced hyperemia of the bone marrow with occasional hemorrhagic foci (Fig. 2). In addition, gas bubbles were present in the diaphysis and epiphysis of the bones, particularly in obese mice. The bubbles appeared as round or oval clear spaces of varying size, equaling in a few instances the diameter of the medullary canal. Often they were irregularly shaped, their smooth outline distorted by bony trabeculae protruding into the bubble (Fig. 3).

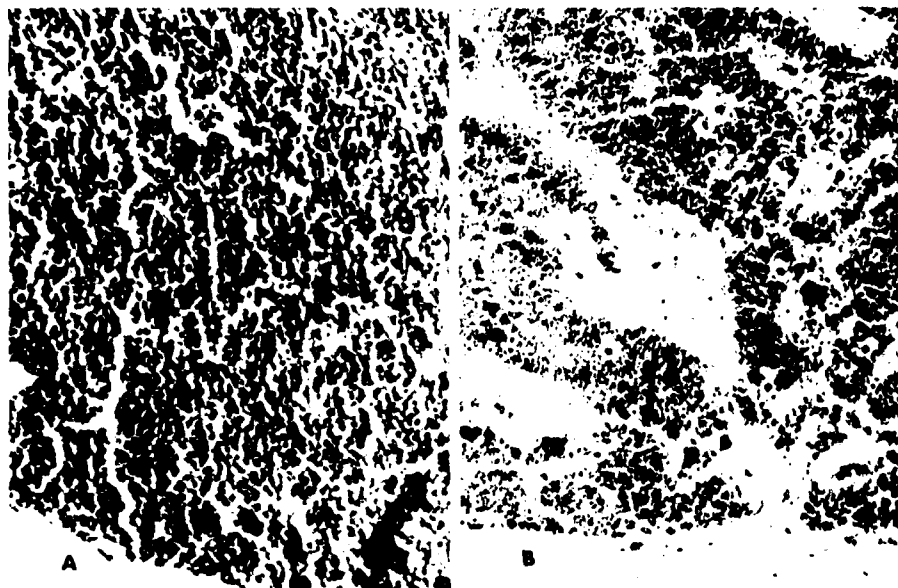


Fig. 2. Tibial bone marrow of a thin mouse. A, Control (not compressed/decompressed). B, 48 hours after exposure to compression/decompression. Note the marked congestion and hemorrhagic foci. (Hematoxylin-eosin, original magnification 25.2x.)

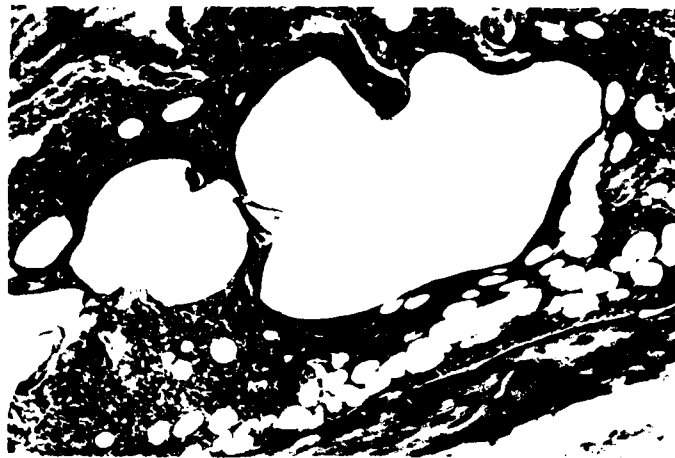


Fig. 3. Femoral diaphysis of an obese mouse 40 min after the last of six exposures to compression/decompression. The large empty spaces are gas bubbles, their spherical or oval shape being distorted by bony trabeculae. (Hematoxylin-eosin, original magnification 25.2x.)

Histologic evidence of osteonecrosis appeared after a period of at least 2 mo following the initial dysbaric exposure. The necrotic lesion always involved the spongy tissue of a part or of the entire epiphysis. In early stages of the lesion, the osteocytes in epiphyseal trabeculae exhibited pyknosis and karyorrhexis and the marrow cells showed indistinct cellular boundaries and loss of nuclear staining.

In more advanced lesions the lacunae in the necrotic trabeculae were devoid of osteocytes and the intertrabecular marrow spaces contained amorphous masses of granular debris (Fig. 4) and sometimes fragments of necrotic bone. Haversian canals, whenever they could be observed in necrotic areas, appeared empty or contained debris from disintegrated tissue. Several microcracks (fissures) were seen between lamellae, usually extending to the surface of the trabeculae (Fig. 5) and, in some cases, resulting in tissue fragmentation. A few microscopic fissures were also seen in control animals with no evidence of osteonecrotic lesion, thus raising the possibility of artifacts or of alterations unrelated to dysbaric exposure and making the significance of this finding questionable.

In some cases necrotic epiphyseal trabeculae appeared fractured and occasionally collapse of the articular surface was observed. In other cases there was erosion of the articular cartilage and of the subjacent bone of the epiphysis with formation of concave defects, sometimes associated with epiphyseal collapse (Fig. 6). These alterations of the articular surface resembled osteoarthritic changes as reported by Sokoloff (1956).

At later stages fibrovascular tissue invaded intertrabecular spaces and replaced necrotic marrow (Figs. 7 and 8). Sometimes vascular connective tissue showing evidence of osteoclastic activity could be seen surrounding partially resorbed bone fragments (Fig. 7). Appositional new bone formation was observed in only few cases. Deposition of new bone on preexisting necrotic trabeculae resulted in thickening of these structures. Figure 8 shows new bone formation in apposition to necrotic bone tissue from which it is sharply demarcated. Figure 8 also shows areas of hematopoietic and adipose tissue elements within the vascular connective tissue, suggesting reconstitution of the necrotic marrow.

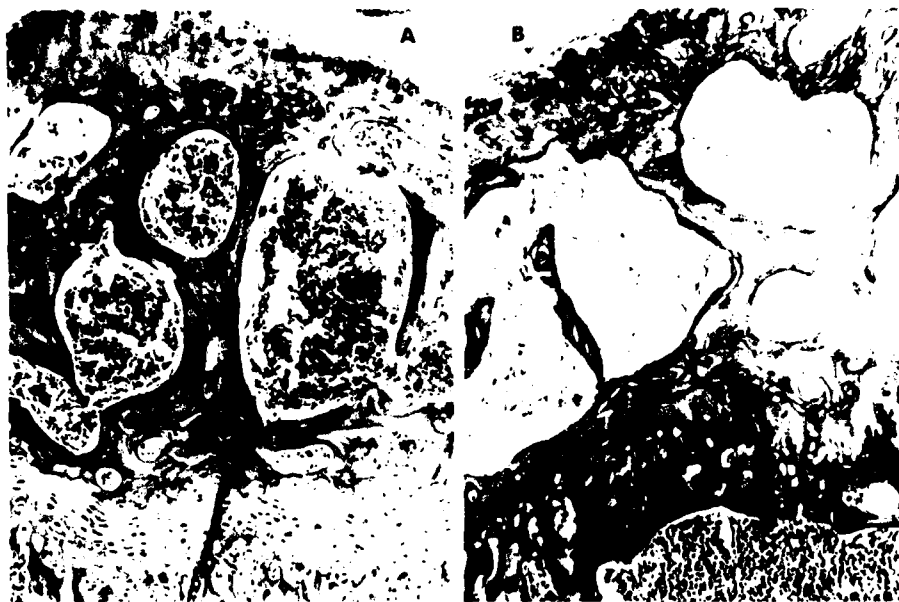


Fig. 4. Epiphysis of the proximal end of the tibia of a thin mouse. A. Control (not compressed/decompressed). B. 5 mo after multiple exposures to compression (rapid)/decompression. Note the necrotic trabeculae with empty lacunae and microcracks and the intervening marrow spaces containing granular debris. Normal bone marrow can be seen in the lower portion of the microphotograph. (Hematoxylin-eosin, original magnification 25.2X.)



Fig. 5. Epiphysis of the proximal end of the tibia of an obese mouse 4 mo after multiple exposures to rapid compression/decompression. Necrotic trabeculae exhibit lacunae devoid of osteocytes and multiple microcracks some of which extend to the trabecular surface. The intertrabecular spaces in the necrotic area contain amorphous masses of granular debris. (Hematoxylin-eosin, original magnification 25.2X.)

Fig. 6. Knee joint of an obese mouse 4 mo after repeated exposures to compression (rapid)/decompression. The epiphysis is collapsed. The articular cartilage and the subjacent epiphyseal bone are eroded with formation of a concave defect. (Hematoxylin-eosin, original magnification 25.2X.)



Fig. 7. Area in the epiphysis of the proximal end of the tibia of an obese mouse 11 mo after hyperbaric exposure. Note fibrous connective tissue surrounding fragments of partially resorbed bone. A few multinucleated osteoclasts can be seen around the necrotic bone. (Hematoxylin-eosin, original magnification 64X.)

INCIDENCE AND LATENT PERIOD

The incidence of dysbaric osteonecrosis was 34.1% in obese and 5.8% in thin animals (Table 2). The lesion was also seen in 4 of 46 control obese mice but in none of 45 control thin animals. Definite osteonecrotic changes became histologically evident after a period of at least 2 mo following the initial exposure to compression/decompression. Table 3 shows that, in a period up to 4 mo after exposure, the incidence was 12.9% in obese and 0% in thin



Fig. 8. Area in the epiphysis of the proximal end of the tibia of an obese mouse 10 mo after hyperbaric exposure. Necrotic trabeculae and subchondral bone with empty lacunae are evident. New appositional bone with lacunae containing viable osteocytes is sharply demarcated from the adjacent necrotic tissue (left). The vascular connective tissue which has replaced the necrotic marrow contains areas with hemopoietic and adipose tissue elements. (Hematoxylin-eosin, original magnification 64 $\times$.)

mice. In animals that died or were sacrificed 4 mo after exposure or later, however, the incidence increased to 47.4% in obese and to 7.4% in thin mice. In fact there was a significant correlation between incidence and time period following exposure. Figure 9 shows this correlation in obese mice subjected to a single dysbaric exposure. These results indicate that the post exposure latent period varies—ranging from 2-3 mo to at least 9 mo inasmuch as the incidence of dysbaric osteonecrosis was higher in the 9-12 mo than in the 6-9 mo period.

DISTRIBUTION

In most cases dysbaric osteonecrosis in mice was observed in the epiphysis of the proximal end of the tibia bilaterally (38%) or unilaterally (62%). The femur was involved in some cases, with the lesion usually localized in the distal end. Osteonecrotic changes in the head of the femur were seen only occasionally. Table 4 shows the distribution of the lesion in the various bones.

INFLUENCE OF OBESITY

There was a striking difference in the incidence of osteonecrosis between obese mice and their thin littermates (Table 5). Obesity also influenced the latent period; osteonecrotic changes appeared earlier in the heavier mice. In animals with an average weight of 78 gm,

TABLE 2
Incidence of dysbaric osteonecrosis in mice

	Compressed/ Decompressed	Controls	Probability
Obese	34.1%(54/158)*	8.7%(4/46)	.001<P<.01
Thin	5.8%(11/189)	0% (0/45)	N.S.†

*(Number of animals with lesions/total number of animals)

† Not significant

TABLE 3
Incidence of dysbaric osteonecrosis in mice in the
early and late periods following hyperbaric exposure

Type	0 - 4 Mo	4 Mo or more	Probability
Obese	12.9%(8/62)*	47.9%(46/96)	$P<.001$
Thin	0%(0/40)	7.4%(11/149)	.1<P<.2

*(Number of animals with lesions/total number of animals)

TABLE 4
Distribution of osteonecrotic lesions

Tibia (<i>Prox. end</i>)	80.8%	(80/99)*
Femur (<i>Total</i>)	15.1%	(15/99)
Femur (<i>Head</i>)	3.0%	(3/99)
Femur (<i>Dist. end</i>)	12.1%	(12/99)
Sternum	2.0%	(2/99)
Humerus (<i>Dist. end</i>)	1.0%	(1/99)
Ilium	1.0%	(1/99)

*(Number of specified bones with lesions/total number of bones with lesions)

20.5% of the lesions appeared within the first 3 mo after exposure (Table 6). None of the mice weighing 54 gm or less developed osteonecrosis during this period.

INFLUENCE OF THE NUMBER OF EXPOSURES

Obese animals subjected to multiple dysbaric exposures had a significantly higher incidence of dysbaric osteonecrosis than animals exposed only one time. In thin mice the number of lesions was too small to reflect the possible effect of the number of exposures on the incidence of osteonecrosis. The number of exposures also influenced the latent period. In mice subjected to multiple exposures (rapid compression), lesion incidence within 3 mo following initial exposure was 25%. In contrast, none of the animals with a single exposure (rapid compression) developed osteonecrosis within the same period.

INFLUENCE OF THE RATE OF COMPRESSION

The incidence of dysbaric osteonecrosis in mice subjected to stage compression, was significantly lower than in animals exposed to rapid compression. Table 8 shows the respective frequencies of lesions in obese and thin mice subjected to multiple exposures with rapid or stage compression.

INFLUENCE OF VARIOUS FACTORS IN COMBINATION

In this investigation obesity, rapid compression, and multiple exposures produced a statistically significant increase in the incidence of dysbaric osteonecrosis. The combined effect of two of these factors was greater than that produced by each of them individually and a combination of three factors influenced the incidence of the lesion to a greater degree than any combination of two factors. The effect of various combinations is compared in Fig. 10. Incidence of osteonecrosis was lowest (0%) in thin mice subjected to stage compression and highest in obese mice subjected to rapid compression and multiple exposures.

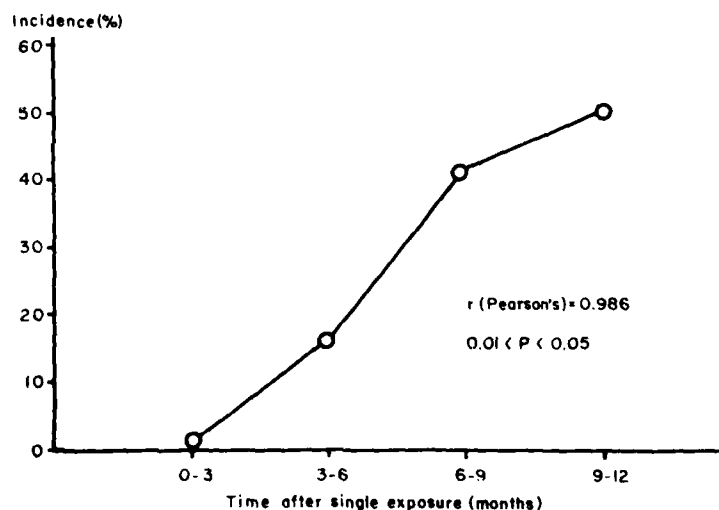


Fig. 9. Correlation of the incidence of dysbaric osteonecrosis with post-exposure interval in obese mice subjected to a single compression (rapid)/decompression.

TABLE 5
Influence of obesity on the incidence
of dysbaric osteonecrosis in mice

Type	Average weight (g)	Incidence	Probability
Thin	24	5.8%(11/189)*	P<.001
Obese	54	26.3%(15/57)	
Obese	78	38.6%(39/101)	

*(Number of animals with lesions/total number of animals)

TABLE 6
Influence of obesity on the latency
of dysbaric osteonecrosis

Type	Average weight (g)	Percent of animals with lesions		Probability
		0-3 mo	>3 mo	
Thin	24	0(0/11)*	100(11/11)	.02<P<.05†
Obese	54	0(0/15)	100(15/15)	
Obese	78	20.5(8/39)	79.5(31/39)	

* (Number of animals with lesions in period/total number of animals with lesions)

† Statistical probability of difference in the latency between the 78- g group and the 24- and 54- g groups combined.

TABLE 7
Influence of the number of hyperbaric exposures on
the incidence of dysbaric osteonecrosis in mice
subjected to rapid compression

Type	Single exposure	Multiple exposures	Probability
Obese	28.3%(19/67)*	47.3%(27/57)	.02<P<.05
Thin	6.7%(6/89)	5%(5/100)	N.S.†

*(Number of animals with lesions/total number of animals)

† Not significant

TABLE 8

Influence of the rate of compression on the incidence of dysbaric osteonecrosis in mice with multiple exposures

Type	Rapid compression	Stage compression	Probability
Obese	47.3% (27/57)*	23.5% (8/34)	.025 $P < .05$
Thin	7.1% (5/70)	0% (0/30)	N.S.†

* (Number of animals with lesions/total number of animals)

† Not significant

The effect of obesity can be seen by comparing columns 1, 2, and 3 with 4, 5, and 6 respectively. Comparison of columns 4 and 6 demonstrates the effect of compression rate; of columns 5 and 6, the effect of the number of exposures.

DISCUSSION

The results presented indicate that dysbaric osteonecrosis can be experimentally produced in mice. Diagnosis was made conservatively. Morphologic changes in osteocytes or bone marrow cells, although they may represent early manifestations of a lesion, did not suffice by themselves to make a diagnosis of osteonecrosis. Nor did the absence of osteocytes in lacunae in focal areas constitute adequate evidence of bone necrosis unless it was associated with other alterations (e.g. necrosis of bone marrow, invasion by fibrovascular tissue, appositional new bone formation, etc.). It is therefore possible that the incidence of dysbaric osteonecrosis has been underestimated because some lesions may have been missed either in their early phases before they were fully manifested or at late stages because of almost perfect restitution of necrotic tissues; the latter, however, is not very likely since *creeping substitution* was seen only rarely. The fact that reparatory changes were infrequent is an indication that in most cases there was a severe defect in blood supply. The high incidence of lesions in the proximal end of the tibia and the distal end of the femur may be related to circulatory peculiarities and postural characteristics of the mouse.

None of the control thin mice developed bone necrosis. The occurrence of lesions in four of the control obese mice could be associated with the obesity. Obesity and hyperlipidemia have been considered predisposing factors in nondysbaric aseptic bone necrosis (Conti and Sciarli 1973; Wellfing 1973). Obese mice have a fatty liver and it has been suggested that the fatty liver is capable of spontaneously releasing embolic fat globules into the circulation (Owens and Worthington 1962).

In animals subjected to compression/decompression, obesity influenced the incidence of osteonecrosis more than any other factor studied in these experiments. It was previously reported that the degree of obesity in mice correlates with their susceptibility to

decompression sickness (Antopol et al. 1964). These correlations, however, do not imply that the delayed dysbaric osteonecrosis is associated with the acute manifestations of decompression sickness. On the contrary, the animals which developed dysbaric osteonecrosis did not exhibit signs of decompression sickness. Dysbaric osteonecrosis, therefore, appears to be independent of decompression sickness, at least in mice. This observation is consistent with the well-known fact that delayed bone lesions have been detected in divers and compressed-air workers who did not manifest acute symptoms and signs of decompression sickness.

The increased susceptibility of obese mice to dysbaric bone necrosis may be related to the high solubility of nitrogen in fat and the higher content of fat in the bone marrow of obese animals. Fatty bone marrow exchanges nitrogen slowly and decompression could cause great supersaturation of dissolved gas in this tissue with the potential of generating gas bubbles over relatively long periods of time. Gas bubbles were seen in the diaphyseal and epiphyseal marrow of obese mice, even after a period of several days following decompression.

It is tempting to apply the above considerations to a possible explanation of the fact that dysbaric osteonecrosis can be produced without preceding manifestations of decompression sickness. *Safe* decompression tables are considered adequate as long as they prevent development of decompression sickness. It is conceivable, however, that safe decompression, while keeping gas-tension levels below those required to produce acute manifestations of decompression sickness, may permit supersaturation of the long half-time fatty bone marrow with subsequent bubble formation. In addition, it is known that gas bubbles may be present

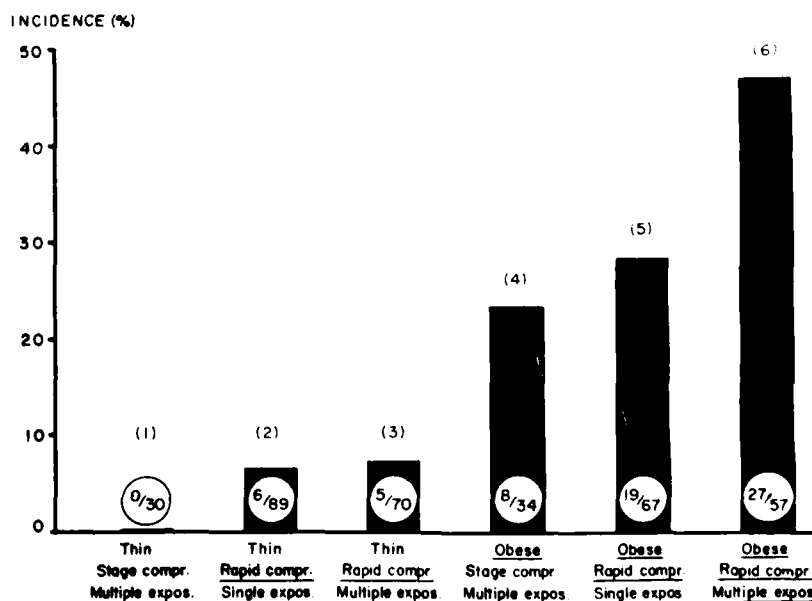


Fig. 10. Influence of obesity, rate of compression, and number of exposures in various combinations on the incidence of dysbaric osteonecrosis. Factors which were shown to increase the incidence are underlined. Comparison of the incidences in columns 1, 2, and 3 with those in columns 4, 5, and 6 shows the influence of obesity. The effect of the rate of compression is shown when columns 4 and 6 are compared and the effect of the number of exposures when columns 5 and 6 are compared.

even after routine asymptomatic decompression (Belinke 1967). These asymptomatic gas bubbles were referred to as "silent." They may be "silent" in terms of decompression sickness but not in terms of dysbaric osteonecrosis. Harvey (1974) has stated that "the low tolerance of bone for inert gas supersaturation may precipitate development of lesions (osteonecrotic) when present day decompression tables are followed." If this is so, decompression tables may require recalculation in consideration of the longer half-time tissues and the occurrence of silent bubbles.

Extravascular gas bubbles released from fatty elements of the bones could, within the rigid confines of the osseous tissue, exert sufficient pressure to compress blood vessels. Initially this would affect the veins, which are more susceptible to pressure, thus resulting in stasis. This is consistent with the hyperemia and hemorrhagic foci observed in the bone marrow of animals that died within 48 hours after decompression. These circulatory alterations could produce anoxia which in turn could precipitate necrotic bone changes. Circulatory impairment and ischemia could also be caused by arterial obstruction produced by intravascular bubbles. It has been reported, however, that only a small percentage of vessels remains blocked after gas embolization (Duff 1954) and arterial air embolism failed to produce bone infarction (Kahlstrom 1939).

These speculations should not limit pathogenetic considerations to the direct effects of gas bubbles. Circulating nitrogen bubbles could trigger a chain of secondary events including aggregation of platelets and erythrocytes, coalescence of unstable lipids and changes in the coagulation mechanism. Any one of these alterations might result in vascular obstruction and anoxia in the bones. Fat embolization could also result from disruption of fatty tissue by expanding gas bubbles. Experimental production of aseptic bone necrosis by fat embolization following intraaortic oil infusion was recently reported by Jones, Sakovich, and Anderson (1974). The possible implication of fat embolization in the pathogenesis of dysbaric osteonecrosis merits further exploration. The hypothesis that embolization may be involved in the mechanism of dysbaric osteonecrosis is further supported by the observation that introduction of artificial emboli (glass beads) into the common iliac artery of rabbits produced femoral osteonecrotic lesions (Walder 1974). Regarding the possible role of thrombotic material, it should be mentioned that administration of anticoagulants and platelet inhibitors in animals did not prevent development of dysbaric osteonecrosis (Smith and Stegall 1974).

Blood-gas interface phenomena can result not only in platelet and lipid changes but also in the release or activation of vasoactive substances (Chryssanthou 1973; Chryssanthou, Waskman, and Koutsoyiannis 1974). It is of interest in this respect that intrarterial administration of vasodilator substances causes a redistribution of blood flow in the limb by increasing the flow through the muscles at the expense of that through the bone marrow (Semb 1971). This decrease of blood flow could render the bone more vulnerable to extravascular or intravascular factors that cause ischemia.

In all of the above postulated mechanisms, the protagonist is the gas bubble, which directly or indirectly causes circulatory impairment. Alternatively there is the possibility that factors other than, or in addition to gas bubble-related events may play a role in the pathogenesis of dysbaric osteonecrosis. It has been suggested that gas-concentration gradients resulting from rapid pressure changes can produce osmotic changes and fluid shifts that could contribute to the production of bone lesions (Hills 1970). Consequently the rate of compression becomes a factor that may influence the development of bone changes. Supporting this hypothesis is our finding that, with rapid compression, the incidence of dysbaric osteonecrosis is higher than with stage compression. It has also been reported that

the severity and frequency of hyperbaric arthralgia are reduced in divers subjected to slow compression rates and that gas-induced osmosis is implicated in this phenomenon (Bradley and Vorosmarti 1974). Another speculation on the pathogenesis of dysbaric osteonecrosis implicates altered immunity and dysproteinemia. This hypothesis of an autoimmune process is based on the latency of bone lesions and on the observation of rouleaux formation, amyloidosis, and long delayed membranous glomerulitis in some animals with dysbaric osteonecrosis (Antopol et al. 1964). Changes induced by high P_{O_2} , which in turn may influence development of osteonecrosis is still another factor that merits consideration.

It is evident from the foregoing discussion that the mechanism of dysbaric osteonecrosis is still unclear. The various concepts that have been presented involve several diverse pathogenetic factors which can be summarized as follows:

1. Factors causing circulatory impairment: *a*, extravascular pressure (e.g. by growing gas bubbles); *b*, vascular obstruction by emboli (e.g. fat, gas bubbles); *c*, vascular obstruction by thrombotic material (e.g. fibrin, platelets); *d*, vasodilator substances causing decreased blood flow in bones.
2. Factors not associated with blockade of blood supply: *a*, gas-induced osmotic shift of fluids; *b*, autoimmunity and dysproteinemia.

Further experimentation and additional clinical and epidemiological studies are needed before the relative importance of the postulated pathogenetic factors can be assessed. It is reasonable at this juncture, however, to propose that the pathogenesis of dysbaric osteonecrosis involves several of the above mentioned factors acting in concert or in sequence.

The findings of the present study regarding the latency in the development of bone necrosis and the influence of multiple dysbaric exposures are consistent with well-known observations on human subjects. The influence of obesity and of the rate of compression on the incidence of the disease in man has not yet been established. Although the results of the present investigation indicating an increased incidence of dysbaric osteonecrosis in obese and in rapidly compressed animals must be extrapolated with caution, they should draw attention to the possible role of these factors in the development of the lesion in humans.

CONCLUSIONS

1. Dysbaric osteonecrosis can be experimentally produced in mice, particularly, in obese strains.
2. There is a latent period ranging from 2 to 9 mo or possibly more.
3. In obese mice the incidence is greater and the latent period shorter than in thin siblings.
4. With multiple exposures, the incidence is higher and the latent period shorter than with single exposure.
5. With stage compression, the incidence is lower than with rapid compression.
6. Dysbaric osteonecrosis in mice appears to be independent of decompression sickness.
7. The pathogenesis of dysbaric osteonecrosis may involve several initiating and contributing factors that act in concert or in sequence.

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Chryssanthou, C. P. 1976. Ostéonécrose dysbarique chez la souris. *Undersea Biomed. Res.* 3(2): 67-83. L'histopathologie de l'ostéonécrose dysbarique et l'influence du nombre d'expositions, de la vitesse de compression et de l'obésité sur la fréquence et la latence des lésions ont été étudiées chez 438 souris (2,205 os examinés). Les animaux ont subi des pressions de 75 psig pendant 2-6 heures (expositions uniques ou multiples). La compression a été soit rapide, soit par paliers; la décompression a été *sans danger*. Après une période de latence de 2-12 mois, 34,1% des souris obèses et 5,8% des souris maigres avaient développé une ostéonécrose épiphysaire tibiale ou fémorale. Les conclusions suivantes s'imposent: 1. l'ostéonécrose apparaît indépendante de la maladie de la décompression; 2. la fréquence de l'ostéonécrose augmente, et la période de latence se raccourcit chez les souris obèses; 3. la fréquence de l'ostéonécrose augmente et la période de latence se raccourcit après des expositions multiples; 4. la fréquence reste plus restreinte avec une compression par paliers; 5. la pathogénèse de l'ostéonécrose peut impliquer plusieurs facteurs (gène circulaire due aux bulles intra- ou extravasculaires, embolies, thromboses, substances vasoactives, osmose gazeuse, autoimmunité) qui agissent ensemble ou en séquence.

dysbarie	ischémie	obésité
os	osmose	vitesse de compression
bulles	embolie graisseuse	plongées répétées
histopathologie	susceptibilité	pathogénèse
	ostéonécrose aseptique	

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DYSBARIC ALTERATION OF THE
BLOOD-BRAIN BARRIER

Blood-brain and blood-lung barrier alteration by dysbaric exposure

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Chryssanthou, C., M. Springer, and S. Lipschitz. 1977. Blood-brain and blood-lung barrier alteration by dysbaric exposure. *Undersea Biomed. Res.* 4(2): 117-129. -- Failure of certain circulating substances to penetrate specific organs led to the concept of blood-organ barriers. Such barriers can be altered by various physical or chemical means. This report concerns modification of the blood-brain barrier (BBB) and blood-lung barrier (BLB) by dysbaric exposure. Trypan blue was intravenously administered to 19 experimental rabbits (subjected to compression-decompression) and to 11 controls (kept at ambient pressure). Gross and microscopic examination and measurements of dye extracted from tissues revealed greater dye penetration into lung and brain of the experimental animals. Dye concentration in brain was 12.10 $\mu\text{g/g}$ tissue in experimental and 2.93 μg in control animals; in lungs it was 935 μg and 434 μg , respectively ($0.01 > P > 0.001$). Increased permeability of BBB and BLB was associated with intravascular bubbles. The mechanism of BBB and BLB alteration may involve chemical agents activated by gas-blood interface or vascular injury produced by bubbles. These observations could have pathogenetic implications in decompression sickness and may suggest new methods for facilitating penetration of therapeutic agents into the brain.

blood-organ barriers
trypan blue
rabbits
permeability

compression-decompression
intravascular bubbles
decompression sickness
pathogenesis

After Goldman (1913) demonstrated the peculiar impermeability of the brain to trypan blue at the turn of the century, the concept of barriers was advanced to explain the observation that certain circulating substances fail to penetrate specific organs or tissues. The presence of the so-called blood-brain barrier (BBB) has been long established. The relative impermeability of other organs or tissues to certain drugs, vital dyes, colloids and other substances led to the postulation of barriers which separate blood from the fetal side of the placenta, the testis, the interior of the eye, etc. The existence of a blood-lung barrier (BLB) was proposed to account for the failure of this organ to stain by intravenously administered chlorophyllin, trypan blue, and tetrazolium salts (Chryssanthou and Antopol 1961; Chryssanthou and Antopol 1963).

The permeability of blood-organ barriers can be altered by various chemical and physical means. Venoms, allergic agents, bacterial products, bile salts, artificially induced seizures, X-irradiation and gas embolization have been reported to alter the BBB (Bouton 1940;

Bjerner, Broman, and Swensson 1944; Broman and Lindberg-Broman 1945; Broman 1949; Clemente and Holst 1954; Eckman, King, and Brunson 1958; Johansson 1975) and bradykinin and bacterial endotoxins were shown to increase permeability of the BLB (Chryssanthou and Antopol 1961; Chryssanthou and Antopol 1963).

The present study is, to our knowledge, the first report concerning alterations of the BBB and BLB induced by exposure to dysbaric conditions.

MATERIALS

Albino female rabbits weighing 3–4 kg were employed. They were housed in metal cages in animal rooms with controlled temperature (65°–68° F) and relative humidity (50%) and were fed Purina Rabbit Chow and water ad libitum. The animals were kept under these conditions for a stabilization period of at least two weeks before they were used.

A hyperbaric chamber (Bethlehem Corporation, Model 1835 HP) with controlled temperature and relative humidity was utilized. The chamber was pressurized with air (dry air cylinders, Matheson Company, Inc.). Solutions of 1000 u/ml sodium heparin (Upjohn Company) and of 2% trypan blue (K and K Laboratories, Inc.) in sterile normal saline were prepared for intravenous injections. The extractant (for trypan blue extraction from tissues or blood) consisted of four volumes of 95% ethanol and one volume of 17% benzalkonium chloride (Zephiran, City Chemical Corp.)

METHODS

The animals were numbered, weighed, and randomly divided into an experimental (subjected to compression-decompression) and a control group (kept at ambient pressure). Both control and experimental animals received an intravenous injection (marginal ear vein) of trypan blue solution (4 ml/kg). The experimental animals were injected within 4 min after decompression to sea level. They were then observed for clinical manifestations of decompression sickness until they died or were killed. The animals which died within 75 min after decompression were not included in these studies. Those which survived for more than 90 min postdecompression were killed by intravenous administration of sodium nembutal. Control animals were killed at intervals corresponding to those of the experimental animals. Just prior to killing, or when death appeared imminent in the experimental group, all animals received an intravenous injection of heparin solution (1 ml/kg) to maintain liquidity of the blood and permit perfusion of tissues. Prior to heparin administration a blood sample was obtained for dye concentration determination. Immediately after death the animals were autopsied and the degree of gross staining of the lungs and brain recorded. The animals were also inspected for the presence of gas bubbles in tissues or in blood vessels. The lungs were perfused with normal saline. Representative portions of lung and brain were fixed in formalin for histologic processing. Tissue sections (5- μ m thick) stained with hematoxylin-eosin, light eosin, and unstained preparations were subjected to light and phase microscopy. The extent of trypan blue penetration into tissues was graded. The remaining lung and brain tissue was frozen for dye extraction at a later time. The data were statistically evaluated (Student's *t*-test).

Hyperbaric exposure

The experimental animals were subjected, one at a time, to 90 psig (202.5 fsw) air pressure for 12 min and then decompressed to sea level in 23 min. Figure 1 shows the dive profile

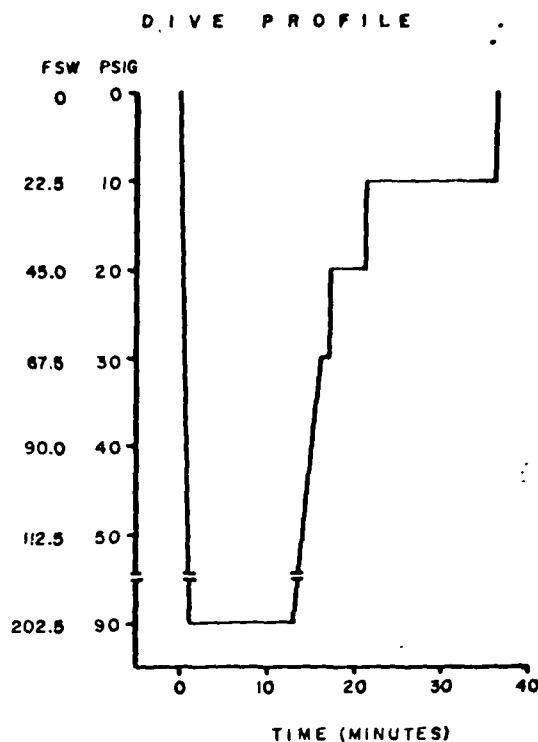


Fig. 1. Compression-decompression schedule used for experimental group of rabbits.

employed. This exposure produced decompression sickness with paraplegia in one third of the animals.

Gross and microscopic grading of tissue staining

The degree of tissue staining was graded grossly from 1+ to 4+ according to the intensity and extent of staining and microscopically from 1+ to 3+ on the basis of frequency of fields revealing the presence of dye in tissue and intensity of staining. The grading was done blindly by two observers.

Perfusion of lungs

The lungs of both control and experimental animals were perfused with normal saline (under 25 mmHg) by means of a catheter inserted into the pulmonary artery. Perfusion continued until the outflow of the perfusate was clear.

Determination of dye concentration

Trypan blue was extracted from tissues or from plasma samples by a method modified from Caster (Caster, Simon, and Armstrong 1953; Caster, Simon, and Armstrong 1954). Frozen

tissues were thawed, minced and homogenized with distilled water. The homogenate (or plasma sample) was freeze-dried and then pulverized with a mortar and pestle to a fine powder. The powder was mixed with the extractant (1 g/10 ml) and shaken for 1 min. The mixture was centrifuged for 3 min and the clear-colored supernatant collected. This procedure was repeated until the final extract contained less than 5% of the total dye extracted. Usually this required 4 to 5 sequential extractions. The collected supernatants were read in a spectrophotometer against the extractant. To correct for interference by other colored material extracted from tissue or plasma, the following procedure was used. The optical density of the extracts was measured at 400 nm and 575 nm. These wavelengths represent the maxima in the absorbance curves of dye-free tissue and trypan blue, respectively. The ratio of the absorbance at 400 nm and 575 nm is constant regardless of concentration. Correction for interfering substances was made by algebraic calculations. The standard consisted of the dye alone dissolved in the extractant. A plot of trypan blue concentration against optical density readings was found to be linear over the range of concentrations measured. The concentration of the dye was expressed as μg of dye/g dry weight of tissue or ml of blood.

Dye levels in plasma

Since diffusion is involved in the penetration of dyes from plasma to the tissues, it is important to know the level of dye in the circulating blood. In preliminary experiments dye concentrations in plasma were determined at various intervals following intravenous administration of 2% trypan blue solution (4 ml/kg). Figure 2 shows the mean plasma levels of trypan blue from 5 to 180 min after dye injection. It can be seen that the curve flattens 75 min after dye administration and remains practically horizontal at the level of about 230 $\mu\text{g}/\text{ml}$. Since there was little variation between individual dye concentration curves, it was assumed that animals which died or were killed at least 75 min after dye injection had approximately equal concentrations of circulating dye. This was confirmed in several experimental and control animals by determining dye concentration in blood samples obtained just prior to death.

RESULTS

Clinical manifestations

Seven of 21 animals subjected to dysbaric conditions developed decompression sickness. The disease was manifested by paralysis of the hind legs, twitching, and severe respiratory distress with panting and gasping. Two of the animals which suffered decompression sickness died a few minutes after decompression and consequently were excluded from the study.

Gross and microscopic examination

Lungs

On gross examination the lungs of all experimental animals appeared stained. In 53% of the animals the staining was intense, with a patchy or diffuse distribution. In the control group the lungs were stained in 82% of the animals, but the staining was usually focal and weak. Only one control animal (9%) exhibited intense staining. Figure 3 shows the gross appearance of experimental and control lungs. Microscopic examination of unstained preparations of exper-

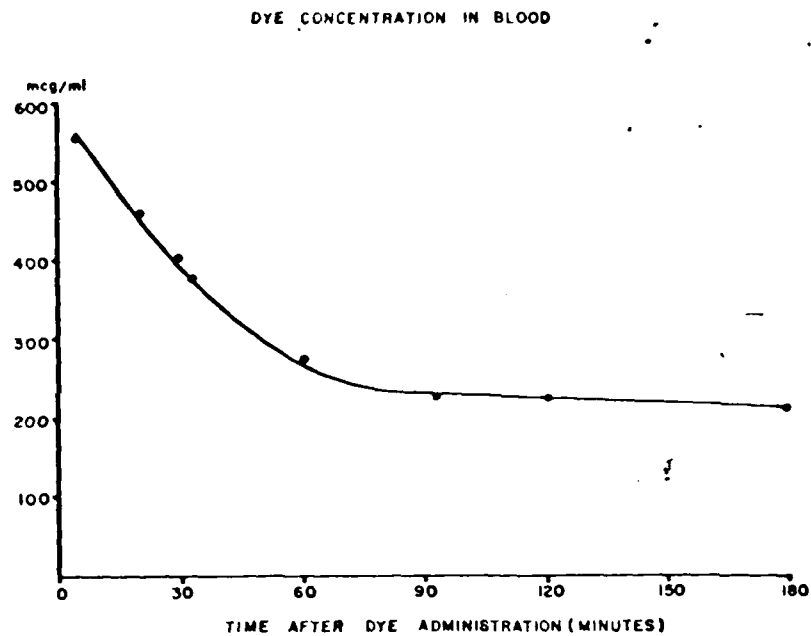


Fig. 2. Mean blood levels of trypan blue at various intervals after intravenous injection of dye.

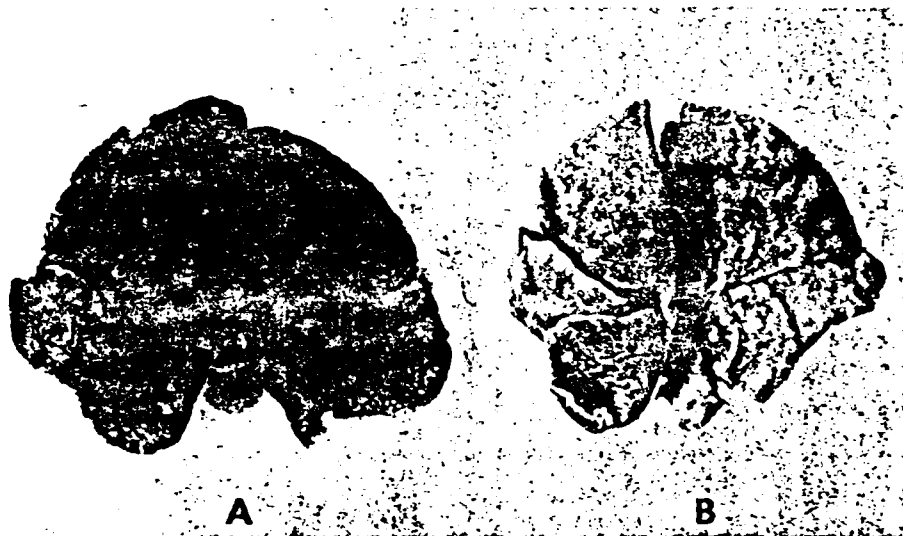


Fig. 3. Lung from rabbit subjected to compression-decompression (A) showing diffuse and intense staining. In contrast, corresponding control lung (B) appears practically unstained. (Experimental and control animals in this figure and in following microphotographs have been injected with same amount of dye and killed after same interval following dye injection.)

imental lungs revealed diffuse moderate staining of the alveolar septa, vascular wall and perivascular and peribronchial tissue with foci of intense dye concentration (Figs. 4 and 5). These focal accumulations of dye consisted of either small, diffusely stained, blue patches or of aggregates of dye granules. In the alveolar septa it was difficult to determine whether the dye granules were in capillaries, in the interstitial tissue, or in pneumocytes. Dye granules were also seen within several alveolar macrophages. Microscopically, control lungs showed no evidence of dye extravasation (Fig. 4), except in one case. Table 1 presents the degree of staining on gross and microscopic examination of individual control and experimental animals.

Brain

Grossly, the cerebral hemispheres of the experimental group exhibited patchy areas of light staining in 84% of the animals. Only one (9%) of the control brains was stained. Microscopically, in unstained preparations the cerebral parenchyma of several experimental animals appeared light blue with intensely stained vessels and focal accumulation of dye granules in the perivascular tissue (Fig. 6). Localization of dye was possible in preparations stained lightly with eosin and in unstained preparations examined with light and phase contrast optics. Microscopic examination of control brains did not reveal the presence of dye (Fig. 6) except in one animal. Table 2 shows the degree of gross and microscopic staining in individual animals.

Dye concentration in tissues

The mean dye concentrations in the lungs and brain of controls and of animals subjected to dysbaric conditions are shown in Table 3. The differences in the mean value between control

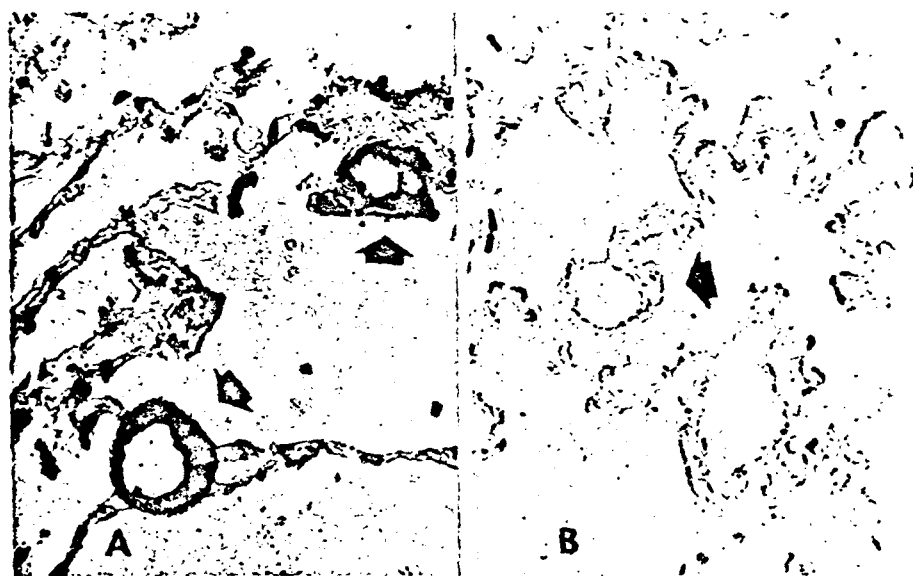


Fig. 4. Lung from a rabbit subjected to compression-decompression (A) exhibiting diffuse moderate staining of alveolar septa with focal concentrations of dye and intensely stained vascular walls (arrows). Compare with corresponding control (B) showing absence of dye from alveolar and vascular wall (arrow). (Unstained preparation, original magnification $\times 256$.)



Fig. 5. Deeply stained vascular wall in lung of experimental animal (A) stands in contrast to the unstained vessel in corresponding control lung (B). (Unstained preparation, original magnification $\times 160$.)

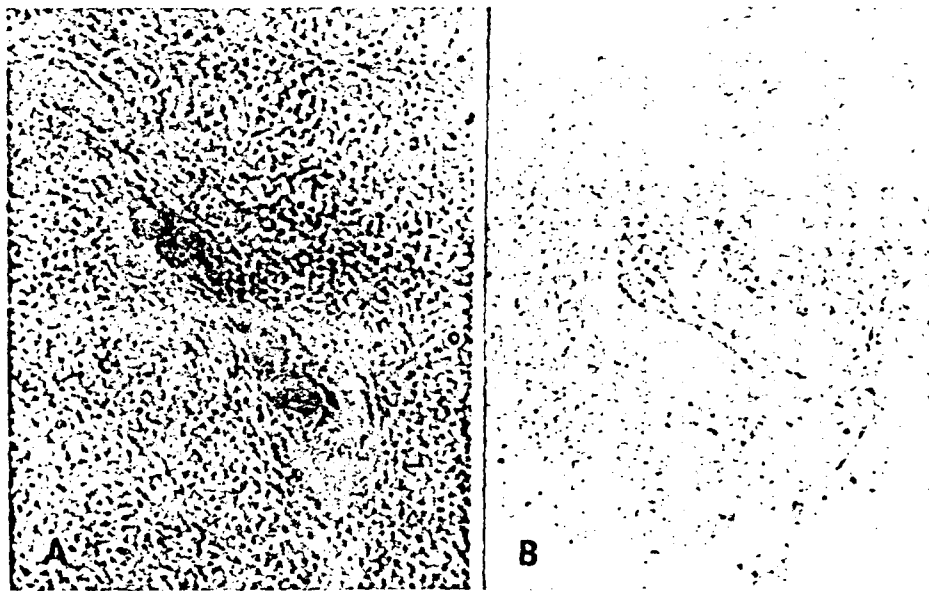


Fig. 6. White matter from cerebrum of an animal subjected to compression decompression (A) exhibiting diffuse moderate staining of parenchyma and intensely stained vessel. Corresponding control (B) showing absence of dye in parenchyma and vessel. (Unstained preparation, original magnification $\times 256$.)

TABLE I

Degree of staining in lung

Compressed - Decompressed			Controls		
Animal No.	Gross	Micro	Animal No.	Gross	Micro
299	++++§	+++	962	+++	-
946	++++	*	300	++	+
939	+++	++	941	++	-
929	+++	+	943	++	-
935	+++	+	947	++	*
940	+++	+	986	++	-
932	+++	*	292	+	-
938	+++	*	942	+	-
952	+++	*	944	+	-
979	+++	*	287	-	-
945	++	+++	291	-	-
298	++	++			
937	++	++			
934	++	+			
936	++	+			
954	++	+			
981	++	+			
989	++	*			
933	+	+			

§See text for grading method; * tissue was not subjected to microscopic examination.

and experimental animals are significant at high levels of confidence. The distribution of individual dye concentration can be seen in Fig. 7. The same figure correlates the level of dye concentration with the presence of grossly visible intravascular gas bubbles and with decompression sickness. In all cases of decompression sickness there were grossly visible intravascular gas bubbles. However, their presence was not always associated with clinical manifestations of the disease. There was some overlap between control and experimental dye concentrations in both lungs and brain. It should be noted, however, that none of the experimental animals with dye concentration values in the control range exhibited intravascular bubbles. The presence of bubbles in blood vessels was always associated with high concentration of dye in the brain and lungs. Furthermore, all animals which manifested decompression sickness had high dye concentrations but the reverse was not always true.

DISCUSSION

Permeability of blood-organ barriers is not an "all-or-none" phenomenon. Most substances, including vital dyes, penetrate these barriers, though sometimes slightly or slowly. Therefore, barrier permeability to a substance should be quantitated and preferably expressed in terms of

TABLE 2

Degree of staining in brain

Compressed - Decompressed			Controls		
Animal No.	Gross	Micro	Animal No.	Gross	Micro
951	+++§	++	941	+	+
940	++	++	287	-	-
945	++	++	289	-	-
298	++	+	292	-	-
937	++	+	300	-	-
938	++	+	942	-	-
939	++	-	943	-	-
946	++	-	944	-	-
929	+	+	947	-	-
935	+	+	962	-	-
952	+	+	989	-	-
981	+	+			
933	+	-			
934	+	-			
989	+	-			
299	+	*			
954	-	+			
979	-	-			
932	-	*			

§See text for grading method; *tissue was not subjected to microscopic examination.

TABLE 3

Concentration of dye in tissue

Tissue	Compressed- Decompressed	Control	Significance
Lung	935 $\pm$ 128*	434 $\pm$ 66	0.001 $<P < 0.01$
Brain	12.1 $\pm$ 1.95	2.95 $\pm$ 0.63	0.001 $<P < 0.01$

Values are mcg/g dry tissue; *mean $\pm$ SEM; significant by Student's *t*-test for small samples of unpaired observations.

DYE CONCENTRATIONS IN TISSUE

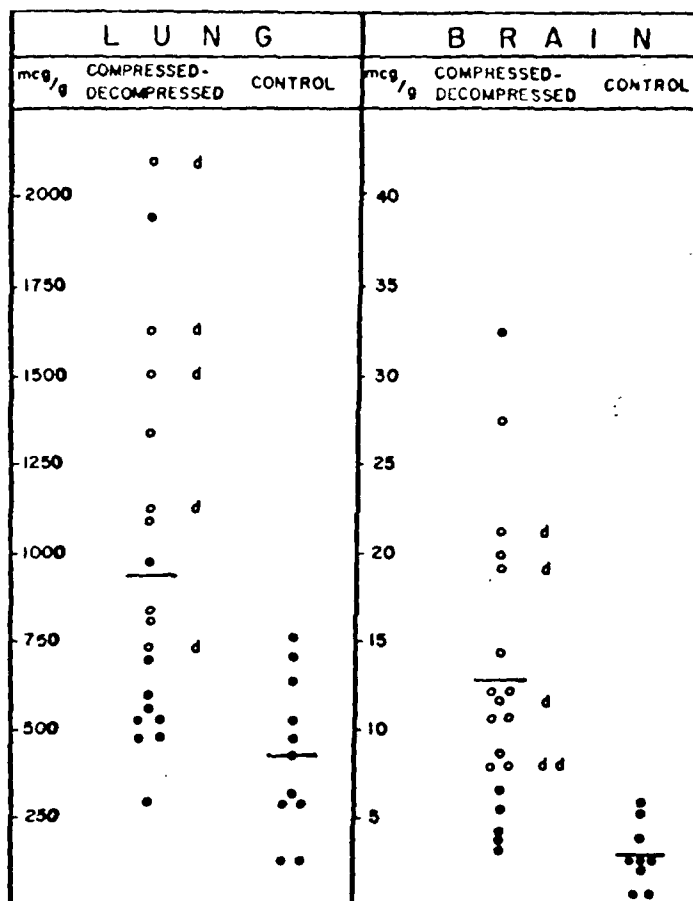


Fig. 7. Dye concentrations in lungs and brain of experimental and control animals. Note that in experimental animals with intravascular gas bubbles (○) dye concentrations are higher than in animals with bubble-free vessels (•). Horizontal bars represent mean values and (d) indicates animals which suffered decompression sickness.

rate of penetration. Comparison of barrier permeability to a circulating dye under different conditions is meaningful only when concentrations of the dye in tissue and plasma are determined and temporal parameters evaluated.

In the present study transgression of trypan blue through the BBB and BLB was quantitated in both control and experimental animals at a time when all animals had approximately equal plasma levels of trypan blue.

The results of gross and microscopic examination and dye concentration determinations show that a significantly greater amount of dye penetrated the barriers in experimental animals

than in controls. This indicates that dysbaric exposure increased the BBB and BLB permeability to trypan blue.

Bouton (1940) observed as early as 1940 that the brain appears grossly stained following air embolization and Broman (1949) showed disturbances in cerebrovascular permeability resulting from either air or fat embolism. It is not certain whether in our experiments barrier alterations were caused by gas or other emboli. There was, however, a definite correlation between grossly visible intravascular gas bubbles and increased dye concentrations in lung and brain. All animals which manifested decompression sickness exhibited increased barrier permeability but alteration of barriers was not always associated with decompression sickness. These observations suggest that gas bubbles can cause changes in barrier permeability even in the absence of clinical manifestations of decompression sickness.

The exact mechanism by which dysbaric exposure alters barrier permeability is obscure. Hypoxia caused by embolization or other circulatory disturbances is not a likely pathogenetic factor since it has been shown that anoxic states of several hours' duration have no deleterious effects on cerebrovascular permeability to trypan blue (Broman 1949). In our experiments anoxia, if present, could not have lasted for more than one hour. Furthermore, changes of the BBB have been reported within a few seconds following intracarotid injection of air, oxygen, or carbon dioxide (Johansson 1975). One could also discount possible effects of vasodilation or changes in blood pH and osmotic pressure since even extreme variations in these parameters failed to alter barrier permeability (Broman 1949). It seems more likely that gas bubbles cause direct mechanical injury of blood vessels or that vascular permeability is altered by the action of chemical agents released or activated in the course of reactions initiated by blood-bubble interface activity (Chryssanthou 1973; Hallenbeck, Bove, and Elliot 1973; Chryssanthou 1974b). Smooth muscle activating factor (SMAF), which was shown to be activated by gas bubbles (Chryssanthou 1974a), increases vascular permeability (Chryssanthou, Teichner, Goldstein, Kalberer, and Antopol 1970), and bradykinin, which has been implicated in decompression sickness and could be activated by gas bubbles (Chryssanthou, Teichner, Goldstein, and Antopol 1973; Hallenbeck et al. 1973; Chryssanthou 1974b), has been reported to alter the BLB (Chryssanthou and Antopol 1963). It is also conceivable that protein denaturation at the blood-bubble interface may result in release of protein-bound dye, thus increasing plasma concentration of free dye.

All these postulated mechanisms implicate gas bubbles. It is still possible that the observed correlation between intravascular gas bubbles and barrier alteration has no pathogenetic significance and that changes in permeability are caused by other factors related to compression or decompression.

In discussing mechanisms of barrier alteration, one should have the anatomical and functional characteristics of the barriers in mind. The BBB is generally believed to be related to the tight interendothelial junctions and the virtual absence of pinocytotic vesicles from brain capillaries (Oldendorf 1974). The nature of the BLB, on the other hand, is still unclear. It is therefore possible that alterations of the BBB and BLB involve different mechanisms.

Changes in the permeability of BBB and BLB may have pathogenetic implications in decompression sickness. It has already been emphasized that all animals which suffered decompression sickness exhibited increase in barrier permeability. Metabolites, released or activated humoral agents, and other plasma components which normally do not penetrate the BBB and BLB may, under dysbaric conditions, gain access to the brain or lung and cause pathologic changes. Parenthetically, it can be noted that the observed dysbaric alterations of the BBB may suggest new methods for administering chemotherapeutic agents, antibiotics or neuroactive drugs which under normal conditions do not penetrate the BBB.

This work was supported by the Office of Naval Research, Department of the Navy, Contract N00014-75-C-0312 and the Lenore Weinstein Fund. The authors wish to express their appreciation to Drs. R. Stenger, G. Goldstein, and I. Feigin for their advice. They also wish to thank Ms. G. Molenge and S. Martin for their technical help, Mr. Osmay Yalis for the photography and Ms. C. Towner for her secretarial assistance. —Received for publication July, 1976; revision received December, 1976.

Chryssanthou, C., M. Springer, and S. Lipschitz. 1977. Altération de la barrière hémato-encéphalique et hémotopulmonaire par l'exposition dysbarique. *Undersea Biomed. Res.* 4(2): 117-129. —La constatation que certaines des substances circulantes ne réussissent pas à pénétrer dans certains organes est à l'origine du concept des barrières hémato-organiques. Ces barrières se laissent altérer par des moyens physiques ou chimiques. Nous rapportons la modification de la barrière hématoencéphalique et de la barrière hémotopulmonaire par l'exposition dysbarique. Des injections intravéneuses de bleu trypan ont été administrées à 19 lapins "expérimentaux" (qui ont subi la compression-décompression) et à 11 animaux témoins, qu'on a gardés à la pression ambiante. Les examens anatomiques et microscopiques, ainsi que la détermination du taux de colorant extrait des tissus, ont mis en évidence une plus grande pénétration du colorant dans les poumons et les cerveaux des animaux expérimentaux. La concentration cérébrale en était 12,10 $\mu\text{g/g}$ tissu chez les expérimentaux, et 2,93 $\mu\text{g/g}$ chez les témoins; la concentration pulmonaire en était 935 $\mu\text{g/g}$ et 434 $\mu\text{g/g}$, respectivement ($0,01 > P 0,001$). La perméabilité augmentée des barrières hémato-encéphaliques et pulmonaires est associée aux bulles intravasculaires. Le mécanisme de l'altération des barrières peut impliquer des agents chimiques activés par l'interface gaz-sang ou des lésions vasculaires dues aux bulles. Ces observations peuvent contribuer à l'élucidation de la pathogénie de la maladie de décompression, et suggérer de nouvelles méthodes pour faciliter la pénétration des agents thérapeutiques dans le cerveau.

barrières hémato-organiques	perméabilité	maladie de décompression
bleu trypan	compression-décompression	pathogénie
lapins	bulles intravasculaires	

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Increased blood-brain barrier permeability to tetracycline in rabbits under dysbaric conditions

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Chryssanthou, C., B. Graber, S. Mendelson, and G. Goldstein. 1979. Increased blood-brain barrier permeability to tetracycline in rabbits under dysbaric conditions. *Undersea Biomed. Res.* 6(4): 319-328. — Alteration of the blood-brain barrier (BBB) by dysbaric exposure may have relevance in several areas of hyperbaric medicine. Drugs administered to persons exposed to dysbaric conditions, e.g., divers, compressed air workers, may penetrate the brain in amounts that could produce toxic or undesirable effects. Modification of the BBB may also have pathogenic implications in decompression sickness. Furthermore, increased BBB permeability to certain potentially useful antitumor agents, antibiotics, and other compounds under dysbaric conditions may provide the basis for a new therapeutic approach. This report concerns the influence of dysbaric exposure on BBB permeability to an antibiotic. Tetracycline (5–40 mg/kg) was intravenously injected in 22 experimental rabbits (subjected to air compression-decompression) and 17 controls (kept at ambient pressure). Fluorescence microscopy and spectrometry revealed significantly greater tetracycline concentrations in 72.7% of the experimental brains. With the 5 mg/kg dose, the mean tetracycline concentration was 0.17 $\mu\text{g/g}$ in control brains and 0.33 $\mu\text{g/g}$ in experimentals. These results indicate that dysbaric exposure increases BBB permeability to tetracycline. It appears that BBB alteration is related to intravascular gas bubbles but is independent of the development of decompression sickness. The conclusions of this investigation are pertinent to brain pharmacotherapy and may provide some new insight into the mechanism of decompression sickness. They also point to potential risks connected with drug administration under dysbaric conditions that can alter BBB permeability.

blood-brain barrier	compression-decompression
barrier alteration	decompression sickness
increased brain permeability	intravascular gas bubbles
tetracycline	rabbits
brain pharmacotherapy	dysbaric exposure
drug risks under dysbaric conditions	

The permeability of the blood-brain barrier (BBB) to vital dyes was shown to increase as a result of exposure to compression-decompression (Chryssanthou, Springer, and Lipschitz 1977) and air embolization (Johansson 1975). The significance of this observation and its relevance to hyperbaric medicine is threefold.

1. A variety of drugs, and particularly those with low lipid solubility or a high degree of ionization, do not readily penetrate the BBB. Exposure to compression-decompression

may, however, by increasing barrier permeability, allow larger amounts of the drug to enter the brain and produce toxic or undesirable effects. Therefore, the possibility of BBB modification under dysbaric conditions should be an important consideration in the pharmacotherapy of divers and compressed air workers.

2. Changes in the BBB may have pathogenetic implications in decompression sickness and other dysbaric disorders.
3. Alteration of the BBB by dysbaric exposure may suggest new methods for therapeutic or diagnostic administration of compounds that, under normal conditions, do not penetrate the BBB or do so to a very limited extent.

The present communication deals with the effect of dysbaric exposure on the permeability of the BBB to tetracycline. This antibiotic was selected because appropriate light stimulation causes fluorescence, which enables easy detection on gross and microscopic examination and measurement by fluorescence spectroscopy.

MATERIALS AND METHODS

Thirty-nine albino female rabbits weighing 2.5 to 5.0 kg were used. They were kept in metal cages in a separate animal room with controlled temperature (65°–68°F) and relative humidity (50%) and fed Purina Rabbit Chow and water *ad libitum*. The animals were used experimentally after a stabilization period of 2–3 weeks. They were then weighed, numbered, and randomly divided into an experimental and a control group. Both groups received tetracycline HCl injected into the marginal ear vein. Tetracycline doses of 5, 10, 20, and 40 mg/kg body weight were administered to 8, 5, 4, and 5 experimental animals and to 6, 4, 3, and 4 controls, respectively. The experimental animals received the tetracycline injection immediately after exposure to dysbaric conditions. They were then observed for clinical manifestations of decompression sickness until they died or until they were killed by sodium nembutal administration 90 min after the tetracycline injection. Control animals kept at ambient pressure were killed after the same post-tetracycline interval as that of the corresponding experimental rabbits. Because of space limitations in the hyperbaric chamber, these experiments were conducted in 16 runs, with 1 or 2 experimental and 1 or 2 control animals in each run.

All animals were autopsied immediately after death. Blood vessels and tissues were examined for gas bubbles. The brain was inspected under ultraviolet light for possible sites of tetracycline fluorescence. Representative sections of the brain were taken for microscopic examination, and the remaining tissue was frozen to be used for extraction and spectrophotometric quantitation of tetracycline at a later time. The gross and microscopic findings and the results of the spectrophotometric determinations of tetracycline concentrations in control and experimental animals were compared and checked for possible correlation with intravascular gas bubbles or signs of decompression sickness.

Dysbaric exposure

The animals of the experimental group (1 or 2 at a time) were placed in a hyperbaric chamber (Bethlehem Corp., Model 1835 HP) with controlled temperature (68°–72°F) and relative humidity (50%) and subjected to a dive profile previously described (Chryssanthou et al. 1977) (Fig. 1).

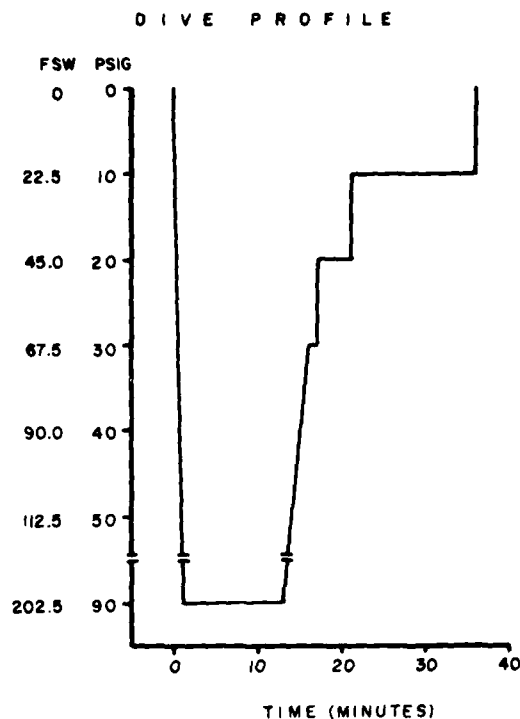


Fig. 1. Compression-decompression schedule used for experimental group of rabbits.

Fluorescence microscopy

Representative samples from various parts of the brain were obtained and unstained frozen sections were examined by a Zeiss photomicroscope under ultraviolet light using BG-12 or UG-2 exciting filters and BG-23 or OG-5 barrier filters. The sections were fixed in absolute alcohol and mounted in glycerine. Under these conditions, tetracycline emits a golden-yellow fluorescence. Evaluation of the degree of tetracycline penetration was done blindly by two observers by grading the sections from 0 to 3+ on the basis of the intensity and extent of fluorescence.

Extraction and chemical determination of tetracycline

Tetracyclines form highly fluorescent complexes with divalent metal ions and barbiturates. These can be extracted from aqueous medium with various organic solvents and read in a fluorescence spectrometer (Kohn 1961). After removal of sections for microscopic examination, the remainder of the brain tissue was immediately frozen until ready to be extracted. At that time the tissue was thawed and homogenized. A weighed amount of ground tissue was extracted by shaking with 0.1 N HCl at 5°C for 1 h. After centrifugation, an aliquot of the supernatant was deproteinized. Sodium barbital and calcium chloride were added, and the

mixture extracted with ethyl acetate. The ethyl acetate layer was withdrawn and read at an excitation wavelength of 390 nm and a fluorescence wavelength of 520 nm. The instrument used was a Perkin-Elmer Fluorescence Spectrometer 204. Comparison was made against standards prepared from tetracycline hydrochloride.

Statistical evaluation

The results of chemical determinations were evaluated by Student's *t*-test and those of the microscopic grading by the chi-square test.

RESULTS

Clinical manifestations

Eight of the 22 animals that were subjected to compression-decompression exhibited signs of decompression sickness, including paralysis of the hind legs and respiratory manifestations with panting and gasping. Six of these animals died; their death was in most cases preceded by severe respiratory distress and convulsions. Table 1 lists the clinical observations in relation to the degree of increased permeability of the blood-brain barrier.

TABLE 1
BLOOD-BRAIN BARRIER PERMEABILITY INCREASE AND MANIFESTATIONS
OF DECOMPRESSION SICKNESS

Animals With Decompression Sickness			Animals Without Decompression Sickness	
Animal No.	E/C Ratio	Signs* of Decompression Sickness	Animal No.	E/C Ratio
9	2.14	PCD	21	6.60
30	2.00	P	8	3.07
4	1.73	RCD	39	2.37
7	1.67	RPD	40	2.04
3	1.67	PCD	23	1.58
19	1.65	RCD	37	1.30
5	1.53	CD	25	1.22
18	0.96	R	13	1.17
			36	1.13
			34	0.78
			29	0.78
			15	0.70
			32	0.67
			27	0.30
Mean $\pm$ SEM**			1.90 $\pm$ 0.47	

E/C ratio = ratio of tetracycline concentration, Experimental/Control; *P = paralysis (usually paraplegia); R = respiratory distress; C = convulsions; D = death; **P > 0.09 (NS).



Fig. 2. Cerebrum from a rabbit that received tetracycline (40 mg/kg) intravenously after exposure to dysbaric conditions. Bright fluorescent foci (tetracycline) are present in frontal and temporal lobes.

Gross examination

Appreciable fluorescence under ultraviolet light was only observed in the brain of a few animals subjected to dysbaric conditions. Some of these brains exhibited foci of intense golden-yellow fluorescence (Fig. 2). In the control animals, fluorescence in the brain was seen only in an occasional animal and was faint and diffuse. In general, however, there were no significant differences in gross fluorescence between experimental and control animals. Intravascular gas bubbles, particularly in the vena cava and the right side of the heart, were seen in most of the experimental animals. In many of them gas bubbles could also be detected in the arterial system.

Microscopic examination

Microscopic examination of unstained frozen sections of brain tissue revealed detectable fluorescent foci in all of the experimental animals but only in a few of the controls. Furthermore, the intensity of fluorescence in brains of animals exposed to dysbaric conditions was significantly greater than in controls (Fig. 3). Fluorescence was assumed to be due to tetracycline, since the excitation light and the filters used produced the expected color characteristics. Fluorescent foci varied in size and brightness and usually appeared as clusters dispersed in

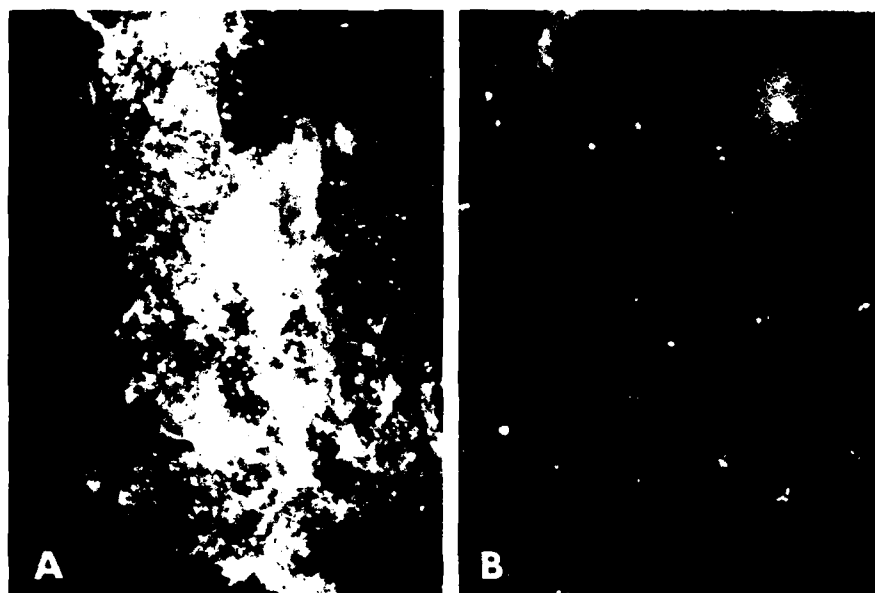


Fig. 3. Section from cerebral cortex of a rabbit subjected to dysbaric conditions and (A) exhibiting clusters of intensely fluorescent foci (tetracycline); in contrast, corresponding section from a control animal (B) kept at ambient pressure shows faint diffuse auto-fluorescence and only a few scattered fluorescent foci. Both control and experimental animals were intravenously injected with same dose of tetracycline (5 mg/kg) and were killed at same interval after injection. (Unstained frozen section, original magnification $\times 64$).

several areas of the cerebrum (Fig. 3). They were also seen in the wall of vessels and in the perivascular tissue (Fig. 4). Table 2 summarizes the grading of fluorescence in the brain of individual control and experimental animals injected with 5 mg/kg tetracycline.

Extraction and fluorescent spectrometry of tetracycline

Table 2 shows the concentration of tetracycline in the brain of individual experimental and control animals after intravenous administration of 5 mg/kg tetracycline. It is evident that in the experimental group, tetracycline levels are higher than in controls, with statistically significant differences. The table also shows positive correlation between tetracycline concentrations determined by fluorescence spectroscopy and the degree of tetracycline fluorescence determined microscopically. Figure 5 presents the mean concentrations of tetracycline in the brains of control and experimental animals after different intravenous doses of tetracycline. It is again apparent that, in animals subjected to compression-decompression, tetracycline penetrated the BBB to a greater degree than it did in controls. Table 1 presents the magnitude of BBB permeability alteration in each experimental animal in terms of the ratio of brain tetracycline concentration in the experimental animal over the concentration in the corresponding control (E/C ratio). It can be seen that in 72.7% (16/22) of the animals, exposure to dysbaric conditions resulted in increased BBB permeability (E/C ratio higher than 1). This effect is statistically significant, with a probability of $0.02 < P < 0.05$. The table also compares the E/C

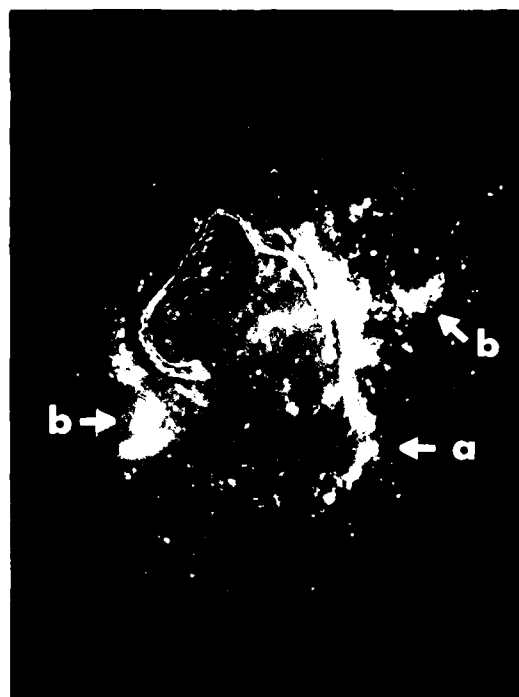


Fig. 4. Cerebral cortex from a rabbit that received tetracycline (5 mg/kg) intravenously after dysbaric exposure. (Auto-fluorescence of elastica outlines a portion of a cerebral vessel.) Bright fluorescent foci (tetracycline) can be seen in wall of vessel (a) and in perivascular tissue (b).

TABLE 2
DEGREE OF PENETRATION OF BLOOD-BRAIN BARRIER BY TETRACYCLINE (5 MG/KG)

Experimental			Control		
Rabbit No.	Fluorescence Microscopy*	Chemical Determination, $\mu\text{g/g}$ tissue	Rabbit No.	Fluorescence Microscopy*	Chemical Determination, $\mu\text{g/g}$ tissue
3	3+	0.35	16	1+	0.23
8	2+	0.43	2	1+	0.21
9	2+	0.30	6	0	0.15
7	2+	0.25	12	0	0.15
5	2+	0.23	10	0	0.14
15	2+	0.16	14	0	0.12
13	2+	0.14			
4	1+	0.26			
Mean**	2.00	0.33		0.27	0.17
$\pm$ SEM	± 0.19	± 0.21		± 0.03	± 0.01

* = Graded 0 to 3+; **0.02 < P < 0.05.

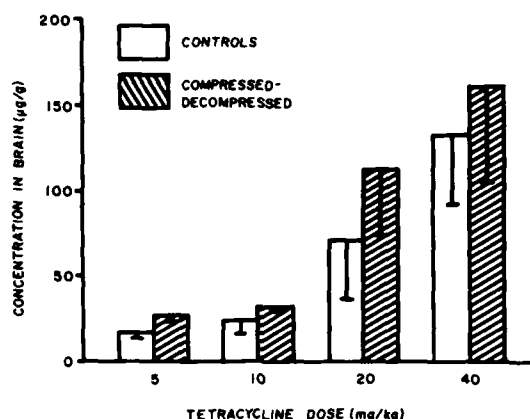


Fig. 5. Effect of a dysbaric exposure on permeability of BBB to tetracycline after intravenous tetracycline administration at various dose levels. Height of columns represents mean concentration of tetracycline in brain, and vertical lines represent SEM.

ratios between animals that manifested signs of decompression sickness and those that did not. There was no significant correlation between BBB alteration and signs of the disease ($P = 0.09$). Of the 16 animals in which exposure to compression-decompression caused increased BBB permeability, 7 developed clinical signs but 9 did not.

DISCUSSION

The results of this investigation suggest that dysbaric exposure can increase the permeability of the blood-brain barrier to tetracycline. The findings are consistent with previous reports that dysbaric exposure and air embolization increase BBB permeability to intravenously injected vital dyes (Johansson 1975; Chryssanthou et al. 1977). The data presented in Table 1 indicate that 7 out of 8 animals that manifested signs of decompression sickness exhibited appreciable increases in BBB permeability. On the other hand, the data show that the BBB was altered by dysbaric exposure also in animals that did not develop decompression sickness. More than half of the animals in which BBB permeability increased did not exhibit any clinical manifestations. In fact, the three animals that exhibited the greatest alteration of the BBB (highest E:C ratio) were entirely asymptomatic.

These observations may be pertinent and significant in several areas. An important aspect of the possible dysbaric alteration of the BBB is the potential danger of toxic or undesirable effects of medication received by persons exposed to significant changes of atmospheric pressure, e.g., divers, compressed air workers. It is important to be aware of the possibility that, because of barrier modification, certain drugs administered to subjects under dysbaric conditions may enter the brain at undesirably high levels. In addition to possible health hazards, increased permeability of the BBB to certain drugs may result in sedative or excitatory effects, in impaired perception or judgment, or in behavioral changes. Such effects could interfere with the subject's performance or even jeopardize his safety.

Modification of the BBB by dysbaric conditions may also provide new insight into the mechanism of decompression sickness. Humoral agents or cellular factors with potential central effects released or activated in the course of the disease and metabolites and neuroactive substances that normally have limited access to the brain may, under dysbaric conditions, penetrate the barrier at higher rates and produce effects associated with the pathogenesis of dysbaric disorders.

Finally, alteration of the BBB by compression-decompression introduces the possibility of a new approach in the pharmacotherapy of the brain. The BBB constitutes a serious obstacle in the administration of potentially useful antitumor agents, antibiotics, and neuroactive drugs that, under normal conditions, do not penetrate the BBB. To pass across the BBB, antibiotics or other drugs should be characterized by high partition parameter values (product of unionized fraction $\times$ partition coefficient), which reflect the ability of a drug to partition between a lipid phase and aqueous solution. There is a general correspondence between partition parameter value and CSF/plasma concentration ratio (Jacobs 1940; Brodie, Kurz, and Schanker 1960). Drugs with low partition parameter values may have to be administered intrathecally into the cerebrospinal fluid to bypass the BBB (Weinstein 1970).

Most efforts to overcome this difficulty have been directed to the synthesis of drugs with properties that would allow greater barrier permeation. This approach, however, did not prove successful in treating meningitis, leukemia, or other brain disorders (Rappaport 1976).

A promising approach to this problem would be to administer drugs under conditions that increase BBB permeability to the administered substance. Diverse chemical and physical means, including bacterial products, bradykinin, gas embolization, hypertonic perfusion, and X rays, have been reported to alter the permeability of blood-organ barriers (Bouton 1940; Broman and Lindberg-Broman 1945; Broman 1949; Clemente and Holst 1954; Chryssanthou and Antopol 1961; Chryssanthou and Antopol 1963; Rappaport, Bachman, and Thompson 1972; Johansson 1975). The observed increase in BBB permeability under dysbaric conditions opens a new avenue of investigation that may lead to effective and safe methods in brain pharmacotherapy. Encouraging in reference to this speculation is the observation that the BBB can be modified by exposure to dysbaric conditions in the absence of clinical signs of decompression sickness. The possibility that the BBB can be altered by dysbaric exposures that present minimal or no risks of producing dysbaric disorders merits exploration.

The mechanism of BBB modification by dysbaric exposure is still obscure. There are observations that implicate intravascular gas bubbles (Johansson 1975; Chryssanthou et al. 1977). According to the findings of the present and earlier studies, however, the presence of gas bubbles is not necessarily associated with clinical manifestation of decompression sickness. Gas bubbles can be "silent" (asymptomatic) and still produce BBB alterations.

This work was supported by the Office of Naval Research, Department of the Navy, Contract #N00014-75-C-0312, and the Lenore Weinstein Fund. The authors thank Ms. G. Molenge, Ms. S. Marrin, and Mr. J. Rice for their technical help, Mr. O. Yalis for the photography, and Ms. E. McManus for her secretarial assistance. — *Manuscript received for publication April 1979; revision received July 1979.*

C. Chryssanthou, B. Graber, S. Mendelson, et G. Goldstein. La perméabilité accrue de la barrière "cérébro-sanguine" des lapins à la tétracycline dans les circonstances dysbares. *Undersea Biomed. Res.* 6: 319-328, 1979. Un changement de la barrière "cérébro-sanguine" (BCS) effectuée par une exposition dysbare peut-être important aux certains domaines de la médecine hyperbare. Les drogues administrées aux personnes qui ont été exposées aux circonstances dysbares, e.g., les

plongeurs, les ouvriers de l'air comprimé, peut pénétrer le cerveau en quantités que peuvent produire les effets toxiques ou malséants. Une modification de la BCS peut avoir aussi des implications pathogènes à propos de la maladie de décompression. De plus, la perméabilité accrue de la BCS aux certains agents anti-tumeur potentiellement utiles, aux antibiotiques et aux autres composés aux circonstances dysbares peuvent fournir la fondation d'une approche nouvelle thérapeutique. Ce compte rendu concerne l'influence de l'exposition dysbare sur la perméabilité de la BCS à une antibiotique. Vingt deux lapins-cobaye sujete à l'air compression-décompression et 17 lapins contrôles maintenus à la pression ambiante ont subi des injections intraveineuses de la tétracycline (5-40 mg/kg). La microscopie fluorescence et la spectrométrie ont exposé les concentrations de la tétracycline en quantité beaucoup plus important dans 72,7% des cerveaux expérimentaux. A une dose de 5 mg/kg, la concentration moyenne de tétracycline a été 0.17 $\mu\text{g/g}$ parmi les cerveaux contrôles et 0.33 $\mu\text{g/g}$ parmi les cerveaux expérimentaux. Ces résultats indiquent que l'exposition dysbare augmentent la perméabilité de la BCS à la tétracycline. Il apparaît que le changement de la BCS se rapporte aux boules intravasculaires du gaz mais est indépendante du développement de la maladie de décompression. Les conclusions de cette enquête se rapportent à la pharmacothérapie du cerveau et peuvent fournir des considérations nouvelles chez le mécanisme de la maladie de décompression. Ils entraînent aussi aux risques potentielles qui se rapportent à l'administration des drogues dans les circonstances dysbares qui peuvent changer la perméabilité de la BCS.

barrière "cérébro-sanguine"
altération de la barrière
perméabilité accrue du cerveau
risques des drogues dans les circonstances dysbares
compression-décompression

maladie de décompression
boules intravasculaires du gaz
lapins
exposition dysbare

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PROGRAM AND ABSTRACTS
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39 MODIFICATION OF THE BLOOD-BRAIN BARRIER BY SMOOTH MUSCLE ACTING FACTOR (SMAF). C. Chryssanthou, R. Kersh* and M. Margiotta*. Beth Israel Medical Center and Mount Sinai School of Medicine of the City University of N.York, N.Y. 10003.

Dysbaric exposure and air embolization increase blood-brain barrier (BBB) permeability to vital dyes and drugs. Modification of the BBB is relevant to the pharmacotherapy of divers and compressed air workers and may have pathogenetic implications in decompression sickness (DS). Chemical mediators released or activated by gas bubbles have been considered as factors in the development of BBB alteration by dysbaric exposure. The previously described smooth muscle acting factor (SMAF), which increases vascular permeability, is activated by compression-decompression in vivo and by air bubbles in vitro. In view of the above, the possible effect of SMAF on the BBB was explored. Each of 8 experimental and 9 control rabbits were intravenously injected with 80mg/kg trypan blue; 30 min. later they received, under nembutal anesthesia, an intracarotid dose of 5mg/kg SMAF (experimentals) or an equal volume of 0.9% NaCl (controls); one hour after dye injection the brain was perfused with 0.9% NaCl to flush out blood and then removed for microscopy and dye extraction. The mean dye concentration in the brain was 12.3µg/g tissue in experimental animals and 3.8µg/g tissue in controls ($P < 0.001$). Dye was microscopically detected in frozen sections of experimental brains but not in controls. These results indicate that SMAF increases BBB permeability suggesting that SMAF may be implicated in dysbaric modification of the BBB. (Supported by the Office of Naval Research, Department of the Navy, Contract #N00014-75-C-0312.)

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BIOCHEMICAL EFFECTS OF PRESSURE, INERT GASES, OXYGEN

REVERSIBILITY OF DYSBARIC ALTERATION OF THE BLOOD-BRAIN BARRIER.
C.P. Chryssanthou, R. Fuhrer* and D. Higgins*. Beth Israel Medical
Center and Mount Sinai School of Medicine of the City University of New
York, N. Y. 10003.

It has been reported that blood-brain barrier (BBB) permeability in rabbits can be modified by exposure to dysbaric conditions. These observations were confirmed by other laboratories in studies on rats and guinea pigs. The present investigation deals with the reversibility of dysbaric BBB alterations. A total of 40 rabbits were subjected to 90 psig air pressure for 12 min and then stage decompressed to sea level in 40 min. They were divided into 4 groups of 13, 6, 5 and 16 animals which were intravenously injected with 2% trypan blue (4 ml/kg) immediately, 6, 16 and 24 hours after decompression respectively. The animals were sacrificed 90 minutes after dye injection. Dye penetration of the BBB was determined by extraction and colorimetric measurements of dye concentration in brain tissue and by microscopic examination of representative brain sections. Mean dye concentration was 26.2 mcg/g brain tissue in animals injected immediately after decompression and 12.2 mcg/g in the group injected 24 hours later ($P < 0.02$). Microscopic examination also revealed lesser penetration of dye with longer intervals between decompression and dye injection. The results suggest that dysbaric alteration of the BBB is reversible. These observations are pertinent to the question of possible risks of toxicity or undesirable CNS effects in divers and compressed air workers receiving drugs. Modification of the BBB by dysbaric exposure and its reversibility may also have potential applications in brain pharmacotherapy. (Supported by the Office of Naval Research, Department of the Navy, Contract #N00014-75-C-0312).

EFFECTS OF HYPERBARIC NORMOXIC EXPOSURE ON BRAIN GLUCOSE PHOSPHORYLATION
AND BLOOD-BRAIN BARRIER GLUCOSE TRANSPORT. T. Obrenovitch* and F. Brue*.
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