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PHYSIOLOGICAL ADJUSTMENTS TO DEEP DIVING IN THE PACIFIC GREEN TURTLE (*CHELONIA MYDAS AGASSIZII*)

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Abstract—1. When the green turtle (*Chelonia mydas agassizii*) is subjected to hydrostatic pressure as great as 19 atm, such as to simulate prolonged deep diving, the bradycardia of submersion is accentuated. As long as 9 min may elapse between heart beats; 4- or 5-min intervals are not uncommon.

2. The lungs are shown to collapse when the turtle is subjected to pressures of 9–19 atm, demonstrating that the surface area across which gas exchange occurs may be reduced at depth.

3. Direct measurement and comparison of the nitrogen tension in carotid artery blood and in tracheal air demonstrate that equilibrium conditions are never attained during a prolonged deep dive, but that enough nitrogen enters the blood to render the green turtle susceptible to gas emboli in the brain after emergence.

INTRODUCTION

A PRIMARY requirement for successful deep diving is relative immunity to caisson disease. In the simplest case, the gases in the lungs diffuse into the blood as a function of time and pressure. They will then partition among the body fluids and tissues. If the animal should rise to the surface too quickly, time might not allow all of the dissolved gases to diffuse from the blood and tissues into the lungs; as a result gas bubbles would form which might cause distress or even death. Supposedly, animals adapted to deep diving are largely free of such difficulties.

One of the first questions about the supposed immunity of natural divers to caisson disease is whether they dive deep enough to be in danger. Ommanney (1932) speculated that finback whales do not dive deeper than 33 m, thus hardly to a depth sufficient to induce caisson disease. Laurie (1933) pointed out that the major food supply of blue whales, namely krill (Euphausiidae), is most abundant at 100 m and, therefore, that they go at least that deep. Laurie also recalled the case of a sperm whale that became entangled in a cable at a depth of 900 M under circumstances indicating that it had been caught while still alive. More recently,

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Heezen (1957) reported a similar occurrence at 1134 m. Howell (1930) recorded a case wherein a harpooned fin whale was killed by impact with the bottom at 550 m. Among the pinnepeds there are reports of a harp seal being caught on a hook and line at 275 m (Nansen, 1925), an Alaskan fur seal at 55 m, a Steller sea lion at 183 m (Kenyon, 1952) and a northern elephant seal at 183 m (Scheffer, 1964). Loggerhead turtles have been captured in nets at 60–80 m (Ingle & Smith, 1949) and Pacific green turtles have been seen feeding at 73–110 m (Curray, personal communication) and at 290 m off Cape San Lucas (Landis, 1965). Experimental studies in which depth recorders were attached have been performed on seals. Scholander (1940) reports a bladdernose seal pup to 75 m and DeVries & Wohlschlag (1964) recorded a Weddell seal at 350 m. Kooyman (1966) measured 381 dives of the Weddell seal, the deepest one to 600 m. Thus, the evidence indicates that air-breathing vertebrates dive deep enough to get caisson disease if pressure and depth are the only factors to consider.

Laurie (1933) calculated, on the basis of the ratio of lung to body weight, that the amount of air taken down in the lungs of whales at each dive is too small to permit caisson disease, even if all of it is dissolved during the dive. This conclusion was also reached by Ommanney (1932) and Irving (1935).

Laurie found that blood taken from blue and fin whales after capture is never supersaturated with nitrogen, whereas there is a slight excess in the urine and in the allantoic fluid. He ascribed these concentrations of gas to the activity of a nitrogen-fixing organism in the blood. Irving (1935) suggested that the accumulation of nitrogen is prevented by a slow diving circulation coupled with an enhanced recovery circulation making possible the effective elimination of nitrogen between each dive. He concluded that "in view of the limited supply of nitrogen and the favorable conditions of the circulation there is no reason why a whale with ordinary respiratory and cardiovascular systems should be in danger of caisson disease".

Scholander's examination of fresh whale blood did not reveal any nitrogen-fixing organisms, though old blood frequently contained numerous bacteria. As a further check for intrinsic factors of protection he compressed blood with air at pressures and duration corresponding to those of actual dives. Decompression bubbles formed just as readily in whale blood as in human or ox blood, demonstrating that whale blood had no specific qualities which provide protection against caisson disease.

Scholander tested the theory that the basis of immunity lay in an inhibited invasion rate of nitrogen into the blood. Subjecting a mouse to 10 atm air pressure for 5 min rarely induced emboli. Repeating the procedure caused caisson disease. Scholander claimed that these findings identified invasion rate as one of the determining factors. Two frogs, one totally submerged and the other breathing nitrogen, were placed in a pressure bomb and subjected to 50 atm pressure for 30 min. The submerged frog was virtually immune while the other one always developed caisson disease. Scholander stated that the difference was because the invasion rate of nitrogen is greatly lowered in the totally submerged frog,

due to the alveolar surface being greatly reduced and to the alveolar walls being thickened by compression. The number of gas bubbles in the blood of the totally submerged frog after decompression is a reflection of the amount of gas carried down in the lungs, establishing the importance of limited lung air in the avoidance of caisson disease. This further implies that the lungs and thorax were fully collapsible. Extreme flexibility is characteristic of the thorax in diving mammals (Howell, 1930; Laurie, 1933; Irving, 1935; Slijper, 1936) and total collapse appears quite possible. Scholander verified, for the seal *Cystophora* and porpoise *Phocaena*, that the thorax could yield to permit fully collapsed lungs without structural damage occurring. Extrapolating these observations on the mouse and frog to the diving mammals, he concluded that the alveoli shrink and thicken under increasing pressure and successively empty their air into the semi-rigid trachea and bronchial tree. The alveolar shrinking and thickening will greatly retard nitrogen invasion. Below the depth of alveolar collapse all the air will be contained in the trachea, making further invasion very slow.

To test this theory in seals, Scholander tied one to a weighted line, lowered it rapidly 300 m into the sea, and then raised it quite rapidly. On emersion it died of caisson disease, showing that the protective mechanism is only relative. In summary, Scholander stated that protection was due to a reduced rate of nitrogen invasion and to a limited nitrogen supply, in combination with the circulatory adjustments postulated by Irving (1935).

MATERIALS AND METHODS

A. *Material*

The turtles were purchased from commercial fishermen in Baja California, Mexico. All had been captured by use of a harpoon with a stop 0·5 in. behind the point to limit penetration of the carapace and thus to avoid excessive injury.

Twenty-two turtles were used in this study, which extended from August 1959 to April 1963. With the exception of three animals, all were kept in indoor tanks at temperatures ranging from 18° to 23°C. The indoor group was fed mackerel with occasional offerings of squid, langostina (*Pleuroncodes planipes*), horsemeat and kelp. The animals stored outdoors refused to take any offered food, apparently preferring the algal growths on the sides of their concrete tank.

B. *Pressure chamber*

Conditions of deep diving were simulated in a pressure chamber (Fig. 1), constructed to permit the sampling of arterial blood and alveolar air and the recording of the heart rate of a turtle, at hydrostatic pressures as high as 22 atm. Compression of the turtle's lungs could be measured directly from the calibrated water reservoir and information concerning lung collapse was yielded by a differential manometer.

The basis of this chamber is two hemispheres, 34 in. dia. To each of these is welded a 4-in. flange by which the halves can be bolted together to form a sphere; a flat $\frac{1}{8}$ in. Neoprene gasket forms the seal. The chamber can be filled or emptied in 6 min, by means of a Jabsco impeller pump, through a manifold entering at the

bottom. Also entering this manifold are the copper tubing connexions to a pressure gauge and to the hand-operated hydraulic pump that is used to increase the pressure. By means of this pump, pressure within the chamber can be increased by small increments at a rather rapid rate. The reservoir feeding this pump is a Lucite tube, calibrated so that the volume of water forced into the full chamber to produce the desired pressure can be measured accurately. This measurement is necessary in the determination of lung volumes. The instrument is closely calibrated, so that corrections can be applied for the important factors of chamber deformation and water compression.

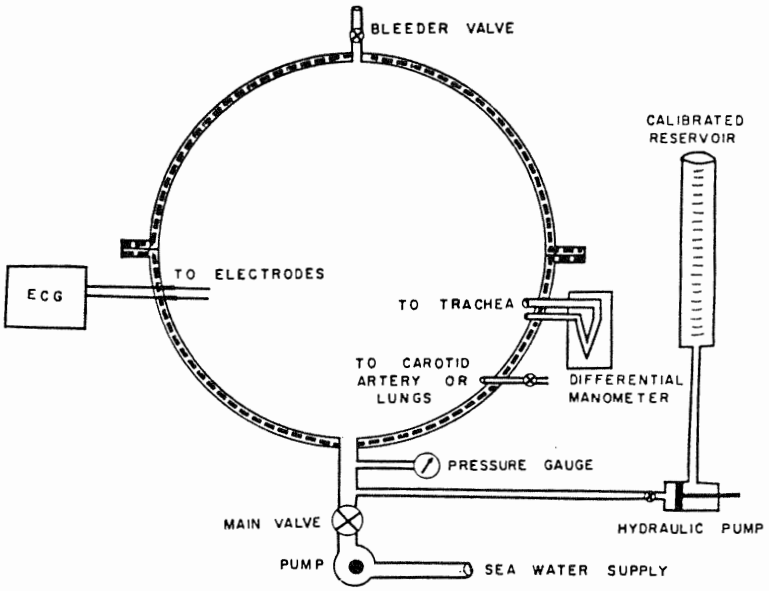

FIG. 1. Diagram of pressure chamber.

A check on the applicability of Boyle's Law to an enclosed gas volume in the chamber was made on a balloon containing a known amount of air. As water was added to the sealed system, the pressure on the balloon was increased and its volume decreased. A logarithmic plot of these two factors against each other was virtually linear, indicating that the pressure chamber volume changes accurately following Boyle's Law over the pressure range utilized.

Alveolar air and arterial blood samples are drawn through a metering needle valve in the wall of the chamber. The chamber wall is pierced by Joy electrical fittings which connect the electrocardiograph to the electrodes on the turtle. The differential manometer is fabricated from a single block of Lucite, the two legs forming a **V**. One leg of this manometer is connected by copper tubing and appropriate fittings to a tracheal cannula in the turtle; the other leg is similarly connected through the pressure chamber wall to communicate freely with the

water contained therein. It is thus possible to measure the pressure differential between the inside of the turtle's trachea and the inside of the tank at any stage of compression.

C. *General procedure*

In all experiments, the turtle, fastened to a weighted board, is submerged by being placed in the pressure chamber which is subsequently pumped full of sea water. The temperature of the water is measured at the beginning and end of each experiment.

In the experiments where the turtle is subjected to a prolonged deep dive, pressure is applied 15 min after submergence, and increased rapidly to the desired level. During the ensuing hour, there is usually a slight drop in pressure for which no adjustment is made. Fifteen minutes before emergence, the pressure is quickly released. The total length of each dive is about 100 min.

D. *Methods in the study of circulation*

Heart rate. The heart rate is recorded between two plate electrodes affixed either to the plastron, one over the heart and the other as close to the left rear leg as possible, or to the carapace, one to either side of the mid-line, with the greatest possible antero-posterior separation. The electrodes are stainless steel plates, 3 cm square, glued onto a 12-cm circle of expanded Neoprene rubber. After washing the plastron in fresh water and drying it, the electrode-bearing pad is glued to the turtle with rubber-base contact cement. This system satisfactorily excludes sea water in all experiments. Insertion electrodes fail to meet this requirement. Sanborn Redux electrode paste ensures proper contact between the electrode and the turtle. The heart rate is recorded on a Gilson Electronic Mini-polygraph. In the experiments performed at pressures above 1 atm, a pre-amplifier is put into the system in front of the Mini-polygraph.

E. *Methods in the study of respiration and gaseous exchange*

1. *Blood gases.* All samples of blood are drawn anaerobically from a cannula filled with physiological saline inserted in the carotid artery. After discarding the first 3 ml to prevent contamination by saline or uncirculated blood, a 1-ml sample is drawn into a 5-ml syringe whose dead space is charged with a solution of 2·0% heparin and 4·0% sodium fluoride, a concentration calculated to yield about 2 mg of heparin per ml of blood. The sodium fluoride is virtually saturated in an attempt to attain a final concentration of 10 mg/ml of blood as recommended by Peters & van Slyke (1932). Hemolysis is not increased by the addition of these substances. Each syringe is sealed with a piece of glass rod and rubber tubing and immediately plunged into ice water, where it is kept until the analyses can be done. After each sample is drawn, a solution of heparin and saline is forced back through the cannula to prevent clotting. At elevated pressures a special stainless steel syringe, with an O-ring seal, driven by a lead screw device, is required to inject the heparin solution.

In experiments at elevated pressure, analysis of the blood is complicated by cavitation. As the blood is drawn, bubbles form in the syringe. The blood gas analyses are done in a van Slyke manometric apparatus, introducing the entire contents of the syringe as each sample. The liquid volume of each sample is determined by weighing the syringe before and after transfer, and multiplying this value by the specific gravity of the blood.

As it is necessary to put a capillary extension on the syringe to introduce the sample into the apparatus, the dead space is about 10 per cent of the sample size with subsequent increase in transfer loss. However, the accuracy of the transfer is greater than 90 per cent and there is no doubt that these values reflect the actual gas content of the blood under the experimentally induced conditions.

As a safeguard against working with anemic animals, experiments requiring blood sampling are rarely repeated on any one animal. The hematocrit is always measured as a check.

2. *Tracheal gases.* Samples of gas are withdrawn from the trachea at suitable intervals before, during and after a dive for composition analysis in the Scholander 0·5 cm^3 gas analyzer. This is done by inserting a thin-walled polyethylene cannula, 1·5 mm o.d., approximately 10 cm into the trachea. The cannula is led through a needle valve in the wall of the pressure chamber which is, in turn, connected to a gas sampling bulb. The gas in the dead space of the sampling system is bled out and ejected to the atmosphere prior to sampling. The size of the sample withdrawn from the turtle never exceeds 3 ml, an amount ample for duplicate analyses of oxygen, carbon dioxide and nitrogen content.

3. *Pressure–volume characteristics of lungs.* It is important to know the pressure–volume characteristics of the lungs because one of the possible sources of protection against caisson disease is thought to be a reduction in the surface area for gas exchange due to lung collapse induced by increased external pressure. The questions that need to be answered are: (1) Does the volume of the respiratory system diminish proportionately to the pressure applied; and (2) how is the distribution of the respiratory gases affected by the application of pressure?

To determine whether the turtle's lungs compress at higher pressures in accordance with Boyle's Law, a series of readings are made of pressure and volume as the pressure is increased to a level assumed to cause lung collapse. Each set of values is then tested in the equation

$$V_1 = (\Delta V - C)\, P_2/\Delta P, \qquad (1)$$

where V_1 is the original volume at atmospheric pressure, ΔV is the volume of water added, C is the chamber correction factor, P_2 is the pressure at which the observation is made and ΔP is the change in pressure induced. If Boyle's Law (of which this equation is a form) is applicable, V_1 will be constant.

4. *Lung collapse.* Scholander's 1940 investigations suggested that protection against nitrogen invasion of the blood and tissues would be virtually assured if the lungs could be fully collapsed. Studies of diving mammals and observations of pressurized frogs implied that the lungs of these animals are collapsible. One

purpose of the current study is to determine directly whether the lungs of the green turtle collapse under increased hydrostatic pressure, and if they do, what effect the collapsing has on the invasion of nitrogen.

The supposition that the lungs are collapsible is tested in two ways. The first is to withdraw gas directly from the lungs, as described in the methods of measuring respiratory volume (Berkson, 1966). When no more air can be extracted, it is assumed that the lungs are either fully collapsed or closed off from the trachea. The second method of causing collapse more closely simulates conditions apt to occur during a natural deep dive. It is based on the rationale that as the hydrostatic pressure is increased, the pressure inside the trachea will be equal to that of the tank as long as the lungs are patent, and free communication between the alveoli and trachea exists. However, should these conditions no longer be satisfied, the tracheal pressure will fall below that of the chamber because the semi-rigid trachea will resist the external pressure. The turtle is subjected to increased hydrostatic pressure until a relatively sudden appearance of a pressure differential between the trachea and the chamber indicates that the volume of gas in the respiratory system has been reduced to the volume of the anatomical dead space. This end-point is determined by use of a differential manometer, one leg of which is isolated in the trachea and the other open freely to the pressure chamber.

Anatomical evidence tends to support the premise that the end-points described above indicate alveolar collapse rather than blockage between the lungs and trachea, for dissection of the respiratory system of a green turtle does not reveal any sort of sphincter or valve arrangement in the bronchi.

RESULTS

1. *Heart rate*

Changes in the heart rate of turtles subjected to elevated hydrostatic pressure were observed and recorded in seven experiments. The general effect of increased hydrostatic pressure is to accent the bradycardia of submersion (Figs. 2, 3). Immediately following the application of pressure, the heart rate drops sharply to 0 and stays there for 2–7 min. The pressure portion of the dive is characterized by an alternation of spikes associated with struggling and periods as long as 9 min of no cardiac activity, indicating that no blood is being circulated during a significant portion of the dive.

As pressure is released and the animal returns to the state of being submerged at atmospheric pressure, there is a sudden increase in heart rate. Whether this is due to the direct effect of pressure release or to the struggling that frequently occurs at this time could not be ascertained. Following this spike, the heart rate drops again to a relatively slow pace, though in no case is it ever as slow as it was when the animal was under elevated pressure. In each of the seven dives the heart rate seemed to increase toward the end of the dive, in three dives while the animal was still under pressure.

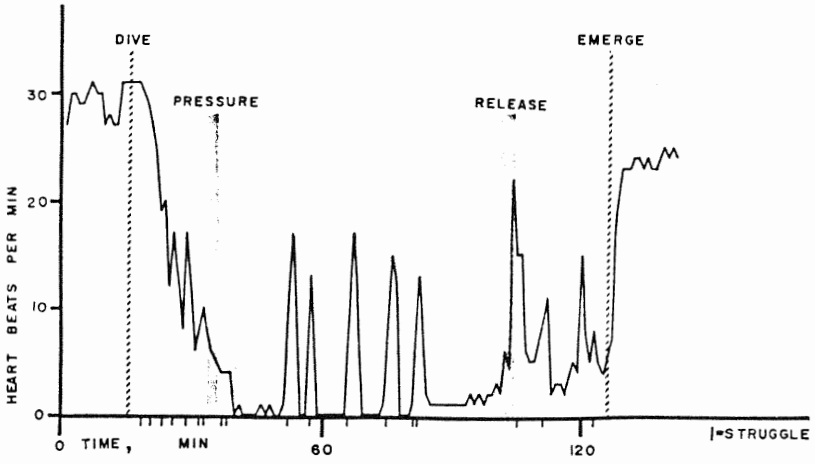

FIG. 2. Heart rate of turtle under 5 atm hydrostatic pressure.

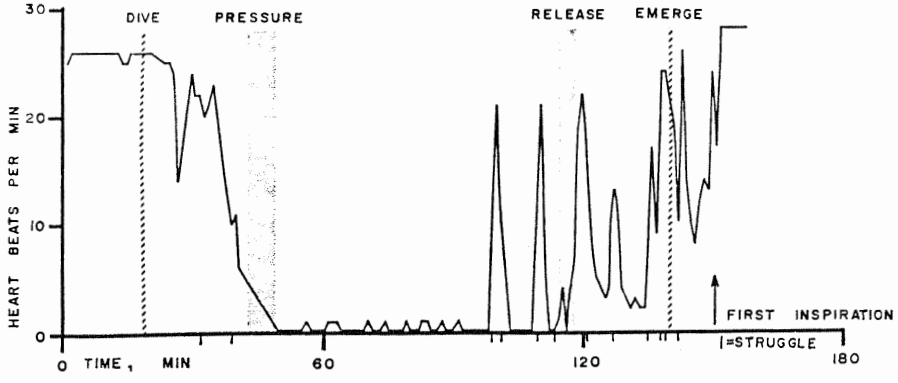

FIG. 3. Heart rate of turtle under 18 atm hydrostatic pressure.

2. *Mechanical responses of the lungs to pressure changes*

a. *Pressure–volume characteristics of the lungs.* The change in respiratory volume as a function of external pressure was studied in ten experiments. Observations were made at various intermediate pressures between 2 and 5 atm during the course of each dive. On standardization to atmospheric conditions the respiratory volumes prove to be quite uniform (Table 1). This uniformity is taken to mean that the lungs compress much like a gas-filled balloon. The small variation, which may easily be within the uncertainty inherent in the method, is remarkable, for it implies that little of the gas in the lungs dissolves out during compression. This may be due to the pressure being increased rapidly, for not more than a few seconds intervened between each of the progressive pressure–volume readings.

TABLE 1—VARIATION IN RESPIRATORY VOLUMES (ml) OBSERVED AT DIFFERENT PRESSURES DURING THE SAME DIVE, STANDARDIZED TO ATMOSPHERIC PRESSURE

Turtle	Weight (kg)	Atmospheres 2·4	3·0	4·1	5·1	Average
XII	17·3	1813	1825	—	1785	1808
		1746	1756	—	1725	1742
XIV	19·5	1805	1821	—	1815	1814
		2830	2830	—	2797	2819
		2587	2611	—	—	2599
XV	17·3	1486	1506	—	1487	1493
		1228	1258	—	1247	1244
		2141	2150	—	2134	2142
XVII	13·2	1659	—	1661	—	1660

b. *Characteristics of lung collapse.* The differential pressure required to collapse the lungs by the air aspiration method was determined four times. The plots of these findings (Fig. 4) very closely approximate each other in that the inflection point of each curve occurs at about the same pressure. The sharp inflection means that an increased pressure differential can extract no more air from the respiratory system, which, in turn, implies that the lungs are either collapsed or closed off. The pressure required to do this is approximately 7 cm of water. As a check for trapped air, a small piece of lung was removed after aspiration and placed in water. In each of three tests the tissue barely floated suggesting that although a small amount of air still remained in the alveoli, extraction was virtually complete.

In ten experiments the lungs were collapsed by the increased external hydrostatic pressure method. Pressure was steadily increased until, at 9–17 atm pressure, the manometer became suddenly unbalanced and the pressure within the trachea became 18–34 cm of water less than the chamber pressure (Fig. 5 presents two typical plots). At low pressure the manometer may be unbalanced to a greater or lesser degree in either direction. This may be due to the turtle exerting such mechanisms as a Valsalva maneuver. However, the tests show that as the pressure is increased the manometer becomes virtually balanced until there is a sudden lag in the tracheal pressure, implying collapse resistance of the airways. Lung collapse at these pressures means that all of the air in the lungs will be forced into the rigid air-passages at 80–160 m depth and hence be restricted from entering the blood and tissues.

The trachea and bronchi dissected out of a 21-kg turtle measured 76 ml in volume. If consideration is made for the small bronchioles and for the bony nasal passages, which were not measured, this figure may easily be doubled. As the total respiratory volume ranges from 1250 to 3250 ml (Berkson, 1966), or eight to twenty-one times as great as the rigid air-passage volume, the lungs

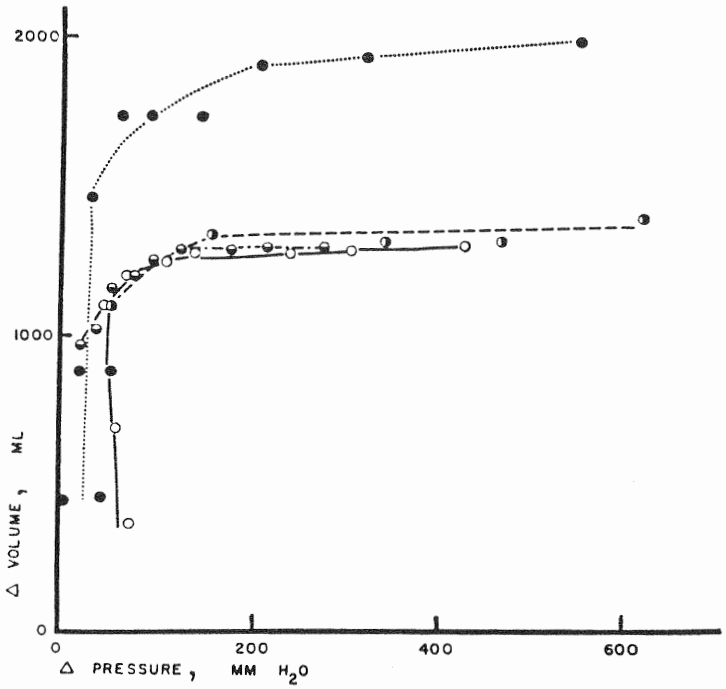

FIG. 4. Changes in lung volume as a function of air aspiration.

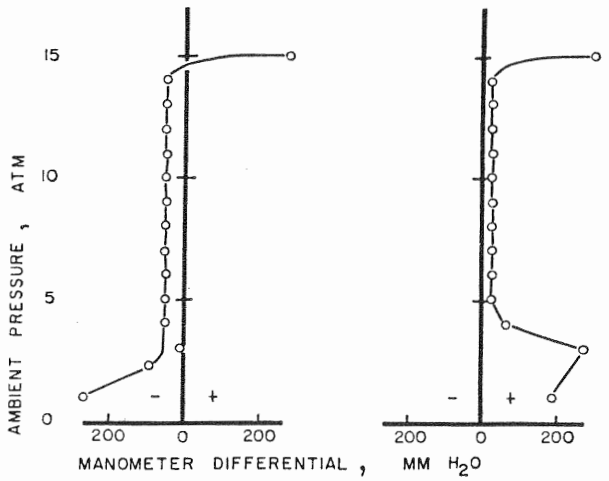

FIG. 5. Pressure differential between the trachea and the tank plotted against absolute tank pressure.

should collapse at 70–200 m depth. The average depth of collapse predicted from tracheal volume is 120 m.

3. *Changes in tracheal air due to pressure*

The component gas tensions of the tracheal air were determined at 2, 3, 5, 9 and 17 atm pressure. Tension values of the various gases were calculated by multiplying concentration by ambient pressure, which tended to drop slightly during the pressure phase of the dive. In each trial the changes in tension that occurred during the dive before pressure was applied were the same as those observed in prolonged diving at 1 atm (see Berkson, 1966).

The effects of 5 atm pressures (shown in Figs. 6, 7) are typical of the changes that occur at the low pressures of 2–9 atm, wherein the lungs are not collapsed. There is little variation in the configuration of the curves at these lower pressures, though the absolute values differ. At 17 atm the lungs are collapsed, and the derived curves (Figs. 7, 8) are different.

One would expect that the tension of the gases would increase in accordance with the pressure applied. In fact, the carbon dioxide tension increases to about one-half to two-thirds and oxygen increases to about one-half of the values expected; nitrogen tension always increases to a value slightly higher than that expected. While under pressure, the slope of the carbon dioxide curve decreases more rapidly at higher pressure. Oxygen also drops; its slope is slightly greater than that of carbon dioxide and is also related to pressure, except at 17 atm when the lungs are collapsed. At this very high pressure, the drop is severely inhibited until the latter part of the dive. The nitrogen tension always drops slightly, seemingly unaffected by increased pressure. On pressure release the tension of each gas drops sharply; carbon dioxide to about 50 mm of mercury regardless of the pressure endured (probably reflecting the saturation tension of the blood), oxygen to an expected asphyxic value except in the deepest dive, and nitrogen to almost pre-dive level. During the 15 min immediately following release but before emergence, the changes in tracheal gas tensions reflect metabolic activity.

4. *Changes in tensions of blood nitrogen due to pressure*

The tension of nitrogen in arterial blood was determined in two animals subjected to hydrostatic pressure assumed to be sufficient to induce lung collapse. The two experiments will be discussed separately because they are not duplicates.

In the first experiment the turtle is rapidly pressurized to 18·7 atm, the pressure required to produce lung collapse as indicated by the manometer method. The blood is analyzed in the van Slyke manometric apparatus. By this method it is possible to measure the total nitrogen present in the blood despite its supersaturation (Fig. 9). Even at almost 19 atm the arterial blood nitrogen tension attained after 30 min is only slightly more than half of the calculated saturation value, implying either lack of equilibrium or admixture with unsaturated venous blood.

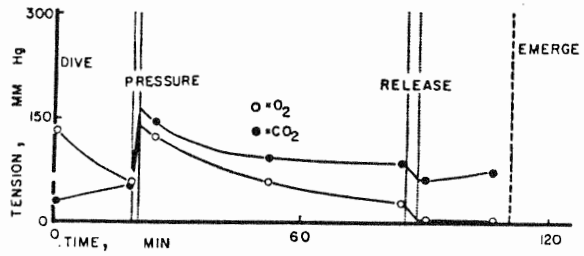

FIG. 6. Changes in oxygen and carbon dioxide tensions in tracheal air before, during and after exposure to 5 atm pressure (lungs not collapsed).

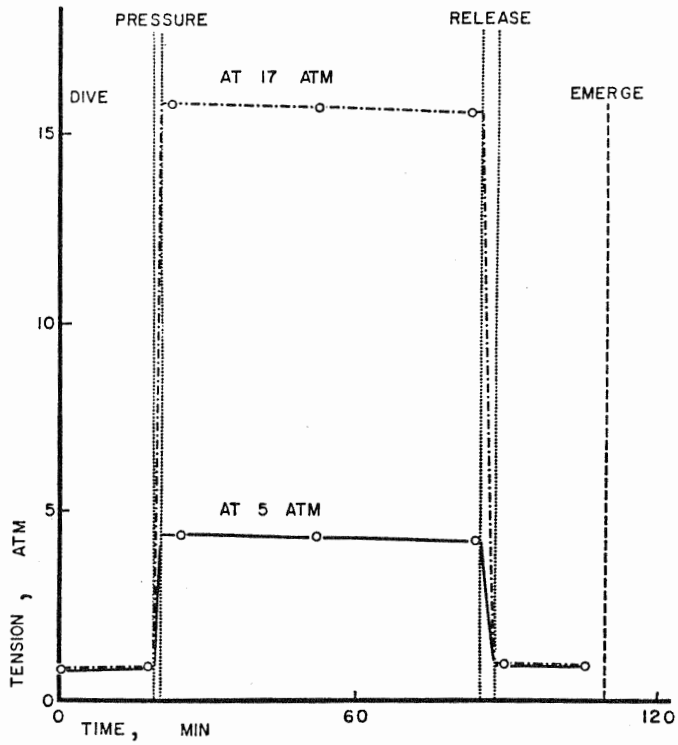

FIG. 7. Comparison of changes in nitrogen tension in tracheal air at 5 and 17 atm.

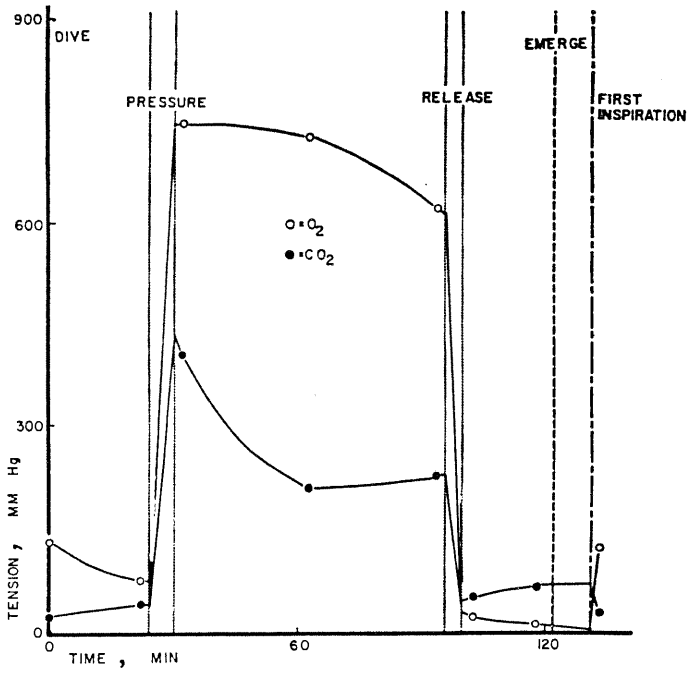

FIG. 8. Changes in oxygen and carbon dioxide tensions in tracheal air before, during and after exposure to 17 atm pressure (lungs collapsed).

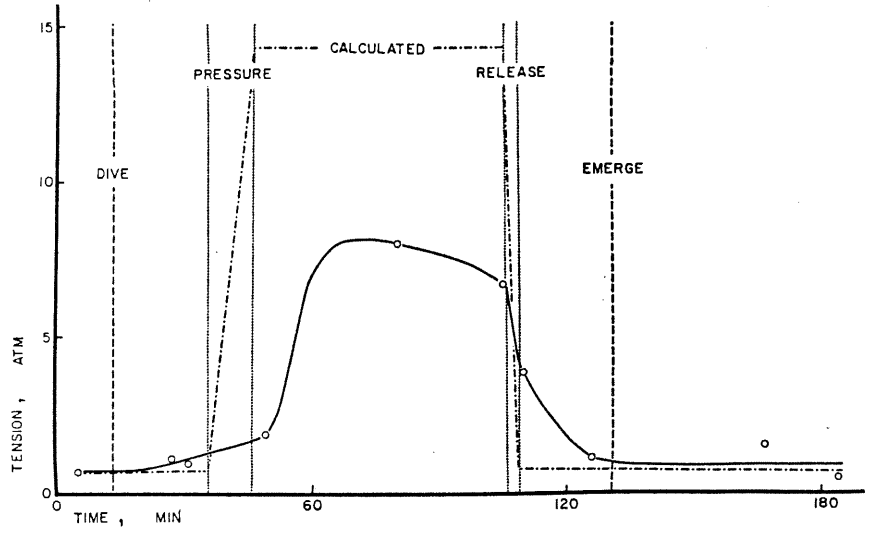

FIG. 9. Changes in nitrogen tension of arterial blood before, during and after exposure to 18·7 atm. Broken line represents calculated nitrogen tension (saturation) at pressure.

In the second experiment, the hydrostatic pressure is increased in a stepwise fashion to determine the relationship between pressure applied and blood nitrogen tension and also to assess nitrogen diffusion qualitatively. The procedure is to double the pressure every 15 min until collapse pressure is attained and to sample at 5 and 15 min of each stage. Thus two samples are drawn at atmospheric pressure, two at 2 atm, two at 4 atm, two at 8 atm, two at 19·4 atm and finally two at atmospheric pressure again. The nitrogen tension is positively correlated with the pressure applied (Fig. 10). At pressures up to and including 4 atm the nitrogen

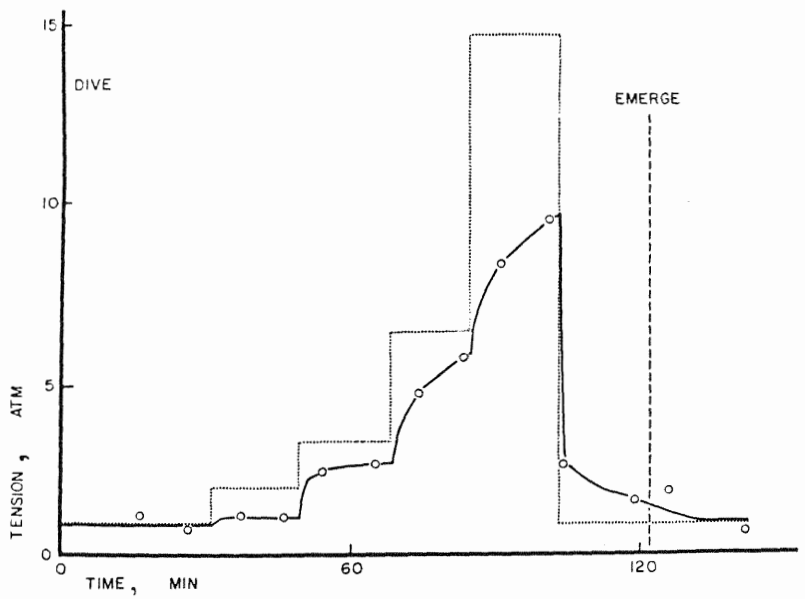

FIG. 10. Changes in blood nitrogen tension induced by increasing the hydrostatic pressure in a stepwise fashion to 19·4 atm. Dotted line represents calculated nitrogen tension (saturation) at each step.

tension reaches its maximum value within 5 min after pressure is applied, and then levels off. Above this pressure diffusion is less rapid, but still the major portion of the ultimately dissolved gas is detectable within 5 min. The most important point is that whereas the nitrogen tension of the blood is significantly higher in each step than saturation of the stage just beneath it, at no time are equilibrium values attained.

Knowing that the tension of blood nitrogen does not reach its calculated saturation value in the deep-diving turtle, it would be of great interest to compare observed nitrogen tensions of the blood and trachea at various hydrostatic pressures. This has been done (Fig. 11), using data from seven experiments. It is immediately apparent that equilibrium does not exist at any pressure and that this lack of equilibrium is especially pronounced at high pressures.

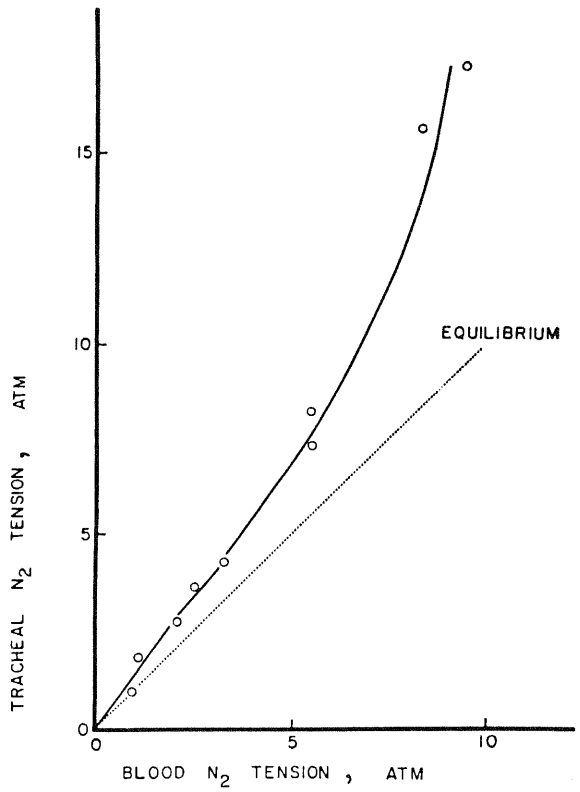

FIG. 11. Comparison of tracheal nitrogen tension with carotid artery blood nitrogen tension of turtles exposed to various hydrostatic pressures.

CONCLUSIONS

The bradycardia of submersion is accentuated when pressure is applied. The number of minutes during the pressure phase of the dive without heart beats ranges from 28 per cent to 68 per cent of the total. This significant reduction in circulation could be a very potent method of reducing the distribution of gases from the lungs throughout the tissues of the body where they could give rise to bubbles, and hence induce caisson disease, on decompression. This sort of adjustment would bear out Irving's (1935) prediction, for it would limit the amount of nitrogen in the tissues to that which could enter without the aid of the circulatory system.

After pressure release, but while the turtle is still submerged, the heart rate immediately increases to a value well above that typical of the same stage of a simple prolonged dive. Once again, this would be advantageous, for the lungs will have expanded to their normal size and the partial pressure of the lung nitrogen will be less than that of the blood and tissues. The enhanced circulation

serves to clear the body fluids of whatever nitrogen was forced in during the dive. Full normal heart function is not yet seen, possibly because the diving bradycardia still is being maintained for energy considerations. If so, a compromise has been effected between the dual needs of the animal to clear nitrogen and to conserve energy.

The pressure–volume characteristics of the lungs provide support for Scholander's hypothesis that the mechanical response of the lungs is of prime importance in the prevention of caisson disease by the retardation of nitrogen invasion. The results demonstrate that the lungs of the turtle may be compressed and collapsed, either by aspiration or by external hydrostatic pressure.

In every pertinent experiment in which analyses were performed, the tensions of each lung gas dropped during the course of the pressure part of the dive. The oxygen and carbon dioxide disappear in excess of or contrary to metabolic dictates and the nitrogen more slowly, as would be expected from its much lower solubility. The fact that the slope of the curve representing nitrogen disappearance from the lungs is no greater at high pressures than at low suggests that nitrogen diffusion may not be as great as would be expected at increased pressures.

The blood nitrogen picture supports the suggestion that the invasion of nitrogen is inhibited. As the pressure is increased, the tension of nitrogen increases in the blood, but not as predicted by Henry's Law, implying some interfering mechanism. In an experiment wherein the turtle was subjected to rapid increase of hydrostatic pressure to 18·7 atm, the nitrogen tension of the blood rose rapidly to a high of only 8 atm. Thirty minutes later the tension had dropped to 6·6 atm (Fig. 9). These values are considerably lower than the calculated saturation tension of 14·5 atm. This animal died several hours after the dive ended. Autopsy revealed numerous gas emboli in the capillaries of the cervical fascia and in the right auricle. In a later experiment the pressure was increased stepwise, until the lungs collapsed (Fig. 10). The nitrogen tension of the blood increased each time the pressure was increased but never reached calculated saturation. This turtle also developed caisson disease. Comparison of observed blood and tracheal nitrogen tensions also shows lack of equilibration at all measured pressures up to 19 atm (Fig. 11).

This consistent lack of saturation may be due to insufficient time for equilibration before the samples were drawn, to the admixture of the carotid artery blood with venous blood, or to an insufficient supply of nitrogen in the lungs to permit saturation. We know from Fig. 9 and from the lower pressures of Fig. 11 that time is not the limiting factor, for the nitrogen tension is seen to reach a maximum and then to drop or level off well below saturation level.

Admixture of carotid artery blood with venous blood may be due to the turtle's three-chambered heart shunting some vena cava blood past the pulmonary circulation or it may be due to the lungs being collapsed, so as to prevent the diffusion of nitrogen into the blood. These two sources of admixture cannot be separated definitely without additional measurements on blood from the vena cava and pulmonary artery and vein. However, it is seen in other parts of this work that the circulation is slowed and that selective ischemia probably occurs

during a dive. These factors will reduce the rate of return and probably lessen the mixing of vena cava blood with arterial blood. If the lungs are patent, the pulmonary vein blood should be fully saturated; if they are partially collapsed this blood should be partially saturated; and, of course, if the lungs are fully collapsed the returning blood will show little increase in nitrogen.

The third possible explanation for the lack of saturation may be that lung nitrogen is limited, and at equilibrium only subsaturation tension is possible. A 20-kg turtle carries down about 1·6 l of nitrogen, enough to yield a blood tension of about 6·7 atm at equilibrium. In the experiment represented by Fig. 9 the observed blood tension was such as to imply that the total nitrogen of the lungs was distributed uniformly throughout the body. However, in view of the extremely slow circulation and the selective ischemia exhibited by the diving turtle it is hard to accept this explanation. The same partial saturation would be seen if the lungs were collapsed, excluding most of the nitrogen from the blood, and the circulation was reduced to a heart–brain–lung system. As other data suggest that these conditions prevail, this explanation is more probable. Total tissue equilibrium is further denied by the data in Fig. 10, which shows that arterial blood was never saturated at lower pressures even though sufficient nitrogen was available, and at lung-collapse pressure the tension was higher than could be reached unless portions of the body were excluded from the circulation. The data shown in Fig. 11 also support this view, for at all pressures, and especially at collapse, the blood nitrogen tension lags behind the tracheal tension, implying exclusion of nitrogen.

On the basis of these findings it is apparent that the turtle has no innate immunity to caisson disease, but that it is protected to some extent against nitrogen saturation. The circulatory adjustments undoubtedly retard nitrogen invasion considerably, but not enough to remove all danger, for during a prolonged deep dive the nitrogen tension in the carotid artery is very high, increasing the possibility of gas emboli in the brain.

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