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BREATHING OF PRESSURE OXYGENATED FLUIDS BY SUBMERGED MAMMALS: A LITERATURE REVIEW

by

P. L. Hendricks

LT MC USNR

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Research Work Unit MF022.03.03-9025.28

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Gerald J. Duffner, CAPT MC USN
COMMANDING OFFICER

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Philip L. Hendricks

Lieutenant, MC, U. S. Naval Reserve

SUBMARINE MEDICAL RESEARCH LABORATORY

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Submitted by:

Thomas N. Markham
LCDR, MC, USN
Head, Military Operations Branch

Reviewed and Approved by:

Charles F. Gell, MD, DSc(Med)
Scientific Director
Submarine Med. Research Lab.

Approved and Released by:

Gerald J. Duffner
Captain, MC, USN
COMMANDING OFFICER

SUMMARY PAGE

THE PROBLEM

To review the literature of work done on submerging lower mammals in liquid to perfect a technique whereby such animals could survive a period of immersion, with the idea that such liquid breathing might possibly become feasible for men.

FINDINGS

The work of twenty-three authors is reviewed and summarized. It is concluded that while the research done up to this point presents very interesting possibilities for the future, these possibilities are limited by too many serious problems (poor CO_2 diffusion, fluid and electrolyte imbalance, fatigue, etc.) to hope for any practical application to man in the near future.

APPLICATIONS

If a feasible technique could be worked out for animals to successfully breathe liquids, it should be possible to extend the process to humans and thus overcome many of the problems of diving compressions and decompression.

ADMINISTRATIVE INFORMATION

This investigation was carried out by the author and reported in partial fulfillment of the requirements for qualification as a Qualified Submarine Medical Officer. His thesis was selected for publication at this time as a Submarine Medical Research Laboratory Report in order to make this information readily available in the literature of Submarine Medicine and for use in the School of Submarine Medicine at the Submarine Medical Center. It has been designated as SMRL Report No. 518, MF022.03.03-9025, and is the 28th report on that Work Unit.

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ABSTRACT

The author reviews the literature on fluid respiration and reports on the publications by twenty-three researchers, describing the various experiments performed, results obtained, problems encountered and possible future applications of this work.

It appears that certain investigators became interested in the mechanism of death by drowning. Work progressed from this to purposely submerging animals in liquids to perfect a technique whereby the animals could survive a period of immersion. The object of this work was to test the feasibility of man's breathing liquid and thus overcoming many of the problems of diving compression and decompression. As work progressed, problems began to arise—poor CO_2 diffusion, fluid and electrolyte imbalance, fatigue, et cetera—which would appear to severely limit the usefulness to man of such a system. More work is needed in order to overcome these major problems before man can apply them to himself if, indeed, he can ever hope to do this.

BREATHING OF PRESSURE OXYGENATED FLUIDS BY SUBMERGED MAMMALS: A LITERATURE REVIEW

Work on the subject of fluid respiration began as an outgrowth of studies on the mechanism of death by drowning. The asasumption that acute death is primarily due to asphyxia was unproved. During drowning, fluid may enter into the air spaces of the lung. Transfer of water and solutes across the alveolar walls would cause alteration in the volume of composition of the body fluids incompatible with life. Recognition of this led to a distinction between drowning in fresh water and in sea water.[1] However, it was not known to what degree fluids aspiration contributed to death or how the chances for survival from near drowning in fresh and salt water differed.

In 1960 den Otter[2] and co-workers attempted to assess the relative importance of anoxia and the sequelae of entry of water into the lungs during drowning in fresh water. In dogs one lung was filled with tap water; the other was ventilated with oxygen to prevent hypoxia. The animals remained in good general condition and the arterial and venous blood pressure remained stable as long as a normal arterial oxygen saturation was maintained. Most of the water introduced into the lung remained confined to the lung tissue as interstitial edema and alterations in plasma electrolyte concentrations were not very marked. The authors concluded that death due to drowning in fresh water is primarily a result of anoxia. However, transfer of water and solutes across the walls of fluid filled alveoli may differ from the transfer occurring during actual drowning. Shunting of pulmonary blood flow through the ventilated lung would tend to diminish such a transfer while gravity would tend to promote a flux of fluid from the air spaces into the lung vessels of an animal not submerged.

Kylstra [3, 4] in 1961 reported on an experiment in which the effects of anoxia and of the presence of fluids in the lungs were observed separately in animals submerged in fluids with different oxygen content under otherwise similar conditions.

Adult, random bred "Swiss" mice of either sex were submerged in tap water, isotonic saline, or water from the North Sea all at 23 to 25°C. The animals were not anesthetized. The interval between submersion and the last visible respiratory movement was referred to as survival time.

In mice submerged in either tap water, isotonic saline or sea water at 23 to 25°C in an open container no respiratory movements were observed after approximately one minute.

Three mice submerged in tap water at 25°C under compressed nitrogen (7 atm.) lived from 4.1 to 5.4 minutes. Three mice submerged in 25°C tap water equilibrated with oxygen at 8 atm. pressure (absolute) lived from 5.7 to 6.1 minutes.

Three mice submerged in sea water at 25°C under compressed nitrogen (8 atm. abs) survived from 4.8 to 8.2 minutes. When submerged in 25°C sea water equilibrated with 8 atm. abs. of oxygen, three survived from 11.0 to 11.5 minutes. Three mice submerged in 25°C saline equilibrated with oxygen at 8 atm. abs. lived from 22 to 40 minutes.

All animals submerged in a fluid not oxygenated died cyanotic. All those submerged in oxygenated fluids had pink skin and mucous membranes to the end.

At autopsy the lungs of tap water, open container drowned animals floated in water and contained gas. The lungs of mice drowned in isotonic saline or sea water under compressed oxygen or nitrogen resembled liver and sank in water. The lungs of pressurized tap-water-drowned animals were either completely filled with fluid or contained varying amounts of fluid and gas.

Oxygen in solution at 8 atm. abs. is apparently readily available for the submerged mammal. Mice submerged in tap water which contained a sufficient amount of dissolved oxygen lived only slightly longer than controls subjected to drowning asphyxia. Thus death could only be attributed to the

sequelae of fluid aspiration; aspiration of tap water killed mice about as quickly as lack of oxygen. The survival time of mice submerged in an open container appeared not to be significantly affected by the composition of drowning medium. The lungs of all mice drowned under pressure had been previously filled with compressed oxygen and this explains the longer survival under pressurized nitrogen than in an open container.

The author states that the experimental results leave no doubt as to the cause of death due to sea water submersion being anoxia. Whether death by drowning in fresh water is caused by lack of oxygen or by water in the lungs or by a combination of these would depend on the amount of water aspirated and the interval between submersion and the time of entry of the water into the lungs. Respiration of isotonic saline appeared to be relatively harmless although the cause of death in this case could not be determined.

It is interesting to note that the author suggests that addition of 9 grams of salt to each liter of swimming pool water might increase the number of successful resuscitations after accidental submersion.

Goodlin[5] in 1962 studying the feasibility of a fetal incubator immersed newborn mice in Hanks basic salt solution under increased oxygen tension and found that they survived up to six times longer than others in the same solution at atmospheric pressure. At the time of death such animals were never cyanotic but appeared very well oxygenated. In a study of 1220 mice, newborn survivals of nine hours occurred at solution temperature of 28°C and an oxygen pressure of 250 psi. Solution temperature of 37°C led to low survival time for mice despite increased oxygen tension. The author suggests that at higher temperature and oxygen tensions there is a very narrow range of oxygen pressure between adequate supply and oxygen poisoning.

In an experiment similar to his first but with slightly different materials Kylstra et al[6] was able to produce longer survival of submerged mice. Adult, random bred "Swiss" mice were submerged in Sterofundin (a balanced salt solution, pH 6.8 containing acetate and lactate) or Sterofundin plus 0.1% T.H.A.M. Seventeen mice submerged in Sterofundin equilibrated with oxygen at 8 atm. abs. and temperature ranging from 6 to 41°C survived up to 4 hours, the maximum survival occurring at 20°C.

Thirty-one mice submerged in Sterofundin at 20°C and oxygen presure from 0.2 to 8 atm. abs. continued breathing for 5 to 247 min. However, when 0.1% T.H.A.M. was added at 20°C and oxygen varied from 1 to 8 atm. abs. the survival time ranged from 4 minutes to 18 hours, survival being a linear function of oxygen tension.

Fifteen mice in oxygenated Sterofundin with 0.1% T.H.A.M. were subjected to uniform compression from 8 to 160 atmospheres by means of compressed oxygen, or nitrogen, or both. Rhythmic respiratory movements were observed at 145 and 160 atm., but in both instances the animals appeared as if deeply anesthetized. Mice that breathed fluid for 10 to 30 minutes were subsequently kept alive for over 2 hours in compressed gas with a high oxygen tension.

T.H.A.M. minimizes hyercapnic acidosis. Addition of this to the fluid medium resulted in markedly prolonged survivals of submerged mice. This would indicate that elimination of CO^2 was deficient under the given experimental conditions. Carbon dioxide retention results in an increase of arterial pCO_2 as a function of time. Resulting shifts of the oxygen dissociation curve of hemoglobin to the right may well account for progressively longer survivals at higher oxygen tensions.

The longest survival of mice was observed at 20°C under 8 atm. abs. of oxygen pressure. According to the authors adverse effects of deep hypothermia probably account for the shorted survivals at lower temperature, whereas at higher temperatures the shorter survival times may have been due primarily to deficient elimination of CO_2.

The authors then state that if humans possess a tolerance to pressure similar to the mice then escape from submarines might be possible at depths far greater than the ones currently considered to be prohibitive. By

using fluid as a respiratory medium instead of toxic or narcotic compressible gases an important limiting factor could be eliminated.

At about this time Bodell and co-workers[7] working on a capillary membrane oxygenator for extracorporeal circulation were having similar problems with CO_2. The elevated O_2 and low pH values they at first obtained were incompatible with life while oxygenation was more than adequate. The adequate $C0_2$ concentration encouraged them to seek ways of removing more CO_2 which they were able to perfect by reengineering the capillary membranes for better gas exchange.

Under ether anesthesia Pegg et al[8] performed tracheotomies on 33 rats, tied them to a lucite frame, placed electrocardiographic leads and placed the rats in a pressure chamber. Desired pressure and solution pO_2 were obtained within three minutes. Solution temperature was held between 30°-37°C. With 16 rats a solution balanced in ionic concentration and osmolarity with rat plasma was used.

Upon immersion of the tracheotomy tube the heart rate dropped approximately 40 percent in five rats. In the same rats irregular respiration during the first three minutes gave way to a respiratory rate of approximately 28/min with prolonged forced respiration. Skin and mucous membranes remained pink. Survival time was less than five minutes at 2.5 atm. (2 rats), rose to 170 and 248 minutes at 10 atm. (2 rats), and dropped to 90 and 135 min. at 20 atm. (2 rats). After 30 min. breathing the balanced solution at 10 atm. (one rat), arterial oxygen saturation was 76%, pCO_2 174 mm Hg and pH 6.61. The high pCO_2 reflected the poor CO_2 carrying capacity of this virtually unbuffered solution. Six other animals were immersed in a solution buffered with 0.4% T.H.A.M. After 30 min. in this solution at 10 atm. (one rat), pCO_2 was 50 and pH 7.00. Arterial oxygen saturation was 24% at 10 atm., 93% at 17.5 atm. and 100% at 20 atm. where foaming occurred.

After 30 minutes in the liquid, rapid decompression (30 sec.), removal from the chamber, and postural drainage to remove most of the 5-7 ml of fluid in the lungs, eight rats quickly resumed normal breathing and had a normal gait. However, within 15 minutes, sero-sanguinous fluid with bubbles appeared in the transparent tracheotomy tube, respiration became slow and labored, mucous membranes were cyanotic, and the rats died despite administration of 100% oxygen or intermittent positive pressure breathing. When the chest was opened, the lungs collapsed completely and small pleural effusions were present. Lung extracts for pulmonary surfactant done in 5 rats had minimum surfaces tensions greater than 18 dynes/cm (normal less than 10 dynes/cm).

The labored respiration and acidosis seen in rats, even in the buffered solution, suggested that they succumb in the liquid from exhaustion from the excessive work of breathing. Breathing of pressure oxygenated solutions is not innocuous, as the author states, as demonstrated by the prompt death usually seen upon return to the gaseous environment. Since the animal is totally submerged, it is subject to breathing contaminants (skin oils, urine, feces) which may damage the lungs. Perhaps retained liquid in the lungs is detrimental and the prolonged survival of the animals breathing saline resulted from its lack of oncotic pressure (provided by the glucose and T.H.A.M. in the other solutions) permitting more rapid absorption from the alveoli. The collapse of the lungs and abnormal surface tensions of lung extracts seen at autopsy of rats removed from the liquid, and their need for continuous positive pressure, suggests that death following air breathing is due to massive atelectasis secondary to washing out of the pulmonary surfactant with consequent increase in surface forces tending to collapse alveoli. A deficiency of this material occurs in idiopathic respiratory distress syndrome of the newborn in which PAS positive staining hyaline membranes are found as was the case with the rats in this experiment.

Evolution is generally considered irreversible, but on the basis of previous experiments Kylstra had demonstrated the adaptability of adult mammals to aquatic respiration. He

took the next step and studied this phenomenon in dogs.[9] A tub filled with saline was placed in a pressure chamber large enough to accommodate the investigators also. A dog was anesthetized, shaved, washed, intubated or tracheotomized and given prophylactic antibiotics. The chamber was pressurized with air and oxygen was bubbled through the saline until saturation. The animal was then submerged and observed through the transparent cover. Jets of exhaled liquid were clearly visible breaking the surface. Fluid breathing was terminated by lifting the dog out of the tub, draining the liquid from his lungs through a hose, and then forceful inflation for several times with air during chamber decompression.

The first mammal to survive breathing was a mongrel dog. His average systolic and diastolic pressures were diminished slightly but stable. The solution was equilibrated with oxygen at 5 atmospheres at 32°C. There was a moderate brachycardia. Respiration was slow and regular, arterial blood remained fully oxygen saturated but arterial CO_2 increased by 8 volumes percent in 20 minutes. The animal was resuscitated after 24 minutes submergence.

It is not difficult to provide adequate oxygen but the amount of CO_2 eliminated in the fluid depends on the partial CO_2 pressure in the alveolar capillaries and the solubility of CO_2 in the alvelor fluid. The isotonic salt solution at 39°C equilibrated with a 40 mm Hg pressure of CO_2 contains about 30 ml. of CO_2 per liter while one liter of air contains approximately 50 ml of CO_2 under similar conditions. Thus, alveolar saline ventilation should be about twice as great as air. However, the viscosity of water is about 36 times that of air at 37°C so that 36 times more pressure is necessary for laminar saline solution flow through the trachea and bronchi. In water, turbulence occurs at flow rates 27 times smaller than in air at 37°C and the pressure required for turbulent flow is much greater. Thus, the work of liquid breathing is likely to be excessive. In the dogs, respiratory pressure fluctuations of up to 40 mm Hg were recorded in the right atrium indicating strenuous inspiratory and expiratory muscular activity. In spite of this, alveolar ventilation was inadequate and CO_2 retention occurred.

In a variation of this experiment[10] hyperbarically bubble oxygenated modified Ringer's solution flowed from an elevated container into the lungs and drained out into a lower reservoir. With an inflow-outflow valve system fluid ventilation resembled pump ventilation with air except that tubal volume displacements occurred by gravity instead of a piston. The CO_2 content of the inspired fluid was negligible. By collecting the expired fluid and determining the oxygen and carbon dioxide content of a mixed minute volume, the rate of oxygen uptake and carbon dioxide elimination was computed and gave evidence that mammalian lungs can function as gills. It was the author's hope that if man were able to breathe oxygenated water instead of an oxygenated carrier gas, exploration of the oceans would no longer be limited by gas toxicity and decompression sickness.

A short review of the work done up to this point is provided by Kylstra.[11] Also noted in this article is the fact that puppies submerged as long as 54 minutes[12] and newborn rats submerged in water at 37°C continue to make respiratory movements—they can survive underwater for at least 40 minutes, recover when taken out of the water and develop normally into adult rats.[13] This ability of newborn mammals to exist temporarily in a completely aquatic environment can partly be explained by their tolerance to anoxia.[14]

Many experimenters have observed that the fetus can make ventilatory movements. The extent to which ventilation occurs is uncertain but it is well known that asphyxia induces ventilatory movements. Howatt and co-workers[15] experimented with fetal lambs in an attempt to find the volumes of liquid displaced, the mechanical limitations involved in breathing liquid and the extent to which amniotic fluid inhaled by the fetus will mix with and displace liquid already in the lung.

After delivery of the lamb by cesarean section, a tracheotomy was performed and a tube inserted and connected to a bottle containing 1-2 liters of normal saline. The umbilical cord was clamped and the lamb began

to gasp between 1 and 2 minutes after occlusion. Usually the largest individual gasps occurred during this period when pleural pressures up to -68 cm water, inspired flow up to 1.5 liters/min., and displaced volume of up to 14.5 ml were recorded in mature animals. After release of the cord the animal usually began to gasp again within a minute—having stopped with prolonged clamping—and then frequently entered a phase of regular liquid breathing lasting up to 20 minutes. During this period breathing sometimes reached sustained rates between 20 and 30/min. The total volume ventilated after release of the cord was usually larger than during the preceding period of occlusion.

Static pressure-volume inflation curves at 10 ml volume increments over the volume range between resting lung volume and the addition of 100 ml of normal saline were approximately linear and gave compliance values between 9 and 15 ml/cm H_2O. Compliance pressure thus represented a very small part of transpulmonary pressure during inspiration. The major part of the pressure was evidently resistive.

It appears to the authors that although the major part of each liquid breath is returned unmixed with lung liquid, a small part of the inhaled liquid does mix with and wash out some of the lung liquid. It was concluded that asphyxial gasping by the fetus causes some considerable admixture of lung liquid with amniotic fluid. Presumably with the passage of time such inhaled liquid would become evenly distributed throughout the lungs by diffusion.

This work demonstrates the known ability of animals to breathe fluids and points out the difficulties which occur—resistance to flow, poor admixture, etc.—which would apply to any experiments with adult mammals breathing liquid. The authors also note that an effect of ventilation with amniotic fluid could be the washout of the pulmonary surface active agent or the inactivation of this material by substances present in the amniotic fluid. This problem was, in fact, one of the first ones noted in the early experiments with mice breathing various fluids.

Kylstra[11] noted a similar problem to the poor amniotic fluid-lung fluid exchange in the low diffusion of CO_2 in water. It is so much slower than in air that the partial pressure at the center of the liquid filled alveoli remains well below the partial pressure in the alveolar capillaries. This results in a large "diffusion dead space" which renders liquid breathing inefficient as compared with air breathing so that greater minute volumes of ventilation are required. Computed total dead spaces for CO_2 in liquid-ventilated dogs ranged from 119 to 274 ml at tidal volumes of 150 to 400 ml. Thus, the elimination of dissolved CO_2 through the lungs of liquid breathing mammals is generally deficient. This problem may be solved, at least in part and temporarily, by the use of appropriate CO_2 buffers. As noted previously, mice and rats submerged in solutions to which T.H.A.M. has been added survive many times longer than in unbuffered solutions.

More light was shed on the subject of fluid dynamics in saline filled lungs by the work of West and associates.[16] They noted that blood flow decreases greatly from the bottom to the top of the upright human lung and in the isolated dog lung. It has been shown to be caused by the hydrostatic pressure differences which are inevitable in an organ which separates blood from gas by a thin membrane. In only two situations would these pressure differences be eliminated; in outer space with the absence of gravity or in a lung filled with and surrounded by fluid with the same specific gravity as blood. Thus, West removed the left lung of greyhound dogs, tied glass cannulae into the pulmonary artery, left main bronchus, and excised left atrium. The lung was placed vertically in a water-tight lucite box. The box was filled with warm saline and the lung was then inflated with warm normal saline. The lung was perfused with venous blood from a second dog via two femoral vein catheters and returned via the jugular. The distribution of blood flow was measured by injecting aggregated I^{131}-labeled albumin into the pulmonary artery and scanning the lung from bottom to top when the particles had been trapped by the small vessels. After equilibrating the al-

veolar saline with more inhaled iodinated albumin and scanning again blood flow per unit alveolar volume could be calculated.

The isolated lung gradually became edematous during the course of perfusion. Sections were taken at the end of the experiments, fixed and stained. The sections showed some interstitial edema but in general the alveoli, blood vessels, and bronchi looked remarkably normal.

Means and standard errors for blood flow for five equidistant points up the lung showed that blood flow was, on the average, about evenly distributed although the vascular pressures were such that in the air-filled lung, the flow distribution would have been grossly uneven due to pressure inside the vessels increasing down the lung while the pressure outside the vessels remains constant. By contrast the saline-filled lung has the same vertical pressure gradient inside as out resulting in an even blood flow.

From the statistics compiled on fluid exchange, it was concluded that ventilation per unit alveolar volume was substantially uniform. However, during the course of rebreathing to equilibrate the I^{131}-labeled albumin, an unexpected delay was noted in mixing of the inspired tracer with the alveolar saline. It was then noted that with no postinspiratory pause, a relatively large proportion of inspired tracer was exhaled early in expiration and that towards the end of a relatively long expiration, the expired radioactivity became very low. Thus the late expired alveolar saline had a tracer concentration far below what would have been expected if mixing within the alveolus were complete.

At this point the authors note that the gross inequality of blood flow and ventilation under increased acceleration is due to pressure differences across the lung tissue secondary to the difference in density between blood and air. A suggested way of abolishing these pressure differences is by replacing air with oxygen-enriched saline for short periods. This expedient may be possible for the very large initial acceleration necessary in future space travel.

The present belief is that the linear velocities of inspired gases or liquids are so small in the terminal airways that mixing of the inspired tidal volume with the alveolar gas occurs only by diffusion. Diffusion is very slow in liquids and in these experiments the flow rates produced laminar flow and weak convection currents with little mixing of fluids.

Diffusion within the airways in the gas phase may cause a barely measurable inequality of ventilation but it is likely to interfere seriously with gas exchange in the saline-filled lung. In a related study, after inhalation of radioactive oxygen, the alveolar concentration at an expired volume of 700 ml was only 11% of the inspired concentration with no postinspiratory pause. After 3 minutes pause the alveolar concentration rose to 31%. If curves are extrapolated to an expired volume of 1000 ml, the alveolar concentration with no pause falls to less than 5% and the diffusion gradient is seen as very large. Diffusion limitations of a similar order of magnitude must occur for carbon dioxide. The mechanical mixing action of the heart within the thorax may be a slight mitigating factor.

Another study in which the pulmonary gas exchange was measured in dogs mechanically ventilated with a hyperbarically oxygenated modified Ringer's solution is reported by Kylstra.[17] The experiments were conducted in a US Navy recompression chamber sufficiently large to accommodate the animal and two investigators. The chamber was pressurized with air, and in most of the experiments the dogs were not submerged but remained exposed to the chamber atmosphere. Twenty-nine mongrel dogs were anesthetized with IV sodium pentobarbital, given prophylactic antibiotics, and intubated. The ventilating fluid was a modified Ringer's solution with 1 g/liter of dextrose added to balance the osmotic effect of the plasma protein. Bicarbonate was omitted to obtain a virtually unbuffered solution.

The oxygenated fluid was made to flow into the lungs by gravity and expired liquid drained into a reservoir, was reoxygenated and reused. The liquid was maintained at 37°C. The dog was allowed to breathe oxy-

gen for 3 minutes to wash out nitrogen. Succinyl-choline was then given IV to prevent further respiratory activity. Motor driven valves alternated the inflow and outflow lines. Fluid volumes were obtained by suspending the dog from a strain gauge force transducer and reading volumes off a calibrated weight record. Liquid ventilation was terminated by stopping the valve motor with the outflow open and, after adequate drainage, the lungs were forcefully inflated several times by the endotracheal tube.

No particular emphasis was placed on resuscitation of the dogs, nevertheless, seven animals survived the procedure and one delivered a litter of healthy puppies 44 days later. These dogs, in good health and without any signs of respiratory embarassment were sacrificed and histologic examination of the lungs performed. There was a remarkable degree of scarring with interstitial fibrosis and thickening of alveolar walls and septa which were very cellular and vascular. These changes were focal and in tissues not involved by this scarring process the alveolar walls were relatively thin but did not possess the delicate appearance of normal lung tissue since there was an increase in cellularity. There were scattered giant cells and masses of eosinophilic material surrounded by small round cells embedded in the scarred areas. Dense eosinophilic membranes in association with chronic inflammatory cells and fibroblasts were seen in alveolar and bronchiolar luminar. Modest emphysematous changes were present just beneath the pleura. Only minor pathological changes were found in the lungs of the other dogs, consisting of small granulomata sometimes associated with amorphous bluish-pink material and giant cells. There were focal pleural thickening and emphysema. It of course is not known which of these dogs represents the expected reaction. No discussion of the possible factors of death was made in the article and any would be pure speculation.

The oxygen uptake through the lungs of the dogs was adequate, ranging from 31 to 93 ml/min at inspired oxygen tensions of 3300 to 3640 mm Hg in the fluid. The arterial oxygen tensions were very low at inspired oxygen tensions of less than 5 atm. The elimination of dissolved CO_2 through the lungs was generally deficient with respiratory exchange ratios ranging from 0.3 to 0.7 at arterial CO_2 tensions of 43 to 80 mm Hg.

Kylstra found, as had West, that net transfer of gases within the air spaces occurs by diffusion and bulk flow but that bulk flow accounts for no more than a fraction of the net transfer of gases within the air spaces. Net transfer of gases within the terminal air spaces and the overall transfer of gases in saline ventilated lungs is primarily diffusion limited.

The gas exchange in liquid-ventilated dog's lungs is similar to the computed gas exchange in a lung model consisting of sperical exchange units in which gas transfer occurs by diffusion only. In this same experiment Kylstra and co-workers mathematically compared gas exchange results to radial diffusion in a sphere. This lung model contains approximately one-half million spherical exchange units each with a diameter of approximately 1 mm. This is comparable to the overall diameter of primary lung lobules.

There did exist a fair degree of positive linear correlation between the measured arterial oxygen tensions and computed theoretical values. Measured gas tensions in artificial blood and alveolar liquid were plotted against computed distances from the center of a hypothetical exchange unit. Tidal volumes and computed total dead space volumes are given. The largest part of those total dead spaces appeared to be the result of diffusion gradients within the exchange units which seriously impair the efficiency of tidal ventilation in liquid breathing.

Unfortunately the complex geometry of the anatomical structure and the lack of precise information regarding their dimensions and the compliances which determine bulk flow patterns appeared to the authors to preclude an analysis of their experimental data in terms of actual diffusion profiles within the exchange units proper. It was concluded that pulmonary gas exchange in liquid-ventilated dogs is diffusion limited and can be described mathematically in terms of radial diffusion in a sphere.

Lukin[18] studied the difficulties which develop during liquid breathing; attempted to determine the survival rate, and made gross and histopathologic exams of nonsurviving animals.

Seven mongrel dogs were partially or totally immersed in Ringers solution plus 0.1% T.H.A.M. as buffer in a procedure similar to the earlier experiments. The average tital volume for liquid breathing was 150 ml. Up to 175 ml of liquid was retained in the lnugs after the experiment, part of which may have passed into the blood.

Two of the dogs survived. One developed temporary apnea during submersion but recovered. The second appeared to do well even though his arterial oxygen concentration fell as low as 13 vol % at one point. Three other dogs developed apnea within 13 minutes and died. The sixth dog developed apparent bronchospasm after 25 minutes and died. The seventh dog developed some bronchospasm but maintained minimal ventilation, however, arterial oxygen was 11 vol % at 30 minutes when the experiment was terminated.

Necropsy was done on four of the dogs. Their lungs were found to be highly edematous and discolored dark red to brownish red, indicating hyperemia and hemorrhage. Histopathologic exam confirmed the finding. No atelectasis was found in any of the lobes examined. The general picture resembled acute pneumonia. The central nervous system was also examined and vascular lesions were found in the spinal cord, brain stem, and cerebral cortex.

At an oxygen tension of 8 atmospheres, hemoglobin in a concentration of 15 gm % can carry about 20 vol % 0_2, and plasma up to 18 vol % giving a total of about 38 vol % for whole blood. The highest levels in the dogs ranged from 26 to 37 vol %. It is a suggested possibility that the CO_2 buildup (up to 72 vol % highest) may be secondary to edema and consequent decreased diffusion. Although hypercapnia is a great problem it was not regarded as a causative factor of death in this experiment. The cause appeared to be a sharp drop in arterial 0_2 concentration to about zero just before death.

The cause of the fatal fall in arterial 0_2 concentration was apnea and/or dyspnea with bronchospasm. Vascular lesions which developed in the lungs and in the medulla were probably the chief causes of the arrest of respiration. It remains questionable whether the pressure of a merely iso-osmotic salt solution in the bronchioles and alveoli is entirely harmless. On the other hand, the hemorrhages observed in the central nervous system point to a systemic vascular reaction typical of various acute stress situations. The authors were unable to draw any conclusions about survival rates.

Another approach to exchanging gases with fluids was experimented with by two different groups at about this time. The thickness of a seawater layer with an oxygen concentration approximating that of the surface (150 mm Hg) varies widely in the oceans, extending from 150 to 300 feet in some areas. Since the partial pressure of oxygen in circulating sea water is higher than that of expired air, the reverse being true with CO_2, a net transfer of the two gases in the desired direction is possible. An apparatus was constructed by Strauss and Moulder[19] to allow a guinea pig to maintain respiratory exchange by means of these diffusion gradients. A teflon membrane was interposed between the guinea pig's expired air and the water. The air above the water was at atmospheric pressure. Fifteen such membrane units were supported on an open frame and each unit was made by placing screening between two Teflon membranes. Expired air flowed between the two membranes which were sealed by autoclavable masking tape. A respiratory valve from the guinea pigs trachea was connected to a manifold at each end of the apparatus. The apparatus was submerged and the guinea pig placed on top of it above the water level. The quantity of oxygen diffusing across the membrane increased until it equalled the amount of oxygen utilized by the animal which occurred after 3½ hours. The respiratory rate rose however, probably secondary to hypoxia and fatigue. It was later calculated that the apparatus furnisher approximately 4 ml oxygen/min.

In the second experiment the apparatus was first flushed with an air-nitrogen mixture of pO_2 108 mm Hg. The respiratory pO_2 rose for the first half hour and thereafter remained stable for 5 hours. The membrane apparatus did allow diffusion of 0_2 and CO_2 at a level permitting animal survival. However sea water contains at best only 9 ml of oxygen per liter and a large quantity of water and a large membrane surface area are needed even for a small animal. Also, if the apparatus were taken below the surface as with a diver, problems of lung compression, gas narcosis, etc., arise.

Another artificial gill was developed by Bodell[20]. Silicone rubber tubing readily allows diffusion of oxygen and carbon dioxide and utilizing this facilitated large areas for gas exchange. The apparatus is similar to a previously described capillary membrane oxygenator for extracorporeal blood circulation.[7] Expired air is passed through the lumen of the tubing, partial pressure differences allowing diffusion of O_2 and CO_2 in and out respectively.

Expired air from rats in a submerged enclosed chamber was circulated through submerged silicon tubing in tap water by means of a roller pump. The animals breathed from 22 hours to 6 days and all survived. The pO_2 decreased and the pCO_2 increased at first but levelled out at 80 mm and 20 mm Hg respectively; pressure sufficient to sustain life. Varying the water flow directly varied the pO_2 and pCO_2. It also became apparent that toxic gases such as ammonia (from urine decomposition) are excreted by this gill.

Howlett[21] and co-workers in 1965 reported on an extracorporeal circulation apparatus using fluid exchange of oxygen rather than gaseous exchange in order to minimize blood trauma. Venous blood entered the bottom of concentric lucite cylinders forming a film on the wall and exited at the top. The spaces between the cylinders were filled with a fluorocarbon fluid containing dissolved oxygen. The blood film was in contact with the fluid and readily took up oxygen from it with minimal blood trauma. The fluid is a fluorocarbon (primarily isomers of $C_8F_{16}O$, labeled FX-80 and supplied by Minnesota Mining and Manufacturing Co.) and used because of its inertness, high oxygen solubility, density much greater than blood and immiscibility with blood. Work on these fluids had been done by various companies as early as 1954.

Oxygen is at least ten times as soluable in silicone and fluorochemical liquids as in plasma or saline. A given volume of silicone oil (polymethylsiloxanes) oxygen saturated at atmospheric pressure contains half again as much oxygen as the same volume of air or whole blood; fluorocarbon liquid under similar conditions contains three times as much. Clark and Gollan[27], on the basis of this information, submerged mice and cats in the two fluids with oxygen bubbled through it at atmospheric pressure. Mice breathing the liquid fluorocarbon for one hour, in contrast to those breathing the silicone oils, survived for several weeks after removal from the fluids. Immersion survival time averaged about 4 hours at 18°C, 40 minutes at 25°C and 15 minutes at 30°C. One animal continued to breathe the liquid for 20 hours at 18°C. The addition of Fluothane arrested swimming motion and increased survival. The cerebral oxygen tension during fluid breathing was roughly equivalent to that observed during air breathing. After the animal is removed from the fluid the brain oxygen level returns to control values. If liquid temperature is increased from 20° to 35° or the pO_2 lowered from 600 to 140 mm at 20° C the recording of brain oxygen falls to zero.

Anesthetized cats were submerged in and spontaneously respired through a tracheal cannula the fluorocarbon liquid with oxygen bubbled through it. Arterial oxygen tensions ranged between 140 and 300 mm using tidal volumes between 12 and 60 cm^3 and endotracheal pressures of 10 to 15 cm water. The arterial pCO_2 increased from 50 to 80 mm and the pH fell from 7.35 to 7.10.

One cat was observed for 5 days following liquid breathing; the animal walked about and drank milk but was in respiratory distress during this time and succumbed within

15 minutes after the subcutaneous administration of 50 mg hydrocortisone, with copious loss of bloody fluid from the trachea. All of the organs were grossly normal except the lungs, which appeared congested when collapsed but normal when inflated. Several of the apparently normal mice sacrificed on the fifth day showed red areas distributed on the lungs in a polka-dot pattern. The observations in mice strongly suggested that the tracheal diameter limits the gas exchange, meeting the requirements only in hypothermia, at a high fluid pO_2, and a viscosity near that of water. In the cat, arterial oxygenation was entirely adequate but, again, carbon dioxide elimination is impaired.

The authors state that fluorochemical liquid respiration should prove to be more efficient than aqueous liquid respiration because of its remarkably higher solubility for oxygen and carbon dioxide, its higher diffusion coefficient for gases, and its somewhat lower viscosity. Whether pulmonary damage observed is due to solvent activity, toxic impurities, chemical interaction, or some other factor is not clear. It is certain that fluorocarbon liquid is superior to the silicone oils.

DISCUSSION AND CONCLUSION

What began as an investigation of drowning blossomed into a field of endeavor aimed toward making gills out of mammalian lungs[23] and overcoming decompression problems by sidestepping them. The experiments reviewed in this paper began with simple submersion experiments and developed into more complex physiological investigation. At first there seemed to be great possibilities for the eventual use of liquid breathing for man in order to overcome decompression problems. Uses were advocated in deep sea exploration, submarine escape, medical research, and even space travel. However as more information is compiled the problems in this concept have become more clearly defined. The problems include fluid and electrolyte imbalance, anoxia, respiratory exhaustion secondary to breathing a very dense medium, breathing of contaminants in the fluids, abnormal surface tension of fluids remaining in the lungs and large diffusion dead space imposing a serious limitation on gas exchange. The organic liquids removed the need for hyperbaric oxygenation but carbon dioxide retention still remains a great problem. The pulmonary damage due to the organic liquids remains a major complication.

The use of gill systems does not seem likely for individuals at present since the required system is too bulky. Application of the gill system could be used for whole undersea environmental systems where bulkiness is not a problem. One might conceive of man breathing fluid into a gill system but again the size would preclude any usefulness for an individual. Also the amount of heat loss from the body through the fluid either with a gill or not is too great for survival. Heating the fluid again presents unit size and weight problem

In conclusion, the work done up to this point presents very interesting possibilities for the future. However, these possibilities are limited by serious problems the solutions to which certainly do not appear to be just over the horizon.

SUMMARY

Certain investigators became interested in the mechanism of death by drowning. Work progressed from this to purposely submerging animals in liquids to perfect the science whereby the animals could survive a period of immersion. The object of this work was to test the feasibility of man's breathing liquid and thus overcoming many of the problems of diving compression and decompression. As work progressed problems began to rise — poor CO_2 diffusion, fluid and electrolyte imbalance, fatigue, etc. — which would appear to severely limit the usefulness to man of such a system. More work is needed in order to overcome these major problems before man can apply them to himself if, indeed, he can at all.

BIBLIOGRAPHY

1. Swann, H. G.: Mechanism of Circulatory Failure in Fresh and Sea Water Drowning. Circulation Research 4:241-43, 1956.
2. Otter, G. Den; Van Nouhys, F.; and Baker, N. J.: Investigation On the Cause of Death in Fresh Water Drowning. Acta Physiol. Pharmacol. Neerl. 9:415-430, 1960.
3. Kylstra, J. A.: Drowning: The Role of Salts in the Drowning Fluid. Acta Physiol. Pharmol. Neerl. 10:327-334, 1962.
4. Klystra, J. A.: Breathing Fluid, Experientia 18; 68, 1962.
5. Goodlin, R. C.: Foetal Incubator, Lancet 1:1356-1357 (June 23) 1962.
6. Kylstra, J. A.; Tissing, M. O.; and van der Maen, A.: Of Mice as Fish. Trans. Amer. Soc. Art. Int. Organs 8:378-383, 1962.
7. Bodell, B. R., et al: A Capillary Membrane Oxygenator. J. Thorac. Cardiovasc. Surg. 46:639-650, 1963.
8. Pegg, J. H.; Horner, T. L.; and Wahrenbrock, E. A.: Breathing of Pressure Oxygenated Fluids, in Proc. Second Symp. on Underwater Physiol., Publ. 1181, Natl. Acad. Sciences — Natl. Research Council.
9. Kylstra, J. A.: Breathing of Pressure Oxygenated Salt Solutions, Dis. Chest 47:157-159 (Feb.) 1965.
10. Kylstra, J. A.; and Lanphier, E. H.: Gas Exchange in Fluid Ventilated Dogs, Fed. Proc. 23:469, 1964.
11. Kylstra, J. A.: Survival of Submerged Mammals, NEJM 272:198-200 (28 Jan) 1965.
12. Edwards, W. F.: De l'influence des agens physiques sur la vie, Paris; Crochard, 1824, p. 654.
13. Fazekas, J. F.; Alexander, F. A. D.; and Himwich, H. E.: Tolerance of Newborn to Anoxia. Am. J. Physiol. 134:281-287, 1941.
14. Glass, H. G.; Snyder, F. F.; and Webster, E.: Rate of Decline in Resistance to Anoxia of Rabbits, Dogs, and Guinea Pigs from Onset to Viability to Adult Life. Am. J. Physiol. 140: 609-615, 1944.
15. Howatt, W. F.; et al: Ventilation of Liquid by the Fetal Lamb During Asphyxia. J. Appl. Physiol. 20:496-502 (May) 1965.
16. West, J. B., et al: Distribution of Blood Flow and Ventilation in Saline-filled Lung. J. Appl. Physiol. 20:1107-1117 (Nov) 1965.
17. Kylstra, J. A.; Paganelli, C. V.; and Lanphier, E. H.: Pulmonary Gas Exchange in Dogs Ventilated with Hyperbarically Oxygenated Liquid. J. Appl. Physiol. 21:177-184, 1966.
18. Lukin, L.: Oxygenation of Blood During Inhalation of a Liquid Medium. Proc. Soc. Exp. Biol. Med. 121:703-707 (Mar) 1966.
19. Strauss, R. H., and Moulder, P. V.: Underwater Breathing Device Utilizing Oxygen Dissolved in Water. Nature (London) 207:532-533, (31 July) 1965.
20. Bodell, B. R.: An Artificial Gill, Surgical Forum 16:173-175, 1965.
21. Howlett, S., et al: Fluid Fluorocarbon as Oxygenator in Experimental Extracorporeal Circulation. Arch Surg. 91:643-645 (Oct) 1965.
22. Clark, L. C., and Gollan, F.: Survival of Mammals Breathing Organic Liquids Equilibrated with Oxygen at Atmospheric Pressure. Science 153:1755-1756 (24 June) 1966.
23. Kylstra, J. A.: Advantages and Limitations of Liquid Breathing, in Proc. 2nd Symposium on Underwater Physiology, Nat. Acad. Sci. National Research Council, C. J. Lambertsen (ed.) Baltimore: The Wiliams and Wilkins Co., 1967, pp. 341-350.

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13. ABSTRACT

The author reviews the literature on fluid respiration and reports on the publications by twenty-three researchers, describing the various experiments performed, results obtained, problems encountered and possible future applications of this work.

It appears that certain investigators became interested in the mechanisms of death by drowning. Work progressed from this to purposely submerging animals in liquids to perfect a technique whereby the animals could survive a period of immersion. The object of this work was to test the feasibility of man's breathing liquid and thus overcoming many of the problems of diving compression and decompression. As work progressed, problems began to arise--poor CO_2 diffusion, fluid and electrolyte imbalance, fatigue, et cetera, --which would appear to severely limit the usefulness to man of such a system. More work is needed in order to overcome these major problems before man can apply them to himself, if indeed, he can ever hope to do this.

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