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CARBONIC ANHYDRASE INHIBITION AS A MODEL FOR GAS TRANSPORT IN Squalus acanthias

E. D. Robin, H. V. Murdaugh, Jr., and J. E. Millen, University of Pittsburgh, Pittsburgh, Pa., and University of Alabama, Birmingham, Ala.

Theoretical analysis of gas transport dictates that passive diffusion of respiratory gases across the gill in aquatic forms will produce $PaCO_2$'s less than 5 mmHg. One mechanism which would permit a higher $PaCO_2$ is the establishment of a positive steady state CO_2 gradient between arterial plasma and expired sea water, a positive K_2 (see Robin and Murdaugh, this issue). It has been established that carbonic anhydrase inhibition in the dogfish is capable of producing $PaCO_2$ greater than 10 mmHg. Therefore the effect of a potent carbonic anhydrase inhibitor, acetazolamide, on K_2 was studied in 14 dogfish. K_2 was calculated from the following relationship:

$$K_2 = PaCO_2 - P_ICO_2 + \left(\frac{\text{cardiac output}}{\text{gill water flow}}\right)\left(\frac{1}{CO_2}\right)\left(C\bar{v}_{CO_2} - Ca_{CO_2}\right)$$

In all studies K_2 values were greater than 5 mmHg and quantitatively accounted for $PaCO_2$'s greater than 10 mmHg. This study verifies the ability of steady state CO_2 gradients to produce high values of $PaCO_2$ in the face of relatively high PaO_2's.

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ENERGY EXCHANGE DURING DIVING IN THE SEAL, Phoca vitulina

E. D. Robin, H. V. Murdaugh, Jr., J. E. Millen, and C. D. Hearn, University of Pittsburgh, Pittsburgh, Pa., and University of Alabama, Birmingham, Ala.

The ability of the seal to tolerate prolonged diving, over 20 minutes, makes the seal an excellent model for studying oxygen depletion. With seals that have been trained to dive under laboratory conditions and to undergo expired air collections one may readily quantitate the control resting oxygen consumption in the animal. A face mask was designed that could be quickly placed over the face of the animal and expired air collected using Rubens Valve and a Douglas Bag. The oxygen debt incurred during diving could be quantitated by multiplying diving time by the control oxygen consumption. Carbon dioxide surplus could be quantitated in a similar manner. Ten studies were performed in 6 seals. It was found that following diving there was an incomplete repayment of oxygen debt. Increase in oxygen consumption over control values following the dive averaging less than 50% of the calculated debt. There was, however, an overshoot of carbon dioxide excretion that averaged approximately 160% of the calculated carbon dioxide surplus accumulated during diving.

This apparent discrepancy with the Law of Conservation of Energy, may be related to several possible mechanisms that include depression of metabolism during diving, variable oxygen binding to some tissue components, increased efficiency of energy utilization during diving, a slow repayment of oxygen debt that could not be detected operationally, or alterations of the ki-

netics pattern of intercellular oxygen association and disassociations produced by the hypoxia of diving.

These results emphasize the importance of intracellular transients in defining respiratory gas exchange and suggest the value of the seal as a model for studying O_2 depletion on a broad evolutionary basis.

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IN VITRO CULTIVATION OF CELLS DERIVED FROM MARINE ORGANISMS

R. W. Schlesinger, T. M. Stevens, and R. E. Schlesinger, Rutgers University, New Brunswick, N. J.

Quantitative cell culture methods have opened new approaches to the study of mammalian cells as isolated organisms or homogeneous populations. In general, these methods have contributed much to our knowledge about physiological, genetic, and biochemical regulatory mechanisms. In addition, in vitro cell cultures have provided ideal systems for the study of virus infection at the cellular and molecular level.

Several investigators have turned their attention to attempts to establish fish cells in continuous culture and to search for virus-like agents in fresh water or marine fishes. The promising results of these efforts have been summarized in a recent Symposium ("Viral Diseases of Poikilothermic Vertebrates," Ann. N. Y. Acad. Sci. 126, 1-680, 1965). Thus far, the greatest success has been obtained with cells from certain fresh water fishes and from a few marine teleosts. It has been the experience of all workers in this field that methods and media developed for cultivation of mammalian cells are, with minor modifications, also best suited to meet the requirements of fish cells. A number of fish viruses have been shown to grow and produce characteristic cytopathic effects in these cultures.

No reports have been published on the successful cultivation of cells derived from the more primitive cartilaginous fishes. Most of our work has been done with first year dogfish embryos (Squalus acanthias), but some success has also been obtained with skate fin tissue.

Embryos are collected aseptically from "candles," washed thoroughly in several changes of phosphate-buffered 0.22M NaCl solution (DF-PBS), and, after removal of the eyes, finely minced with scissros. The mince is thoroughly washed in $Ca^{\#}$- and $Mg^{\#}$-free PBS and then once or twice in a 0.025% trypsin solution in $Ca^{\#}$- and $Mg^{\#}$- free PBS (DF-trypsin). The thorough washing is essential to prevent the formation of mucous "ropes" which would otherwise entrap the cells during trypsinization.

The washed mince, suspended in 5 to 10 vol. of DF-trypsin, is placed in a fluted flask on a magnetic stirrer and agitated for repeated periods of 10 to 20 minutes each. The temperature is maintained at 18°C or less. The product of the first trypsinization period is discarded. Cell suspensions from subsequent periods are pooled, filtered through multiple layers of sterile gauze, and centrifuged. The cell sediment is resuspended in nutrient medium (DF medium), and the density adjusted to 0.25-1 x 10^7 cells/ml. Four ml portions of this suspension are seeded in 30 ml plastic tissue culture flasks (Falcon).

After much trial and error, the following medium has given the most promising results: