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

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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.

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Calcification In Arthropod Skeletons I.

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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-

monstrated for Mg, Sr, Ba, Pb, Ag, Cu, and uranyl radical. The inorganic portion of the skelton seems to be primarily calcium carbonate with small amounts of phosphate and magnesium. Layers of the skeleton in which $CaCO_3$ crystals form, become non-metachromatic (i.e. unstained), while adjacent non-calcified zones (containing the Millon-positive material) exhibit a purple metachromasia when treated with toluidine blue at pH=4.9 and .60. The metachromasia exhibited is not resistant to alcohol but persists in 80% acetone. In the epicuticle calcospherites consisting of radially oriented microcrystals are found, while in the endocuticle crystals with angles approximating those of calcite have been noted in the developing cuticle. In the mature cuticle the small angular crystals have given rise to solid abutting masses of crystal. It is suggested that the Millon-positive zone acts as a cation pool until the metastable condition necessary for calcification of the epicuticle and endocuticle is attained.

Calcification In Arthropod Skeletons II.

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The Decapod crustacean *Pagurus acadianus* Benedict in the intermolt stage was utilized in this study. The integuments of the cephalothorax, abdomen and cheliped were removed to distilled water and scraped free of soft tissues with the aid of Pyrex glass wool. The cephalic portion ("cephalic shield") of the cephalothorax, along with the adjacent cervical grooves and linea anomurica (of Bouvier) was separated from the rest of the cephalothorax (the epibranchial and epicardial portions) which is designated the "thoracic shield" for convenience. The abdominal cuticle was isolated after removal of the first three abdominal segments, as well as the penultimate segments and all appendages. The upper and lower surfaces of the hand were separated from the digit of both chelipeds. Total dry weight was obtained after removal of alcohol and acetone soluble lipids and pigments. Trichloracetic acid (10%) was utilized to demineralize the tissue. The TCA extracts taken from the "cephalic shield" averaged 64% of the total dry weight. Analysis of the TCA extract above yielded the following approximate values: inorganic PO_4^{-3}, 4.5%; 34% Ca^{++} (Cal-red method); 50% CO_3^{-2} (as $BaCO_3$); and the other 11% consisted of Mg^{++}, some amino acids, and a small amount of unidentified material. We thus felt justified in referring to the TCA extract as the "mineral fraction". Extraction of the organic residue with hot water yielded a water-soluble protein fraction (Arthropodin); while extraction with 5% KOH for 18-24 hrs. at room temperature produced the alkali-soluble partial protein hydrolysate and saponifiable lipid fraction; while the washed residue was primarily chitin.

In order of their decreasing mineralization the samples can be arranged as follows: (A) cheliped, (B) cephalic shield, (C) thoracic shield, and (D) abdomen. Preliminary analyses of samples (A), (B) and (C) are listed below: