

Calcium and Electric Potential across the Clam Mantle

Abstract. An excised clam mantle develops a potential difference, shell side positive, when both surfaces are bathed by tap water or physiological saline solution. The magnitude and sign of the potential are sensitive to the calcium concentration in the bath solution. Bubbling carbon dioxide through the solution bathing the shell side increased the potential.

It was reported some years ago that the mantle of several fresh-water lamellibranchs developed a potential difference between the two faces when the preparation was bathed with tap water (1). More recent work has shown that transepithelial potentials are generated as a concomitant of the active transport of ions—sodium transport in the case of the frog skin and toad bladder and chloride movement across the vertebrate gastric mucosa (2). The mantle in certain mollusks has been implicated in the mobilization of calcium for shell formation (3), and it seemed that this ion might be related to the potential difference described by Lund. Such a relationship would be important for at least two reasons. It would afford a new preparation for studying the means by which bioelectric phenomena are developed. More important, perhaps, it would provide a system for studying the movement of calcium by living membranes. Since the preparation seems to be virtually unknown, we wish to direct attention to it here by reporting some experiments that show that the potential difference is dependent on the calcium ion content of the medium.

When a mantle is excised and mounted so that it separates two chambers containing bathing solutions, a potential difference can be measured across the epithelium. The shell side is positive to the body side, and the magnitude is of the same order of size as potentials reported for a variety of epithelia (that is, 30 to 70 mv). The potential falls more or less rapidly, but not to zero, so that after 1 to 2 hours a small, stable potential is generated, usually 2 to 10 mv, with the shell side of the preparation positive. Although this is less striking than the initial potential difference, its stability made it more useful as an experimental variable, and the experiments reported below concern this quantity.

The gross composition of the bathing solutions does not seem to change the electrical picture qualitatively. Lund used tap water on both sides of the mantle, while we have used a clam Ringer's solution and occasionally a sodium-free "choline Ringer's"; in all cases an initial potential difference was developed which decreased toward a

final, stable value. However, the stable potential difference was exceedingly sensitive to calcium ion concentration in the bathing solution. Figure 1 shows how this potential difference varied as a function of calcium in the body-side solution. Neither sodium nor potassium affected it in this fashion. It is important to note that the concentration in the other (shell-side) solution also caused the potential difference to change, in this case to decrease as a function of concentration and even to reverse polarity.

The data also show, however, that the mantle does not act simply as a calcium electrode, for the potential difference changed less than the theoretical 29 mv per tenfold change in concentration.

One other interesting phenomenon is the change in potential difference brought about by bubbling CO₂ through the shell-side solution. Incorporation of 5 percent of CO₂ in the oxygen used to aerate caused a marked increase in the magnitude of the potential difference. Bubbling the gas through the body-side solution had no effect. The presence of carbonic anhydrase in the oyster mantle was reported by Wilbur and Jodrey (4), and carbonic anhydrase is also present in the clam mantle (5). Its role in the CO₂ effect has not been assessed.

The possible participation of calcium in the genesis of the potential difference was suggested by the fact that the mantle (at least in the oyster) transports calcium from body to shell side, as shown by the excellent work of Wilbur's group. Exploration for a CO₂ effect was suggested by the fact that carbonate is

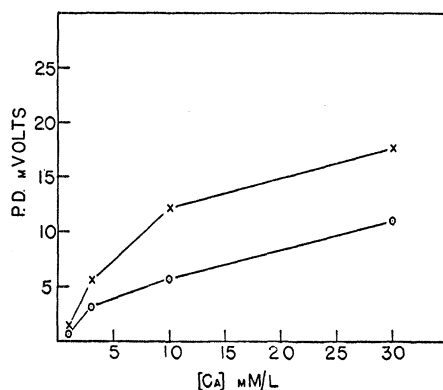


Fig. 1. Mantle potential (in millivolts) as a function of calcium concentration. The mantle was excised and equilibrated in a physiological saline containing CaCl₂ (1 mmole/liter). After 4 hours it was mounted in the experimental chamber with the same saline bathing both surfaces. Changes in calcium concentration were made by replacing aliquots of the saline with a volume of stock CaCl₂ solution sufficient to maintain isotonicity. The results of two different experiments are shown.

required for shell formation. However, the exact role of these substances in the bioelectric phenomenon and the way they are handled by the mantle are still unknown (6).

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References and Notes

1. E. J. Lund, Ed., *Bioelectric Fields and Growth* (University Cooperative Society, Austin, Tex., 1947).
2. V. Koefoed-Johnsen and H. H. Ussing, *Acta Physiol. Scand.* **42**, 29B (1958); C. A. M. Hogben, *Am. J. Physiol.* **180**, 641 (1955).
3. L. H. Jodrey, *Biol. Bull.* **104**, 398 (1953).
4. K. M. Wilbur and L. H. Jodrey, *ibid.* **108**, 359 (1955).
5. Unpublished experiments.
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Delayed Effects of Nicotine on Timing Behavior in the Rat

Abstract. Hungry rats were trained to time precisely by rewarding with food those lever responses spaced 20 to 22 seconds apart. Injections of nicotine disrupted the timing behavior slightly, but pronounced delayed effects occurred 3 and 4 days after the drug injection and following a temporary return to base-line performance.

Research in psychopharmacology has largely taken the direction of determining the immediate effects of drug administration on behavior. Latent or delayed actions upon behavior have not been extensively investigated. Because of this, it frequently has been assumed that recovery of base-line performance following a drug injection indicates that the action has been dissipated and that subsequent dosing may be safely undertaken. The errors inherent in such a procedure are illustrated by the findings of the present investigation, which demonstrate clear-cut behavioral effects of nicotine several days after injection and following a temporary return to base-line behavior.

The behavior under investigation involved a precise timing discrimination (1). Hungry rats were first trained to space their lever-pressing responses at least 20 seconds but no more than 40 seconds apart in order to obtain a drop of sweetened, condensed milk. The 20-second period of eligibility (20-second limited hold) after the 20-second timing period had elapsed was progressively reduced over many weeks of training until a 2-second limited hold was in force. The final experimental conditions for assessing the effects of the drug