and calcium, a major chemical constituent of seawater. Great sensitivity is possible, because of the large increase in abundance of calcium in passing from freshwater to marine salinities. With trace element indicators the range of abundances is small, so the sensitivity is low, and a complex chemistry controls their incorporation into the sediment. (iv) The chemical technique for extracting the phosphate and determining the different fractions is simple and inexpensive.

It seems likely that the iron- and calcium-phosphate fractions formed in ancient sediments in much the way that they do today, if the composition of the ancient seas was similar to those of the present. Once the mixed calcium-iron-phosphate crystallized, the insolubility of the various components in ordinary pore solutions should prevent any alteration in composition during lithification. Of course, the extent to which sedimentary phosphate may be used to estimate paleosalinities in sediments that undergo progressive metamorphism needs to be investigated. Also, once the applicability of the method to a variety of sedimentary rocks is established, the possibility exists that it may be used to study the paleosalinities of ancient seas where the composition, compared to recent oceans, is open to question.

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14. Details of the chemical relationship involved in the transition from iron phosphate to calcium phosphate are in preparation for publication elsewhere.
15. The results were obtained while I was supported by FWPCA research grant WP-00651-04.

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## Gastropod Urosalpinx: pH of Accessory Boring Organ while Boring

Abstract. *Recent development of a microelectrode has enabled the first continuous recording of the* p*H of the secretion of the normally functioning accessory boring organ of the shell-boring predatory snail* Urosalpinx. *The recording was made in an incomplete borehole in a glass-shell model. The minimum* p*H recorded was 3.8; hitherto the secretion had been considered neutral.*

Penetration of hard, densely mineralized, calcareous substrates by lower plants and invertebrates is common (*1–3*), but the mechanisms by which this is effected are little understood. Those hypothesized are mechanical, chemomechanical, and chemical.

Carriker *et al.* determined that the boring of holes in the shell of bivalve prey by predatory muricid and naticid gastropods, to obtain food, consists of two alternating phases: (i) chemical, in which an accessory boring organ (ABO) or demineralization gland, secretes an uncharacterized substance that etches and weakens the shell at the site of penetration, and (ii) mechanical, during which the radula rasps off and swallows some of the weakened shell as minute flakes (*1–5*). The boring process is not visible from outside the snail and prey.

Recent determinations of the concentration of hydrogen ions in the surface fluid of forcefully extruded and of excised ABO's, and of ABO homogenates, of muricid snails with minute strips of short-range *p*H papers, the color being verified under the microscope against standardized buffer solutions checked with a *p*H meter, consistently gave reactions close to neutrality (*3*). These results confirmed earlier observations (*6*) of the ABO's of naticid snails, with the exception of Schiemenz's (*7*), who obtained a red color when blue litmus paper was applied to the intact exposed organ of a dying *Natica*. On this basis he suggested that the secretion is acid, but could not say how acid. Development by Charlton (*8*) of a special glass microelectrode has enabled for the first time continuous recording of the *p*H of the secretion of the intact, normally functioning, muricid ABO in an incomplete borehole in prey shell. The microelectrode discloses that, during this phase of boring, the *p*H of the secretion of the ABO drops to a minimum of 3.8. We now report the results of these studies.

We used large muricid snails, *Urosalpinx cinerea follyensis* Baker from Wachapreague, Virginia, ranging in height from 33 to 40 mm; they were maintained in the laboratory in running sea water (salinity, 32 per mille; *p*H 8.2; temperature, about 18°C). In this medium they bored holes (about 1 mm in diameter) in the valves of and fed actively on local Cape Cod oysters, *Crassostrea virginica* (Gmelin).

A recently improved glass-shell oyster model permitted observation of the boring process, as well as access to the extended ABO through a pore (1 mm in diameter) in the glass plate covering an artificial, incomplete borehole (approximately 1.2 mm in diameter and 1 to 2 mm in depth) in the shell at the glass-shell juncture (Figs. 1–3). Models were filled with live, actively metabolizing, de-shelled young oysters and placed among snails in running sea water. In time, snails located the incomplete boreholes, apparently attracted by oyster metabolites diffusing from the boreholes through the glass-shell interface, and bored in them.

The ventral surface of the foot was tightly appressed to the glass and shell

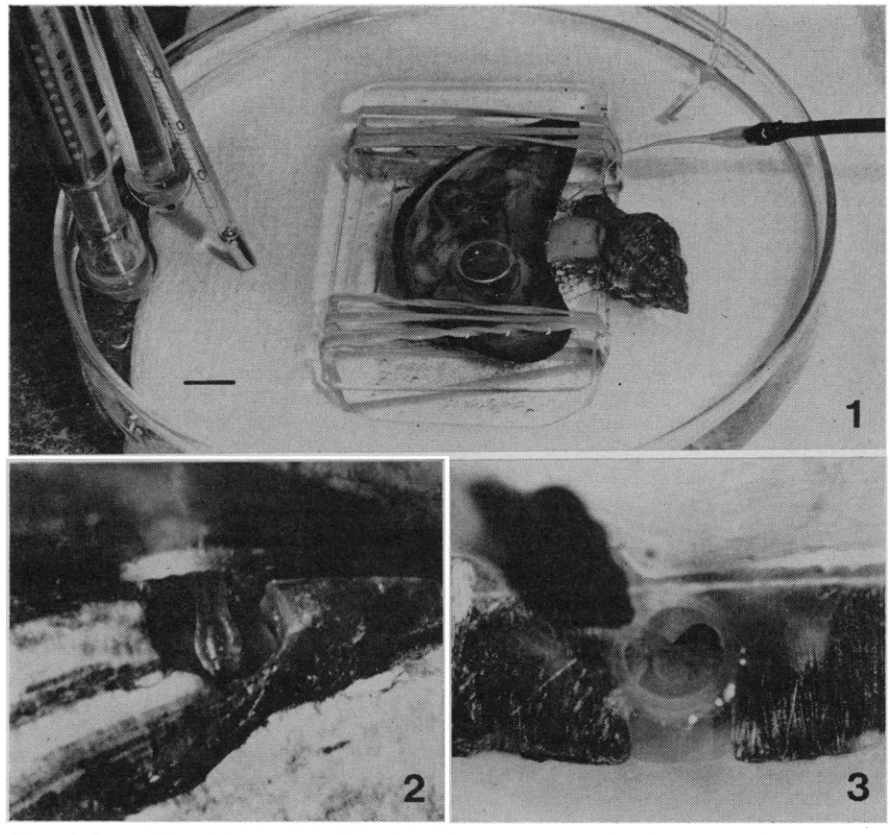
surfaces around the borehole (Fig. 3); this prevented dilution by ambient sea water of the secretion within the borehole. Once a snail was actively boring, it was transferred on the model to a shallow dish of sea water at room temperature and under a binocular microscope (Fig. 1).

Charlton's glass microelectrode and reference electrode were placed in the sea water around the model, and the leads were connected to a Beckman Zeromatic *p*H meter and strip-chart recorder; the electrodes of a standard Beckman research *p*H meter were inserted in the sea water near the model for standardization. The *p*H-sensitive glass bulb on the tip of the microelectrode was 0.27 mm in diameter (Fig. 2); a glass stem, 1.0 mm long, joined the terminal bulb of the microelectrode to the lead by means of a flexible plastic tube that permitted ready manipulation of the microelectrode from the sea water into the borehole without danger of breakage. During operation the reference electrode was located in the sea water close to the snail. The glass microelectrode was standardized against *p*H 4.0 and 7.4 Beckman buffers and the sea water (*p*H 8.0) before each recording.

The moment a snail withdrew its proboscis, and before it inserted the ABO, we lifted the microelectrode gently from the sea water and suspended it in the borehole in the position shown in Fig. 2. At the close of each recording (usually of less than 1 hour) the microelectrode was again checked against the buffers and sea water. Presence of the microelectrode in the borehole did not seem to bother the snails; although they rasped at the electrode bulb and occasionally took it into the buccal cavity, it was not harmed.

The *p*H of the sea water in the assembled model before entrance of the ABO varied from 6.2 to 7.4, depending on the *p*H of the sea water surrounding the de-shelled oysters—which in turn depended on the condition of the oysters. The ABO assumed various positions in the borehole relative to the microelectrode, ranging from no contact of the secretory epithelium with the bulb to the bulb's complete envelopment. During full envelopment, which occurred at nine different times with different snails during the course of the observations, the *p*H ranged from 3.8 to 4.1. As degree of contact between the secretory epithelium and the bulb decreased, the *p*H rose; in one instance, for example, where full contact of the secretory epithelium with the microelectrode registered a *p*H of 4.1, 0.2 mm away from the epithelium the sea water in the borehole gave a reading of 4.6. Contact of the ABO stalk epithelium with the bulb elicited no change in *p*H.

It was possible to obtain four complete continuous recordings of the *p*H of the ABO secretion during (i) inser-




Figs. 1–3. Fig. 1. The assembled glass-shell oyster model, with glass microelectrode and reference microelectrode for *p*H, standard *p*H electrodes, and the snail in position. Bar, 1 cm. Fig. 2. Bulb of glass microelectrode within an incomplete borehole (1.2 mm in diameter), seen from the point on the side of glass-shell oyster model at which snail approaches model; *p*H-sensitive glass tip on bulb of electrode is 0.27 mm in diameter. Fig. 3. Incomplete borehole in shell of glass-shell oyster model, with ABO of snail extended into it from the side and touching glass microelectrode which enters from above through a hole in glass plate. Scale, as in Fig. 2.

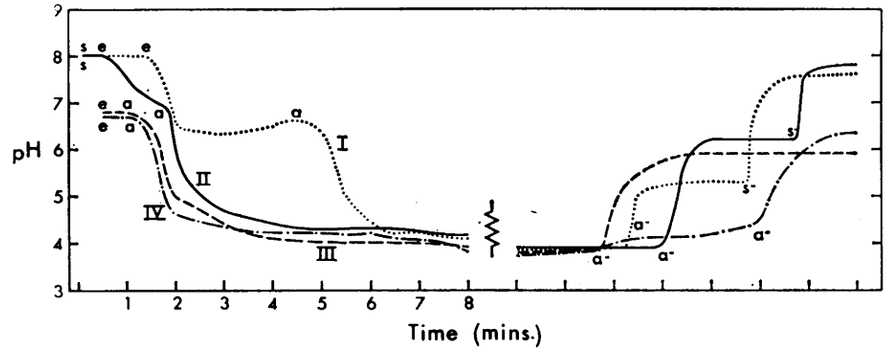



Fig. 4. Continuous strip-chart recordings of *p*H of secretion of ABO's in boreholes. Abbreviations: *s*, microelectrode in sea water outside glass-shell oyster model; *e*, electrode inserted into incomplete borehole; *a*, ABO extended into borehole and contacting sensitive tip of microelectrode; *a*–, ABO withdrawn from borehole. Curves *I* and *II* were taken during the fall in normal boreholes in the shell; *III* and *IV*, during the spring in paraffined boreholes. Break in center of curves indicates duration of ABO's contact with microelectrode; apart from a slight continuous fall in *p*H, there was no change in the curve; the interval for curve *I* was 8 minutes; *II*, 40 minutes; *III*, 4 minutes; *IV*, 6 minutes.

tion of the ABO into the borehole, (ii) full envelopment of the bulb by the ABO secretory epithelium, and (iii) withdrawal of the ABO from the hole (Fig. 4). The period of the ABO in the borehole generally corresponded to that recorded with microhydrophones in earlier studies (*8*). Recording *II* (Fig. 4) is representative of the four *p*H curves. At the start of the observations the *p*H of the sea water in the dish was 8.0. As the snail withdrew its proboscis from the borehole, we inserted the microelectrode (Fig. 4, *e*) and the *p*H dropped to 6.9. In 1.3 minutes the ABO was extended into the borehole and made contact with the bulb (Fig. 4, *a*). In the ensuing minute the *p*H dropped rapidly to 4.7 as the secretory epithelium completely enveloped the bulb. During the subsequent 46 minutes the *p*H continued to drop slowly to a minimum of 3.9. Then the ABO was withdrawn (Fig. 4, *a*–) into the foot, which remained closely appressed over the borehole. As the ABO was retracted, it drew sea water from the cavity of the model across the glass-shell juncture to replace it, and the *p*H rose within 1 minute to 6.2, a *p*H lower by 0.7 than that of the sea water in the borehole when the ABO had first entered. During the steep rise of the *p*H curve, the proboscis was typically inserted into the borehole, exploring and rasping. Two minutes later we moved the microelectrode from the borehole to the sea water around the model (Fig. 2, *s*), and the *p*H rose to 7.5; during the following minute, while we flushed the viscid secretion from the bulb, the *p*H rose again to 8.0—that of the sea water. Full envelopment of the bulb by the secretory epithelium would correspond to full appression of the secretory epithelium to the bottom of the borehole during penetration, and thus the *p*H of the secretion, except for the neutralizing action of the $CaCO_3$ of the shell, should be comparable under both circumstances.

In a number of recordings made during the spring (Fig. 4, *III* and *IV*), the shell material within and in the vicinity of the borehole was impregnated with hot paraffin to eliminate ionization of the $CaCO_3$; in these recordings the minimum *p*H when the ABO was fully around the microelectrode bulb was 3.8, lower by 0.1 *p*H than the minimum values obtained in the unparaffined boreholes.

The *p*H of the fluid on the surface of ABO's, extruded from the amputated feet of snails pinned upside down, ranged from 6.3 to 7.2. When the microelectrode was pressed into the ABO, the *p*H dropped to a minimum of 6.0, but results varied widely, probably as a function of the physiology of the ABO at the time of extrusion.

A snail frequently extends a portion of the propodium of the foot into the the borehole in an exploratory manner after withdrawal of the ABO and before insertion of the proboscis. Contact of the propodial epithelium with the microelectrode did not alter the *p*H appreciably; nor did contact with the integument of the proboscis. On the other hand, when four different snails on separate occasions took the bulb of the microelectrode into their buccal cavities for periods ranging from several seconds to 1 minute, the *p*H fell from 7.2 to 6.7, from 6.7 to 6.1, from 7.2 to 6.7, and from 7.1 to 6.4, respectively—falls varying from *p*H 0.5 to 0.7. This finding suggests that secretions from the salivary glands or the accessory salivary glands, or both, which empty into the buccal cavity, may be slightly acidic.

We conclude that secretion of the normally functioning ABO in the borehole is distinctly acid, a finding contrary to earlier reports (*2–4, 6, 9*) but consistent with the earliest hypothesis (*7*), and acidifies sea water in the borehole over a short radius around the ABO. The acid, still uncharacterized, may play a part in the weakening of shell during boring; active excised ABO's etch polished shell when appressed to it (*4*), though bubbles of $CO_2$ have not been observed in such preparations. The presence of acid does not necessarily rule out the possibility that enzymes or chelating substances, or both, also may be involved in shell destruction. The failure of most earlier attempts to detect convincing acid reactions with *p*H papers was due to the insensitivity of the method and the fact that forcefully extruded and excised ABO's produce no, or minimal quantities of, acid. Apart from slight acidity in the buccal cavity, no acid was detected elsewhere in the snail.

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## Carotenoid Biosynthesis in Rhodospirillum rubrum: Effect of Pteridine Inhibitor

Abstract. *A known inhibitor of pteridine utilization (4-phenoxy,2,6-diamino pyridine) blocks the synthesis of colored carotenoids in the photosynthetic bacterium* Rhodospirillum rubrum. *In many ways the effect is similar to the inhibition of the synthesis of colored carotenoids by diphenylamine. This inhibition is probably independent of other effects of pteridine on photosynthetic electron transport since it is not as readily reversible as the total inhibition of photosynthetic activity by pteridine analogs.*

Purple photosynthetic bacteria normally contain unsaturated methoxylated carotenoids such as spirilloxanthin or spheroidene (*1*). In a wide variety of microorganisms, including photosynthetic bacteria, diphenylamine inhibits the synthesis of the dehydrogenated colored carotenoid pigments, causing the accumulation of the more saturated phytoene with lesser amounts of