## The Direct Demonstration Of Bicarbonate Excretion Across The Gill Membranes Of Squalus Acanthias

Eugene D. Robin, Philip A. Bromberg, and Mrs. Selma Sapira
University of Pittsburgh Medical School

A number of different lines of evidence indicate that bicarbonate excretion in the elasmobranch occurs primarily across the gill membranes and not by means of the renal tubule. However experimental attempts to rigorously demonstrate this excretion have been generally unsatisfactory because of technical problems. The basic difficulty has been the problem of measuring relatively small changes in bicarbonate concentration occurring in relatively large volumes of seawater. These problems were solved by using a modified divided box with the anterior chamber being composed of a plastic envelope of a relatively small volume. In addition, a technique was developed which permits the continuous infusion of bicarbonate solution into the ventral aorta through an indwelling polyethylene catheter. This technique permitted the continuous infusion of relatively large amounts of $NaHCO_2$ and simplified analytical problems. Four separate studies were performed and significant increases of $CO_2$ tension and of bicarbonate concentration of anterior compartment seawater during bicarbonate infusion were demonstrated by direct measurement. The increased $CO_2$ tension presumably reflects $CO_2$ generated as the infused bicarbonate is buffered inside the fish and the increased $HCO_3^-$ presumably reflects direct excretion of this ion across the fish gill membrane.

(This work supported by grants from the National Institutes of Health, United States Public Health Service (H-5059) and an institutional grant from the American Cancer Society (IN-58).

## Calcification In Arthropod Skeletons I.

Leo Schatz and G. Bevelander
New York University College of Dentistry

The Decapod crustacean *Pagurus acadianus* Benedict bears a cephalothorax and abdomen which may be sectioned without prior decalcification. The skeleton contains both calcified and non-calcified zones. Preliminary studies reveal the presence of chitin in combination with tightly bound, as well as loosely bound protein moieties. In regions which will undergo calcification, histo- and microchemical tests reveal a Millon-positive substance which reacts like tyrosine or one of its derivatives. The Millon-positive substance is resistant to prolonged extraction with borate buffer at pH=9.2, or with hot water (90°C.), but is extracted, along with tightly-bound protein by 24 hr. treatment with 5% NaOH at room temperature, and subsequent rinsing with water. The Millon-positive regions exhibit strong cation binding activity, even after pre-treatment with dilute HCl to remove preformed carbonate and phosphate. Ion-binding was de-