ability of these ions to substitute for sodium in the generation of many bioelectric potentials that normally are a consequence of an increase in sodium permeability (*7*, but compare *8*). We have found also that continuous perfusion with potassium-free seawater could reduce the RP and that increasing the concentration of extracellular calcium ions fivefold over that in normal seawater could also significantly reduce the RP. Furthermore, each of these procedures (that is, replacement of sodium or potassium or an increase in calcium) usually depolarized the cell by some 10 to 20 mv, altered the *I-V* curves similarly to bright light, that is, from multirectifying to singly rectifying and abolished the spontaneous "bumps." When lithium or Tris was substituted for sodium, these latter changes occurred simultaneously with the abolition of the RP and were similarly transient. That is, during the period when the RP was abolished, the *I-V* curve was similar to that of a highly light-adapted cell, while during the phase of partial recovery of the RP in sodium-free solutions, the *I-V* curve resembled that of a partially light-adapted cell.

Our observations show that the CIM hypothesis is inadequate to explain the generation of receptor potentials in *Limulus* photoreceptors. Within the physiological range of membrane potential, light and current are essentially equivalent in these cells, in that membrane conductance is highly dependent on membrane potential but relatively independent of whether that potential is evoked by light or extrinsic current. It cannot be true both that there is such an identity in the action of light and current and that the RP is the result simply of a permeability (conductance) increase. Also, removal of either sodium or potassium ions from the fluid bathing these cells has qualitatively similar effects on their response and membrane properties. It is highly unlikely that the RP is caused by an increase in permeability to both of these ions, since it is abolished by removing either sodium or potassium ions from the external medium.

It is conceivable that conductance changes of a more complex nature could generate the RP directly. For example, illumination might lead to an increase in sodium conductance and a decrease in potassium conductance of exactly the right magnitude to duplicate the underlying nonlinearity of the cell membrane (Fig. 2). Such a mechanism, however, would require a number of *ad hoc* hypotheses. Among these would be that the increased sodium permeability requires the presence of extracellular potassium and the decrease in potassium permeability requires the presence of sodium in the medium (*8*). While we cannot categorically reject such a complex mechanism, we believe there is a more parsimonious way to account for our results (*6*).

We conclude, therefore, that the response of photoreceptor cells in *Limulus* is not generated by a conductance increase mechanism as has been generally supposed. Because of the basic similarity of the action of light and current in the physiological range of membrane potential, the cell membrane appears to possess a source of current which has the properties of a high source-impedance, constant-current generator. Moreover, light appears to control this source of transmembrane current. Because of the underlying nonlinearity of the cell membrane, a light-evoked depolarization of the cell is accompanied by a secondary increase in conductance. The identity and properties of the primary, light-dependent current-generator are considered elsewhere (*6*).

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A Role for the Sodium Pump in Photoreception in Limulus

Abstract. *The membranes of photoreceptor cells in* Limulus *have an electrogenic sodium pump which contributes directly to membrane potential and whose activity is changed by light. These light-induced changes in pump activity underlie the receptor potential.*

In the accompanying paper we presented evidence that the receptor potential (RP) in *Limulus* ventral-eye photoreceptors is not generated by a conductance increase mechanism; rather, the conductance changes associated with the RP appear to be secondary and a consequence of the basic nonlinearity of the current-voltage (*I-V*) characteristic of the photoreceptor membrane (*1*). We noted that some complicated conductance change mechanism might explain our results, but that they can be accounted for by a simpler hypothesis. We were led to our hypothesis, in part, by the basic similarity of action of light and current in the physiological range of steady-state membrane potential; and we suggested that the photoreceptors have a current generator in their membranes which is altered by the absorption of light. Our hypothesis is that the ventral-eye photoreceptors have an electrogenic sodium pump which contributes directly to steady-state membrane potential and which is the current generator altered by light.

Recently, a number of excitable cells have been found to have an electrogenic sodium pump (*2–8*). By definition, a pump is electrogenic if the ratio (β) of the potassium ions actively transported into the cell to the sodium ions actively transported out of the cell is other than one (*3, 4, 9*). This imbalance of ions moving through the pump constitutes a source of current which either hyperpolarizes or depolarizes the cell membrane directly, depending upon whether β is, respectively, less or greater than one. In any steady-state, of course, the current must be counterbalanced by an equal current moving in the opposite direction through the passive ionic pathways lying in parallel with the pump across the cell membrane.

An apparently obligatory component of the sodium pump mechanism is a Na^+,K^+-dependent adenosine triphosphatase, which, by catalyzing the hydrolysis of adenosine triphosphate (ATP),

provides the energy necessary to drive the pump (*10–13*). This system has a number of characteristics: (i) a requirement for intracellular sodium (*4, 14*); (ii) a requirement for extracellular potassium, which may be partially replaced by other ions (for example, Rb^+ and Cs^+) (*6, 12–14*); (iii) a reduction in activity by increased intracellular calcium (*2, 15*); (iv) a reduction in activity by sufficient cooling (*2, 7, 13*); and (v) specific inhibition by the cardiac glycosides (for example, ouabain) (*11–13*).

For the sodium pump to be implicated in a physiological process it must be demonstrated that the process is altered by procedures which correspondingly alter the activity of pump adenosine triphosphatase (*4*). When this process is presumed to involve an electrogenic component of the sodium pump, the contribution to membrane potential generated by the pump should, for example, be abolished by pump inactivation (*2–7, 16*). Thus, in some cells, an electrogenic sodium pump contributes significantly to steady-state membrane potential (*2, 7*), posttetanic hyperpolarization (*3, 5, 6*), and some postsynaptic potentials (*16*).

In the *Limulus* ventral eye, removing sodium or potassium or increasing calcium in the extracellular medium depolarizes the membrane by some 10 to 20 mv (*1*). We assume that the changes in concentration of sodium and calcium in the extracellular fluid lead to similar changes in their concentrations at the inner surface of the membrane. In addition, when the preparation is either cooled to below 2°C or treated with 1 m*M* ouabain in seawater, the membrane also depolarizes by some 10 to 20 mv. Since each of these procedures should inactivate pump adenosine triphosphatase, we suggest that the depolarizations observed are a result of removal of the electrogenic component of the sodium pump.

Further evidence suggesting an electrogenic pump are our findings that the membrane potential dependence upon temperature (about 1 mv/°C) exceeds that attributable to the Nernst relation (*2, 4*) and that injection of sodium ions intracellularly through a micropipette hyperpolarized the membrane by several tens of millivolts (*2*). These data indicate that the steady-state membrane potential in the dark can lie at a value more negative than that brought about solely by the ionic concentration gradients acting across the passive membrane conductances and are consistent with our hypothesis that ventral-eye photoreceptors have an electrogenic sodium pump which contributes directly to steady-state membrane potential ($\beta < 1$).

With regard to the RP, we have found that those procedures which reduce or abolish the pump adenosine triphosphatase activity correspondingly reduce or abolish the RP and affect the *I-V* curves similarly to light. Those procedures are perfusion of the extracellular fluid with solutions (*1*) free of sodium or potassium or with a high concentration of Ca, cooling the preparation to below 2°C, or treating the cell with ouabain (Fig. 1). At the time the RP is abolished by ouabain the cell is not completely depolarized, and the RP cannot be returned by hyperpolarizing the cell with current; but a spike similar to that found in untreated cells (*17*) can be evoked by electrical stimulation.

The above data, plus the reversibility of all the effects of all agents except ouabain, suggest that the procedures used to inactivate the pump do not produce any nonspecific damage to the membrane. In addition, injection of intracellular sodium can lead to an increase in the magnitude of the RP of cells perfused extracellularly with a sodium-free solution; the effects of removal of extracellular potassium can be partially counteracted by replacement with Rb^+ or Cs^+, and the dependence of the membrane potential upon temperature is altered similarly by bright light and by ouabain.

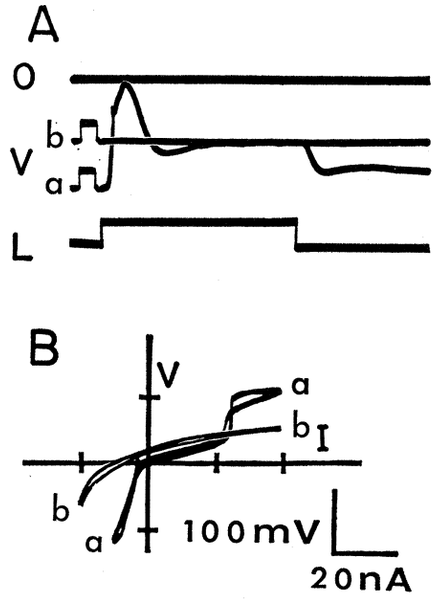

Fig. 1. Effect of ouabain on photoreceptor cells in the ventral eye of *Limulus*. Membrane potential and receptor potential (A), and current-voltage curves (B), obtained in seawater (*a*) and after treatment with seawater containing $1 \times 10^{-3} M$ ouabain (*b*). In (A), *O* is zero membrane potential; *V*, intracellular potential; *L*, the monitor of light stimulus. Calibration in *V* is 10 mv, 100 msec. In (B), *V* is potential; *I*, current; origin, at resting potential in the dark.

We take our results as evidence that the RP is brought about by a change in the activity of the electrogenic sodium pump. The nature of this change is unknown but may involve a change either in the pump's rate, electrogenicity (β), or direction of pumping. Of these three possibilities, we believe a change in β, probably an increase, is the most likely, although there may be either direct or secondary changes in the rate of pumping. We doubt that light reverses the direction of the pump since we have observed that these photoreceptors can be stimulated for hours with intense lights without deterioration, and others have found that light has no significant effect on the hydrolysis of ATP in vertebrate photoreceptors (*11, 18*).

Our observation (*1*) that replacement of sodium with lithium or Tris abolishes the RP only transiently, but markedly reduces the cell's sensitivity to light, suggests that lithium, or potassium (or both) may partially, but rather ineffectively, substitute for sodium in activation of adenosine triphosphatase (*3, 19*). Our observation that the iontophoretic injection of sodium into cells bathed in a sodium-free medium produces an increase in the RP is difficult to reconcile with any hypothesis that the RP is generated primarily by changes in the membrane's sodium conductance; however, we would expect, from our hypothesis, that intracellularly injected sodium ions would reactivate the pump and lead to a larger RP.

The degree of correlation between the effects of light and of pump inactivation on the membrane *I-V* characteristics and on the RP is high, even when these effects are transitory (*1*). These findings warrant further study, particularly with reference to the mechanisms underlying the *I-V* characteristics. If the changes in the *I-V* curves produced by light and by pump inactivation actually represent changes in the sodium pump, as we propose for the RP, the *I-V* characteristics may be, in

part, a measure of the potential dependence of the activity of the electrogenic sodium pump (*5, 20*).

Finally, although some of our results showing the qualitatively similar action of light and pump activity are steady-state measurements, our evidence suggests that changes in pump activity are also necessary for the production of the graded component of the RP transient. There may, however, be components of the transient which do not involve the pump per se but are secondarily activated by the changes in pump activity induced by light.

In summary, we have presented evidence consistent with the hypothesis that *Limulus* ventral-eye photoreceptors have in their membranes an electrogenic sodium pump, that this pump has some of the characteristics of a high source impedance current generator, that this pump contributes directly to the steady-state membrane potential, and that changes in pump activity underlie the receptor potential evoked by light. Specifically, we suggest that light alters the electrogenicity of the pump, perhaps by affecting the affinities for ions of the pump's sodium or potassium sites (or both) which are thought to lie, respectively, at the inner and outer surfaces of the membrane (*12–14*). Should our hypothesis prove correct, then the question of how light evokes potential changes in photoreceptors may become the question of how the light-induced conformational changes in rhodopsin molecules, lying at sites in or on the photoreceptor membrane (*21*), can alter the activity of those presumably nearby membrane molecules which make up the sodium pump machinery.

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Short-Latency Antidiuresis Following the Initiation of Food Ingestion

Abstract. *A factor associated with the ingestion of food is shown to produce a short-latency antidiuresis. Animals consuming large quantities of a highly palatable solution during a period of food deprivation exhibit an antidiuresis immediately following the initiation of eating. The rapidity of the response raises the possibility of a signaling factor separate from postingestional influences.*

In the present study we investigated the time course of antidiuresis initiated by the ingestion of food in awake unrestrained animals. It is well known that the postprandial phase of food ingestion results in an antidiuresis. A recent report by Stacy and Brook (*1*) describes this phenomenon in sheep. Earlier, Jacobs (*2*) reported a decrease in urine production during food ingestion in rats, but the latency of the antidiuresis was not studied.

Experimental subjects were food-deprived animals that overhydrated themselves by consuming large quantities of a highly palatable mixture of 0.125 g of saccharin (sodium-*o*-benozoic sulfimide) and 3.0 g of glucose (S + G solution dissolved in distilled water to a total volume of 100 ml). This technique avoids the complication of release of the antidiuretic hormone associated with the stress imposed by gastric loading. We had demonstrated in an earlier report (*3*) that when rats have food freely available they ingest the S + G solution during a 24-hour period in amounts equal to 60 percent of their body weight and that food deprivation increases the intake to approximately 90 percent of body weight.

A total of 29 male Holtzman albino rats (80 days old at the start of the experiment; average weight, 300 g) were used. They were housed in individual cages and maintained on 12-hour cycles of light and dark in a room with a constant temperature of 22°C. The experiment was performed twice, the first time with seven experimental and five control animals and later with nine experimentals and eight controls. Since the results were indistinguishable, they have been combined. Initially, animals were provided with Purina Lab Chow pellets without restriction and the highly palatable solution of S + G. After 4 days of exposure to food and S + G solution the rats were deprived of food for 4 days. Daily measurements of fluid consumption were obtained. Spillage was collected in plastic cylinders mounted under the drinking tubes. Following the 4th day of total food deprivation the subjects were divided into two groups matched for consumption of the S + G solution during the preceding 4 days. The solution was removed and the 16 experimental rats were provided with Purina Lab Chow pellets.

Measurements were made of the volume and concentration of total solids in each urine sample obtained from both groups during the 3 hours following food presentation to the experimental animals. During this period no fluid was available to the animals. Each urine sample was collected in a syringe from a cardboard covered with Saran Wrap placed under the cages and the time of urination was recorded. A num-