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 CO_2 through the shell-side solution. Incorporation of 5 percent of CO_2 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 CO_2 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 CO_2 effect was suggested by the fact that carbonate is 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*).

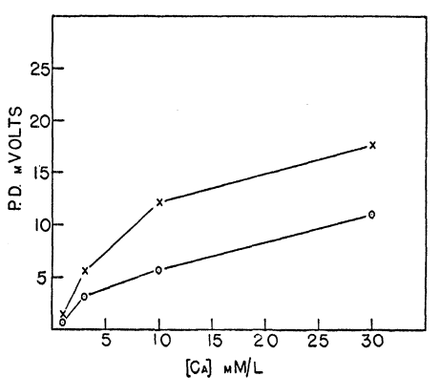

Fig. 1. Mantle potential (in millivolts) as a function of calcium concentration. The mantle was excised and equilibrated in a physiological saline containing $CaCl_2$ (1 mmole/liter). After 4 hours it was mounted in the experimental chamber with the same saline bathing both surfaces. Changes in calcium concentration were made by replacing aliquots of the saline with a volume of stock $CaCl_2$ solution sufficient to maintain isotonicity. The results of two different experiments are shown.

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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

required that responses be spaced at intervals of 20 to 22 seconds in order to produce reinforcement. Responses which occurred either before or after the 2-second "pay-off" period were not reinforced, and merely initiated a new timing cycle.

Three male albino rats that had been feeding ad libitum were gradually starved to 80 percent of their original body weights, and were then maintained at the reduced weights by limited feedings immediately after each experimental session (*2*). The animals were trained in sound-resistant chambers containing a lever in one wall and an automatic feeding device that presented the 0.1-ml liquid reward for 4 seconds. All experimental sessions were of 2 hours' duration.

The timing behavior in this situation is conventionally described by a distribution that gives the relative frequencies of the times between successive responses (interresponse times). For this study the interresponse times were grouped into 2-second categories and cumulated over the 2-hour sessions. In addition, records were taken of the average daily response rate and the total number of reinforcements earned.

With sufficient training, the timing behavior sharpens so that a clear-cut peak in the interresponse time distribution occurs at the reinforced interval, or, for many animals, at the interval just preceding the reinforced one. A series of nicotine bitartrate injections, administered no more frequently than once a week, was initiated when the interresponse time distributions had stabilized and the total number of reinforcements obtained in each session was roughly constant.

Figure 1*a* presents the interresponse time distributions for the series of experimental sessions preceding and following the largest dose of nicotine given. Examples of the base-line performance may be seen in the predrug histograms. The large proportion of interresponse times in the 0- to 2-second category is typical, and represents, for the most part, multiple bursts of responses, but the remainder of the distribution gives a good picture of the timing behavior. The distributions may be seen to peak at, or just prior to, the rewarded interval (white column), and to decline sharply at either side.

Table 1. Total number of reinforcements obtained in the daily sessions indicated for four nicotine test series.

Drug dose* (mg/kg)	Number of reinforcements			
	Predrug	Day of drug injection	Day 1 postdrug	Day 4† postdrug
		Rat C-15		
0.1	59	50	65	36
0.15	40	40	53	14
0.25	47	40	62	25†
0.2	39	39	55	39
		Rat C-27		
0.1	60	39	72	29
0.15	56	26	68	26
0.25	48	61	57	32†
0.2	71	39	38	30
		Rat C-29		
0.2	81	23	81	38
0.15	64	78	72	55
0.25	54	61	65	46†
0.1	69	59	73	55
Grand mean	57.3	46.2	63.4	35.4
Predrug (%)	100	81	110	61

* Doses are listed in chronological sequence. † Values are number of reinforcements on day 3, rather than day 4, postdrug.

The crosshatched distributions were obtained on the day that the drug was administered; these distributions differed in no significant way from those obtained on the previous day, suggesting that the drug in this dosage has little or no effect on the timing behavior (*3*). For the two sessions following the drug day the timing behavior remained intact and perhaps even improved as indicated by an increase in the total number of reinforcements obtained (Fig. 1*c*). On the third day, however, the delayed effect occurred. This effect revealed itself as a flattening of the interresponse time distributions, an increase in the average response rate (Fig. 1*b*), and a marked decrease in the total number of reinforcements obtained (Fig. 1*c*). At this time the animals were observed to be somewhat agitated in the experimental chamber, but not in the home cages. No such agitation was observed on the day of the drug injection.

The reproducibility of the delayed nicotine effect is shown by the data presented in Table 1; the total number of reinforcements earned is used as an index of timing performance. In nearly every series the frequency of reinforcement on day 4 (or day 3) postdrug is seen to be depressed well below (on the average, 40 percent) the levels obtaining before or shortly after the injection. It also may be observed that the delayed effect (as well as the direct effect) of the drug tends to decrease with repeated dosing. Finally, it should be pointed out that the behavioral base line is a critical determinant of the drug effect, and particularly, in the present situation, it may be that a less stringent timing requirement might not reveal a similar magnitude of action.

Long-term biochemical changes have been described which result from single small doses of nicotine, and some of these may be related to the present behavioral findings (*4*). The changes reported in blood glucose levels, epinephrine output, and blood plasma potassium levels would appear to deserve further correlational investigation (*5*).

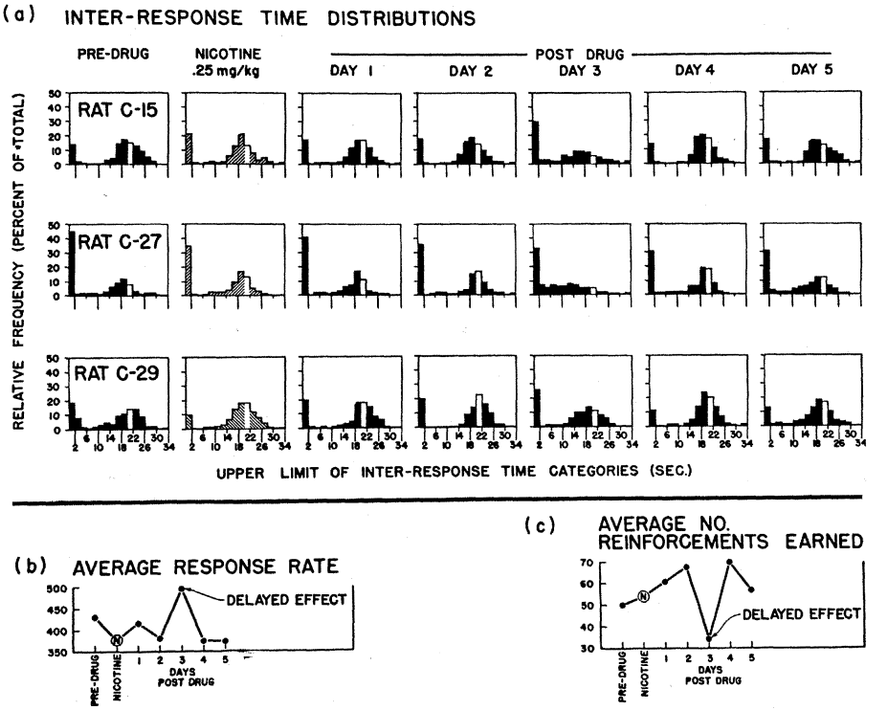

Fig. 1. Immediate and delayed effects of nicotine on three measures of timing behavior. (*a*) Individual relative frequency distributions of interresponse times for each day in the series. The white columns indicate the reinforcement interval. (*b*) Daily response rates averaged for three animals throughout the series. (*c*) Daily number of reinforcements earned, averaged for three animals throughout the series.

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1. B. F. Skinner, *The Behavior of Organisms* (Appleton-Century, New York, 1938), p. 306; M. Sidman, *Science* **122**, 925 (1955).
2. A fourth rat died on the second day following the first nicotine injection.
3. Since the graphed data represent a third cycle in the experiment, the rats may have acquired a tolerance for the drug and therefore show little if any immediate nicotine effects.
4. T. Sollmann, *A Manual of Pharmacology* (Saunders, Philadelphia, 1957), p. 454.

5. The authors wish to express gratitude to Larry Stein for his critical reading of the paper.

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Autoradiographic Investigation of Incorporation of H^3-Thymidine into Cells of the Rat and Mouse

Abstract. The application of H^3-thymidine results in labeling of those nuclei of cells in which deoxyribonucleic acid (DNA) is synthesized during the interval between application and the sacrifice of the animal (*1–3*). This paper reports autoradiographic investigation with H^3-thymidine of rats and mice. This method permits a more exact statement of the number of dividing cells than does the microscopic estimate of mitosis. The latter method is practically impossible in tissues with small fusiform cells. Moreover, it is possible to obtain information about the relative time of DNA synthesis in different cells.

Adult rats and mice received 460 and 50 μc, respectively, of H^3-thymidine by intraperitoneal injection, and were sacrificed after 90 and 60 minutes, respectively. Autoradiograms were prepared from 5-μ, paraffine-imbedded slices, by use of the stripping-film method (Kodak autoradiographic plates AR 10), or with liquid emulsion (Ilford G 5). Exposure times were up to 20 days.

The autoradiographic blackening was exclusively limited to a certain percentage of nuclei and matched the distribution of the chromatin in the nuclei. Nucleoli with a diameter above 2 μ appeared as white spots within the silver grain covered chromatin. A remarkable result was that the nuclei either were labeled rather uniformly or were absolutely free from silver grains. For that reason the percentage of labeled nuclei in a section was independent of the exposure time.

The relative numbers of labeled nuclei (H^3 index) and of mitosis (mitosis index) were determined for different tissues and the percentages these formed of the total number of examined nuclei were calculated. Table 1 gives the results.

The examined tissues can be classified in three groups with a distinct difference in the percentage of H^3-labeled nuclei. These three groups correspond to the scheme given by Cowdry (*4*).

Fixed postmitotic cells (central nervous system, musculature). No labeled nuclei are found in ganglionic cells, in cells of striated muscle, or in cells of the heart muscle. However, in all three tissues there are some mesenchyme cells with labeled nuclei. Likewise, some labeled nuclei were found in the choroid plexus and in cells of the subependymal regions of the brain. Contrary to the case of striated muscle, there are few labeled nuclei in the cells of smooth muscle.

Reversible postmitotic cells (parenchymatous organs). In the liver the distribution of labeled nuclei does not favor certain lobes nor parts of lobules. Liver cells with two nuclei were rather often seen in which both nuclei were equally covered with silver grains. The percentage of labeled Kupffer cells is markedly higher than that of the labeled epithelial cells of the liver. In the kidney the labeling of the nuclei occurs considerably less often in the epithelial cells of the canaliculi than in the endothelial cells of the glomeruli and of Bowman's capsule. No preference of labeling is given to any parts of the canaliculi. There are no accumulations of labeled nuclei in the different layers of the adrenal cortex. In the pancreatic epithelia and in the islets of Langerhans the number of the labeled nuclei corresponds with the number in the organs mentioned above.

Accumulations of labeled nuclei are found in the peribronchial tissue of the lungs. Within the bronchial tubes the labeled epithelial cells belong partly to the basal and partly to the upper layer of cells of the ciliated epithelium. Labeled nuclei are only sporadically distributed in the single-layered pleural endothelium. There are increased numbers of labeled pleural endothelia over subpleural inflammatory infiltrations. There is a remarkably high number of labeled nuclei of alveolar cells.

It is remarkable that in all parenchymatous organs the number of labeled cells is much smaller in the parenchyma than in the interstitial connective tissue and reticuloendothelial system.

Vegetative intermitotic cells (epithelium of skin and mucous membranes, lymphatic tissues, testicle, connective tissue). In the abdominal skin and in the esophagus, labeled nuclei are found in the basal cellular layer only. In the stomach, labeled cells appear more often in the lower third of the gastric foveolae than in the glands. Only very few labeled nuclei are seen in the Brunner's glands of the duodenum. Almost every other cell of the Lieberkühn's crypts is labeled. The epithelia of the villi are free of labeled nuclei. Similar statements are true for the jejunum and the ileum. In the colon the major density of labeled cells is found just above the bases of the crypts. Some nuclei of large basophil stem cells were labeled in the germinative centers of the spleen and of the lymphatic glands. Few labeled nuclei of large basophil reticular cells were detected in the peripheral zones of the lymphatic follicles and of the Malpighian corpuscles. Accumulations of labeled nuclei are specially found in the sinus of the spleen and originate in the relatively small nuclei of the prolymphocytes (*5*). Here, according to the more solid arrangement of the chromatin, the silver grains are closer

Table 1. Percentage of nuclei labeled by H^3-thymidine and percentage of mitosis, for cells of various organs.

Organ	Kind of cells	H^3 index (% of labeled nuclei)	Mitosis index (% of mitosis)	H^3 index ÷ mitosis index
	Group 1			
Central nervous system	Ganglionic cells	0		
Heart muscle; skeletal muscle	Cells of striated muscle	0		
Gastrointestinal tract	Cells of smooth muscle	0.28		
	Group 2			
Liver	Liver epithelia	0.4	0.04	10
	Kupffer cells	1.2		
Kidney	Epithelia of canaliculi	0.6	0.05	12
	Endothelia of glomeruli	4.0		
Suprarenal gland	Cells of the cortex	0.4	0.04	10
Pancreas	Pancreatic epithelia	0.83	0.073	11
	Cells of Langerhans' islets	0.8		
Lung	Surface cells of alveoli	1.8		
	Bronchial epithelia	1.0		
	Group 3			
Abdominal skin	Basal layer	4.0	0.52	8
Esophagus	Basal layer	20.0		
Duodenum	Epithelia of mucous membrane	17.0	1.1	15
Jejunum	Epithelia of mucous membrane	15.0		
Colon	Lieberkühn's crypts	8.2	0.72	11