PRL Control

THE PHYSIOLOGICAL RESEARCH LABORATORY

Scripps Institution of Oceanography

-A brief synopsis of research activities-

Scientists in the Physiological Research Laboratory (PRL) concentrate on behavioral, physiological, and biochemical adaptations of both marine and terrestrial organisms. The members of the laboratory engage in projects with other domestic and foreign institutions and with the U.C.S.D. School of Medicine.

The research objectives of PRL are comparative in nature and general themes of basic similarity of life mechanisms as well as specific adaptations of individual organisms are sought from the diversity of life forms which are studied. Extremes of environment in which organisms are found specify the variety of conditions to which life forms have adapted; thus, a spectrum of thermal conditions, the great depths and pressures of the deep seas, the contrast of breathing water and air (or, both), and the nature of the modifications imposed by the return of air breathing vertebrates (marine reptiles, penguins, marine mammals) to a life at sea all constitute challenges to which our researchers and their students respond. To put it simply: our goal is to distinguish the nature of specific adaptations which allow survival in a given environment from those more general mechanisims which are required by a broad spectrum of organisms in diverse environments. Toward this end we share the conviction that knowledge of behavior and conditions under which organisms live in nature is a prerequisite for our laboratory work. Thus, the laboratory is engaged in field activities in such diverse environments as the tropics, the arctic and antarctic seas, and the oceans overlaying the deepest depths of the earth. The land based laboratory at Scripps Institution of Oceanography offers us the opportunity to apply the perspective acquired in the field to investigations requiring facilities not available elsewhere.

In what follows, selected projects illustrate the scope of interests of PRL researchers and their students and post-doctoral colleagues.

THE DEEP SEA IN THE LABORATORY

Physical factors in the sea influence both the distribution of oceanic life and its evolution. The importance of salinity and of temperature as significant parameters for oceanic life has been long recognized. The importance of pressure, however, has not been widely appreciated nor as well-studied as other physical factors. Indeed, a role for pressure as an environmental influence on life processes was widely doubted prior to PRL researchers, Dr. A. A. Yayonos' pioneering efforts which succeeded in recovering deep sea bacteria from depths of 11,000 meters (1,100 atmospheres). His recovery and success in culturing these organisms in the laboratory under conditions of temperature and pressure characterizing their home environment, demonstrated that they could not reproduce except under high pressure. This observation firmly established the phenomenon of barophilism. Since this initial success, Dr. Yayanos has extensively studied the physiology of deep sea bacteria grown in the laboratory under simulated deep-ocean pressures and temperatures. Since temperature and pressure together influence biological structures and biochemical reactions, bacteria have been isolated from habitats differing in temperature in pressure. Most of the deep ocean has a temperature that approximates $2^{O}C$. Among the notable exceptions to this are the waters near hydrothermal vents, the Mediterranean Sea ($T + 13.5^{O}C$), the Sulu

Sea ($T = 9.8^{o}C$), the Halmahera Basin ($T = 7^{o}C$), the Celebes Sea ($T = 3.9^{o}C$, and the deep waters near Antarctica ($T = 0.5^{o}C$).

In the fall of 1985 Dr. Yayanos, Ronald Van Boxtel (Staff Research Associate), and two graduate students, Edward DeLong and Leon Zwacki went on an expedition to the Eastern Mediterranean Sea aboard the French Research Vessel Le Noroit. The Eastern Mediterranean Sea has depths to 5,000 meters and a temperature at this depth of $13.5^{o}C$. Studies completed on cultures show that the bacteria are barophilic; however, they cannot grow at $2^{o}C$ at any pressure. Thus, the physical conditions of temperature and pressure act in concert to make habitation of deep cold ocean niches of such bacterial impossible.

In the spring of 1986 Dr. Yayanos and several SIO co-workers joined the SIO Research Vessel Thomas Washington for Leg 8 of the Papa-Tua Expedition for a 4,700 nautical mile trip in the Western Pacific Ocean. Sampling was done in the Halmahera Basin, Celebes and Sulu Seas and in two trenches. Studies of these organisms are currently underway and should lead to a firmer understanding of how temperature and pressure together influence the distribution and evolution of oceanic life.

Graduate students Linda Lutz and Edward DeLong have discovered some surprising aspects of deep sea bacteria. Linda Lutz has found that some deep-sea bacteria possess the ability to repair, by a photoreactivation mechanism, their DNA damaged by exposure to ultraviolet light. Since deep-sea bacteria presumably are never exposed to visible light, it seems paradoxical that they should possess such a mechanism. Her studies may help us to better understand both the nature of photoreactivation and the evolution of deep sea bacteria. She has also found that both excision repair and photoreactivated repair of DNA are pressure-adapted processes in these deep-sea bacteria. Edward DeLong has discovered that many deep-sea bacteria make polyunsaturated fatty acids as part of their membrane phospholipids. While he is primarily studying the importance of polyunsaturated fatty acids to membrane function in a high pressure environment, he has noted that they are essential nutrients for deep-sea animals and that they are too refractory to be supplied only from primary production in shallow parts of the ocean. He has thereby established that there is at least one critical role for deep-sea bacteria in the deep-sea food web.

Dr. Yayanos recently recovered two giant isopods (Bathynomus giganteus) in one of his insulated traps from the Sulu Sea (2,500 meters). The site of their capture was found and mapped with Seabeam by John Abbot. The animals survived transit and were maintained for another five weeks at atmospheric pressure. Observations indicated that decompression had adversely affected their nervous system. Development of high pressure aquaria may enable more protracted study of such rare and seldom caught deep-sea animals. The field and laboratory work shows the value of insulated high-pressure traps, the usefulness of Seabeam in finding and describing deep-sea habitats, and the need of high pressure aquaria for maintaining deep-sea animals.

MARINE MAMMALS - CONSUMMATE DIVERS

Studies of free-ranging behavior, physiology, and hydrodynamics of seals and sea lions by PRL researcher, Dr. Gerald Kooyman, have been the leading factor in expanding our knowledge and understanding of the successful invasion of the sea by air breathing mammals.

The long standing concept that protracted submersion was accompanied by switching metabolic pathways from those dependent on oxygen to anaerobic pathways which may sustain energy production in the absence of oxygen, has been shown by Dr. Kooyman and his students to be an exceptional finding. Kooyman took advantage of a rare ecological setting in the antarctic to evaluate this concept. By capturing Weddell seals and transporting them by sledge to remote areas on an ice flow, he could provide a single isolated breathing hole which had been augered in the ice sheet. The animals were allowed to use this access to the water, a locality to which they returned to breath between dives. Wearing a time depth recorder designed at PRL, the animals provided a long term (up to two weeks) record of their diving activities. Blood samples could be acquired at the breathing hole from catheters in their superficial veins. The animals are deep divers, frequently working at 200-400 meters. Diving times, ranging from a few minutes to 25 min in duration, were shown to be sustained on oxygen reserves since blood analysis revealed that lactic acid, a certain indicator of anaerobic metabolism, had not accumulated. Rare dives, which exceeded 25 minutes in duration, were terminiated with excess lactic acid in the blood. Such dives are likely to be associated with attempts to escape the area, temporary loss of the position of the breathing hole, or avoidance of preditors. Previous studies of forced diving in the laboratory were invariably associated with excess lactic acid whether submersion time was long or short. Subsequent studies of free ranging animals wearing time depth recorders show that over 98% of dives are oxidative in nature. Breath holding time and O_2 reserves in man is limited to around 2 minutes in duration. Kooyman accounted for the protracted oxidative diving time of the Weddell seal through a combination of factors: large blood volume and hemoglobin concentration, high concentration of an O_2 binding pigment, myoglobin, in the muscles, and streamlining, low drag ratio, and effecient, low energy cost locomotion.

Studies of various species of seals and sea lions in the outdoor marine tanks at PRL and in the SIO water flume allow measurements of drag and energy costs of locomotion at various swimming speeds up to the maximum sustainable rates. These studies, in conjunction with field observations, indicate that energy costs of underwater locomotion are optimized at specific speeds which are most frequently used in nature.

Deep divers, such as Weddell and Elephant seals, routinely exceed depths from which emergence is expected to be associated with massive formation of Nitrogen bubbles in blood and other body fluids. Such depths would also crush the chest wall of typical mammals, including man. The avoidance of these fatal consequences is accounted for by the absence of a rigid chest wall which allows progressive collapse of the lungs before a large quantity of Nitrogen enters the blood stream. The collapse forces the compressed gas into the rigid upper airways where it can not be transported to the blood. Dr. Kooyman's studies of blood oxygen levels, during compression of seals up to 15 atmospheres, show that Nitrogen concentration does not reach sufficient levels to produce bubbles.

Dr. Kooyman and his group continue to develop more sophisticated and smaller recorders which store information on velocity, depth and duration of underwater time. Such devices do not encumber normal behavior and may be deployed for weeks at a time in freely ranging animals. Such studies occur in such diverse areas as the California offshore islands, Alaska, the coast of Africa, Galapagos Islands, Peru and the Antarctic and Arctic. These observations are providing much new information on underwater foraging behavior, energy requirements and distribution. Together with the laboratory studies at PRL, this interplay of field observation and laboratory investigation is expanding our appreciation of the adaptations which have accompanied the invasion of the sea by the consummate divers we know as marine mammals. Such studies are also being applied to marine turtles and birds. Dr. Kooyman has recently shown that the giant Leatherback sea turtle exhibits a suite of behaviors, including time and depth of diving, which closely resembles the Weddell seal. It will be a great interest to discover the similarities and differences in adaptations which have allowed these independent invasions of the depths of the ocean by air breathing animals of disparate evolutionary origins.

CONTRASTS IN BREATHING WATER AND AIR AND THEIR IMPLICATIONS FOR HEART SURGERY

The breathing of water by fishes imposes the necessity of ventilating great quantities of this medium across the gills because water contains a much lower concentration of oxygen than does air. Water also has a high capacity to take up the carbon dioxide (CO_2) produced by metabolism as the blood passes through the gills. The result is the maintenance of very low CO_2 tensions and content in the blood of fishes. This is in sharp contrast to air breathers whose lungs and blood exhibit CO_2 tensions and contents which are around 15 times those of fishes. Fishes have a limited capacity to alter the CO_2 tensions in blood while air breathers can regulate CO_2 tensions by altering the level of lung ventilation. High rates of ventilation result in "blowing off" excess CO_2 while low rates cause elevations in CO_2 tenions of the blood. In man the level of acidity (pH) of blood and body fluids is closely guarded by regulatory mechanisms which regulate CO_2 tensions in blood by fine tuning of the lung ventilation. Hyperventilation results in reduction of CO_2 tensions in the lungs and blood and alkalosis (a rise in pH). Simply stated, CO_2

is an acidifying agent and its regulation, through ventilation, stabalizes our blood pH at a value of about pH 7.4 in man. Departures from this value are of clinical concern because pH abnormalities lead to dire biochemical consequences.

Fishes, kept at various temperatures, exhibit specific blood pH values at each temperature. Higher pH values are associated with low temperature. Thus, unlike mammals, which maintain body temperature at a constant level, fishes exhibit an inverse relationship between pH and body temperature. Having no capacity to regulate CO_2 tensions, their regulation of pH is dependent on cellular mechanisms which take up or extrude bicarbonate ions, an alternate method of pH regulation.

These observations led PRL Director, Dr. Fred White to investigate the regulation of pH in air breathing vertebrates which normally experience wide swings in body temperature. He found that reptiles alter blood pH in a fashion similar to fishes. The mechanism for this regulation was found to be the control of CO_2 tensions via ventilatory adjustments. Low temperatures were associated with relatively higher ventilation rates, lower CO_2 tensions, and as in fishes, higher pH at low temperatures. At temperatures equivalent to that of man, the acid-base state of the blood was found to be the same for turtles, lizards, and man. Biochemical studies by SIO researcher, Dr. George Somero, showed that enzyme activities were maintained at optimal levels at all temperatures at pH's achieved by fishes and reptiles. This is associated with temperature related effects of hydrogen ion concentration (pH) on these crucial functions.

The introduction of hypothermia to reduce metabolism and blood flow requirements in the early 1950's led to the current practice of utilizing low temperature in man for the repair of virtually all cardiac abnormalities subject to surgical correction. Dr. White found that there was universal control of the blood pH and CO_2 tensions in these patients at values considered normal for normothermic body temperature. This led him and his associates to raise the question of the efficacy of this practice in the light of the comparative physiological and biochemical studies in lower animals at varying temperatures. Studies by Dr. White and surgical colleagues at U.C.L.A. and U.C.S.D. provided evidence that the functional integrity of the mammalian heart and kidneys was significantly improved when the CO_2 tensions were adjusted to achieve a pH-temperature relationship similar to that found in fishes, amphibians and reptiles. Of special importance was the demonstration by White and surgeon Julie Swain, that the complications associated with cardiac excitability (fibrillation), an often life threatening situation, were greatly reduced in occurrence when the lessions of comparative physiology were applied. Clinical resistance to these pH regulation strategies was strong and vocal owing only to the broadly held concept that what is normal at $37^{\circ}C$ should be normal at all temperatures. However, early clinical trials in a few surgeries led first to the conclusion that the pattern of acid-base management of cold-blooded animals did no harm in hypothermic man. More recently, clinical investigations in large series of patients undergoing cardiopulmonary bypass and divided into groups subject to classical pH regulation and the more unconventional pattern of lower animals, has convincingly verified the experimental investigations of Dr's. White and Swain. Patients whose blood pH followed the pattern of primitive vertebrates exhibited a much lower incidence of cardiac fibrillation. Other clinicians in Europe have reported fewer post surgical acid-base complications. There is currently an increasing appreciation of these lessons from comparative physiology and biochemistry in surgeries in Europe, Japan, and the United States. It is now becoming clear that patients are benefiting from these comparative studies, a matter of concern to the approximately 300,000 people in the U.S. who are annually taken into the chilly world of fishes and turtles for the repair of life threatening cardiac abnormalities. These findings also emphasize that comparative studies, while revealing specific adaptions to environments as distinct as water and air, can also be revealing of general requirements of life forms, including those of man.

A FINAL COMMENT:

This account has emphasized only three areas of concern to PRL researchers. Other studies focus on a range of topics including the marvelous salt excreting nasal glands of marine birds, the biophysics of bubble formation, temperature regulation, gas exchange in air breathing fishes, cardiovascular function in sharks, and energetics of swimming in tuna fishes which maintain swimming muscles at high temperatures. It is our hope that the reader will have gained some pleasure from this brief report, and perhaps experienced some of our excitement as we anticipate our future research and teaching opportunities at Scripps Institution of Oceanography.

Fred N. White

Director, Physiological Research Lab.