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ABSTRACT

Attempts to define the no-decompression air limits for miniature swine were complicated by individual variance and bends tolerance development, but, based solely on the appearance of clinical signs, they appear more resistant to bends than man except perhaps at depths of 140 feet or greater. Although an 80% incidence of bends was observed in 100 feet/90 minutes, 140 feet/60 minutes and 180 feet/30 minutes, miniature swine generally tolerate dives to 60, 100, 140 and 180 feet for bottom times three or more times longer than corresponding no-decompression limits for man. They typically exhibit temporary signs of middle ear distress almost exclusively on ascent; attempts to relieve this by myringotomy failed due to the peculiar anatomy of the porcine ear canal.

Miniature swine suffer severe gas embolism of the thoracic caudal vena cava and pulmonary artery in the absence of bends signs; marked embolism is recorded in swine performing 180 feet/15 minutes no-decompression profiles. Threshold bottom times for pulmonary artery gas embolism in miniature swine, using the Doppler flowmeter as an embolus detector, are 5 minutes or less at 100 and 180 feet. There is, therefore, virtually no doubt that human divers experience pulmonary embolism following no-decompression dives which are considered "safe."

Unmodified existing Doppler flowmetry equipment cannot detect emboli smaller than about 50 μ m diameter in the caudal vena cava under physiologic blood flow conditions. For a large number of reasons, several of which cannot be eliminated under in vivo conditions, existing Doppler apparatus is unsatisfactory for estimating the size of passing gas emboli. A theoretical basis is presented for the development of a more sensitive embolus detector using resonant pulse-echo ultrasonics.

INTRODUCTION

The need for a suitable animal model for hyperbaric research is well recognized. In the field of diving technology alone, the development of safe decompression procedures is expensive, time-consuming, dependent on maintaining a pool of human volunteers, and must be considered hazardous. Many aspects of decompression sickness research cannot be studied on human subjects at all, at least with the technological means currently available. Animal models used extensively in the past include laboratory rodents, dogs, goats and small subhuman primates, none of which seem to respond to hyperbaric excursions as does man. Specifically, they are more resistant to bends, in a way suggesting body size and cardiovascular makeup--i.e., total blood perfusion characteristics--play a dominant role in bends susceptibility between species. Hyperbaric research, then, would seem to demand at the outset that the animal model be of a size comparable to adult man and, to whatever extent possible, share anatomic and physiologic cardiovascular characteristics with man. Such an animal must also be easily obtained at reasonable cost and be sufficiently suitable for laboratory use as regards standardization, domestication, tractability, trainability and tolerance of major surgical operations.

The Pacific Northwest Laboratory, Battelle Memorial Institute (Battelle-Northwest, BNW), has been performing a number of biomedical studies utilizing miniature swine for over a decade, and since 1966 has been conducting research with this species relative to the artificial heart program of the National Heart and Lung Institute. Because of their similarities to man regarding size and cardiovascular physiology, not to mention other factors, they continue serving as experimental models for this work and may serve in more advanced research on cardiac assist devices in the near future. These similarities, plus their relatively high fat/lean ratio compared to man, suggested their possible use as human analogs in hyperbaric research.

It has been generally believed for some time that most of the signs and symptoms of decompression sickness, including such delayed sequelae as aseptic bone necrosis, are a result of gas bubbles formed in blood or tissues during and following decompression from a hyperbaric environment.

Theoretical considerations certainly allow for the formation of intracorporeal gas bubbles under such conditions, as well as for many of the symptoms and signs recorded during actual cases. In peracutely fatal cases of decompression sickness, gross widespread gas embolism has been verified in both animals and man, though peracute deaths have occurred in which such lesions have not been demonstrable. In the latter cases, where postmortem examination has been promptly and properly performed, it has been suggested⁽¹⁾ that gas embolism did exist to a degree which would have been asymptomatic in normal individuals but which proved lethal because of extant subclinical patent foramina ovale or pulmonary arteriovenous shunts, permitting small emboli to escape lung clearance and enter the left circulation. Other explanations for bends symptoms have been offered, including fat embolism, peripheral rouleaux formation and venous sludging, intravascular clot formation and circulation, and extravascular (intracellular) gas bubble formation, to name a few; however, it is generally accepted that gas embolism is the prime suspect and worthy of extensive laboratory and clinical investigation.

Much research at BNW, including those artificial heart studies in progress since 1966, has required the telemetric measurement of blood flow rates in selected vessels of free-ranging animals, for which the ultrasonic Doppler blood flowmeter was selected and used extensively. It becomes immediately apparent to anyone working with the Doppler flowmeter that the instrument is exquisitely sensitive to the presence of gas bubbles in the fluid circulating through its transducer, whether that fluid is blood or a blood-substitute suspension used for bench testing. In mid-1967 the question arose whether gas bubbles formed in vivo by rapid decompression might be detectible with this instrument, assuming they did in fact circulate in suitable large vessels following such decompression. Simultaneously the search for an animal analog to man for hyperbaric research led us to consider evaluating the miniature swine for this purpose. With internal research funding, pilot studies were initiated.

In November of 1967 a miniature swine with a Doppler flow transducer on the thoracic caudal vena cava was subjected to a severe hyperbaric excursion (180 feet/60 minutes, linear decompression to surface in 6 minutes, air atmosphere) throughout which caudal vena cava blood flow was telemetered to

a recording system outside the hyperbaric chamber. Emboli were indeed detected, beginning 2 minutes after beginning decompression (120 feet depth) and becoming increasingly severe until collapse of the animal at 10 minutes postsurfacing, by which time the blood flow signal had become virtually obliterated by embolic artifacts. This experience was reported⁽²⁾ as were subsequent experiments in which peripheral venous emboli were detected atraumatically with transcutaneous Doppler flowmetry during and following hyperbaric excursions using miniature swine, pygmy goats and dogs.⁽³⁾ During these early experiments it came to our attention that similar, apparently independent experiments using sheep were underway at Virginia Mason Research Center in Seattle, the first published account of which was basically in agreement with our observations.⁽⁴⁾ In May of 1968 we performed our first (and only) series of trials on human subjects at the reservoir behind Smith Mountain Dam near Roanoke, Virginia, through the cooperation of Andre Galerne and volunteer divers of International Underwater Contractors, Inc. Peripheral veins (radial, jugular, femoral) were monitored transcutaneously following actual working dives and during one dry chamber simulated dive (180 feet/60 minutes, air, standard table decompression). Emboli were never detected, even though the diver performing the dry chamber dive suffered bends requiring recompression, and the trials largely served to point out practical difficulties involved in monitoring human subjects. These and other animal experiences were subsequently reported.⁽⁵⁾

At about the time of the Smith Mountain Dam trials, discussions were well underway with the Bureau of Medicine and Surgery (BuMed) regarding U. S. Navy support for contract research directed toward evaluation of the miniature swine as an analog to man for hyperbaric research and the use of the Doppler flowmeter as a detector of decompression-produced gas embolism. This finally culminated in the award of Contract No. N00014-69-C-0350, administered through the Office of Naval Research (ONR), in May of 1969. Originally designed as a three-year program, this contract was terminated in May of 1971 shortly following a complete revision of experimental plan by the sponsors, with the concurrence of BNW. A final report on the research performed during the two fiscal years follows.

OBJECTIVES

The miniature swine⁽⁶⁾ attracted attention as a possible animal analog to man for hyperbaric research because of its similarities to man in size and cardiovascular physiology and because of its relatively high fat/lean ratio, the latter of potential importance particularly in saturation exposures. If the similarities between this species and man extend to their response to hyperbaric excursions, the miniature swine may qualify as a valuable animal model for basic research in hyperbaric medicine or even as a stand-in for man during the early phases of development of decompression tables for man. One specific objective of our research program was to attempt to define the no-decompression limits for this species through dry chamber air dives, using as a guide the no-decompression limits for man as published in the U. S. Navy Diving Manual for 1963.⁽⁷⁾

Following the discovery that the ultrasonic Doppler blood flowmeter^(8,9) was capable of detecting gas emboli in animals undergoing decompression,^(2,3,4,5,10) questions arose whether asymptomatic gas emboli, if they exist, can be detected prior to the onset of bends symptoms. Besides its obvious applications to animal research on the kinetics of bubble formation, the instrument may be useful for monitoring decompression on an individual basis or for objectively assessing the embolic status of human subjects suffering decompression sickness. Questions also arose as to the threshold size of embolus detectible with this instrument, and whether some characteristic of the signal produced by an embolus might give a measure of its size. The second objective of our research program, stated broadly, was to continue our earlier work on the use of the Doppler flowmeter in these regards.

During the first one and one-half years of the contractual period, research emphasis was two-fold according to experimental planning agreed upon by BNW, BuMed and ONR: (1) To evaluate the miniature swine as an analog to man for decompression research through attempts to determine the no-decompression limits at three depths, tentatively set at 60, 140 and 190 feet. The latter depth was changed to 180 feet following the suggestion of and data received by Surgeon Commander J.S.P. Rawlins, R. N., with whom the original plan had been discussed during an earlier meeting with

Dr. Tor Richter (then Director, BuMed). (2) To ascertain, first through bench studies and subsequent in vivo investigations, the threshold size of gas embolus detectible with the Doppler flowmeter under actual or simulated blood flow situations and the adaptability of this instrument as an in vivo bubble-sizer.

Six months into the second fiscal year the above objectives were modified according to sponsor wishes. Experimentation with unmodified Doppler flowmeters as bubble-sizers was discontinued, as was theoretical and preliminary experimental studies on improving bubble sensitivity of this instrument. Studies of alternate ultrasonic methods for bubble sizing and detection in vivo were discontinued, and further work on establishing no-decompression bottom time limits for miniature swine was terminated. The new objectives outlined included the determination of no-decompression limits for miniature swine at 100 and 180 feet based not on clinical signs but on the appearance of gas emboli in the pulmonary artery as detected by the telemetric Doppler flowmeter. In addition, in those animals which displayed pulmonary embolism or clinical signs, pulmonary artery embolic status would be monitored during recompression and stage decompression following standard treatment tables for man. An extension of this work would have included the development of modified treatment tables for the miniature swine in the event that the standard procedures for man did not eliminate pulmonary embolism, presumably for future comparison with similar tables developed for man when and if reliable methods for transcutaneous detection of pulmonary artery emboli in man become available.

A proportionate amount of work relative to this new research plan involved extension of the program into the third fiscal year, as only six months remained following revision of plan before termination of the fiscal year in progress. Laboratory work on this revised program was further curtailed by the announcement, three months later, that the program would not be so extended, requiring a rather premature stoppage of experimentation.

MINIATURE SWINE RESPONSE TO HYPERBARIC EXCURSIONS

Objectives

The objective was to attempt to determine the no-decompression limits of adult miniature swine at three depths, comparing this to the no-decompression table in the U. S. Navy Diving Manual (1963) for man.⁽⁷⁾

Methods

Twenty-six miniature swine were used to perform a total of 108 pig-dives designed to evaluate their response to such excursions in comparison to man. (This number does not include those relatively mild excursions with instrumented animals conducted in connection with the study on pulmonary artery embolism, covered later in this report.) Sixteen were males (ten castrated), ten were females, and all were of the Hanford Miniature (HMS) or HMS-Labco Cross strains.⁽⁶⁾ Twenty-four weighed between 105 and 136 pounds, the others weighing 92 and 154 pounds; no correlation between bends susceptibility and weight was evident. One hundred of the 108 pig-dives were performed by normal, unoperated animals; eight pig-dives were performed using animals on whose thoracic caudal vena cava had been implanted a perivascular Doppler flowmeter transducer. Depths simulated by dry chamber air dives included 60, 100, 140 and 180 feet, the latter substituted for the originally planned 190 feet on the recommendation of Surgeon Commander J.S.P. Rawlins, R. N., who cited a 12% incidence of bends in R. N. divers performing 180 feet/20 minutes no-decompression excursions.

All dives were of the no-decompression type, with linear ascent and descent at 60 feet/minute and bottom times up to 180 minutes. Surface intervals always exceeded 48 hours. Animals were coaxed to enter and leave the chamber without rough handling, and postdive observations were made as the animals moved freely in an outside walkway or in their pens. Close observation of behavior continued for two hours postdive, with intermittent checks made for the following 24 hours. Husbandry, including feed composition and amount, was virtually identical for all animals.

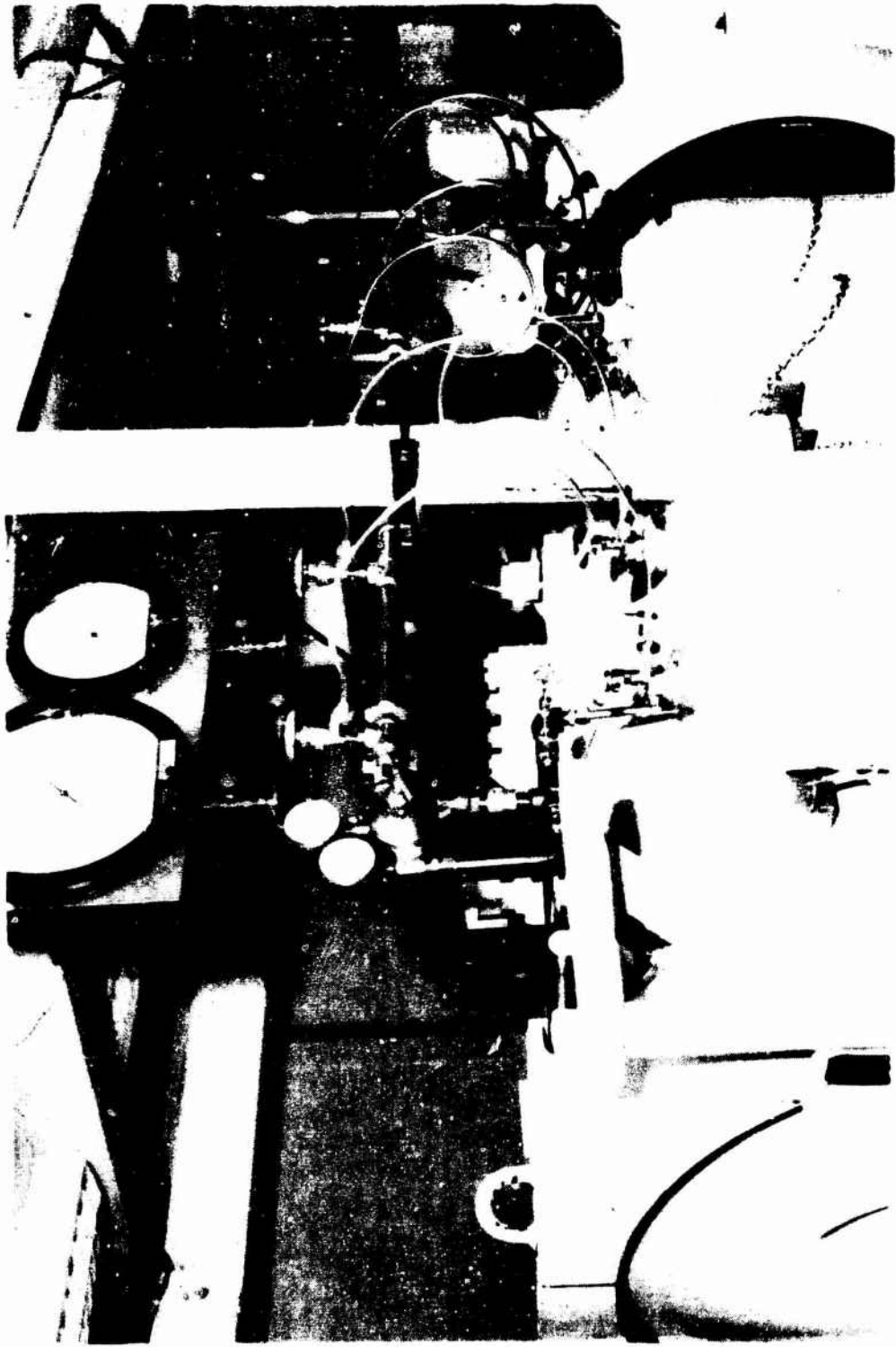


Figure 1. Hyperbaric chamber and cylinder manifold air supply used in miniature swine studies.

Since animals cannot relate symptoms, assessment of their physical condition is always made on the basis of signs presented. A number of different signs were commonly observed (Table I), and it became necessary to define what sign or set of signs constituted decompression sickness. As we gained experience in the response of this species to hyperbaric excursions, the following were defined to be diagnostic signs of decompression sickness: (1) Lameness, muscular fasciculation or weakness in any limb, persisting for 15 minutes or longer and relieved by recompression, and/or (2) Acute, progressive respiratory distress, with or without cyanosis, and/or (3) Central neurologic signs, e.g. dysmetria, paralysis, convulsions, unconsciousness. Temporary ataxia, incoordination, loss of balance, head shaking and tilting, and vomiting were interpreted as being of vestibular origin, a result of relative pressure changes in the middle ear, and were not considered signs of bends in the absence of other signs.

Results

A summary of the 108 pig-dives and signs observed is given in Table I. Omitting the 180-foot bounce dive, the results of 107 pig-dives may be summarized further as in Table II, where bends is defined as above.

TABLE I. SUMMARY OF CLINICAL SIGNS OBSERVED IN 108 PIG-DIVES

Depth (Feet)	Bottom Time (Minutes)*	No. of Dives Performed	Signs (Number) and Remarks
60	60	16	Ascent head shaking (13), pruritus (6), vomiting and lethargy (1), ataxia (1).
	90	2	No signs.
	120	9	Ascent ataxia (3), vomiting (2), ascent head shaking (6).
	180	2	Ascent ataxia (1), ascent head shaking (2).
100	60	8	Vomiting (3), ascent head shaking (5), ascent ataxia (2). Quick recovery without treatment in all cases.
	90	6	Ascent head shaking (5), ascent ataxia (3), vomiting (2), muscular tremors (2), rear leg lameness (5). FIVE BENDS, treated successfully by recompression.
140	15	15	Ascent head shaking (13), ascent ataxia (5), descent head shaking (2), pruritus (1), vomiting, lethargy and ataxia following surfacing (1).
	30	9	Ascent head shaking (7), ascent ataxia (5), pruritus (2), persistent vomiting (1), total hearing loss (1), temporary inability to rise following surfacing (2).
	45	4	Ascent head shaking (3), ascent ataxia (3), descent ataxia (1), muscular tremors after surfacing (2), vomiting and ataxia (2), persistent lameness (2). TWO BENDS, successfully treated by recompression.
	60	5	Bottom ataxia (2), ascent head shaking (2), vomiting (2), pruritus (2), rear leg weakness and lameness (1). Also two peracute deaths preceded by ataxia, dysmetria, dyspnea, convulsions, hematemesis, paralysis and unconsciousness. FOUR BENDS, only two successfully treated by recompression.

TABLE I. (Cont.)

Depth (Feet)	Bottom Time (Minutes)*	No. of Dives Performed	Signs (Number) and Remarks
180	3	1	Bounce dive on animal habitually showing ataxia and vomiting. No signs observed.
	10	5	Ascent head shaking (4), vomiting (1).
	15	2	Ascent ataxia (1), vomiting (1), descent ataxia (1), bottom ataxia (1), persistent incoordination (1). Both Doppler flowmeter preparations, many caval emboli detected. No treatment required.
	20	19	Ascent head shaking (8), ascent ataxia (4), pruritus (3), ascent unconsciousness (temporary) (1), persistent ataxia, vomiting and lethargy (2), progressive lameness (2), temporary lameness (1), one Doppler preparation (anesthetized) revealed massive gas embolism of thoracic caudal vena cava. THREE BENDS, all treated successfully.
	30	5	Ascent head shaking (1), temporary rear leg lameness (not treated) (1), frothy ascitic fluid at necropsy (1), progressive dyspnea (4), restlessness (1), cyanosis (4), progressive signs of lameness, ataxia, incoordination, weakness, paralysis and death in spite of recompression (3), vomiting (1). FOUR BENDS, three died in spite of recompression and one successfully treated.

* Bottom time is defined as period between beginning descent and beginning ascent. Ascent and decent rates 60 feet/minute.

TABLE II. BENDS INCIDENCE IN MINIATURE SWINE

<u>Depth (Feet)</u>	<u>Bottom Time (Minutes)*</u>	<u>Number of Pig-Dives</u>	<u>Number of Bends Cases</u>	<u>%</u>
60	60	16	0	0
	90	2	0	0
	120	9	0	0
	180	2	0	0
100	60	8	0	0
	90	6	5	83
140	15	15	0	0
	30	9	0	0
	45	4	2	50
	60	5	4	80
180	10	5	0	0
	15	2	0	0
	20	19	3	16
	30	5	4	80

* Bottom time is defined as period between beginning descent and beginning ascent. Ascent and descent rates 60 feet/minute.

Discussion

It must be stressed that milder symptoms, such as those reported by human subjects, may have been experienced by these animals. For example, pruritus may or may not indicate "skin bends;" attempts to resolve this by recompression gave highly equivocal results. Drooping ears, lethargy and salivation probably indicate nausea. Certain characteristics in countenance or demeanor often gave us the impression that the animal was suffering from confusion, mild vertigo or deep pain somewhere other than in the limbs. Total deafness was confirmed in at least one case, but subtler abnormalities such as partial deafness and tinnitus can be diagnosed in animals only with great difficulty if at all. Mild visual defects are equally difficult to detect. Thus, our conclusions must properly be based on clinically observable signs only, through experienced personnel working with swine as test animals for decompression procedures could undoubtedly attune themselves to more subjective indicators of decompression sickness in this species.

In addition, the picture is not as clear as Table II might imply. Marked individual variations are seen between animals, so the number of pig-dives are inadequate for computing statistically significant incidence limit figures, e.g. exposures producing a specified incidence of bends in the population. Two pig-dives at 180 feet/15 minutes without signs of bends, for example, is of little significance. The most striking example of individual variation was seen when two littermates of equal size performed a 140 feet/60 minutes excursion together in the chamber. Forty minutes after reaching surface one exhibited hematemesis and dysmetria, rapidly progressing to collapse and clonic convulsions. The littermate showed no signs whatsoever. Both were returned to the chamber where, despite recompression to 200 feet, the first animal died. At no time did the littermate seem disturbed.

It is strongly evident, as well, that experienced animals (those having experienced a half dozen or more recent dives) are more resistant to bends than are neophytes. For example, the first fifteen 180 feet/20 minutes profiles were performed by five animals, each of whom had recently completed 3 dives each at 60 and 140 feet (without bends). Although no signs of bends were observed following these 180-foot excursions, three of the subsequent four trials of this profile--using neophyte pigs--produced bends. If this phenomenon could be unequivocally confirmed, an animal model is available for the study of acquired bends resistance. In the meantime, however, such a profound dependence on prior diving experience confounds progress in defining the no-decompression limits for this species. Probit analysis is not acceptable for statistical evaluation in view of this experience-dependent response.

Pigs exhibited signs of ear pain and vestibular disturbance (head shaking, temporary ataxia or loss of balance) almost exclusively during ascent. Such signs were seen to some extent on virtually all ascents, whereas similar signs appeared during only 4 of the 108 descents and in only 2 of the 26 animals. Since man, if he has difficulty at all, typically suffers most during descent, it appears there is a difference in pharyngeal/eustachian/middle ear architecture between the species. We have performed no comparative anatomic study.

Procedures were considered which would alleviate or eliminate this problem, specifically bulla osteotomy and myringotomy. The osseous bullae of swine, however, are difficult to approach surgically, lying medial to the mandibular rami and surrounded by vital structures. Furthermore, they are composed mostly of spongy bone rather than being hollow (as in the dog, for example), so that relief through trephination would certainly jeopardize the auditory ossicles and inner ear structures. Assuming this could be accomplished, maintaining the opening in communication with ambient atmosphere on a long-term basis is fraught with problems.

Myringotomy is a rather simple procedure in most animals and man but is made difficult in the swine because of the peculiar anatomy of their external auditory canal. The canal courses downward, inward and forward from the external meatus, surrounded for the first few centimeters by a cartilagenous tube and for the next few centimeters by bone. The canal then turns inward at an angle of about 60° to its original direction, continuing to the tympanum with its wall surrounded by bone. This angulated osseous canal makes it impossible, for example, to examine the tympanum with ordinary otoscopes. For one to perform a myringotomy without damage to the auditory ossicles (or deeper structures), and be reasonably certain of that fact, major surgery is required. Either a lateral ear resection would have to be performed, cutting away cartilage and bone to expose the entire vertical portion of the canal, or else a large opening would have to be trephined in the bony canal at its angle to enable insertion of instruments. Neither approach seemed attractive in view of the objectives of this research program, and "blind" myringotomies were attempted.

Measurements on carcasses established a total depth from external meatus to tympanum of about 10 cm. Since hot-wire probes of proper flexibility and elasticity were not immediately available, hyfrecation probes were fashioned from spring steel wires and heat-shrinkable tubing. With a 10-cm. depth mark on the probe, it was inserted along the lateral wall of the canal until gentle pressure did not further its progress, at which point it was assumed that the blunted probe tip was against the ventral edge of the tympanic membrane. The probe was then connected to a hyfrecator unit and one to three seconds of high current applied. The



Figure 2. External auditory canal of the miniature swine, following lateral resection. The probe marks the external meatus, and the pointed centimeter rule indicates the beginning of the bony portion of the canal. The horizontal portion of the canal appears about 2 cm deeper, as indicated by the arrow.

procedure was then repeated on the opposite ear, and, in three and ten days, the animal subjected to non-bends producing dives known to produce marked signs of middle ear distress (determined individually beforehand). In all cases, no relief from middle ear distress was seen, and attempts to develop simple myringotomy procedures for diving miniature swine were abandoned in favor of more important program objectives.

All animals which died as a result of their hyperbaric excursion were immediately examined at necropsy. All had widespread and massive gas embolism of both arterial and venous systems, usually to the extent that the blood was foamy in appearance. No grossly visible vessels were free of embolization. One animal, ascitic as a result of caval stenosis under an implanted Doppler flowmeter transducer, was sacrificed immediately after displaying transient postdive lameness in one rear leg (180 feet/30 minutes). No intravascular emboli were grossly visible, but the accumulated ascitic fluid was covered with foam.

Conclusions

If one defines bends in miniature swine as above, the response of that species to simple no-decompression air dives is apparently unlike man except perhaps at depths greater than 140 feet. Although clear signs of bends were produced in 80% of the profiles 100 feet/90 minutes, 140 feet/60 minutes and 180 feet/30 minutes, the miniature swine seems to tolerate dives to 60, 100, 140 and 180 feet for bottom times three or more times longer than corresponding no-decompression limits for man with only temporary signs of distress, if any at all. Approximately 120 planned dives, intended to investigate bottom times at 100, 140 and 180 feet at and below the estimated 50% incidence points, were not conducted because of program revision at this point, and requisite data permitting statistical definition of a 10% incidence exposure for each depth are not available.

Miniature swine commonly exhibit temporary signs of ear distress, ataxia, vomiting and incoordination during hyperbaric excursions, probably because of an inability to voluntarily open the eustachian tubes. That these signs occur almost exclusively on ascent suggests a basic difference in pharyngeal/eustachian/middle ear anatomy when compared to man.

POSTCAVAL GAS EMBOLISM IN MINIATURE SWINE
DURING AND FOLLOWING SEVERE HYPERBARIC EXCURSIONS

Objectives

Though not specifically stated as an objective in the program statement of work, some early chamber dives were performed with miniature swine having a Doppler flowmeter transducer implanted on the thoracic caudal vena cava. The objective was to monitor caval embolism during exposures less severe than those we had studied on internal funding prior to receiving USN support.

Methods

Five of the miniature swine used in the studies summarized in Tables I and II of the preceding section were operated and Doppler flowmeter transducers implanted around the thoracic caudal vena cava through a routine right thoractomy (see Appendix). The transducers, 13 mm I.D., were coupled to the vessel with sterile agar (and, ultimately pleural fluids) and the leads delivered percutaneously at the dorsal midline between the scapulae. Although the transducers seemed to fit well at surgery, early stenosis and closure of the vessel occurred in most animals. The use of larger (16 mm I.D.) transducers is advisable, however, lack of good fluid coupling in the immediate postoperative period precludes early flow detection with such oversized transducers.

The Doppler flowmeter telemetry unit is connected to the percutaneous transducer leads and secured to a lambswool-backed leather harness especially designed for miniature swine.⁽¹¹⁾ The FM signal carrying audio flow information is transmitted from the animal to a receiving antenna within the chamber or through the viewing ports to outside receiving antennae, all antennae connected to the input terminals of a remote FM receiver. Thus the animal is allowed free movement within the chamber as blood flow data is recorded on magnetic tape and monitored aurally.

Results

An anesthetized animal so prepared was moved directly from the operating table to the hyperbaric chamber and subjected to a 180 feet/20 minutes excursion (Rawlins' dive). Severe caval gas embolism was recorded beginning

prior to reaching surface (first bubble heard at approximately 60 feet depth during ascent), becoming progressively more severe until periodic loss of flow signal suggested "foam flow." The animal was sacrificed immediately and gross, widespread venous gas embolism confirmed at necropsy. Arterial embolism could not be confirmed.

A second animal, fully recovered from transducer implant surgery, was similarly prepared and subjected to a 180 feet/15 minutes excursion. Again, caval gas embolism was easily detected, with approximately three emboli/second recorded 10 minutes after reaching surface. No signs of bends were seen. This was repeated one week later using the same animal, with the same results. This time the animal was recompressed to 100 feet and stage decompressed according to standard air treatment tables (schedule 1-A, page 1.6.2, U. S. Navy Diving Manual, 1963⁽⁷⁾). During stage decompression the rate of embolic signals was reduced but not eliminated; typically, flow audio was relatively artifact-free while the animal lay quietly and embolic "showers" followed body movement.

Similar observations were made with one of the other Doppler instrumented animals using the same profile. The remaining two operated animals were lost to the study because of percutaneous lead failure.

Discussion

These findings, coupled with the fact that sixteen of nineteen 180 feet/20 minutes excursions produced no signs of bends, left little room for doubt that rather severe venous embolism can exist in the absence of bends signs. Whether such gross venous embolism can exist in the absence of bends symptoms remained (and remains) unknown, however, subsequent experiments on pulmonary artery embolism during much milder hyperbaric excursions suggests quite strongly that it does. These experiments are discussed in the following section.

Conclusions

Miniature swine can suffer severe gas embolism of the thoracic caudal vena cava, hence of the pulmonary artery, without signs of bends. Marked embolism of this vessel is noted in miniature swine performing 180 feet/15 minutes no-decompression profiles, less severe than the 180 feet/20 minutes profile reported as causing a 12% incidence of bends in Royal Navy divers.



Figure 3. Doppler instrumented miniature swine in hyperbaric chamber, showing girth harness holding telemetry unit.



Figure 4. Doppler instrumented miniature swine free-ranging after hyperbaric excursion in chamber, showing girth harness holding telemetry unit.

PULMONARY ARTERY EMBOLISM IN MINIATURE SWINE
UNDERGOING MILD NO-DECOMPRESSION HYPERBARIC EXCURSIONS

Objectives

According to sponsor revision of experimental plan midway through the second fiscal year, work to that point was to be discontinued and efforts made to study the relationship between pulmonary artery gas embolism and various profiles, beginning with definition of the no-decompression limits for miniature swine at 100 and 180 feet depths using sustained pulmonary artery gas embolism as the end point indicator. It was well established by that time (see preceding section) that rather severe embolism could exist in the absence of clinical signs, and it was presumed that a no-decompression curve more similar to that of man might result if pulmonary embolism, rather than clinical signs, were the basis for establishing "safe" limits. On completion of this task, it was suggested to extend the data to 140 and 60 feet depths and, using profiles producing mild embolism, investigate the efficacy of recompression (according to standard treatment tables) in reducing or eliminating embolism. Obvious extensions of this work were to include the development of air treatment tables based on pulmonary artery embolic status in the miniature swine.

Methods

A total of five miniature swine were operated and Doppler transducers implanted around the pulmonary artery via left thoracotomy through the fifth or six intercostal space (see Appendix). Following three weeks recovery, simulated air dives were performed with unanesthetized animals to depths of 100 and 180 feet for varying bottom times as pulmonary flow signals were telemetered from within the chamber to a remote FM receiver and recorded on magnetic tape. Ascent and descent rates were always 60 feet/minute; surface intervals were always 48 hours or more.

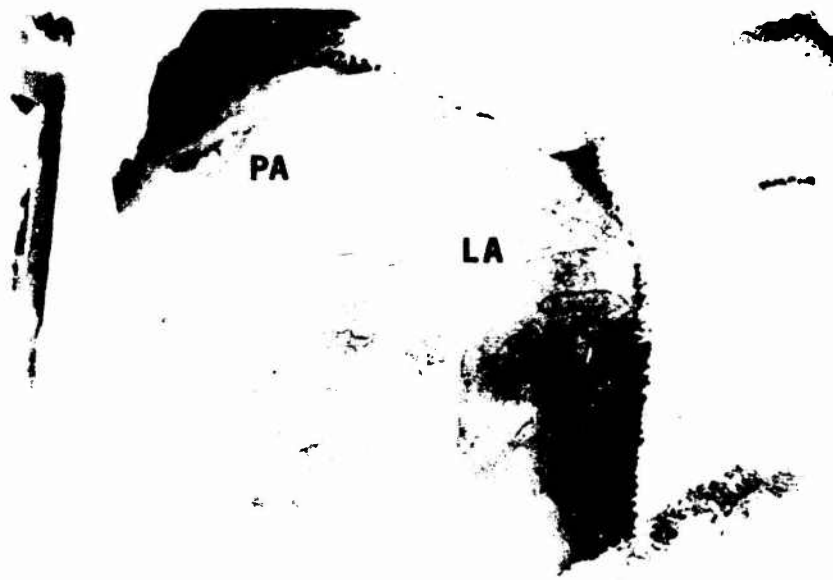


Figure 5. Surgical exposure of base of heart through left thoracotomy, prior to incising pericardium.



Figure 6. Surgical exposure of base of heart through left thoracotomy, following incision and reflection of pericardium. The pulmonary artery (PA) and left atrium (LA) are the main structures presented. The pulmonary artery originates just below the pulmonary valve region (PV).

Results

Ten simulated dives were performed by one animal and five with another. One was sacrificed during surgery following pulmonary artery rupture, a second died about 8 hours postsurgery of unknown causes, and a third recovered uneventfully but did not produce usable Doppler signals (intermittent open circuit subcutaneously). A rather high-pitched flow audio in the two successful preparations indicated a degree of stenosis under the flow transducer, which in fact increases the sensitivity of the instrument to embolus passage: a larger percentage of bubbles must pass through the acoustic field of the transducer, and smaller bubbles occupy a larger proportion of the flow stream than if the artery is not so constricted.

The first series of dives was terminated because of deteriorating flow signal quality suggesting transducer failure coupled with progressive pulmonary artery stenosis. The latter preparation failed due to broken percutaneous leads below skin level (found at subsequent necropsy to be at the level of the rib cage). The results of these 15 trials are summarized in Tables III and IV.

Discussion

It is immediately noted that pulmonary embolism occurred following exposures which, as far as miniature swine and bends signs are concerned, were extremely "safe." Furthermore, embolism was produced by 100-foot dives having bottom times as short as 5 minutes, one-fifth the no-decompression limit for man at this depth! While we expected to detect embolism at bottom times comparable to or slightly less than the human no-decompression limit, such extreme results were not anticipated. It is further noted that the 100 feet/20 minutes profile, considered "safe" for human divers, produced moderately severe pulmonary embolism in the miniature swine.

Although the animals typically exhibited signs of ear distress during ascent, no signs of bends were ever seen throughout the experiments. Pulmonary stenosis, which progressively altered the average flow velocity as indicated by the flowmeter signal, was eventually confirmed at necropsy: The lumen diameter of the pulmonary artery was reduced to about half its normal

TABLE III. SUMMARY OF TEN DIVES: PULMONARY GAS EMBOLISM, ANIMAL 3586

Dive	Depth (Feet)	Bottom Time (Minutes)*	Pulmonary Artery Gas Embolism	Remarks
1	180	6	+++	Many large bubbles heard. Three hours later, bubbles heard only if animal coaxed to move about.
2	180	3 ("bounce")	--	Predive monitoring with animal moving about revealed no persistent embolism from 5 Jan.
3	180	6	+++	Performed 15 minutes after bounce dive; to confirm gross embolism detected 5 Jan. First bubbles easily detected at 30 feet depth during ascent.
4	100	20	+++	Frequent bubbles with "showering" following body movements.
5	180	4.5	+	Only occasional bubbles heard, only following body movements. Could detect none 5 minutes after surfacing.
6	100	10	++	Seeking bottom time threshold for 100 feet depth.
7	100	5	--	Pig fell during ascent and flowmeter leads broke. Hookup delayed monitoring until 5 minutes after reaching surface.
8	100	7	++	Signal loss during ascent; bubbles heard immediately on reaching surface.
9	100	5	++	Bubbles heard at 40 feet during ascent; continued for over 8 minutes, at which time flowmeter disconnected.
10	100	5	+	To resolve difference between 22 Jan and 27 Jan runs: bubbles definitely heard.

* Bottom time defined as time between beginning descent and beginning ascent. Ascent and descent rates 60 feet/minute.

TABLE IV. SUMMARY OF FIVE DIVES: PULMONARY GAS EMBOLISM, ANIMAL 3829

Dive	Depth (Feet)	Bottom Time (Minutes)*	Pulmonary Artery Gas Embolism	Remarks
1	100	5.5	--	No bubbles heard during subsequent 2 hours monitoring.
2	100	7	++	Frequent bubbles heard during body movements; none heard if animal lying still.
3	180	3 ("bounce")	--	Occasional periods of noisy signal, but no embolic artifacts detected.
4	180	4.5	+	Poor signal quality, but bubble artifacts heard during animal movements. Similar to dive #5, animal 3586 (Table III).
5	180	5	+?	Very poor signal quality with brief periods of clear Doppler audio depending on attitude of animal; replaying of tape strongly suggested occasional embolic artifacts during some of these relatively noise-free periods.

* Bottom time defined as time between beginning descent and beginning ascent. Ascent and descent rates 60 feet/minute.

value under the flow transducer. One attempt to avoid this by using an oversized transducer (22 mm I.D.) was thwarted when the transducer body broke during implantation and another of that large size was not immediately available. This stenosis produced no discernible refractile signs, however, and--since pre-dive flow signals were clear--could not have been the source of the artifacts so typical of gas embolism.

It was intended to begin with mild profiles which did not produce detectible pulmonary embolism and "work the animal up" to bubble-producing exposures, establishing a no-decompression threshold for pulmonary artery embolism in "worked up" miniature swine. Since the pulmonary artery carries total venous return, it is reasonable to assume that venous embolism, if it occurs anywhere in the body and the emboli are not subsequently resorbed, will be most sensitively detectible in that artery. Such logic is supported by findings elsewhere.⁽¹²⁾ This, coupled with the accidental pulmonary stenosis increasing instrument sensitivity to bubble passage, perhaps accounts for our misjudgement. The starting exposures agreed upon by the sponsors and BNW investigators turned out, to our surprise, to produce embolism.

There is very little doubt that the audio artifacts heard on the Doppler signal are due to gas emboli. Relatively large masses of fat or blood clots would be required to produce such distinct and characteristic artifacts against a blood flow background, and it is difficult to see how, in repeated dives on the same animal, such severe embolism by particles other than gas bubbles could exist without producing clinical effect or enduring lesions recognizable at necropsy, neither of which has been seen. Nonetheless, it must be admitted that we have not proved that the artifacts detected are indeed gas bubbles, and Smith and Johanson⁽¹²⁾ have theorized that delayed Doppler artifacts, at least, i.e. those appearing hours after surfacing, are intravascular clot emboli. Whatever the Doppler artifacts are, however, they are not there prior to decompression.

One can hardly escape the suspicion, if not the conclusion, that similar embolism must exist in human divers undergoing no-decompression excursions which are considered "safe." There is virtually no doubt, therefore, that caval embolism produced by decompression can exist in man in the absence of

bends symptoms, and that it probably always does whenever that man has experienced a no-decompression dive to 100 feet or more on the no-decompression limit curve. However, of course, this has yet to be verified and its significance evaluated.

The problem to date in verifying or refuting such conjectures in human subjects is one of instrumentation, discussed later in this report. Until this situation is improved, an expedient might be found in correlating pulmonary embolic status of large experimental animals with bends incidence in human divers undergoing identical hyperbaric excursions. Embolism in an animal of similar size and cardiovascular makeup may indicate that the same thing is happening (or about to happen) in his human chambermate.

Conclusions

Threshold bottom times (time between beginning descent and beginning ascent, ascent and descent rates 60 feet/minute) for pulmonary artery gas embolism in miniature swine, using the Doppler flowmeter as a detector, are approximately 4 minutes at 180 feet and 5 minutes at 100 feet. This conclusion must be qualified by recognizing that (1) only two animals were involved in the experiments, and (2) the emboli detected have not been directly proved to be gas bubbles. The likelihood that similar embolism exists in human divers undergoing marginally "safe" no-decompression dives is extremely strong.

DOPPLER FLOWMETER STUDIES

The Doppler flowmeter system evaluated in these studies was of the Franklin type,⁽⁸⁾ using 13 mm I.D. perivascular transducers with a nominal crystal resonance at 10 MHz. The flowmeter electronics (crystal exciter, tuned radio frequency receiver/demodulator, frequency modulated transmitter) were copies of types in use at Franklin's laboratory at Scripps Clinic and Research Foundation, La Jolla, California, purchased by BNW for earlier research programs from Parks Electronics Laboratory, Beaverton, Oregon. A superior Doppler flowmeter having a modified exciter section was later purchased from Pierson Laboratories, San Diego, California, on funds provided by Contract No. N00014-69-C-0350 (ONR). The transducers, polystyrene molds containing PBZ-10 crystals with PVC-insulated 26-gage stranded copper leads, were copies of those in use by Franklin and purchased by BNW from Nortec (Division of Scientific Advances, Inc.), Richland, Washington.

Threshold for Embolus Detection

Objectives

The objective was to determine the approximate threshold bubble size detectible by the unmodified Doppler flowmeter under in vivo conditions.

Methods

Graded microbeads and microballoons of glass were injected via the femoral vein of anesthetized miniature swine on whose thoracic caudal vena cava had been implanted a 13 mm I.D. Doppler flowmeter transducer. The microbeads and microballoons were suspended in physiological saline and injected at rates which, except for the smallest group (37-43 μ m diameter), produced individual Doppler "chirps." Practical procedure difficulties were encountered (electrostatic clumping of the particles, catheter blockage, retention of particles in the catheter or veins by adhesion to the walls, presence of microscopic gas bubbles in the carrier fluid) but were solved for the most part. Frequent injections of saline alone were made to produce "control signals" (verifying that sounds heard were actually due to

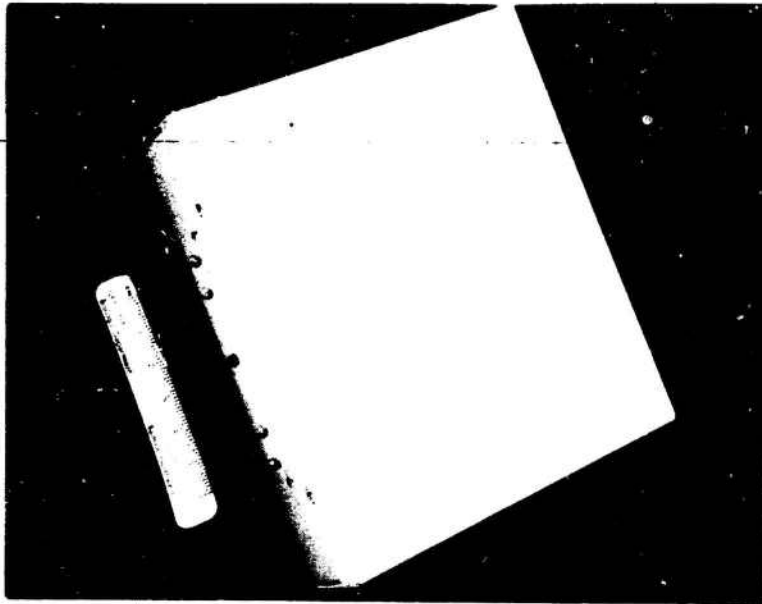


Figure 7. Doppler flowmeter telemetry unit, Parks Electronics Laboratory type (purchased 1967). Case holds batteries.

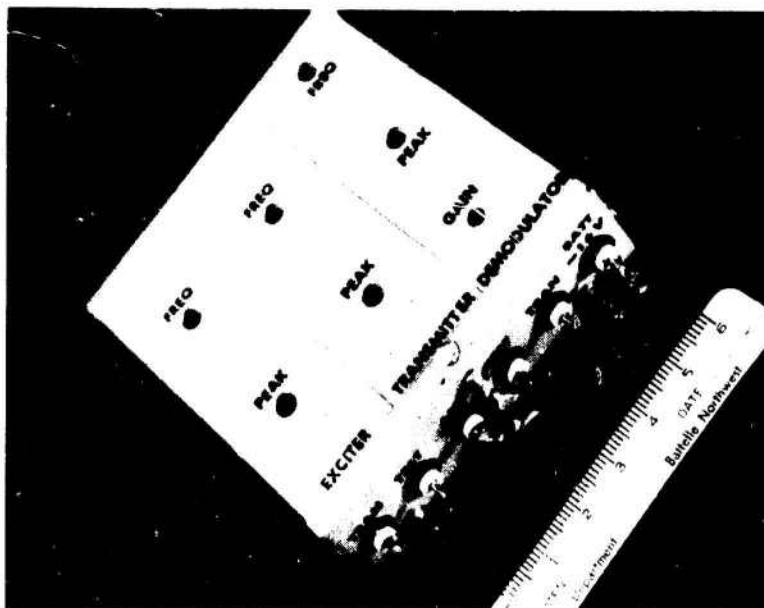


Figure 8. Doppler flowmeter telemetry unit, Pierson Laboratories type (purchased 1971). Case does not hold batteries.

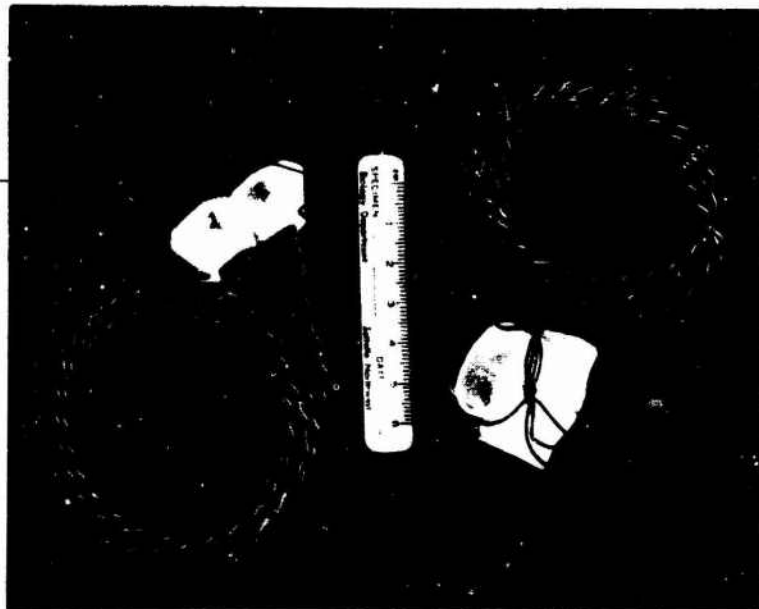


Figure 9. Doppler flowmeter transducers, perivascular type, with polystyrene molded bodies and PVC-insulated leads, for surgical implantation on selected vessels for flow measurement and embolism detection.



2 3 4 5 6 7 8 9

Figure 10. Doppler flowmeter transducer, transcutaneous type, with machined lucite body and PVC-insulated leads, for atraumatic (external) sensing of blood flow and embolism.

particles) and to wash away adherent particles. All batches of graded beads/balloons were characterized by measurement of large numbers using a microscope with a reticule eyepiece, and ranged from 37-43 μm to 400-550 μm diameter.

Results

It was noted early that solid glass microbeads did not produce a Doppler signal as large as hollow glass microballoons of the same size range, indicating forced oscillation and reradiation of ultrasound from the hollow particles was in play (though not necessarily resonance). Under physiologic conditions of caval blood flow, individual glass microballoons of 80-150 μm diameter were very easily detectible in the caudal vena cava using a 13 mm I.D. perivascular transducer, producing large, obvious, individual "chirps" on the blood flow audio background. Microballoons of diameter in the vicinity of 40 μm were virtually impossible to detect individually in the presence of blood flow. Injected in concentrated batches, however, their passage through the transducer field is clearly signalled by an increase in flow volume and a "ragged" quality to the audio signal.

Discussion

The most serious defect in these experiments, of course, is that glass balloons or beads were used instead of actual gas bubbles. Resonant frequency of these particles was not determined and, in any event, would not have been very precise due to size distribution in each graded batch (histograms ranged from left- to right-skewed Gaussians with typical half-maximum widths of 20%) and microscopically visible irregularities in balloon wall thickness. Although we know we are far from the resonant frequency of similarly sized gas bubbles (approximately 0.6 μm diameter for resonance at 10 MHz, resonant frequency for 100 μm bubbles in blood ca. 0.8 MHz at one atmosphere absolute ambient pressure), we do not know how far we were from resonance of the glass microballoons. Thus, it is impossible, from these experiments, to state with confidence the threshold for gas bubble detection in vivo. It must be kept in mind, of course, that threshold size decreases with vessel size, since an embolus of given size will occupy a larger proportion of the blood

flow stream in the transducer field. This further complicates the problem of defining threshold size of gas bubble detection by Doppler flowmetry.

Conclusions

Using a 10 MHz Doppler flowmeter system with a 13 mm I.D. perivascular transducer on the thoracic caudal vena cava of miniature swine, glass microballoons of diameter 80-150 μm are easily detected as individual artifacts on the blood flow background signal. Glass microballoons smaller than about 50 μm diameter cannot be individually detected under such conditions. No extrapolation of these results to gas bubbles in vivo can be made, however, it is conjectured that those results would not differ greatly.

The Doppler Flowmeter as a Bubble Sizer

Objectives

The objectives were to determine whether existing Doppler flowmetry equipment can be used to give an estimate of gas embolus dimensions, first through in vitro studies, then in acute in vivo preparations, and finally in unanesthetized instrumented animals during actual decompression.

Methods

Graded microballoons were used in bench experiments to determine first whether a correlation exists between embolus size and the Doppler signal it produces. Filtered, outgassed 35% glycerin solution (having rheologic similarities to whole blood but producing no background flow signal) was flowed through silastic tubing on which had been placed a perivascular Doppler flowmeter transducer (13 mm I.D., 10 MHz nominal resonance). Gravity flow was first used, replaced later by more satisfactory apparatus consisting of a two-fingered roller pump which produced nearly nonpulsatile flow in closed loops of silastic tubing containing the glycerin solution and microballoons. Because of the difficulty of outgassing the fluid in the silastic tubing, several loops were made with each loop containing a specific grade of microballoon from 37-43 μm to 400-550 μm diameter. Ordinary centrifugal or other constant flow pumps proved unsatisfactory

because of vibrations transmitted to the Doppler transducer through the silastic tubing and fluid, however, the roller pump produced clear signals with negligible background noise. These signals were displayed on an oscilloscope and recorded on magnetic tape. In addition, a half-wave rectifying circuit was used to display the envelope of each microballoon signal on a fast-response strip chart recorder, the area under which was proportional to the total energy scattered by the "embolus".

A variance in signals corresponding to the variance in microballoon size within each graded batch was anticipated, however, it was soon evident that much larger variations existed. In fact, a large number of observations on a single microballoon in its own closed silastic loop confirmed that both maximum Doppler signal amplitude and signal energy in each "chirp" varied from some maximum value to vanishingly small. Furthermore, the distribution of values was so broad that no useful correlation could be made between one size batch and another. Signal characteristics were dependent on other factors such as flow velocity, as is discussed later in this section.

It was decided to further constrain microballoon passage through the transducer field, since the Doppler signal will obviously be different depending on where in the ultrasonic field the particle passes and whether it moves through this field at some angle with the axis. Thick-walled tubing (12 mm O.D., 2 mm I.D.) was interposed in the silastic loop, ensuring that the microballoons would travel near and parallel to the axis of flow and through the center of the common acoustic field of the two transducer crystals. Again, however, the signals produced were of widely varying size.

Microscopically, it was known that the microballoons differed not only in size within a graded batch but in shell thickness. Also, shell thickness was not always uniform over an individual microballoon. Suspecting irregular scattering characteristics due to these variations and assymetries, apparatus was constructed which produces gas emboli of known and constant size, ejecting them at regular intervals on the axis of a moving fluid stream.⁽¹³⁾ At this point in time, however, the experimental plan was revised and no further work on Doppler bubble sizing was performed.

Results

A great number of practical problems in attempting to use the Doppler flowmeter as a bubble sizer were identified, not all of which can be eliminated with existing Doppler equipment. Complicating factors encountered during these experiments included:

1. Artificial emboli (plastic or glass beads or microballoons) have a distribution in size about a mean value depending on how much time and expense is devoted to producing finely graded batches; histograms on several batches ranged from left- to right-skewed Gaussians with half-maximum widths of ca. 20%. Furthermore, microballoons generally have varying wall thickness and individual assymetries which can influence scattering.
2. Beads or balloons tend to clump together due to electrostatic effects; this is easily minimized by prewashing with detergent.
3. The common acoustic field of the two Doppler transducer crystals is an ill-defined region in the vicinity of the vessel axis; all other things equal, like size bubbles traversing the vessel will produce different signals depending on their location in the lumen. Related to this is the fact that solid microbeads, hollow microballoons, and probably gas emboli are influenced by gravitational effects and tend to roll along vessel walls at low flow rates.
4. Bubbles may pass through the Doppler transducer field at angles to, rather than parallel to, the lumen axis. The component of velocity toward either crystal is therefore variable even at constant flow rates and, as covered in the next paragraph, this affects signal size.
5. The tuned radio frequency receiver in existing Doppler flowmeters does not have a flat frequency response curve; it is not designed to be an "amplitude" device, since flow velocity is related only to the frequency of the audio output. Thus, the size of signal produced by a moving embolus varies with flow velocity.

6. Exciter output and TRF receiver gain vary with supply voltage; peak-to-peak amplitude of the final audio output may vary up to 8% with only a 3% change in supply voltage. Thus, batteries are unsatisfactory and must be replaced with a well-regulated low voltage power supply, sacrificing the convenience of portability.
7. Exciter frequency also changes with supply voltage; proportionate changes in the Doppler shift frequency will also occur. This effect is negligible when using the instrument as a blood flowmeter, however, it may complicate attempts to relate spectral characteristics of the audio with bubble size.
8. Capacitive coupling exists between the transducer leads to a great extent, so that slightly different lead orientations will affect flowmeter output. Shielded leads or coaxial cables, besides reducing power to the exciter crystal and TRF receiver input, do not eliminate this problem entirely.
9. If a telemetric flowmeter is used, variations in the gain of the transmitter and FM receiver will affect signal size. In such systems it is also necessary to flatten the overall response curve by adjustment of the receiver controls.
10. When working with very small (under 50 μ m) beads/balloons/bubbles, the water/glycerin carrier must be carefully filtered and out-gassed to eliminate false signals due to microscopic gas bubbles.

Discussion

Some of the complicating factors above can be controlled in vitro, however, some cannot. In vivo, of course, only a few can be controlled (e.g. items 6, 7, 9), many cannot (e.g. items 3, 4, 5, 8). The most obvious new complication which arises in vivo is the presence of a large blood flow background signal. Parenthetically, it has been verified that the ear can discriminate better than moderately sophisticated electronics equipment--bubbles can be heard in the presence of blood flow which are not visually detectible in the audio signal displayed on a memory oscilloscope screen.

Obviously there is little sense in constructing elaborate apparatus for the sole purpose of avoiding in vitro problems which cannot be avoided in vivo. While redesigned Doppler instrumentation and more sophisticated signal analysis apparatus could eventually prove that Doppler-shifted ultrasound scattered from emboli may give reasonable estimates of their size, the effort and expense might be better devoted to the development of alternate methods of bubble sizing.

Conclusions

Existing Doppler flowmetry equipment is unsatisfactory for the estimation of gas embolus size in vivo. The advisability of modifying this instrument for that purpose--rather than developing other methods--is questionable.

Pulse-Echo Resonance: An Alternative Method of Gas Embolus Detection

Theoretical analyses⁽¹⁴⁾ investigating the Doppler flowmeter limitations demonstrated that a resonance pulse-echo technique is several orders of magnitude more sensitive for bubble detection than is the Doppler technique. The bases for this result are the great increase in scattering of sound by resonant (oscillating) bubbles^(15,16) and the $1/\lambda^4$ (Rayleigh) dependence of scattering on the wave length of incident sound.^(17,18,19)

The scattering cross section for flexible spheres is^(18,20)

$$\sigma_s = \frac{64\pi^5 r_0^6}{9\lambda^4} \left[\frac{\bar{x} - x}{\bar{x}} + \frac{3\bar{\rho} - \rho}{2\bar{\rho} - \rho} \cos \theta \right]^2$$

where

- r_0 = radius of scattering sphere,
- \bar{x} = adiabatic bulk modulus of internal medium of sphere,
- x = adiabatic bulk modulus of suspending medium,
- $\bar{\rho}, \rho$ = densities of sphere and medium, respectively, and
- θ = angle between incident and scattered wave.

Erythrocytes are biconcave discs ca. 4 μm in radius, randomly oriented in blood. Assuming they behave approximately as spherical scatterers, the above formula can be used to establish estimates of background "noise" limiting the threshold size for bubble detection. For gas bubbles in blood, near resonance, the scattering cross section is⁽¹⁶⁾

$$\sigma_{\text{res}} = \frac{4\pi r_0^2}{\left(\frac{f_0^2}{f^2} - 1\right)^2 + K^2}$$

where

K = damping constant,⁽¹⁷⁾ the value of which varies from 0.03 to 1.0 as a function of frequency,

r_0 = "resonant bubble" radius, and

f_0 = resonant frequency

The resonant frequency is related to the oscillating bubble radius r_0 by $f_0 r_0 = 326$ cm/sec for air bubbles in water at one atmosphere pressure (simplified Minnaert formula⁽¹⁵⁾).

The results of calculations based on the above formula and supporting information^(15,16,17,18,19,20) are summarized in Table V.

TABLE V. COMPARISON OF NONRESONANT AND RESONANT SCATTERING OF ULTRASOUND BY RED CELLS AND BY ONE GAS BUBBLE, IN BLOOD

Frequency	10 MHz		0.815 MHz (Resonant Frequency)	
	Red Cells at $5.8 \times 10^8/\text{cm}^3$	One 4 μm Air Bubble	Red Cells at $5.8 \times 10^8/\text{cm}^3$	One 4 μm Air Bubble
Scattered Intensity	$2.9 \times 10^{-3} I_0$	$1.8 \times 10^{-10} I_0$	$1.28 \times 10^{-7} I_0$	$1.06 \times 10^{-4} I_0$
<u>Signal</u> "Noise"	0.62×10^{-7}		0.83×10^3	

With decreasing frequency f , the scattering of red cells in blood decreases as f^4 , while simultaneously scattering of small bubbles approaches resonance. The great increase in bubble detection sensitivity and the capability of increasing the signal (bubble) to noise (blood cells) ratio many orders of magnitude suggests that a pulse-echo technique using frequencies far below that of existing Doppler equipment would enable detection of gas emboli in vivo which are not detectable with existing equipment. Although these theoretical predictions have not been experimentally confirmed, they form a sound basis for encouraging development work along these lines.

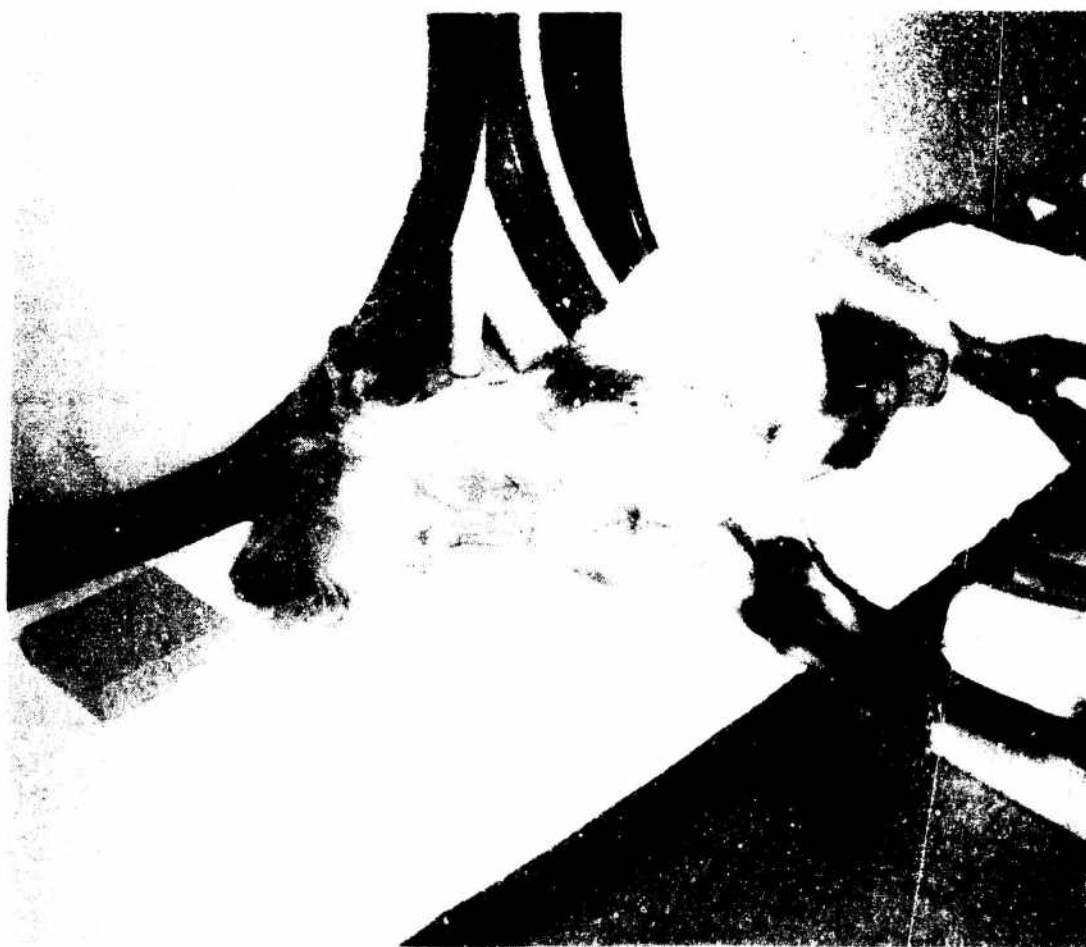


Figure 11. Transcutaneous telemetric Doppler flowmetry used on anesthetized pigmy goat during hyperbaric excursion, with transducer monitoring jugular vein.

REFERENCES

1. Fryer, D. I., presented to the AIRCENT Medical Congress, Fontainebleau, December, 1962.
2. Gillis, M. F., et al., Nature 217: 9 March, 1968.
3. Gillis, M. F., et al., Science 161: 9 August, 1968.
4. Spencer, M. P., et al., Bull. Mason Clinic 22: March, 1968.
5. Gillis, M. F., et al., J. Occup. Med. 11: May, 1969.
6. Bustad, L. K., et al., Swine in Biomedical Research, Pacific Northwest Laboratory, Richland, Washington. Lib. Cong. Cat. Card 66-23515. Frayn Printing Co., Seattle, Washington, 1966.
7. U. S. Navy Diving Manual, NAVSHIPS 250-538, Superintendent of Documents, U. S. Government Printing Office, Washington, D. C., 1963.
8. Franklin, D. E., et al., Amer. J. Electronics 5: 1966.
9. Gillis, M. F., Proc. Ann. Conf. Eng. Med. Biol. 9: November, 1967.
10. Spencer, M. P., et al., J. Occup. Med. 11: May, 1969.
11. Karagianes, M. T., J. App. Physiol. 25: November, 1968.
12. Smith, K. H., et al., Technical Report on Contract (ONR) N0014-69-C-0402 to the Office of Naval Research, June, 1970.
13. Carstensen, E. L., et al., J. Acous. Soc. Am. 19: 1947.
14. Spalek, J., Battelle-Northwest internal report on detection and nature of intravascular bubbles associated with decompression, 1971, Pacific Northwest Laboratory, Richland, Washington.
15. Horton, J. W., Proc. Ann. Conf. Eng. Med. Biol. 10: 1968.
16. Urick, R. J., Principles of Underwater Sound for Engineers, McGraw-Hill, 1967.
17. Reid, J. M., et al., Eighth Int. Conf. Biomed. Biol. Eng.: July, 1969.
18. Rschevkin, S. N., A Course of Lectures on the Theory of Sound, The Macmillan Co., 1963.
19. Morse, P. M., et al., Theoretical Acoustics, McGraw-Hill, 1968.
20. Gosling, R. G., et al., Conference papers, Ultrasonics for Industry 1969, London, October, 1969.

APPENDIX

THORACOTOMY IN THE MINIATURE SWINE

APPENDIX

THORACOTOMY IN THE MINIATURE SWINE

Selected animals, preferably females or early-castrated males in the 100-170 lb. weight range, are fasted not less than 24 hours prior to surgery. No preanesthetic medication is necessary, although certain phenothiazine-derivative tranquilizers (e.g. Acepromazine^r maleate, Ayerst Laboratories, Inc., N. Y.) intramuscularly in low doses may make the animal easier to handle where experienced personnel are not available. Generally, however, such drugs should be avoided. Their effect in swine is somewhat unpredictable, particularly if given to excess, and certain "calming" drugs used on other species are not safe (notably morphine and phencyclidine hydrochloride, the latter better known as Sernylan^r, an excellent hypnotic for primates).

The animal is restrained in dorsal recumbency, preferably in a trough designed for this purpose, and a paramanubrial puncture of the anterior vena cava performed with a 3" 15-gage thinwall needle connected to a syringe containing 4% thiamylal sodium (Surital^r, Parke-Davis, Detroit). This is given to effect (10-15 cc/100 lb.), the syringe removed from the needle, and the vein catheterized with polyethylene tubing (Polymedic^r PE 90, Clay-Adams, Inc., N. Y.). Atropine (Tromet^r, atropine methyl nitrate, Amco Drug Prod. Co., Inc., North Olmstead, Ohio) is given via the catheter and a syringe containing thiamylal sodium attached to maintain light anesthesia during the surgical preparation period.

The lateral chest is close clipped, vacuumed and scrubbed, after which the animal is moved to the operating room and placed on the operating table (adjustable through-type) in dorsal recumbency. With a long-blade laryngoscope the trachea is intubated with a tracheal catheter having an inflation cuff. Infating the cuff, the animal is reoriented in lateral recumbency, the legs bound loosely to the table, and the venous catheter connected to an intravenous drip bottle containing fluids appropriate to the occasion. For relatively uncomplicated operations, such as implanting Doppler transducers on the vena cava, aorta or pulmonary artery, physiological saline is usually sufficient, though one may wish to switch to dextran if the operation

becomes prolonged. Surgical anesthesia is attained during final skin field preparation (antiseptic wash) and draping, for which halothane/oxygen has proved to be an excellent mixture.

Except in mature boars, old or fat animals, one can usually locate the caudal limit of the scapular cartilage by palpation. A short, deep incision in this vicinity is made, serving to test the depth of anesthesia and verify that the surgeon is starting in the right spot. Assured that the incision lies over the dorsocaudal limit of the scapula, the surgeon continues the incision caudoventrally parallel to the underlying ribs to the level of the costochondral junction. Continuing deeply, this approach exposes ribs 6-8, any of which may then be removed from its periosteum and excised for a wide exposure of the central thorax, appropriate for access to both the aorta and posterior vena cava if the thoracotomy is performed through the left side. To expose the base of the heart (pulmonary artery) the initial incision should be made one or two ribs forward (on the left side), so that the dorsal limit of the incision is limited by the presence of the scapula. One need not remove a rib to gain sufficient field for pulmonary artery isolation. The posterior vena cava can be reached through either a left or right thoracotomy.

The posterior vena cava is easily isolated from its mediastinal membrane. The aorta may be isolated by careful dissection, ligating and transecting the required number of pairs of intercostal branches which leave its right dorsal surface and other small variables, the most troublesome of which being a small bronchial branch leaving its right ventral aspect near where the pericardium and aortic adventitia become contiguous. The hemiazygos vein, seen crossing the left aspect of the aorta about 8-10 cm distal to the aortic arch, may be sacrificed without detrimental effect, as may not less than six pairs of intercostal arteries. In this manner it is not difficult to isolate up to seven inches or more of thoracic aorta.

The pulmonary artery presents itself as the largest vascular structure in the field, curving over the crown of the heart away from the surgeon. The pericardium is liberally incised and reflected, and the left atrium retracted caudally to expose this vessel for isolation. Gentle and careful sharp dissection is required to separate the pulmonary artery from its

attachment to the underlying epicardium from near its origin to the aorta, during which rough technique can result in a virtually irreparable tear in the pulmonary artery wall.

The pericardium should be closed to prevent postoperative strangulation of the left atrium, however, no other internal repairs need be made before closing the chest wall. Adjacent ribs are pulled together and fixed with three loops of 28-gage surgical stainless steel. Subcutaneous tissues are closed with 3 non-boilable medium chromic gut and the skin edges approximated with any suitable suture material. Prophylactic administration of antibiotics is advisable if permitted by experimental procedure.

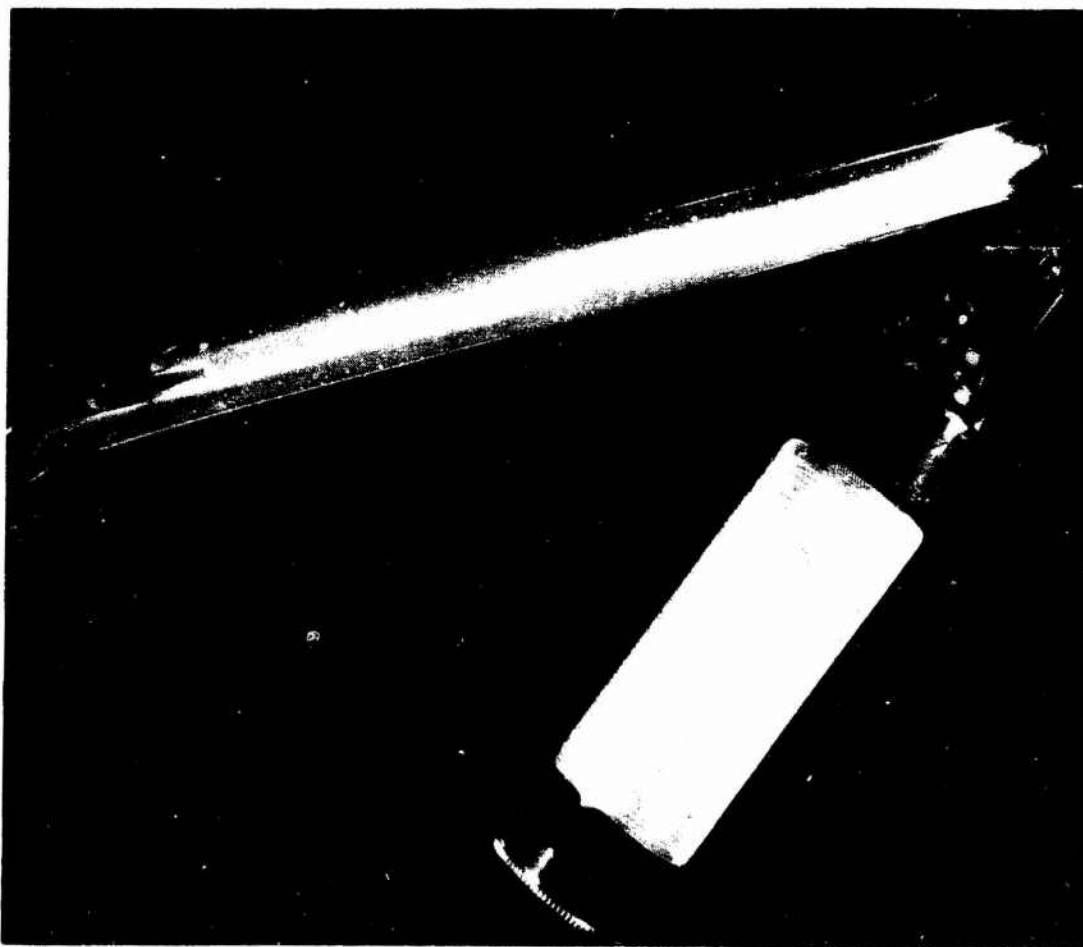


Figure 12. Long-blade laryngoscope specially constructed for use on miniature swine.