blood erythrocytes had lost an R^1 gene complex. In the absence of other information, it could have been assumed from this study that the locus for the Rh blood group is either on the short arm of chromosome No. 1 or the short arm or proximal portion of the long arm of No. 7, because, along with an Rh locus, these segments had been deleted from the major population of the man's dividing blood cells. But Ruddle *et al.* (*2*) have already deduced that the Rh locus is on chromosome No. 1, although evidence available until now had pointed to the long arm of this long chromosome as its position (*2*). Because the chromosome rearrangement in our patient resulted in deletion of a segment of chromosome No. 1, our present observations are well explained by the hypothesis that the Rh locus is located on the deleted segment, that is, on the distal segment of the short arm at some point between band 1p32 and the end of the arm (*3*).

Douglas *et al.* (*4*), using cell hybridization methods, presented evidence that PGM_1 is on 1p somewhere distal to band 1p32. The PGM_1 locus was not deleted by the chromosome rearrangement in the mutant clone of our patient, whereas the Rh locus was. We conclude therefore that the Rh locus lies distal to PGM_1 (*5*).

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3. It is of additional interest that the patient's Rh-negative erythrocytes gave double-dose rather than single-dose serological reactions with antiserum to c and antiserum to e. If our hypothesis is correct, the Rh locus when hemizygous is fully expressed as though two genes were present.
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5. The inability to rule out mosaicism at the Duffy and the ABO loci in this patient makes it impossible to conclude from the present study alone that these loci are not on one of the deleted segments of chromosome No. 1 or No. 7. [However, Duffy has, as just stated, already been assigned to the No. 1 (*4*).] Demonstrable absence in our patient of mosaicism at the MNSs locus eliminates the possibility that it is on either of the deleted segments. Furthermore, deletion mapping in a child with a constitutional chromosome imbalance has shown that the MNSs locus is on the proximal portion of the long arm of No. 2. [J. German and R. S. K. Chaganti, *Science* **182**, 1261 (1973)].
6. We thank J. Fleming and J. Jensen (cytogenetics); and M. Blank, R. Øyen, S. Self, and J. Tegoli (immunohematology) for technical assistance. Supported from PHS grants HD04134, HL09011, AM11188, and CA12779, and by grants from the American Cancer Society and the John A. Hartford Foundation.

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Calcification on an Unstable Substrate: Marginal Growth in the Mollusk Pecten diegensis

Abstract. *Observations of the growing margin of* Pecten diegensis *show that calcification can occur on an unstable substrate, although this initial shell material is quite disordered. Later growth on the inner surface of the disordered material soon becomes ordered, which suggests that the calcification process benefits from the stability of a mineralized substrate, but does not utilize the substrate as a physical or stereochemical template.*

The formation of mollusk shells has been the object of speculation and study for a very long time, but the process is still imperfectly understood (*1*).

Investigations of the mineralogy and structure of shells, culminating in the classic work of Bøggild (*2*), have shown that many different structural arrangements of calcite and aragonite are utilized by mollusks, and that most shells are built of two or more layers with different structures.

Shells grow by the accumulation of new material on a substrate which usually consists of the parts of the shell that are in contact with the tissues of the mantle. The new shell material is usually of the same mineralogy and structural arrangement as the older shell which serves as its substrate; this led to the concept that the substrate plays the dominant role in the calcification process, serving as a crystal template to direct the precipitation of calcium carbonate from a solution maintained by the mantle tissues.

This concept has proved to be overly simplistic; it is now recognized that the organic component of molluscan shells is a precursor to mineralization, and that it may serve as a matrix to control the nucleation, mineralogy, and growth direction of the calcium carbonate (*3–5*). It also appears, based on experiments in shell regeneration, that the mantle can vary the type of organic

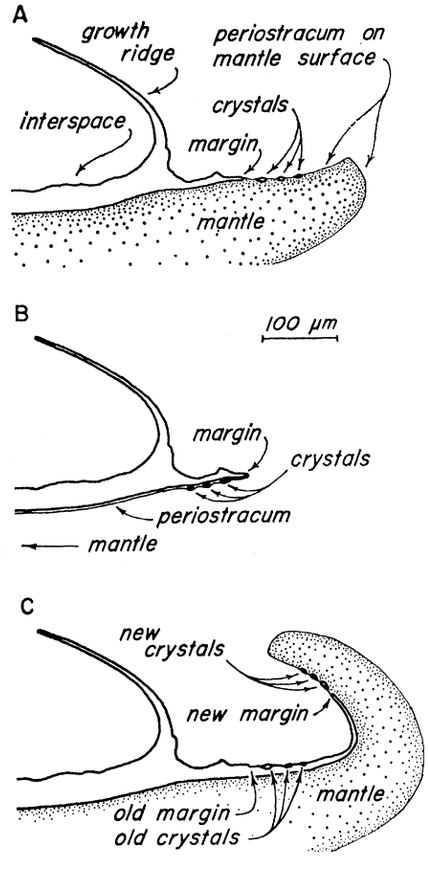

Fig. 1. Growth of the shell margin in *Pecten diegensis*; scale as shown. The direction of growth is to the right. (A) Mantle extended, with crystals forming under a thin periostracum on its surface and eventually coalescing to form the margin of the growing shell. (B) Mantle withdrawn into the shell but retaining contact with the periostracum; crystals not yet integrated into the margin are carried under the shell by the periostracum. (C) Several hours later; crystals observed in (A) and (B) have coalesced (in the same relative positions) into solid shell, and the mantle has moved out and up to begin forming a growth ridge. The interpretation is based on time lapse photomicrography and radial sections through shells.

matrix produced and thus determine the shell structure (*6*). As presently understood, then, the mantle tissues are highly involved in the calcification process.

Despite this, the shell substrate may still exert a strong influence on the growth process. Detailed electron microscopy has shown that a fluid layer separates the mantle and the substrate, and that the organic matrix forms within this fluid (*5*). Because the organic matrix later provides orientation to new crystal growth, it must be oriented itself, and the substrate, with its relatively flat, stable surface, and with the possibility of stereochemical orientation, seems a more satisfactory source of this orientation than the only other candidate, the mantle surface.

Nonetheless, the remainder of this report will be devoted to the suggestion that the main contribution of the substrate toward an orderly shell structure is in providing a stable foundation, and that the orientation of new calcification must originate elsewhere.

The evidence for this interpretation is derived from observations of the marginal growth of *Pecten diegensis*. The shell margin of this bivalve tapers to a thin edge, so that the marginal growth is initiated beneath the periostracum rather than against existing shell material; moreover, this periostracum is extremely thin and has little inherent stability. In this situation the initial calcification is virtually independent of substrate control and itself becomes the substrate for further calcification.

Two approaches were used; in one, the growing margin was observed in vivo by means of time lapse photomicrography; in the other, the outermost shell structure was examined through scanning electron microscopy (SEM). The specimens were obtained from waters off Santa Catalina Island, California (*7*).

The time lapse study found the process of marginal growth in *Pecten diegensis* to be very lively. The mantle edge would extend well beyond the calcified margin of the shell, maintaining a position of moderate stability; small crystals could be observed forming on its outer surface, apparently under a very thin periostracum (*8*), and coalescing into the advancing margin of the calcified shell (Fig. 1A). At frequent intervals the mantle apparently became disturbed and retreated within the shell; at such times the position of the fully calcified shell margin could be precisely determined, and the isolated crystals beyond this margin could not be seen (Fig. 1B). On the return of the mantle from within the shell the crystals could be seen to have returned to the same relative positions, suggesting a firm attachment to the periostracum, and after a time they could be seen to become incorporated into the advancing margin (Fig. 1C).

Figure 1 also shows that two growth forms result from this process; one, the interspace, forms the true outer surface of the shell, with subsequent calcification occurring on its inner surface; the other, the growth ridge, is abandoned by the mantle and receives no further calcification.

Characteristics of these two growth forms were then examined by SEM and compared with the time lapse observations. Figure 2 shows a number of sections through the outer portions of a shell of *Pecten diegensis*. Figure 2D shows the general structure and orientation. Here the direction of growth is

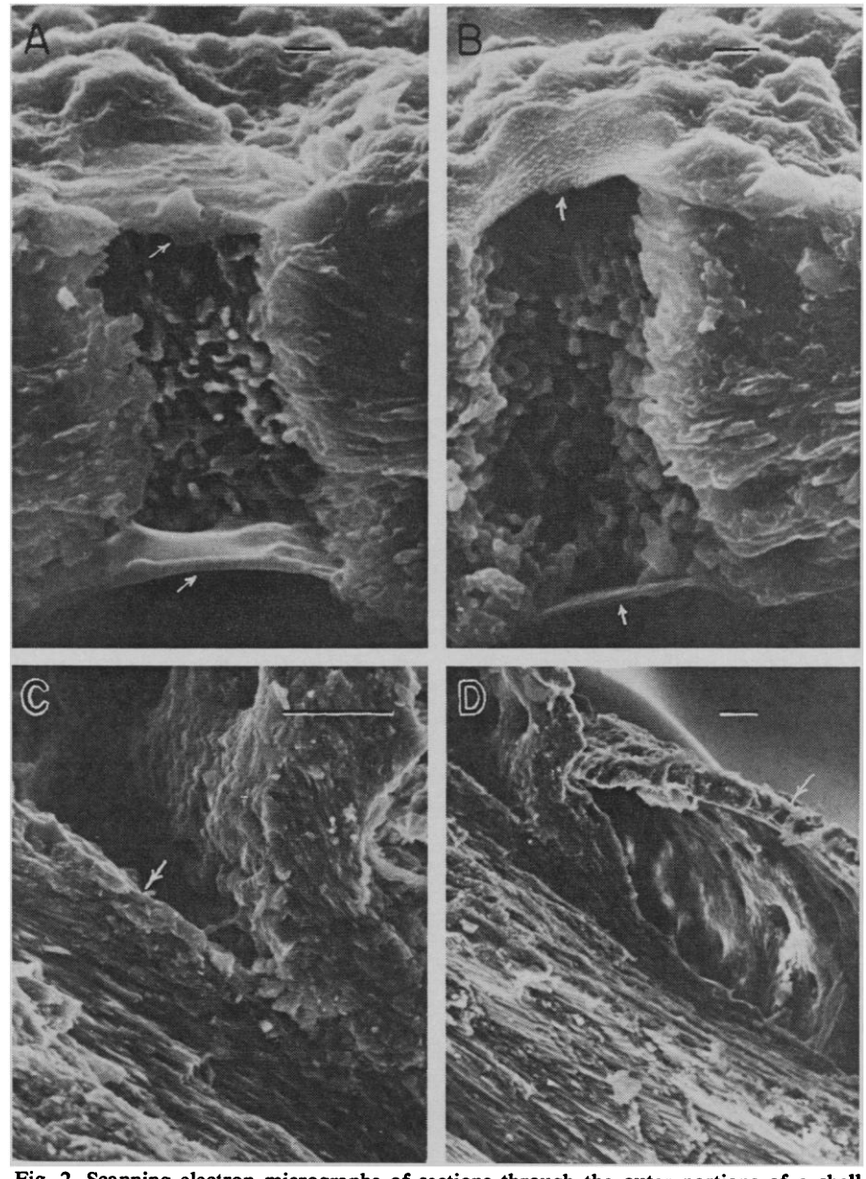

Fig. 2. Scanning electron micrographs of sections through the outer portions of a shell of *Pecten diegensis*. (A) and (B) Sections through pores in a growth ridge [similar to the one indicated by the arrow in (D)]; the arrows indicate the periostracum where it continues across pore openings. The outer surface, at the top, is the site of earliest calcification. Scale bar, 1 μm. (C) Enlargement of the upper left corner of (D). The arrow points to the outer surface of the solid shell; crystals formed at the surface show random form, while crystals formed beneath, with the outer crystals as a substrate, become more regular and elongate. Scale bar, 10 μm. (D) General view of the outer shell structure. The direction of growth is to the upper left; the growth ridges here curve forward, rather than backward as illustrated in Fig. 1 (*9*). Scale bar, 10 μm.

to the upper left, and the growth ridges curve forward rather than backward as in Fig. 1B (*9*). The arrow indicates a section through one of the numerous pores found in the growth ridges; Fig. 2, A and B, are enlargements of similar sections through such pores.

The pores seen in Fig. 2, A and B, are not open to the surface, but are sealed on each end by a thin layer (arrows) of some material. Shells treated with Clorox (*10*) have open pores, indicating that this material is organic. This layer is the periostracum; it is present on the outer (upper) surface as the initial shell layer, and on the inner surface as an artifact of the calcification process, left behind by the retreating mantle (see Fig. 1B).

The upper surface of the growth ridge seen in Fig. 2, A and B, is the first part to be calcified; the periostracum here appears draped over crystals arranged in seemingly random fashion. Beneath this surface the crystallization becomes more uniform, with a suggestion of layering; this is particularly prominent in Fig. 2B. In general the layering marks the development of the calcite laths typical of the outer shell layer, but at least one layer (best seen about 2 μm from the base of the pore at the right side of Fig. 2A) may represent a brief interruption in calcification due to a retraction of the mantle. Much the same sequence can be seen in the region of the interspace; in Fig. 2C (an enlargement of the upper left corner of Fig. 2D) the crystals at the outer surface of the shell (arrow) are irregular; only after 5 to 10 μm of shell thickness have accumulated do the lath-like crystals typical of the outer shell layer begin to form.

These SEM observations are in good agreement with time lapse observations. The irregular, apparently random, crystal arrangement near the surface probably reflects the disruption of the earliest crystallites during mantle movements, before their incorporation into the stable shell margin. Similarly, the uniform, lath-like crystallization encountered a few microns below the shell surface probably reflects the influence of a stable shell on subsequent calcification.

This interpretation of marginal growth in *Pecten diegensis* has implications for the role of the substrate in general. First, it appears that substrate stability is a requirement for orderly shell growth; but if stability is lacking shell growth can still proceed in a disorderly manner. Second, it does not seem likely that even orderly shell growth requires anything beyond stability (and, of course, isolation from seawater) from its substrate; this conclusion is based on the observation that if the crystals in the outer, disorganized zone were directing the orientation of new crystal growth, the new growth would itself be disorganized; instead, it becomes quite orderly as soon as the substrate becomes stable.

These implications lead in turn to the conclusion that the mantle itself, acting through the organic matrix, provides the continuity and orientation to the new growth. If so, we come full circle from the early views of shell calcification as an essentially inorganic precipitation to the point of recognizing it to be a highly sophisticated biochemical process.

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7. They were dredged from a depth of approximately 50 m from the R.V. *Velero IV* on 1 and 2 July 1970, through the courtesy of D. S. Gorsline, University of Southern California.
8. The periostracum was not visible at the resolution of this apparatus.
9. In most specimens of *Pecten diegensis*, first formed growth ridges curve forward while later ones curve backward.
10. Clorox is a commercial solution of 5.25 percent sodium hypochlorite; this has the useful property of dissolving organic matter without harming calcium carbonate.
11. Supported by NSF grant GB-20692. The time lapse work was done at Kerckhoff Marine Laboratory, Corona del Mar, California, through the courtesy of the Division of Biological Sciences, California Institute of Technology. Instrument time on the SEM and valuable assistance were graciously provided by J. W. Schopf, University of California, Los Angeles (NSF grant GA-23741).

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Suppressing Drosophila Circadian Rhythm with Dim Light

Abstract. Drosophila *larvae were reared and allowed to pupate in continuous bright white light. The pupae were then transferred to a much dimmer blue light. In continuous blue light of intensity below 0.001 erg per square centimeter per second, adult flies emerged in pulses 24.7 hours apart, each pulse occupying about 6 hours. But in continuous light of intensity exceeding 0.1 erg per square centimeter per second, they emerged at a steady rate. This intensity range from effective darkness to effective light is roughly from starlight to moonlight. Inside this range, the emergence peaks broaden for about a week with little change of period.*

In a wide diversity of organisms, physiological and behavioral activities exhibit an approximate 24-hour periodism even in the absence of external cues. Although these rhythms are only circadian when isolated from light or temperature cycles, they synchronize to the earth's rotation when exposed to the day-night cycle. In many organisms, circadian rhythms decay in continuous light. For example, as little as 0.2 erg cm^{-2} sec^{-1} of blue light (450 nm) suffices to eliminate circadian variations of growth rate in the fungus *Neurospora* within 3 days (*1*).

I measured the threshold for photosuppression in the fruit fly *Drosophila pseudoobscura*, using the circadian rhythm of eclosion of pupal populations (*2, 3*). Chandrashekaran and Loher (*4*) had already found that 0.3-lux white light [containing about 0.2 erg cm^{-2} sec^{-1} in the effective band, 400 to 500 nm (*5*)] suffices to eliminate circadian variations of pupal eclosion rate in *Drosophila* populations within 3 days. The rate of damping is about the same even under bright light. How much light is needed for this photosuppression? Extrapolation from measurements of rephasing by a single light pulse of 100 erg cm^{-2} sec^{-1}, by means of a theoretical model of clock kinetics, suggested that as little as 0.01 erg cm^{-2} sec^{-1} might suffice, and that under suppressing intensities there should be at most a slight increase of period (*2, 6*).

I reared and harvested *Drosophila pseudoobscura* pupae under continuous bright white light, then transferred them to dim light (400 to 500 nm). After 3 to 8 days, depending on the age of the individual pupa at harvest, metamorphosis terminated in eclosion. These emerging flies were automatically recorded hourly for 7 days in the 12 independent experiments