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CALCIFICATION IN MOLLUSCS. III. INTAKE AND DEPOSITION OF Ca^{45} AND P^{32} IN RELATION TO SHELL FORMATION[1]

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The problem of shell formation in molluscs has been the subject of numerous studies in the past and it is now generally conceded that there are two distinct phases which occur during the elaboration of the shell: (1) the formation of a protein membrane (periostracum); and (2) the mineralization of this membrane which results in the production of the calcified exoskeleton. It is generally agreed that the protein complex which becomes incorporated into the shell is elaborated by the distal portion of the mantle. Some of the details of this process are still somewhat obscure. Similarly, the factors involved in the deposition of calcium carbonate are also in several respects still subject to conjecture and speculation.

In a comprehensive review dealing with calcification in molluscs, Robertson (1941) has summarized a number of concepts which attempt to explain calcification in these organisms: Calcium carbonate is separated from the blood by certain cells at the mantle edge. The carbonate in a colloidal form is then liberated between the epithelium and the periostracum and subsequently undergoes crystalization in this matrix.

According to DeWaele (1930) the calcium carbonate of the shell is separated from the blood and from a so-called extra-palleal fluid, which, it is held, is a solution containing protein, carbon dioxide and calcium carbonate. By physico-chemical processes, the calcium carbonate forms as a result of the escape of carbon dioxide. Robertson further states that the greater part of the calcareous material of the shell in marine molluscs is absorbed directly from sea water as Ca and bicarbonate ions. This author's own experiments, however, fail to show a direct uptake of calcium ions from sea water in a number of gastropods and bivalves. It has further been suggested by Baldwin (1935) and by Freeman and Wilbur (1948) that metabolic carbon dioxide may be the primary source of the carbonate radical.

In regard to the source of calcium which is utilized in the formation of the shell, Orton (1925) observed that shells continue to grow in the English oyster in the absence of food. Similar observations were recorded for the American oyster by Galtsoff (1934) who states in this connection that the amount of calcium utilized is many times greater than could be stored in tissues. Fox and Coe (1943) in their studies on Mytilus also deduce from their observations that the amount of calcium obtained from food alone does not account for the amount deposited in the shell. It has also been shown (Bevelander and Benzer, 1948) that when the calcium

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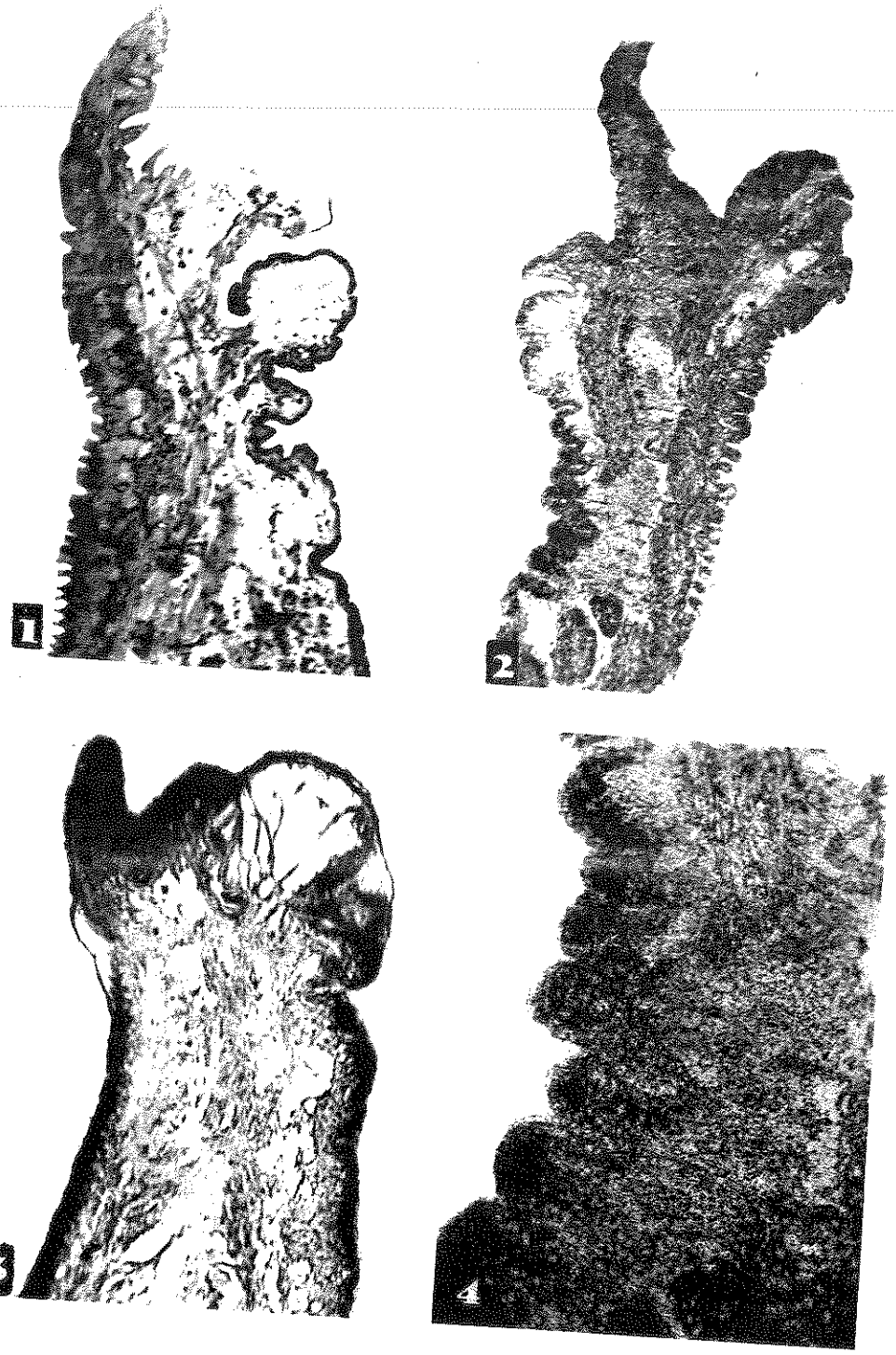

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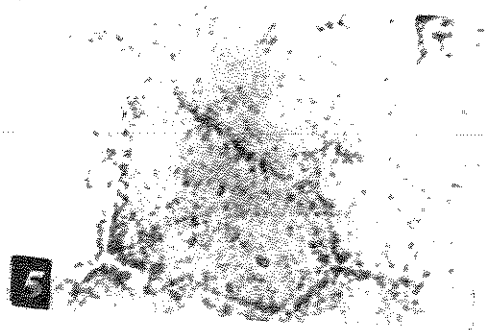
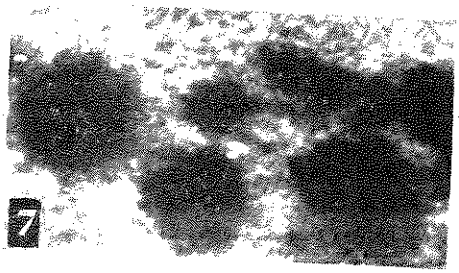
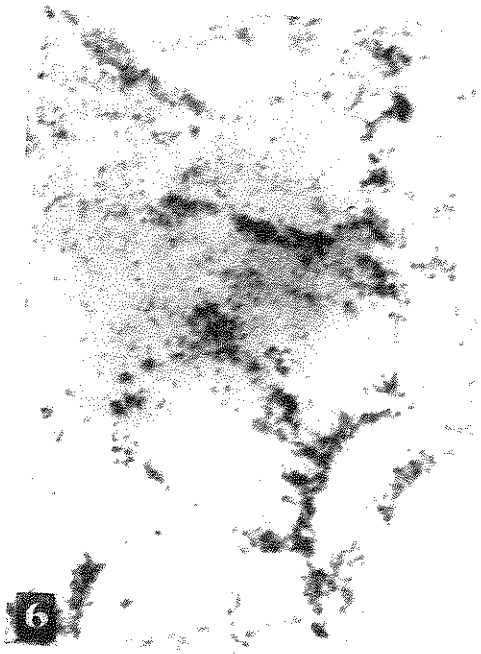
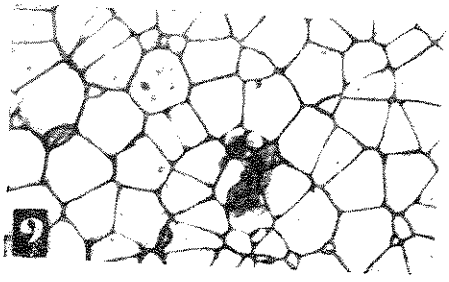






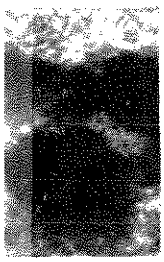


FIGURES 5 AND 6. Radio-autographs of crystals of the shell of Pinna to show localization of P^{32} in the periostracum.

FIGURE 7. Radio-autograph of crystals of Pinna showing localization of Ca^{45} within the crystals. Extraneous blackening of photo due to over-exposure.

FIGURE 8. Large fragment of shell of Pinna showing differential uptake of Ca^{45} in regenerating shell.

FIGURE 9. Surface view of shell of Pinna to show relation of crystals (light areas) to the surrounding periostracum shown as dark lines surrounding each crystal.



marily to the inner surface of the mantle. Examination of Figure 4, which is a photograph of this same area taken at higher magnification, shows that P^{32} is localized in regions of the mantle below the surface epithelium. The P^{32} observed in the mantle occupies a position in this organ which corresponds to the location of the mucus glands. Inasmuch as the method of processing the tissues involved several exposures to aqueous solutions, a procedure which tends to eliminate water-soluble phosphate from the tissues, it appears that the phosphate which remained in the mantle which was subsequently localized in the radio-autographs is an organic phosphate complex.

The sites in which alkaline phosphatase occurs in mantle tissues of molluscs were also ascertained in a variety of species. The localization of this enzyme in the mantle is fairly well exemplified in the fresh water specimen Anodonta which we have chosen to illustrate. Reference to Figure 1 shows that phosphatase is present on the entire inner surface of the mantle. The enzyme is further confined for the most part to the epithelial cells which form the surface of the mantle. The numerous amoebocytes found in this structure also reveal the presence of phosphatase.

Shell:

The localization of Ca^{45} in the shell of Pinna which was laid down under the experimental conditions already described, is shown in Figures 7 and 8. Figure 8 is a reproduction of a radio-autograph of a fairly large piece of regenerated shell which shows an overall black appearance which is lighter in the thinner and more recently formed margin. When selected regions of this shell are examined microscopically, one observes that the Ca^{45} is confined to the areas occupied by the crystals (Fig. 7), while the adjacent peripheral area occupied by the periostracum (see Fig. 9) is relatively devoid of Ca^{45}.

The identification of P^{32} in the regenerating shell of Pinna was ascertained in a manner similar to that which was utilized in connection with Ca^{45}. Reference to Figures 5 and 6 showing several crystals of the shell of Pinna grown in the presence of P^{32} indicates the precise localization and the details of structural variation of the crystal. Insofar as one can evaluate by the method utilized, P^{32} comes to be localized in the protein membrane (periostracum) which encloses the crystals. The effect thus produced by the introduction of P^{32} in the growing shell examined by radio-autographic methods is virtually a negative image of the picture produced by the addition of Ca^{45}.

Discussion

It was pointed out in our introductory remarks that the elaboration of shells in the mollusc consists in the formation of a protein membrane, the periostracum, and a concomitant or subsequent mineralization of this membrane. From observations based upon several studies made on the organic component of the shell, it appears highly probable that this protein complex may be actively involved in the formation or growth of the mineral crystals which come to be incorporated in the shell. Since our observations on this latter topic are still incomplete we have for the present confined our remarks to some aspects of shell formation which are concerned more intimately with the role of some inorganic constituents of the shell.

Although several studies suggest that the calcium utilized in the formation of the shells of marine molluscs is derived in part from the sea water in which the

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animals live (Orton, 1925; Galtsoff, 1934; Fox and Coe, 1943), a number of factors in regard to the source, transport and utilization of calcium still remain obscure.

By using labeled calcium we have shown that calcium ions present in the water are ingested by the organism and are localized in several organs. Of particular interest is the observation that relatively large amounts of labeled calcium are concentrated on the periphery of the mantle. A somewhat similar observation has been recorded by Haysi (1938) and also by Trueman (1942). The method of converting the calcium to calcium oxalate as used by these authors, however, is capricious and subject to error in localizing the calcium crystals.

It was also shown that the labeled calcium which was placed in the water (as calcium chloride) was rapidly incorporated into the mineral component of the shell. Control experiments, by means of which detached pieces of shell were placed in the Ca^{45} solutions, did not reveal the localization of Ca^{45} in the crystals. It is apparent, then, that the mollusc can and does utilize calcium in an ionic form in the production of the mineral component of the shell.

The localization of phosphate and phosphatase in the mantle of the mollusc is not only intriguing but is also a tempting subject upon which to speculate. Manigault (1939) has stated that a direct correlation exists between the mantle phosphatase activity and calcium precipitation in the shell, and further, that phosphatase serves as a transfer agent in the mobilization of calcium. The validity of this concept still awaits confirmation. It has been shown that phosphorylation does occur as a result of the action of mantle phosphatase upon mantle mucus (Bevelander and Krimsky, 1949). The significance of this reaction in relation to shell formation, however, is at present unknown.

Trace amounts of phosphorus have been recorded for mollusc shells (Turek, 1933). It seems fairly evident from our observations that the phosphorus in the shell is confined to the organic constituent of the exoskeletal complex.

Summary

1. Both fresh water and marine molluscs take up labeled calcium and phosphorus from the water.

2. Labeled calcium is concentrated on the periphery of the mantle and also is incorporated into the crystals of calcium carbonate in the newly formed shell.

3. Labeled phosphate was localized on the inner margin of the mantle in the region of the mucus glands. It was also incorporated in the periostracum surrounding the crystals.

4. The phosphatase and phosphate present in the mantle are concerned with a phosphorylating process; the significance of this phenomenon in relation to mineralization is not clear.

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