mosome No. 2 would be 33 percent and, by subtraction, that it is on No. 4, 67 percent. A further calculation involving assumptions about the genetic distance between the break points of the inversion and the translocation on the long arm of the No. 2 chromosome does not appear to refine this estimate. The data suggest that the most probable location of the MN locus is on the distal segment of the long arm of chromosome No. 4, in a region which was perhaps deleted in the child reported by German *et al.*

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References

1. J. German, M. E. Walker, F. H. Stiefel, F. H. Allen, Jr., *Science* **162**, 1014 (1968).
2. L. R. Weitkamp, M. K. Janzen, S. A. Guttormsen, H. Gershowitz, *Ann. Hum. Genet.*, in press.

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Photoreception in Limulus: Role of an Electrogenic Sodium Pump?

Smith *et al.* (*1, 2*) reported electrophysiological observations on the photoreceptor cells in the ventral eye of *Limulus*. They maintain that their results cannot be explained by the conventional sodium permeability increase mechanism, but that a sodium pump with varying electrogenicity must be invoked. However, they do not appear to have taken into account the very much higher ratio of surface to volume of the *Limulus* rhabdome (*3*), as compared to that of the nerve axon. Whereas the internal ion concentrations in the axon change very little after an impulse (*4*), stimulation of a photoreceptor cell can cause very substantial changes in ion concentrations in the photoreceptor elements (*5*).

The normal resting potential (− 60 mv) and the peak receptor potential (+ 30 mv) in the *Limulus* photoreceptors (*1, 6*) can be readily derived from the Goldman equation

$$E = -\frac{RT}{F} \ln \frac{[K_i] + a[Na_i] + b[Cl_o]}{[K_o] + a[Na_o] + b[Cl_i]}$$

By substituting Adams and Hagins' values for the internal and external ion concentrations in squid photoreceptors (*7*) and values of $a = 24 \times 10^{-4}$ and $b = 0.14$ for the resting squid axon (*8*), we calculate − 65 mv for the resting potential. If we assume a sodium permeability increase upon light stimulation, and take $a = 10$ (*4*, p. 42) then a value of + 30 mv is obtained for the receptor potential (RP). The ion concentration changes, resulting from the large surface area of the membrane, would then preclude total recovery, and in the light the potential would remain about zero (*1, 6*). The essential role of the sodium permeability increase is supported by the fact that the RP is abolished by tetrodotoxin (*9*) and by the removal of sodium ions from the bathing solution (*1, 2*). As might be expected from the Goldman equation, the absence of external chloride ions has little effect on the resting potential or the RP (*1*).

Cooling the preparation, removing potassium ions from the bathing solution, or adding ouabain or calcium have essentially similar effects; they all result in an inhibition of the sodium pump, and in photoreceptors this inhibition would lead to a rapid change in ion concentrations and a depolarization of the membrane potential. Axon studies have shown that a depolarization also leads to an increased conductance (*4*, p. 64) thereby explaining why ". . . those procedures which reduce or abolish the pump adenosine triphosphatase activity correspondingly reduce or abolish the RP and affect the current and voltage (I–V) curves similarly to light" (*2*). Because of the concentration changes, the decrease in resting potential with temperature will be larger than expected from the temperature factor in the Nernst equation.

Finally, the observed net efflux of K^+ upon illumination of photoreceptor cells (*5, 10*) is difficult to reconclude with a sodium pump of constant activity but decreasing electrogenicity (*2*). Therefore, we conclude that the permeability increase theory is strengthened rather than weakened by the observations of Smith *et al.* (*1, 2*). The molecular mechanisms which might explain such a permeability increase have been discussed (*10*).

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References

1. T. G. Smith, W. K. Stell, J. E. Brown, *Science* **162**, 454 (1968).
2. T. G. Smith, W. K. Stell, J. E. Brown, J. A. Freeman, G. C. Murray, *ibid.*, p. 456.
3. M. F. Moody, *Biol. Rev.* **39**, 43 (1964).
4. A. L. Hodgkin, *The Conduction of the Nervous Impulse* (Liverpool Univ. Press, Liverpool, 1964).
5. S. Buckser and H. Diamond, *Biochem. Biophys. Res. Commun.* **23**, 240 (1966); G. Duncan, F. J. M. Daemen, S. L. Bonting, *Fed. Proc.* **28**, 331 (1969).
6. R. M. Benolken, *Biophys. J.* **1**, 551 (1961).
7. R. G. Adams and W. A. Hagins, *Biol. Bull.* **119**, 300 (1960).
8. A. Strickholm and B. C. Wallin, *J. Gen. Physiol.* **50**, 1929 (1967).
9. R. M. Benolken and C. J. Russell, *Science* **155**, 1576 (1967).
10. S. L. Bonting, *Curr. Top. Bioenergetics* **3**, 351 (1969).

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Duncan and Bonting (*1*) do not consider those observations which are fundamental evidence against the permeability increase theory or, as we called it, the conductance increase mechanism (CIM). For example, they fail to show how our finding that, at any given level of steady-state membrane potential in the physiological range, the membrane conductance was the same or less in the light than in the dark is consistent with their theory. We interpreted our results to mean that there was no primary conductance increase with light and that the conductance increase observed was secondary and ascribable to the membrane's nonlinear current-voltage characteristic (*2*). Thus, we see the theory envisaged by Duncan and Bonting as inconsistent with these and other data reported in our papers (*2, 3*).

Duncan and Bonting suggest that the relatively high ratio of surface to volume of *Limulus* photoreceptor cells plays an important role in receptor potential (RP) mechanisms in that light leads to large changes in the intracellular ionic concentration of photoreceptors. Their evidence (*4*), however, involved a radioisotopic study of whole frog retinas, and, therefore, any ionic concentration changes observed cannot be referred specifically to the effect of light on photoreceptors alone but would also include changes in other retinal cells. The preceding remark also applies to Duncan and Bonting's subsequent comment on potassium efflux.

Duncan and Bonting cite Benolken's (*5*) and our (*2*) work as evidence that, in the light, the membrane potential remains near zero. Neither Benolken (*5*) nor we (*2*) have reported that steady light completely depolarizes a *Limulus* photoreceptor. In the steady-state the membrane potential is always inside negative (*2, 5*). Also, no one has ever reported the abolition of an RP in a *Limulus* photoreceptor with tetrodotoxin. Benolken and Russell indicated that tetrodotoxin reduced only the transient component of the RP, but required concentration a 100-fold greater than those sufficient to block all-or-none action potentials (*6*). Others, how-

ever, have found tetrodotoxin without effect on the *Limulus* RP (*7, 8*).

In addition, Duncan and Bonting quote our studies on the effects removing sodium from the extracellular fluid as evidence supporting their theory. They overlook our finding that the abolition of the RP is only transitory, even when the sodium substitute is a relative impermeant ion [for example tris(hydroxymethyl)amino methane and choline] (*2*) and they fail to show how this observation is consistent with ". . . the essential (*sic.*) role of the sodium permeability increase . . ." (*1*).

Because one can calculate from the Goldman equation any membrane potential desired by an arbitrary selection of the values for permeability coefficients and ionic concentrations, it is obvious that for such calculations to be relevant to any particular cell, one must employ values derived from the cell in question. Duncan and Bonting, however, have employed permeability and concentration values derived from squid photoreceptors and axons, which our preliminary results indicate are significantly different from *Limulus* photoreceptors. Furthermore, one must show that the light-induced permeability changes must reproduce the observed conductances. This Duncan and Bonting have failed to do. Moreover, it should be clear that the membrane conductance observed at any level of depolarization in the light should be greater than the conductance observed at that same level in the dark, if the permeability change caused the membrane potential changes which our results show clearly is not the case (*2*, figs. 1 and 2). This is to say that one must take into account the potential dependences or nonlinearities of the membrane characteristics in employing the Goldman equation. This also Duncan and Bonting have failed to do. We question, therefore, the relevance of their calculations.

Duncan and Bonting attempt to explain our results on the effects of inhibition of the sodium pump on the RP in terms of a depolarization of the membrane resulting from rapid changes in intracellular ionic concentration (*1*). First, our evidence is against a rapid change in ion concentration causing a depolarization. We observed that pump inhibition led to an intitial partial depolarization and abolition of the RP in a matter of minutes, which we ascribed to the inactivation of an electrogenic pump (*3*), followed over a period of hours by a steady and complete depolarization which may be due to changes in ionic concentration of the kind proposed by Duncan and Bonting. Second, they propose that a depolarization with an increase in sodium conductance like that found during the production of an axonic action potential (*9*, p. 64) explains why pump inactivation reduces or abolishes the RP. In doing so, they imply that the RP is generated by the same membrane mechanisms as an all-or-none spike; however, it is well known and generally accepted that there are fundamental differences in the membrane mechanisms involved in graded, nonelectrically excitable, nonpropagated responses such as the RP or the end-plate potential and in all-or-none, electrically excitable, propagated responses like an action potential (*10*). Moreover, the data do not support Duncan and Bonting's explanation. Depolarization of the photoreceptor with current, by an amount equal to or even greater than that produced by pump inactivation, does not abolish the RP (*7*), as we also observed.

But even if the *Limulus* RP were similar to a spike, which it is not, our results contradict Duncan and Bonting's explanation. For example, increase in sodium conductance (sodium activation) associated with the onset of a spike is followed by a sodium inactivation which persists if the membrane potential remains depolarized, but this inactivation is removed if the membrane is hyperpolarized and the response is once again capable of being evoked (*11*). Therefore, in our experiments where the pump was inactivated with ouabain, the membrane partially depolarized, and the RP abolished, we should have been able to reestablish the RP by hyperpolarization. This was not possible (*3*). We actually performed the above experiment to rule out another possibility, namely, that pump inactivation had led a redistribution of ions such that the resting potential became equal to the equilibrium potential of the RP.

In conclusion, we see neither Duncan and Bonting's comment (*1*) nor our observations (*2, 3*) as supporting the CIM as the basis for the *Limulus* RP. As we noted in our papers, however, some complicated but as yet unformulated conductance change mechanism may underlie the RP (*2, 3*). Nonetheless, we still feel that the available data are accounted for more simply by alterations in an electrogenic sodium pump (*3*).

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References

1. G. Duncan and S. L. Bonting, *Science*, this issue.
2. T. G. Smith, W. K. Stell, J. E. Brown, *ibid.* **162**, 454 (1968).
3. T. G. Smith, *et al.*, *ibid.*, p. 456.
4. S. Buckser and H. Diamond, *Biochem. Biophys. Res. Commun.* **23**, 240 (1966). The other articles cited [G. Duncan, F. J. M. Daemen, S. L. Bonting, *Fed. Proc.* **28**, 331 (1969); S. L. Bonting, *Curr. Top. Bioenergetics* **3**, 351 (1969)] were not available at the time of writing this reply.
5. R. M. Benolken, *Biophys. J.* **1**, 551 (1961).
6. R. M. Benolken and C. J. Russell, *Science* **155**, 1576 (1967).
7. R. Millechia, J. Bradbury, A. Mauro, *ibid.* **154**, 517 (1966).
8. J. Dowling, *Nature* **217**, 28 (1968).
9. A. L. Hodgkin, *The Conduction of the Nervous Impulse* (Liverpool Univ. Press, Liverpool, 1964).
10. H. Grundfest, *Physiol. Rev.* **37**, 337 (1957); *Cold Spring Harbor Symp. Quant. Biol.* **30**, 1 (1965).
11. A. L. Hodgkin and A. F. Huxley, *J. Physiol.* **116**, 497 (1952).

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Selenodetic Implications of Mascons

The discovery by Muller and Sjogren (*1*) of mass concentrations at the circular maria on the moon has elicited a barrage of comment and interpretation in terms of lunar geologic evolution (*2*). Here I present the implications of the mascons for the selenodetic data analysis itself, as conducted at several space centers in the United States. For methods used in the past, the results are quite discouraging.

Plans for the Lunar Orbiter mission provided for two methods of data analysis. The first method was that used for the earth satellites and consisted of estimating harmonic coefficients from the long-term variation in the orbital parameters (*3*). Unfortunately, the methods so suitable for sparse sightings, spread over a year or more for a rapidly spinning planet, were disappointing when applied to voluminous data packed into a few weeks or months about a relatively static body. Thus, most emphasis has been given to the second method—the "direct method"—in which the orbit parameters and coefficients of a truncated harmonic