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:

Mineral Fraction (% of dry weight): (A) 83-97%, (B) 61-67% (C) 19-25%.

PO_4^{-3} inorganic as % of mineral: (A) 2.-2.4%, (B) 4.0-4.9%, (C) 7.3-8+%.

Organic Fraction (% of dry weight): (A) 12.4-15%, (B) 33-39%, (C) 74-80%.

It is natural to expect that if highly mineralized areas yield lower values for organic residue, all determinations of organic fractions calculated on the basis of total dry weight will be low for cheliped and high for abdomen, as indeed they are. When calculated on the basis of % of organic residue a different pattern becomes apparent. On the latter basis the harder structures demonstrated significantly lower Arthropodin values; the KOH extractable fraction was approximately the same order of magnitude in all the tissues (with some decrease in values for the cheliped); but with the KOH residue (chitin) the highest value was found in the cheliped, with significantly lower values in less mineralized areas.

The present work indicates that harder more mineralized regions exhibit lower concentrations of inorganic phosphate, lower Arthropodin values, significantly higher chitin content, but that alkali-extractable material does not differ to any marked degree among the skeletal tissues tested.

Quantitative Study Of Free Amino Acids In The Blood Of Marine Arthropods [1]

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The purpose of these investigations was to compare the amino acid contents of marine arthropod blood with that of insects, especially the cockroach, *Periplaneta americana*. The latter species had been used in attempts to cultivate insect cells *in vitro*, and amino acid analysis had been carried out in the hope that clues to the development of suitable media might be obtained. Because of the obvious difficulties in obtaining adequate quantities of insect blood, it was thought that marine arthropod blood might be substituted if similarities in amino acid composition were found.

Blood samples obtained from *Limulus polyphemus*, *Homarus americanus*, and *Cancer irroratus* were deproteinized, concentrated, and analyzed for free amino acid by chromatography on Moore-Stein ion exchange columns (Moore, S., Sparkman, D., and Stein, W., 1958, *Analytical Chem., 30*, 1185). Only the acidic and neutral amino acids were studied, because time and facilities did not permit setting up a second column for the basic amino acids. These will be analyzed later.

The following amino acids were found in all three species: aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine. Under the ex-