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General Discussion: Early Receptor Potential

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(Discussion of Papers by Drs. K. T. Brown, R. A. Cone, and W. L. Pak.)

J. Y. LETTVIN: This early RP discussed by Brown, and Cone and Pak is a pregnant finding, and I should like to comment on it using more positive language than one should. There are two phases to this phenomenon, an early positive one that, while sensitive to temperature, is not as markedly so as the later negative one. That the positive phase persists still at −35 C argues that it is rate-governed not by the moving about of molecules but rather by a change in the molecules themselves —say the establishment of dipoles by the breaking of chemical bonds and subsequent uncoiling or bending that might not only be itself directly affected by temperature, but also might be damped by the viscosity of the medium around it. That the negative phase is suppressed below freezing argues that it is probably rate-governed by the moving about of molecules, by rearrangement at least. We can make an analogue of this early RP by using photosensitive electrodes in salt water. Here one can show that there is a relatively temperature-stable photoelectric current during a flash, and that this is followed by a temperature-sensitive relaxation of the Helmholtz double layer at the electrode-saline interface. (Please do not misunderstand; this is only an analogy—I am not calling the early RP an artifact.) There is no need to go into the details of how such a model can be made to yield analogous electrical records.

If the generating mechanism of the early RP lies wholly within the rods, it would be decoupled from the outside by the cell membrane. But current must flow one way across the membrane of the outer segment and the other way across the membrane of the inner segment and more proximal parts, otherwise there would be no appreciable external signal. The membrane, if it is at all like nerve membrane elsewhere, acts as a pure capacitance for very short transients; for longer transients the membrane resistivity is involved. One would not expect the positive transient of the early RP to be much affected by depolarization of the cell membrane since it lies within the band where current is carried mainly capacitatively across the membrane, and capacitance is not much changed with depolarization. But the negative phase of the early RP, being later and longer, ought to be enhanced (the external current flow ought be larger) by diminishing the membrane resistivity (as by depolarization at least of the inner segment), with external media rich in K^+ and poor in Na^+.

I have thought, incidentally, that a complementary experiment to heating the retina would be to bathe it in citrated fluids, or media bearing a Ca^{++} chelate, for this would also disrupt the cell membrane and ought not to do much harm to the pigments. From my prejudice I would predict that this method would also abolish the early RP.

Cone has demonstrated clearly that the early RP varies with the amount of visual pigment that can be taken as bleached by a flash, following the model of Wald, Durell, and St. George that bleaching occurs if a pigment molecule absorbs one quantum, but the molecule is reset if it absorbs two. Furthermore, the early RP has, as he shows, the same action spectrum as visual pigment. If we take bleaching visual pigment as the generator of the first phase of the early RP we are then in a position to doubt the odd picture of the outer segment of a rod that the electron microscopists have been proposing. Their notion, briefly, is this: The outer segment has a smooth envelope of cell membrane that surrounds a stack of discs which, although originally developed as invaginations of the cell membrane, have pinched off from it (like mitochondria and cell vacuoles) and lie as independent flattened sacs. This description applies particularly to the outer part of the outer segment, for at the inner part some of the invaginations have not been pinched off. For the most part, however, the microscopists maintain that there is no continuity between the cell wall and the membrane of the discs, and insist that this independence could not arise as a fixation artifact. But the physiological implications of such a picture are dreadful, and on a physiological basis alone many of us have been disposed to reject these findings. For if photopigments are applied to the disc surface as to the inside of the cell wall in the outer segment, how do they signal that they are bleached? Since there is no external path for current flow between disc and inner segment, the amplification to be had by the gating of ionic fluxes cannot work as it does for Hagin's study (this volume) on the retinular cells of cephalopods. The discs, in effect, are Faraday cages. Yet Brown's pictures clearly demonstrate a sink in the outer segment (see my comment on his paper) and a source in the inner segment during the later transients after the early RP in his isolated receptor

retina. This signal current (the late RP) does flow between outer and inner segments exactly as in Hagin's studies. Is it likely that pigment which is attached to the cell wall of the outer segment uses a different system of communication to the inner segment than that which is attached to the discs?

I suppose that one can make up the usual *ad hoc* hypotheses involving diffusion to the cell wall of the products of bleaching occurring at the discs, but that is clearly makeshift.

The very existence of the early RP bolsters the physiological argument against the pictures got with the electron microscope. If every pigment molecule bleaches in the same way, then, to the degree that these molecules are ordered the accompanying dipole changes will be ordered. For were the dipoles not ordered in some way no early RP could be recorded externally. The less the visual pigment is ordered the larger must be the dipole strength attributed to the bleaching of each molecule. As you have seen from Cone's discussion, if one assumes that all molecules are ordered, there is still a large shift set up by each bleaching event. If the discs lie as separate elements inside an envelope, then, if the pigment were ordered with respect to the disc surface by symmetry, no external signal could occur from heavy sudden bleaching. This is because for every dipole set up on one face of the disc, an oppositely oriented one would be set up on the other face, and the two would tend to cancel each other. On this view the only pigment that could contribute to the early RP would be that attached to the surrounding cell wall. If you assume uniform distribution of pigment along all membrane in the outer segment, then the pigment attached to the cell wall is much less than 1% of the total. Thus we would have to have absolutely enormous dipoles set up by each molecule bleaching. If the discs are indeed independent flat sacs, the only way to make every bleaching molecule contribute a moiety to the early RP is to assume an absolute spatial ordering. You would need to assume either that pigment molecules are attached only to one face, say the outer one, of each disc, or else that pigment molecules are attached one way to the outer face of each disc and the other way round to the inner face. In this way the dipoles set up would be lined up spatially within the outer segment, and constitute a kind of electrostatic electric organ lying totally inside the enveloping membrane of the outer segment. But such asymmetry is extremely wild as a supposition. What could possibly distinguish an outer face from an inner face of a free floating disc, given that the disc arises as a pinched off invagination of the same membrane? There are more subtle electrical arguments against this view, but it would be expanding this comment unconscionably long to discuss them.

The alternative, of course, is that pigment molecules are everywhere attached in the same way to a continuous surface of membrane. That *is*, we must suppose that the interior of each disc is still connected to the external medium. Under such conditions, and given the high resistivity of cell membrane, external electrodes see in parallel all the dipoles set up by bleaching. The dipoles are ordered in parallel electrically by the membrane as a boundary. To the extent that this is true, that the displacement currents from all bleaching molecules add with respect to external electrodes, so too one would expect the later ionic currents across the membrane also to be seen in parallel by external electrodes. Rods would not differ much from retinular cells on this account.

Cone's experiments on abolishing the early RP by heat sufficient to hurt the cell membrane but not denature the pigment tend to support the notion that the ordering necessary for the early RP is related somehow to membrane. That is why I suggested the Ca^{++} deprivation at normal temperature. One would not expect abolition of the early RP except as the pigment molecules are electrically ordered with respect to membrane, rather than spatially ordered with respect to each other.

I do not say that the image of independent discs in the outer segment is proved wrong by studies on the early RP, any more than it is disproved by other physiological comment. But in any form-function argument, one ought give some weight to function, and in the light of what Brown, and Cone and Pak have shown, it might be worth while for electron-microscopists to take still another look.

J. R. PLATT: It is interesting to see this early RP from the larger point of view of physical chemistry and biochemistry. The initial event in photoreception is a monomolecular excitation. The later event is the appearance of a propagated membrane or neuron potential, perhaps signaled by the appearance of the *a*-wave and *b*-wave in the ERG. Between these events there is both a physical gap, and a gap in our knowledge. It is a "space gap" of many microns, comparable to the length of the outer segment or perhaps of the whole rod or cone cell; it is a "time gap" of several milliseconds; it is a "linearity gap" between the quantum-proportionality of the initial event and the nonlinearity of the later ones; and, in explanation of all these, it is an "amplification gap" of the order of 10^5 between the quantum event and the later signal, according to Cone's results. The similarity to a "photomultiplier" amplifier is thought-provoking, as Wald has emphasized. This is true regardless of whether the amplification mechanism is largely chemical,

perhaps through a chain of enzymes with multiplied turnover, in the model suggested by Wald, or whether it is partly chemical by a factor of 10^3 up to the level of membrane-signaling, as suggested by Hagins, with the remaining 10^4 provided by ordinary membrane amplification. In either case, the first amplification steps of the transduction process are probably quite different from the more familiar neural or synaptic amplification or propagation processes.

It is evidently of the greatest importance in the next few years to find some new ways of detecting and studying these earliest stages of amplification. It might be done by trying to interrupt them and looking for the chemical intermediates, or by seeing if intermediates can be found which will trigger the later stages; or it might be done by putting in localized millisecond electrical pulses, say with a microelectrode stimulator in the outer segments, to see at what point in space an electrical trigger becomes effective, and whether the early RP and the *a*- and *b*-waves can be elicited in this way and, if so, whether their latencies and amplitudes depend on the electrical trigger in the same way as on a photo-trigger.

But in studying these first stages in the transduction and amplification process, the early RP may be one of the most valuable external indicators. Up to now, the detection of molecular changes in millisecond times has been done primarily by looking for spectral changes or the appearance of ESR spin resonance signals. The detection of millisecond electrical potentials represents an important addition to this armamentarium, and one that is very much cheaper and simpler to use. In fact, it seems likely that many chemical reactions or molecular changes in oriented systems will produce charge-separation and voltaic effects detectable when pulse-initiation can be done. Conceivably, the "positive" and "negative" (or "R_1" and "R_2") components of the early RP represent distinct chemical steps such as a monomolecular change in rhodopsin configuration followed by a diffusion-step or a change of charge in some of the first reaction products, if the activated opsin becomes an enzyme, as Wald has suggested; if so, the effect of chemical reagents on the steps, as detected in the early RP, will help to determine what molecules and reactions are involved.

Evidently, it would be valuable to look for early millisecond potentials for detecting and studying early molecular changes in *every photobiological reaction*—I need not name them here—and indeed in every in vitro photochemical reaction in which the reactants can be oriented with respect to the electrodes. And in addition, it would be valuable to look for such early potentials in *every sensory receptor* where pulse initiation either by light or electrical or mechanical pulses is practical.

G. WALD: In view of the doubts that have been expressed concerning the significance of the early RP, photochemically and physiologically, I should think it might be a good idea to look for it in chloroplasts. They resemble the outer segments of rods and cones in being lamellated structures, in which photosensitive pigments are arrayed in an oriented fashion, so that the whole structure is quasi-crystalline, an approach to the solid state. I would suppose such a structure to be highly polarized in its electrical responses. Hence it would not do to look for an early RP in a collection of randomly oriented chloroplasts, as in the usual leaf. In the moss *Funaria* the chloroplasts migrate in the light to take up specific positions in the cells; whether the individual chloroplasts then assume the same orientation, I do not know. Short of that, I should think one would have to probe individual chloroplasts with microelectrodes. Another point: there is a photochemical process in chloroplasts whose rate declines only slowly with fall of temperature, so that it still goes appreciably at −80 C. This is the photoconversion of protochlorophyll to chlorophyll (J. H. C. Smith and A. Benitez, 1954. Effect of temperature on the conversion of protochlorophyll to chlorophyll A in etiolated barley leaves. Plant Physiol. *29*: 135–143.) Chloroplasts ready to go through this conversion might make particularly apt objects in which to look for the early RP.

K. T. Brown: This same thought has occurred to me, and I have already made preliminary attempts to elicit the early RP from chloroplasts. It seems quite possible that the early RP represents a type of response which is widespread in the biological world. So having found it in the monkey, it seemed logical to me to search for it next at the opposite extreme, namely, in chloroplasts. These attempts have not been successful thus far, but I am continuing on this problem, and I am convinced that the problem of orientation of the chloroplasts is crucial for recording any response which may be present.

In a more general vein, I think it will be fruitful in the near future to explore the types of biological preparations in which the early RP occurs. Such work should give strong clues concerning the significance of the early RP, and may extend the preparations in which it is available as a research tool. It seems to me that at least two main lines of work may be considered. One is to explore the range of biological photochemical reactions which are followed by the early RP, and it is here that the chloroplast experiment applies. Another is to explore the kinds of receptors, other than photoreceptors, in

which the early RP occurs. The photoreceptor may be considered as a specialized chemoreceptor in which the photopigment serves the function of absorbing light and giving rise to a chemical event, which serves as the stimulus for the electrical response. Since the early RP is the end-product of a chemo-electric transduction in the photoreceptor, it may occur and have similar significance in other types of chemoreceptors. Since the vertebrate photoreceptor is a ciliated type of receptor, this raises the possibility of a different type of generalization among receptors. The steps in excitation are not well understood for any ciliated type of receptor, because the receptors which have yielded to detailed analysis have all been of the nonciliated type. The only potential which seems comparable to the early RP is the cochlear microphonic, which also occurs in a ciliated receptor. Thus we have suggested that the early RP and cochlear microphonic may be generated in fundamentally the same manner, and may represent a type of response which is a link in the chain of excitatory events in all ciliated receptors. These possibilities seem to deserve special attention, since investigations along these lines may lead to some unifying principles in the excitation of receptors of different types.

Note added in proof:

During the past summer I have been studying the toad eye, both the isolated retina and the eye cup after removing the retina. The latter preparation has revealed a very rapid biphasic response from the pigment epithelium-choroid complex; this response is probably generated by the pigment epithelial cells. This new finding has been published in *Nature*, Sept. 18, 1965. In very recent observations, which I have made collaborating with Peter Gage, the second phase of this new response has been abolished by lowered temperature, but the first phase survives into the sub-zero range. This strongly indicates that the first phase of the new response is comparable to the first phase of the early RP. Thus the early RP appears to be a type of response which is not unique to vertebrate rods and cones. These new findings strongly suggest that the early RP, or at least its first phase, is a type of response occurring in a wide variety of photosensitive cells.