titer was also lower in a group of patients with the last-mentioned diseases. Antibodies to double-stranded RNA were found in 54 percent of 61 SLE serums tested. These antibodies were also found in a low incidence in patients with rheumatoid arthritis, infectious mononucleosis, chronic active hepatitis, and in patients chosen at random as controls.

Radioactive labeled DNA was precipitated with ammonium sulfate (*10*) in order to determine whether antibodies to single-stranded DNA were also detectable by this method. This test revealed an even higher incidence of antibodies to single-stranded DNA than did the hemagglutination assay. However, further studies are required to define this system, since antibodies which react with single-stranded DNA are not as easily inhibited in the ammonium sulfate system as in the hemagglutination assay.

Comparison of the titers of antibody to single-stranded DNA was made in nine patients with SLE during the stages of active disease and during periods of no evident clinical activity. Although the titers of antibody to native DNA and serum complement were closely correlated with clinical exacerbations, antibody to single-stranded DNA persisted during quiescent periods in eight of nine serial studies over periods of 1 to 2 years. Figure 1 illustrates the course of one patient whose serum showed three peaks of antibody to single-stranded DNA. Two peaks occur during periods when antibody to native DNA is not detectable and serum complement is normal. Although the participation of antibody to single-stranded DNA in an antigen-antibody complex system cannot be excluded, the ubiquitous distribution of these antibodies would suggest either that they are not components of an immune complex system of significant cytotoxicity or, alternatively, that circulating single-stranded DNA appears infrequently. Certain periods are noted when a rise in titer of antibody to single-stranded DNA corresponds with an increase in the titer of antibody to native DNA. Our studies are directed toward identifying circulating single-stranded DNA before or after these peaks of antibody activity.

The data presented indicate that a variety of antibodies to polynucleotides (native DNA, single-stranded DNA, and double-stranded RNA) are found in patients with SLE. Although the factors eliciting the formation of these antibodies are unknown, viral nucleotides or breakdown products of DNA may serve as antigens in a variety of diseases as well as SLE. It is possible that a unique determinant of altered DNA occurs in subjects with SLE or that a specific genetic predisposition exists for the formation of antibodies to native DNA.

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30 June 1969; revised 7 October 1969

Buoyancy Control in the Freshwater Turtle, Pseudemys scripta elegans

Abstract. *Freshwater turtles* (Pseudemys scripta elegans) *significantly corrected experimental displacements of their specific gravity. By reciprocally changing the volumes of lung air and stored water, they set their buoyancy and maintained their body volume. The cloacal bursae may be the active site for water storage and exchange in this mechanism.*

The ability of turtles to control their specific gravity has been considered for many years (*1*) but neither a clear experimental demonstration of control nor an adequate description of the mechanism has been given. Control is most apparent to the observer in the semiaquatic freshwater turtles which, despite their heavy shells, maneuver easily through the water, choosing at one moment to float at the surface and at another to rest on the bottom. To confirm experimentally the existence of control in such a turtle, I displaced the variable suspected of control (specific gravity initially, (ii) attach disturbance (weights or floats) and then noted whether the animal could restore the displaced variable to normal.

Female turtles of the species *Pseudemys scripta elegans*, weighing from 400 to 600 g, were studied. The experimental procedure to test for buoyancy control was to (i) determine specific gravity initially, (ii) attach weight or float, (iii) place the turtle in deep water (25 cm or more) for 18 hours (*2*), and (iv) determine final specific gravity. To measure specific gravity, I weighed each turtle in air (to 1 g) on a triple-beam balance and in water (to 0.1 g) by placing the turtle in a wire cage, submerged in distilled water, suspended from the below-table hook of a top-loading balance. Specific gravity was experimentally increased by taping a lead weight to the plastron (approximately doubling the turtle's weight in water) and was decreased by taping a Styrofoam float to the carapace (about counterbalancing the weight in water). Two groups of eight turtles each were tested with both weights and floats. Turtles in the first group were intact; those in the second, or cloaca-occluded group, each had a soft rubber plug tied securely into its cloaca.

The intact turtles significantly adjusted their specific gravity toward the initial value in response to both weights and floats; the cloaca-occluded turtles successfully compensated only for the floats (Table 1). These results confirm the existence of buoyancy control in this species and also suggest the mechanism. In each adjustment the body weights in water and in air

changed in parallel; for example, in response to the lead weights, each intact turtle lost an average of 19 g in water and 24 g in air. I interpret these changes as an addition of 19 ml of lung air (*3*) and a loss of 24 ml of stored water, respectively. As a consequence, despite these sizable shifts in air and water, the body volumes of the turtles remained relatively constant.

The observed effects on specific gravity resulted almost entirely from the turtles' adjustment of their weight in water or, presumably, of their lung volume. The correlation between lung volume and specific gravity was established by measuring these variables (Fig. 1). Lung volume was measured in a whole-body plethysmograph (*4*). Normally the specific gravity of *Pseudemys* freely swimming in deep water was between 1.003 and 1.006. The wider range in this group was produced by inducing compensations (as described above) or by keeping the turtles in shallow water, where buoyancy control was erratic. The lung volume of these turtles varied from 2.6 to 16.9 percent of the body volume.

The shift in water volume, though not significantly affecting buoyancy by itself, was nonetheless necessary for buoyancy control. This was demonstrated by the failure of the cloaca-occluded turtles to adjust to lead weights, an operation which in the intact turtles involved a loss of stored water. It is known that turtles can move water in or out of the cloaca (*5*), presumably to or from the cloacal bursae, a pair of sacs which open the dorsolateral wall of the cloaca. In the cloaca-occluded turtles this water exchange was prevented. Compensation for the floats was possible, however, because water could be added to the body through the mouth.

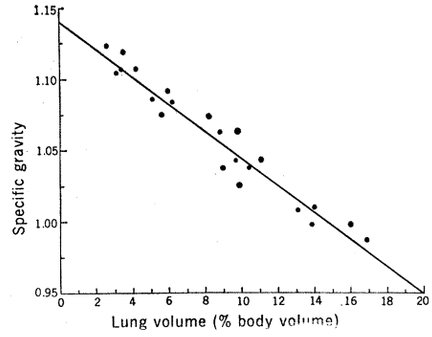

Fig. 1. Relation between lung volume and specific gravity ($r = -0.98$). Twenty-two determinations are shown from 13 turtles. Body volume (in milliliters) was assumed to equal numerically the difference (in grams) between the weights of the turtle in air and water ($y = -0.0095 \times +1.14$).

To determine the site of water uptake and storage in intact turtles, water uptake was induced by experimentally decreasing lung volume, thus simulating the buoyancy adjustment to floats. In this procedure, I submerged turtles for 2 hours after I exposed the animals to an atmosphere of 100 percent oxygen for 1 hour. Lung volume invariably decreased during this apneic period, in one case from 51 ml to 3 ml. Turtles ejected a large volume of water from the cloaca during the first few breaths after this procedure. In six turtles killed and dissected without being allowed to breathe, the cloacal bursae were abnormally distended with clear water, averaging in volume 8.3 ml per 100 g of body weight. Small volumes of water (less than 1 ml per 100 g) entered the gastrointestinal tract and, based on two of the experiments in which the turtles were submerged in water dyed with phenol red, little or no water passed directly into the urinary bladder.

The obligatory adjustment in water volume which accompanies a change in lung volume appears to be due to the restraint on variation in body volume imposed by the turtle's shell. Apart from the normal cyclic variation associated with respiration, substantial variations in body volume are evidently not tolerated, in view of the adverse effect on internal pressure. This principle of maintained volume (or pressure) may explain the seemingly incongruous finding (*6*) that musk turtles, starved for many months, nevertheless maintained constant weight when kept in water.

The shell of the freshwater turtle, because of its high specific gravity (1.444 ± 0.058; $N = 11$), has further significance for buoyancy control. While the shell averaged only 30 percent of the turtle's weight in air, it accounted for about 75 percent of the weight of the turtle in water (weight corrected for lung volume), thus explaining the high specific gravity (1.14) of the turtle after such correction (Fig. 1). Consequently, in order to achieve neutral buoyancy, the turtle must have an unusually large lung volume (14 percent of body volume).

The cloacal bursae, shown to be the site of water storage, are concluded to be the site for active, short-term adjustments in water volume. However, a larger volume of fluid is commonly located in the urinary bladder. This site apparently acts as a relatively static depot involved in large or long-term buoyancy adjustments. The function of the cloacal bursae and urinary bladder together is analogous to that of the ballast tanks in a submarine.

The function of the cloacal bursae has been disputed for many years. Suggestions include underwater respiration (*7*), soil moistening prior to egg-laying (*8*), and ion conservation (*9*). A possible role in buoyancy control was suggested (*1*) but not proved. In 1936 Lüdicke (*10*) observed that during breathing the lungs and cloacal bursae of *Emys orbicularis* expanded and contracted concurrently and proposed that the function of the cloacal bursae is to regulate lung volume. This function is consistent with the role I propose

Table 1. Specific gravity adjustments in intact and cloaca-occluded turtles in response to attached weights and floats. Specific gravity (total) includes weights and floats, the other values do not. Changes are differences between initial values and final values after 18 hours. All values are means ± S.E. Significance of changes in specific gravity was determined by the *t*-test.

Treatment	Weight in		Specific gravity	
	Air (g)	Water (g)	Net	Total
		Intact turtles		
Floats				
Initial	489 ± 18.9	19.1 ± 4.2	1.040 ± 0.008	0.982 ± 0.007
Change	+17.8 ± 4.2	+15.6 ± 2.2	+0.033 ± 0.004*	+0.031 ± 0.004*
Weights				
Initial	499 ± 19.8	22.2 ± 2.3	1.046 ± 0.005	1.104 ± 0.005
Change	−24.2 ± 2.7	−18.6 ± 2.9	−0.039 ± 0.006†	−0.038 ± 0.006†
		Cloaca-occluded turtles		
Floats				
Initial	485 ± 21.2	27.8 ± 3.6	1.061 ± 0.007	1.000 ± 0.006
Change	+11.5 ± 2.4	+14.0 ± 1.9	+0.035 ± 0.006*	+0.030 ± 0.004*
Weights				
Initial	478 ± 19.6	25.9 ± 2.6	1.058 ± 0.005	1.118 ± 0.005
Change	+5.9 ± 2.0	+0.9 ± 2.8	+0.004 ± 0.006	+0.004 ± 0.006

* $P < .01$. † $P < .001$.

that these structures serve in buoyancy control.

The occurrence of cloacal bursae among various turtle families has been surveyed (*11*) and is of some interest in regard to possible buoyancy control. In general, cloacal bursae are found in semiaquatic, freshwater turtles (such as *Pseudemys*) but are absent in the highly aquatic, bottom-dwelling soft-shelled turtles (Trionychidae), in shallow-water mud and musk turtles (Kinosternidae), and in the terrestrial tortoises (Testudinidae). In none of the latter families would buoyancy control represent a particularly useful adaptation. However, cloacal bursae are also absent from the marine turtles although these forms would be expected to possess some buoyancy control.

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Respiration and Deep Diving in the Bottlenose Porpoise

Abstract. *A bottlenose porpoise was trained to dive untethered in the open ocean and to exhale into an underwater collecting funnel before surfacing from prescribed depths down to 300 meters. The animal was also taught to hold its breath for periods up to 4 minutes at the surface and then blow in the funnel. Alveolar collapse is probably complete at around 100 meters, and little pulmonary respiratory exchange occurs below that depth. Thoracic collapse was observed visually at 10 to 50 meters and by underwater television to 300 meters.*

Sperm whales appear to be able to dive to at least 1500 m (*1*), and Weddell seals are known to reach 600 m (*2*). In spite of being air breathers, these true marine mammals have considerable three-dimensional freedom in the sea. To make such dives, they must be able to tolerate the pressures of these depths (60 to 150 atm), and they must also be able to hold their breath much longer than land mammals. We have studied these problems with a trained porpoise in the open sea.

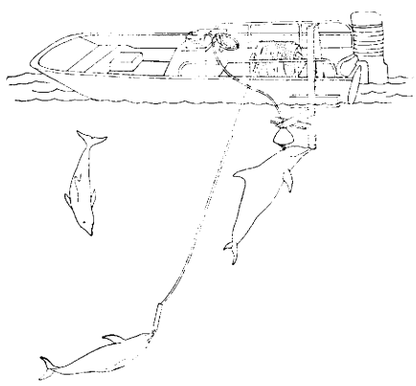

Fig. 1. The experimental setup for deep-diving experiments in the open ocean. The porpoise dives down when the go signal is turned on. He pushes the plunger on the end of the diving test switch, turning the go signal off, and then returns to exhale into the funnel before surfacing.

Scholander (*3*) proposed that alveolar collapse would occur in diving mammals at a depth of about 100 m. Such collapse prohibits gaseous exchange during deep dives and possibly protects the animal from decompression sickness and nitrogen narcosis.

Fiebiger (*4*) suggested that the unique, smooth muscular sphincters of cetacean bronchioles functioned to entrap air in the alveoli. This air would remain in contact with respiratory epithelium during deep dives. We tested both hypotheses in a species that has these sphincters (*5*) and provided further information on diving depths of porpoises, a point of contention (*6*, *7*).

The experiments were carried out with a male bottlenose porpoise *Tursiops truncatus*, 2.25 m long and weighing 138 kg. This animal ("Tuffy") has participated in numerous studies, including the Navy Sealab II experiment (*8*, *9*), and has been employed to find and mark underwater equipment containing acoustic beacons. For this study two tasks were taught. The first required the porpoise to dive on acoustic command to a switch located at the end of a cable. Tuffy was required to press the switch turning the sound off and to return to the surface and exhale into an inverted water-filled funnel with the large opening about 50 cm below the surface (Fig. 1). He was also taught to breath hold just under the surface, again in response to a sound, and to exhale into the funnel on command. Thus expired air could be collected from any depth or duration of dive that the porpoise was willing to make.

Tuffy was trained to work with divers on the ocean bottom, and we took advantage of this to have him swim rapidly back and forth between two divers at 20 m depth so that expired air could be collected after exercise at that depth. He could also be commanded to come to, and exhale into, the funnel on any breath after a deep dive or after a surface breath hold. Thus we collected air samples of breaths from the 3rd to the 15th after a dive, from the 3rd to the 15th after a surface breath hold, and from random breaths during normal leisurely swimming. We also interrupted hyperventilation, which occurred in anticipation of deep dives, to collect expired air samples.

For deep-diving experiments Tuffy was released from his pen to swim beside a small outboard-powered boat to a diving site up to 8 km offshore. The porpoise usually took up a position in the boat's stern wave and thus actually "surfed" for most of the trip.

The deep-diving device consisted of an acoustic beacon, off switch, temperature sensor, and pressure transducer in a housing at the end of 308 m of five-wire marine cable. A control box in the

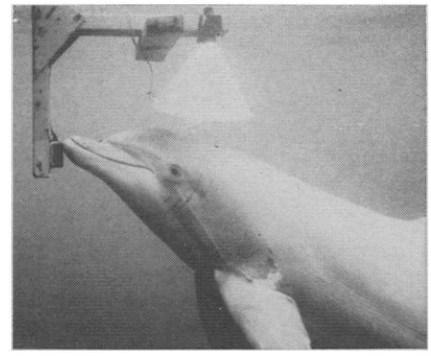

Fig. 2. Porpoise exhales into the funnel just below the surface.