Deep Body Temperature of Untethered Dolphin Recorded by Ingested Radio Transmitter

Abstract. *Information concerning body temperature was recorded by radio transmission from within a dolphin,* Tursiops truncatus, *swimming freely in a pool. The temperature varied according to the activity of the animal and appeared to oscillate when the dolphin was returned to the pool after a brief period outside it. The transmitter was passed approximately 17 hours after it had been swallowed. A new type of antenna was used with the circuit. The transmitter can be adapted for telemetering heart beat, movement, and other physiological variables from aquatic animals.*

One of the more interesting groups of animals is perhaps the least susceptible to study by the usual laboratory methods. The physiology of diving mammals is often followed by taking them from their watery medium and preventing the free motion and diving so basic to their activities. It thus seems important to follow their internal processes by radio telemetry. Telemetering of inside information from humans and other animals in a relatively normal psychological and physiological state is highly developed; information on this subject has been reviewed (*1*) and brought up to date (*2*). The shielding effect of the sea-water medium offers a special challenge in the case of such animals as whales, dolphins, and seals. In this report I describe an experiment with a dolphin, *Tursiops truncatus*, which indicates that it should be possible to record any variable for which there is a sensing transducer; this is the first such experiment conducted on a marine mammal.

The telemetry of physiological information by ultrasound was rejected because of the disturbing effect it would probably have on the normal activities of animals which are known to have good high-frequency hearing associated with sonar and communication activities. Use of frequencies above the animals' normal sound range yields restricted transmission distance with a remaining possibility of sensation due to nonlinearities in the ear. Radio signals are attenuated by passage through sea water or tissue, with signals falling to about one-third in a distance in meters equal to 240 divided by the square root of the frequency in cycles per second; in addition to this shielding effect, there is the usual geometrical decrease with distance, which, for a dipole source, is with the cube of the distance. To obtain reasonable range, very low frequencies are desired, but it is known that the physiological effects of electric currents steadily increase as one approaches 100 cy/sec.

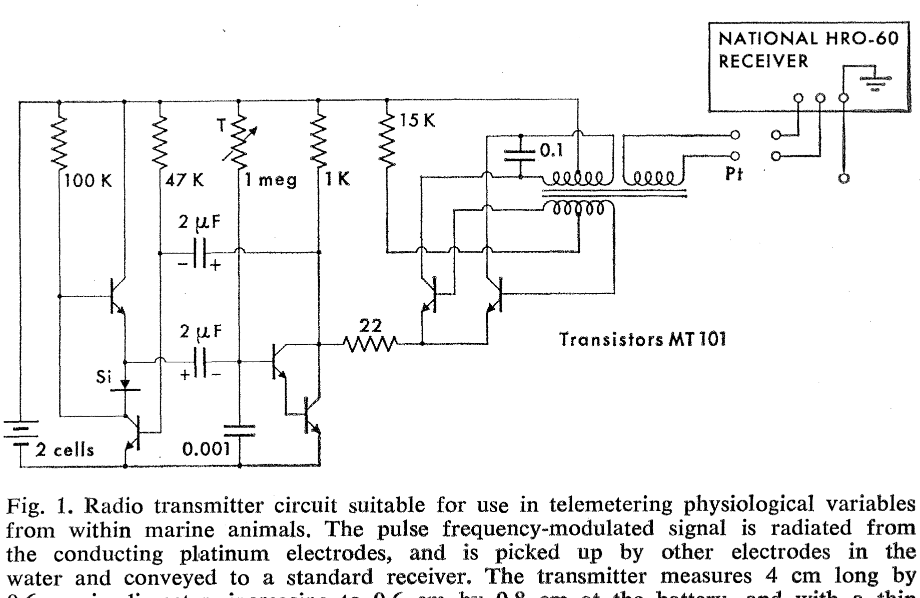

Fig. 1. Radio transmitter circuit suitable for use in telemetering physiological variables from within marine animals. The pulse frequency-modulated signal is radiated from the conducting platinum electrodes, and is picked up by other electrodes in the water and conveyed to a standard receiver. The transmitter measures 4 cm long by 0.6 cm in diameter, increasing to 0.6 cm by 0.8 cm at the battery, and with a thin flexible wire to increase antenna size extending two more centimeters. A similar temperature-sensing circuit with loop antenna has been used to track and study a marine iguana on the Galapagos Islands.

Because of the narrow gastrointestinal tract of the dolphin, the signal in the experiment reported herein was radiated from a pair of electrodes rather than from the usual wire-coil antenna (*1*). To minimize the possibility of ventricular fibrillation or sensation by the animal, the frequency used was approximately 55 kcy/sec. Because of the nonlinear rectifying action of cell membranes, it might be expected that any amplitude modulation in the signal would be sensed, but at these higher frequencies the current is bypassed by the shunting capacity of the membrane. When the circuit of Fig. 1 was applied to the human tongue, its signal could be sensed when operating at 20 kcy, but not at higher frequencies. During the development of this circuit, a number of preliminary experiments were conducted in a tank with *Lagenorhynchus*, which displayed no tendency to avoid such a transmitter, and even showed a tendency to eat the test apparatus out of my hand.

Final testing was done by placing the complete circuit in my mouth and recording the signal while swimming in various parts of the 245-kl porpoise tank at Steinhart Aquarium in San Francisco. Reliable transmission was found over the whole 13.5-m length of the tank. A standard receiver that covered this low-frequency band was used. A good signal was picked up both by shielded and unshielded single turn 2-m loops submerged in the water; signal strength was somewhat reduced when the loop was brought out into the air. A stronger signal was obtained by simply hanging the bare-ended antenna leads down into the water a meter or so apart; this arrangement sensed the electric current distribution set up in the water, and was the one used in the actual experiment. The use of a separate ground electrode minimized noise.

The transmitter consisted of two parts (Fig. 1). The left half of the circuit was a so-called multivibrator whose period between switchings was controlled by the temperature-sensing thermistor *T*. This multivibrator switched on and off the 55-kcy oscillator that constituted the rest of the circuit. The oscillator was switched on for about 20 msec and then off for approximately a second, depending on temperature. This multivibrator produced a time interval that was almost independent of voltage and transistor characteristics. The entire circuit, exclusive of battery and output transformer, occupied a cylinder measuring 0.6 cm in diameter and 1.0 cm long. The output transformer contained eight turns on the base winding, six turns on the collector winding, and three turns on the output winding, all longitudinally wound as a torus on a 0.5 cm diameter tube of Ferroxcube 3B measuring 2 cm long. The transmitter ran for about 35 hours on two Mallory 312 mercury cells; two silver oxide cells such as Eveready S13E would have given slightly more voltage. The transmitting antenna consisted of two platinum wire loops, 0.3 cm in diameter and spaced about 8 cm apart, the second being placed at the end of a springy, insulated wire, for flexibility in passing through the intestine. The components were potted in epoxy resin and wrapped in two layers of 0.025-mm-thick etched Teflon. The ends were sealed with silicone rubber.

The signal from such a transmitter is best heard if the receiver is set for "continuous wave" operation in which a local oscillator combines its output with the arriving signal to give an audible-frequency pulse. The signal could be detected from all regions of the tank, with a weak signal appearing for certain orientations of the transmitter; omnidirectional schemes usable with four electrodes have been discussed (*1*, *2*). The pitch or intensity of the audible signal does not affect interpretation of the temperature indication of the signal. In the dolphin experiment, the signal was timed by using a stop watch to determine the period of a few dozen cycles. By timing a number requiring over 30 seconds for their occurrence, it is unlikely that the observation process can introduce an error comparable with the basic accuracy of the circuit. Timing can be automated by the use of suitable circuits (*1*), and must be if this type of transmitter is used to telemeter more rapidly changing physiological variables.

A 125 kg female *Tursiops truncatus* (named Doris) was made available at Point Mugu in an 8-m circular tank filled to a depth of 1.2 m with ocean water. The transmitter was inserted into a dead fish through the gills, and the dolphin readily swallowed it whole. The transmitted signal could be detected immediately as the animal went about its various activities.

The temperature of the transmitter and surrounding fish soon came to equilibrium at 37.6°C, while the temperature of the water was 13.7°C. During this period, when the transmitter was in one of the first stomachs, the swallowing of a new, relatively cool fish caused a transient drop in indicated temperature of approximately 2°C. This form of transmitter thus provides a method to study the feeding habits of these animals.

During the next 3 hours after feeding, the water temperature increased by 1.6°C, and the animal's temperature remained constant within 0.2° at 36.7°C. The dolphin, which was being used by some other workers for a series of tests, was then conditioned for an hour. During this time its temperature remained fixed at 1.1°C below the previous temperature, while the water temperature increased 0.2°C. A half-hour after termination of this activity, the temperature returned to its previous level. At this time the dolphin was removed from the pool on a stretcher and laid on the ground, where its temperature increased 0.8°C in 25 minutes. Rectal temperature showed a sharp gradient and was 36.3°C at 25 cm. The dolphin was then returned to the pool, where temperature rapidly decreased by 3.0°C, perhaps because of the vasodilation which occurs when these animals are out of the water. Twenty minutes later, the indicated temperature had gone up 1.4°C, and then in another 20 minutes, it had dropped by the same amount. It then rose to 35.3°C, a value about 0.8°C below the value before removal from the pool. It seemed as if several cycles of damped oscillation in a temperature regulator were being observed, though passage of the transmitter past local warm regions, or the swallowing of cool liquids could not be ruled out entirely. The temperature of the water, which was then 15.5°C, decreased steadily during the next 9 hours to 13.6°C, while the dolphin's temperature increased by 0.8°C. It is not yet known whether this increase was due to a change in position of the transmitter within the body, or an overall rise in body temperature. The experiment ended at about 3 a.m.

As the dolphin circled about the tank, variations in the strength of the signal could be detected easily. Approximately 17 hours after the start of the experiment, the transmitter was expelled, the sudden drop in temperature producing a dramatic change in signal, which thereafter was of constant amplitude. There is little information on the time it takes for an identifiable object to pass through the gastrointestinal tract of a dolphin. This particular object was about 8 cm long, partly rigid and partly flexible, and was about as thick as a lead pencil. Passage time may not be constant; I have observed that radio transmitters have remained in my stomach for 12 hours before passing on, which is longer than the usual emptying time.

The type of antenna used gave a strong signal in a very small space. The signal not only was detected everywhere in the pool, but could still be received easily even when the dolphin was placed in a canvas stretcher on the dry ground a meter away from the pool. The signal was sensed whether the receiving antenna electrodes were still in the pool or lying on the ground near the dolphin. Essentially the whole animal becomes the transmitting antenna, and this may offer a very effective means for telemetering physiological information from within land animals as well as aquatic animals. At considerably higher frequencies, the "skin effect" may spread the current path and thus make an animal's body in air a more efficient radiating antenna (after suitable impedance matching with a slightly higher voltage source). The ratio of the numbers of turns of the primary and secondary coils on the transformer was chosen to match the electrical resistance of gastric juice and thus give a good power transfer into it. An equally good output circuit is a constant-current network consisting of a series-tuned circuit, with the electrodes connected across the inductance.

If the core of the transformer in Fig. 1 is split and stuck together again with silicone rubber, it serves as a microphone which modulates the frequency of the transmitter; it thus can simultaneously transmit heart beat if the oscillator is biased so the signal never drops to zero. We have built (*3*) implantable accelerometers to monitor motion, oxygen electrodes to study breath holding, subcutaneous microphones suitable for monitoring turbulence and its control, other microphones adequate to monitor sonar and communication activities, ultrasonic probes that can monitor supersaturation and decompression sickness (*4*), and all of these transducers can be used to modulate the transmitter circuit in Fig. 1. These transducers can be inserted through a hypodermic needle or trochar into inaccessible regions and the leads run to a transmitter to avoid the anesthetic problem (*3*).

The output of the circuit of Fig. 1 can be recorded directly by a magnetic tape recorder, so that a buoyant self-detaching recoverable unit can be used to handle the problem of an animal freely roaming the ocean (*5*). Circuits also exist that are suitable for relaying the internal signal by burst transmission each time the animal surfaces for a breath, though present technology demands that, in general, preprocessing of the information should be done so that essentially the result of the experiment is transmitted, rather than all of the detailed data. For example, in the case of heart-rate diving reflex, this means continuously energizing the external booster transmitter, which would be situated on the dorsal fin to minimize body turbulence, to radiate a frequency-modulated signal proportional to the recent derivative of instantaneous heart rate (*3*). Experiments on many species indicate that multiple transmitters can work simultaneously at different frequencies so that it is possible to record any of the physiological variables as a function of depth, velocity, or temperature, by merely making a separate simultaneous recording of this other variable.

R. STUART MACKAY

Space Sciences Laboratory and Division of Medical Physics, University of California, Berkeley 4

References and Notes

1. R. S. Mackay, *Science* **134**, 1196 (1961).
2. ———, *New Scientist* **19**, 650 (1963).
3. ———, in *Proc. 1st Internat. Cetacean Research Conference, Washington, D.C., August, 1963*, K. Norris, Ed., in press.
4. ———, in "Proc. 2nd Symp. Underwater Physiol., Washington, D.C., Feb. 1963," *NAS–NRC Publ. 1181*, C. J. Lambertsen, and L. J. Greenbaum, Jr. Eds. (National Academy of Sciences–National Research Council, Washington, 1963), p. 41.
5. Electronic recording can be supplemented in the case of depth by attaching to the pointer of a pressure gauge a radioactive pellet, in which case degree of blackening on an adjacent film indicates percentage of time spent at different depths.
6. I thank F. Jenkinson, H. Fishman, M. Bohrod, and E. Woods for technical assistance, E. Herald and the California Academy of Sciences for making available facilities for the preliminary experiments, T. Lang, F. G. Wood, and S. Ridgeway of the Naval Ordnance Test Station and Naval Missile Center for placing at my disposal the facilities for the final experiment, Doris for not chewing her fish, and Jean Mackay for help in taking the data. This investigation was aided by NASA grant SC-5861.

17 March 1964

Pluripotent Stem Cell Function of the Mouse Marrow "Lymphocyte"

Abstract. *Bone marrow from normal and polycythemic mice was filtered through glass wool columns to remove cells other than "lymphocytes." For a given number of nucleated cells, filtered marrow was more efficient than the original marrow in repopulating the spleen of an isogenic recipient previously exposed to lethal irradiation. The proliferative capacity of both the filtered and unfiltered marrow suspensions appeared to be a constant function of the number of small and medium "lymphocytes" present and not of any other cell type.*

Normal bone marrow contains stem cells which are capable of continued proliferation. These may be characterized by their ability (i) to regenerate the hemopoietic tissues of an animal exposed to sublethal irradiation (*1*), (ii) to repopulate, on transplantation, the depleted hemopoietic sites of recipients exposed to lethal irradiation (*2*), and (iii) to reestablish erythropoiesis after the disappearance of recognizable red cell precursors from the marrow of animals made polycythemic by erythrocyte transfusion (*3*). The morphological and functional characterization of the stem cells has long been the subject of controversy (*4*). Studies of the cellular composition of regenerating marrow and of marrow in young animals have suggested, however, that in guinea pigs (*1, 5*), rats (*6*), and mice (*7–9*) the hemopoietic competence of the marrow depends on its content of small, round, mononucleated cells rather than on its content of reticulum cells (*10*) or of granulocytoblastic and erythroblastic elements (*3, 8, 9*). The small round cells in question are lymphocytic in appearance (*1, 4–11*), and are capable of incorporating tritiated thymidine in the nucleus (*1, 8–11*) and of proliferating *in situ* (*1, 8, 10, 11*). Collectively, these findings constitute strong evidence for the concept that the marrow "lymphocyte" is a hemopoietic precursor cell. They do not exclude, however, the possibility that marrow "lymphocytes" may instead exercise some yet undefined trophic or other function that affects hemopoietic cell production by different cell types, although certain of the cited data are not easily reconciled with such an interpretation (*8, 11*).

In this report we describe an experiment in which the competence of mouse marrow "lymphocytes" to act as hemopoietic stem cells was tested directly. This was accomplished by using a glass-wool filtration procedure (*12*) for removing from a suspension of donor marrow cells most of the other elements (Table 1). The proliferative capacity of the cells in the filtrate was assayed by measuring their ability to promote hemopoietic repopulation in irradiated recipient mice. The results were consistent with the interpretation that marrow "lymphocytes" behaved as pluripotent stem cells.

Marrow from long bones of 12-week-old C3H/DiSn or (C3H/Anf × C57 BL)F_1 female mice was suspended in Tyrode's solution to give a concentration of 10^7 nucleated cells per milliliter. Aliquots of the marrow suspensions were filtered twice at room temperature through columns of tightly packed glass wool, prepared according to the description of Hildemann *et al.* (*12*). Original and filtered marrow-cell suspensions were diluted with chilled Tyrode's solution to the desired concentration (10^5 to 3×10^5 cells per milliliter), and 1- to 2-ml injections were given in the tail vein of isogenic recipient females (five to seven animals per group) exposed a few hours earlier to 900 r of 300 kv (peak) total-body x-irradiation.

Aliquots of the diluted cell suspensions were centrifuged for 10 minutes at 1800*g* in a refrigerated centrifuge, and the resulting pellet was smeared on glass slides with a sable brush for differential cell counts. The smears were stained with benzidine for hemoglobin and counter-stained with Wright-Giemsa stain to facilitate distinction between small erythroid cells and "lymphocytes." The total cell yield and the degree of lymphocyte enrichment were related to the compactness of the glass-wool columns; the more compact the column, the lower the yield of cells but the higher the percentage of "lymphocytes" in the filtrate (Table 1, experiment 1). When prospective donor mice were made poly-