and reversals of the major components in the data, but direct manipulation of stimulus phase is necessary for adequate interpretation. It has often been suggested, in accounting for phenomena such as Benham's Top, that the red system is somehow "faster" than the green system.

This suggestion could account for the chromatic effect reported here. Consider the case where a red stripe alone is presented as a square wave at 7 hz. The "faster" red response could allow the system to respond maximally to the beginning and ending of each red presentation, and indeed in this condition we get a train of 14 waves comparable in amplitude, and consequently a large 14-hz component. Conversely, when a green stripe alone is presented, the "slower" green response is not yet completed when the stimulus ends; this could result in a smaller response to the following absence of green, which in turn would give the obtained large 7-hz component.

Such an interpretation is tentative beyond the obvious reasons, since it is restricted to the stimulation frequency used. Psychophysical research offering support for a temporal interpretation also suggests that the effect would vary with the frequency of stimulation (*9*). It has also been reported that with nonalternating stimuli, amplitude of the fundamental frequency component of the VER varies with wavelength of the stimulus and of the surround field (*10*).

Regardless of the ultimate efficacy of our (or any) temporal interpretation, the use of temporal chromatic alternation (successive contrast) allows the study of a given chromatic VER response almost per se. This strong conclusion is based on the large and consistent differences found between red-to-green and green-to-red interactions, and is presumed to reflect different red and green processing systems with different properties.

N. W. PERRY, JR.
D. G. CHILDERS, J. C. FALGOUT
Visual Sciences Laboratory, University of Florida, Gainesville 32601

References and Notes

1. E. P. Johnson, L. A. Riggs, and A. M. L. Schick [in *Clinical Electroretinography*, H. M. Burian and J. H. Jacobson, Eds. (Pergamon, Oxford, 1966), pp. 75–91] first reported use of the stimulus alternation procedure to elicit photopic electroretinograms. Others to report use of the procedure to elicit VER's include D. Regan and H. G. Sperling [*Vision Res.* **11**, 173 (1971)]; J. C. Armington, R. Marsetta, and A. M. L. Schick [*ibid.* **10**, 227 (1970)]; and Riggs and Sternheim (*2*).
2. L. A. Riggs and C. E. Sternheim, *J. Opt. Soc. Amer.* **59**, 635 (1969).
3. Riggs and Sternheim (*2*) stated (p. 637), "Furthermore, there are changes of phase and shape in addition to the measured changes of amplitude, of waves resulting from various wavelength pairs." These changes were not shown or elaborated upon further, but they probably were not similar to the effect we report here (L. A. Riggs, personal communication). In our method we used only temporal modulation of the stimuli (successive contrast) rather than the temporal and spatial modulation (successive and simultaneous contrast) used by others (*1*). In fact, we assume that our use of only successive contrast allowed the effect to be seen so clearly and consistently. Riggs and Sternheim (*2*) also stated that they obtained more consistent VER's by placing stationary black stripes in front of the boundaries between the alternating color stripes. This, in effect, reduced simultaneous contrast.
4. The data presented in Figs. 1 and 2 are from one subject and were replicated in additional sessions. The other subject's data were similar in all significant aspects.
5. Different methods can yield different heterochromatic "matches"; for example, see R. M. Boynton and P. K. Kaiser [*Science* **161**, 366 (1968)]. In addition, heterochromatic flicker photometry is difficult for the subject at the relatively low rate of 7 hz. J. B. Siegfried, D. I. Tepas, H. G. Sperling, and R. H. Hiss [*ibid.* **149**, 321 (1965)] found the VER minima at the heterochromatic match point, but D. Regan [*Vision Res.* **10**, 163 (1970)] subsequently found the VER minima displaced off the match point. Our results correspond more closely to Regan's, perhaps because we also examined the VER frequency components rather than amplitude of the gross waveform. We could shift the point where the two fundamental VER frequencies were approximately equal (for example, Fig. 2, A and B) and obtain even smaller VER's with monochromatic alternation (for example, *M* condition in Fig. 1, A and C) by varying luminance of either color channel slightly (< 0.1 log unit). This extreme VER sensitivity to both luminous and chromatic contrast suggests that a "minimum response" criterion would be appropriate. Average retinal illuminance of the stripes was 56 trolands, measured by the method described by G. Westheimer [*ibid.* **6**, 669 (1966)].
6. The subject viewed the alternating stripes for approximately 30 seconds before each VER was summated, in order to minimize transient effects produced by unstable fixation. Consequently, a given VER component could not be directly linked to either the red-to-green or the green-to-red alternation.
7. M. Ikeda and R. M. Boynton, *J. Opt. Soc. Amer.* **52**, 697 (1962).
8. D. Regan and C. W. Tyler, *Vision Res.* **11**, 1307 (1971); P. L. Walraven and H. J. Leebeek, *J. Opt. Soc. Amer.* **54**, 78 (1964); H. de Lange Dzn, *ibid.* **48**, 784 (1958).
9. D. G. Green, *Vision Res.* **9**, 591 (1969); D. H. Kelly, *J. Opt. Soc. Amer.* **52**, 940 (1962); S. H. Bartley and T. M. Nelson, *J. Psychol.* **50**, 323 (1960).
10. D. Regan, *Vision Res.* **8**, 149 (1968). We have subsequently collected data over a wide frequency range of stimulation, and although results are complex and analyses incomplete, it is apparent that the red and green systems have different and varying resonant frequencies as measured with the VER and successive contrast.
11. Supported in part by NIH grants EY 00077 and EY 00581. We thank J. McCoy and O. Halpenny.

24 May 1972

Pumps or No Pumps

The article by Culliton (*1*) would lead one to believe that active transport occurs in all living cells and that the membrane is the rate-limiting factor for the entrance of ions and other solutes into the living cells. The definition of active transport is met when it is demonstrated that an ion is moved against an electrochemical gradient through a semipermeable membrane. This phenomenon has been clearly demonstrated to occur across cellular material such as the frog skin; and the ions and water are in solution on both sides of the "membrane." The movement of substances from within a given cell against an electrochemical gradient is postulated to occur because of an energy-requiring, membrane situated pump. These ideas are fundamental to the biologists' view of the living cell and are an integral part of a general theory which is termed the membrane or ionic theory.

The pump concept was first postulated explicitly by Dean in 1941 to account for the apparent movement of sodium against an electrochemical gradient. The creation of the pump concept is a natural consequence of a fundamental, yet unproved, postulation that the proteins, ions, and water are in free solution within the cell. In fact, there is no definitive experiment published, to my knowledge, that shows sodium or any other ion to move from a free solution of low concentration within a cell to a free solution of high concentration outside the cell. Part of the confusion that exists is that a rigorous demonstration of active transport is lacking; and the failure to demonstrate this phenomenon, even in extruded squid axons, has been replaced by criteria other than net movement of ions against an electrochemical gradient. For example, ouabain sensitivity and the presence of sodium-potassium activated adenosine triphosphatase have been substituted for active transport.

Furthermore, according to Ling, the energy requirements of the sodium pump alone are excessive. Ling's calculations on the energy requirements of the sodium pump have gone unchallenged—perhaps they were overlooked (*2*). Consider the following: (i) Assume first that ions and water are in a free solution, that the pumps are 100 percent efficient, and that Ling's calculations are incorrect. (I must add that I think the available evidence demonstrates that all these assumptions are incorrect, but I present them so that I may ask a question which perhaps will be answered by the scientific community.) (ii) Proponents of the pump concept argue that from 9 to 23 percent of the cellular metabolic energy is required for the operation of the sodium pump (*3*).

The question is, then, whether the cell can provide the energy necessary to pump potassium, calcium, magnesium, sugars, and amino acids if it takes 10 percent of the energy to run only one pump?

Recently, some alternative hypotheses, often referred to, in general, as the minority view of the cell, have been proposed. Among them the most systematic one is the association-induction hypothesis proposed by Ling. According to this hypothesis, the cell is considered a highly complex system, wherein many of the biological processes are controlled by interaction of ion, water, and macromolecule. The intracellular concentration of any ion will be determined by its association energy (attraction) to macromolecular fixed charged sites and the solvent properties of the cellular water. For example, potassium is preferentially associated with the fixed charges on proteins within the cell, and the sodium concentration is low, primarily because the cellular water is more structured than ordinary water and therefore less sodium is dissolved in it.

Another point of the association-induction hypothesis is that the surface of most cells possesses a net negative charge. Therefore, the cellular potential is a phase boundary potential and not a membrane potential. The postulation of a net negative charge at the surface leads to the prediction that permeation of certain ions into the cell are surface limited (that is, they interact with the fixed negative charges on the surface) and others are bulk phase limited (that is, the major resistance to movement into the cell is the structured water and fixed sites within the cell and not the membrane). That diffusion into smooth and skeletal muscle is surface limited for potassium and bulk phase limited for sodium has been borne out experimentally (*4*).

As a proponent of the minority view, I argue that the physical state of the ions and water within the cell are all important and intimately involved in the mechanisms that regulate the internal environment. Certainly the minority view is not proved; however, a large body of evidence has accumulated in support of this view (*5*). Much of the recent evidence questions the validity of the fundamental assumptions of membrane theory. In fact, the factualness of statements like "The plasma membrane regulates that [the internal] environment, acting as a gatekeeper to allow, through various mechanisms of active and passive transport, the passage of ions, of nutrients, and other chemicals into and out of the cell" (*1*) may be considered inaccurate, or at best, unproved. No one questions the importance of cell membranes—just some of the impossible roles postulated for them.

C. F. HAZLEWOOD
Department of Pediatrics,
Baylor College of Medicine,
Houston, Texas 97025

References and Notes

1. B. J. Culliton, *Science* **175**, 1348 (1972).
2. G. N. Ling, *A Physical Theory of the Living State* (Blaisdell, Waltham, Mass., 1962), p. 217, table 89; *Physiol. Chem. Phys.* **2**, 242 (1970).
3. R. D. Keynes and G. W. Maisel, *Proc. Roy. Soc. London* **142**, 383 (1954); J. Brinley and L. J. Mullins, *J. Gen. Physiol.* **52**, 181 (1968).
4. A. W. Jones and G. Karreman, *Biophys. J.* **9**, 884 (1969); G. N. Ling, *Int. Rev. Cytol.* **26**, 1 (1969).
5. For example, F. W. Cope [*Biophys. J.* **9**, 303 (1969)] proposes a solid-state physics concept of the living cell; R. Damadian [*ibid.* **11**, 739, 761, 773 (1971)] proposes a cellular ion-exchange resin concept; and C. F. Hazlewood, B. L. Nichols, D. C. Chang, B. Brown [*Johns Hopkins Med. J.* **128**, 117 (1971)] and M. Neville [*Science* **176**, 302 (1972)] propose an association-induction hypothesis.
6. Supported by the Robert A. Welch Foundation and PHS research grant RR-0188.

8 June 1972

Eutrophication and Phosphate Detergents

A report by Mitchell entitled "Eutrophication of lake water microcosms: Phosphate versus nonphosphate detergents" (*1*) contains both errors in logic and factual misrepresentations. The ecological significance of several waste water treatments—a synthetic waste effluent treatment, treatment of the waste effluent with a phosphate detergent, and two treatments of waste water with nonphosphate detergents—were assessed in terms of the resulting algal diversity as determined by Shannon's diversity index:

$$H_1 = -\Sigma P_i \log P_i$$

where $P_i = n_i/N$, n_i is the population of the *i*th species, and N is the population of the total community. Mitchell states that this index "ranges from zero for unialgal populations to unity for very diverse communities." The lower limit of this index is indeed zero: the upper limit is not unity. Pielou (*2*) has demonstrated that the greatest value of H_1 is a nondecreasing function of the number of species. In my own experience with Shannon's index using logarithms to the base 10 (*3*), I have found that H_1 is usually greater than unity for natural communities. With logarithms to other bases (*e* or 2), the index is even greater for the same community. It is possible to obtain index values as low as those reported by Mitchell but these must be the result of either extreme dominance or very small samples. A statement of sample size or calculation of "evenness" or "equitability" would have clarified this.

Mitchell then attributes the statement "the diversity of a lake's algal community diminishes with eutrophication" to Wilhm and Dorris (*4*). This particular paper, in fact, makes no mention of algae, lakes, or eutrophication. It is concerned only with the diversity of benthic macroinvertebrates in polluted streams. Following that, Mitchell states: "Thus, oligotrophic lakes would probably have diversity indices of from 0.7 to 1.0 and, as the lakes become eutrophic, the diversity index would drop to 0.3 or less." By prefacing the statement with "thus," Mitchell conveys the impression that the idea originated from the work of Wilhm and Dorris (*4*). This is not the case. They proposed no such classification.

The appropriateness of Shannon's diversity index in this study is questionable. In a study of artificially enriched ponds, Ewing and Dorris (*5*) found that "diversity did not parallel nutrient concentration." The appropriateness and meaning of most popular diversity indices is also very much in question (*6*). Their indiscriminate use should be discouraged.

The reader is deceived into thinking that these microcosm studies in some way represent actual conditions in real lakes. The use of microcosms to simulate lakes has many faults but I will not discuss these. Rather, I would like to direct attention to the effective microcosm concentrations of phosphate and nitrate. From table 2 of Mitchell (*1*) we see that the simulated waste effluent without detergents after activated sludge treatment contains 790 μg per liter of phosphate phosphorus and 10 mg per liter of nitrate nitrogen. The phosphate detergent plus waste water effluent contains 2800 μg per liter of