ANNUAL PROGRESS REPORT

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Contractor New York University NR 163 - 159

Principal Investigator: Gerrit Bevelander

Assistants Shirley Motzkin

Title of Project: Research on Calcification in Molluscs

Objectives: Correlation of the structure of the mantle and the periostracum under normal growth conditions and experimental situations to ascertain more precise information on the elaboration of this protein matrix; tests for various components of the mantle and periostracum, an attempt to ascertain interrelations between structural composition of the mantle, periostracum, and the mineralization of this structure by the use of isotopes and histochemical tests.

Summary of Results: July 1, 1953 - January 30, 1954

I. Electron Microscopy

During the past several months specimines of the organic matrix of the mollusc shell have been prepared, sectioned and photographed with the electron microscope. This operation required a considerable amount of preliminary exploration in technical methods before satisfactory preparations could be obtained. The material examined consisted of a number of diverse species and several developmental stages of this tissue.

The matrix or periostracum of the mollusc shell appears at high magnification to be composed of a fibrous material (schleroprotein) which is lacking in any characteristic structural features such the periodicity observed in collagen fibers.

A study of this tissue shows that each different species thus far examined has a unique structural pattern. For example, the arrangement of the periostracum of Venus mercenaria is obviously different and characteristic when compared with a member of the mytilus family, such as Mytilis edulis which is also characteristic of this particular species. It would appear from our studies as far as they have been carried, that different species of molluscs exhibit characteristic specificity in regard to the structure of the periostracum, a finding which may be utilized in taxonomic studies in the future. Further, a parallelism exists between the specific differences exhibited in the structure of the periostracum and the observed differences in the structure of collagen fibrils which have been examined in a wide variety of animals.

II. Free Amino Acid Determinations of Mollusc Tissues:

Our interest in the protein component of calcifying and calcifying tissues prompted us to undertake an analysis of the free amino acid composition of several mollusc tissues and organs. This study was carried out by means of two-dimensional paper chromatography. Some of the results obtained in this study are shown in the following table:

Muscle	Vesalius gill mantle hepato-pancreas	periostracum
aspartic	aspartic	
glutamine	glutamine	
Taurine	taurine	taurine
asparagine/glycine	asparagine/glycine	asparagine/glycine
alanine		
histidine		
valine		
arginine		
lysine		
proline		
serine	serine	
methionine	methionine	

Comparing the various organs and tissues in regard to free amino acid composition, the data show a relatively large number of amino acids to be present in the mollusc muscle, relatively few in the gill, mantle and hepato-pancreas, and only three or four in the periostracum. The number of free amino acids present in the periostracum are much fewer in number than the observed amino acids detected following hydrolysis.

III. Comparison of some factors observed during normal growth, repair and regeneration:

In all three varieties of shell construction the basic mechanism for elaboration of shell is similar. It consists of the elaboration of a protein matrix and a mineralization of this substance.

During normal growth, a protein matrix is elaborated by the free mantle surface and part of the surface lying adjacent to the shell. Both the prismatic and nacreous portions of the shell are laid down and mineralized simultaneously.

Studies concerned with the regeneration of the shell was observed following removal of parts of the outer margin and shows that shell does tend to grow to its original size and shape. The regenerated shell has the composition of the pris-

matic or outer layer. In other words, the regenerated shell is derived from those parts of the mantle exclusive of the area between the outer and inner fold of the mantle.

Repair phenomena studied by means of attaching glass windows to areas in which the shell was removed and also by means of the "mantle-cover slip technique" indicates that repair in various parts of the shell can and does take place with a rate which depends upon environmental factors and species differences. In repair of the shell, we have observed that the chief source of crystaline material deposited is derived from amoebocytes. These cells appear at the site of injury (or irritation) in large numbers, fabricate minute crystals, and extrude them upon a substrate. The crystals subsequently form calco-spherites, regular or irregular crystal forms and eventually repair the damaged area effectively.

IV. Histochemistry:

In our histochemical studies we have attempted to ascertain histochemical location and sequence of events as they appear to be related to the formation of the mineralized shell.

Examining the source and composition of the matrix, we have observed the following: The matrix is a protein, roughly divisible into an acidophilic and a basophilic fraction. In the formative state we observe in some of the secreting cells the simultaneous occurence of phosphatase, ribonucleic acid and complexes of acid and mucopolysaccharides. In the mantle proper several types of glandular tissue is present. These glands secrete different varieties of mucus. One group of these glands appears to fabricate crystaline calcium carbonate. These structures are abundant in mucoproteins, acid polysaccharides, ribonucleic acid,

glycogen and phosphate.

On the basis of information available from these studies, it appears that one common factor observed in connection with mineralization is the presence of a protein-polysaccharide complex. Whether the polysaccharides are the calcium target, combining with calcium ions as has been suggested in the mammal awaits further study.

In connection with our observations relating to crystal formation we have observed that crystals are fabricated in the glands of the mantle, in the amoebocytes and in other regions. Parts of the periostracum are derived from the mucus glands and when this material is extruded on the mantle surface it contains calcium carbonate particles. The other portions of the periostracum which are derived in the outer fold of the mantle may also have minute mineral particles embedded in it as as it is secreted.

The initial situation just described, however, does do obtain for long. We have observed in normal and experimental conditions that "crystal growth" occurs in the animals' environment. That is, in an aqueous media, in contact with the mantle tissues. Whether the initial mechanism responsible for producing the almost submicroscopic calcium carbonate particles which arise in the mantle are subject to the same metabolic events as the growing crystal which is embedded in the matrix subjected to fresh or sea water in contact with the mantle tissues can only be speculated upon at this time. We have observed that the nature of the substrate upon which crystal growth takes place does influence both size, shape and regularity of the crystals.

Plans for Immediate Future:

At present we are continuing our studies relating to several histochemical features of the mantle and periostracum. This work consists in the main in trying to ascertain more precisely the nature of the chemical constituents by means of fractionation, enzymatic and other chemical methods.

During the summer months of 1954, further exploratory and confirmative studies are planned. In addition to further histochemical evaluations, we are considering additional isotope-trace studies in an attempt to get a more accurate concept of the role of the polysaccharides in the role of calcification. We further plan to study the amoebocytes as a single cell which has the ability to elaborate, transport and give up carbon to particles. We are also planning to extend our experimental studies on regeneration and repair.

Long Range Program:

The projection of a long term program dealing with the problem of calcification in invertebrates will of necessity as the problem unfolds encroach upon a number of related fields of investigation and involve the need for diverse methods of attack.

First a judicious knowledge and use of various forms have and will aid in this study. The development of experimental methods like those used in our regeneration and "cover glass culture" methods are also necessary to further this work. Further, studies are needed to trace the intake of metabolites, to follow and ascertain their role in the elaboration of protein and mineral components of the shell. At the present, a promising approach to this study appears to be in the utilization of the molluscan amoebocytes since these cells elaborate calcium

carbonate crystals. This would involve the use of morphological, chemical, enzymatic and tissue culture techniques. The use of isotopes in ascertaining the source, metabolism, transport and eventual deposition of the metabolite pool would be a possible and valuable approach to this work.

Reprints and Publications on Calcification in Molluscs.

1. Interrelations between protein elaboration and calcification in molluscs. Anat. Rec., V. 117 No. 3, pp. 568-569, 1953.
2. Free amino acids in the shark, lobster and clam. Anat. Rec., 117, No. 3, pp. 635-636, 1953.
3. In preparation:
   (1) A histochemical study of the mantle with special reference to calcification.
   (2) Electron microscope studies of the matrix of the molluscan shell.