nity.) (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.

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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.
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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 ith 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

phosphate phosphorus and 9.5 mg per liter of nitrate nitrogen. These are diluted with "oligotrophic" lake water in a 1 to 9 ratio. The resulting concentrations (accounting for initial concentrations in the lake water) are as follows: waste effluent, 70 μg per liter of phosphate phosphorus and 1.045 mg per liter of nitrate nitrogen; phosphate detergent plus waste effluent, 280 μg per liter of phosphate phosphorus and 0.995 mg per liter of nitrate nitrogen; nonphosphate detergent plus waste effluent, 50 μg per liter of phosphate phosphorus and 1.145 mg per liter of nitrate nitrogen. In Vollenweider's compilation of information regarding eutrophication (*7*), there is only one lake with nitrate levels higher than these—Lago d'Orta, Italy. It is heavily polluted by industrial wastes containing large amounts of ammonium salts. The phosphate concentrations produced by the phosphate detergent and waste effluent exceed those in all but the most grossly polluted lakes of the world (*7*). Even the western basin of Lake Erie contains only 120 μg per liter of phosphate phosphorus during the period of highest concentration (*8*). Phosphate concentrations in the microcosm treated with only the synthetic waste exceed the highest concentrations recorded for Lake Washington (*9*), a well-known case of cultural eutrophication. These concentrations may be high enough to produce initial toxicity to algae conditioned to an oligotrophic lake. With such gross excesses of phosphate and nitrate, this experiment has no meaning for the study of lake eutrophication.

Further criticism of this paper might be possible if sufficient data had been presented on sampling of the microcosms, nutrient levels in the microcosms during the experiment, measurements of total phosphorus, species identifications of all algae instead of just the dominant ones, and chemical content and contribution of the mud, to name a few.

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13 December 1971

I thank Godfrey for so ably reemphasizing the prime point of my report: domestic waste water is such a rich source of nutrients that the simple elimination of phosphates from detergents is unlikely to significantly decrease the rate of eutrophication caused by the resulting waste waters. Godfrey is quite correct regarding the upper limit of Shannon's diversity index. I am at fault for not having emphasized that my observation was based on experience, not on mathematics. I should have said "The index ranges from zero for unialgal populations to near unity for the most diverse planktonic algal communities that I have encountered during my several years of observation in the natural environment and in the laboratory." According to the Shannon formula, a diversity index of unity requires the presence of at least ten algal genera, and even this number is not sufficient if all ten do not have identical populations. In my experience it is very rare to find identical populations for planktonic algae, although enumeration of the total biota present may give much higher diversity indices.

Some information relating to the "richness" and "evenness" of the microcosms is given in my report (table 3 and the fourth paragraph from the end). Obviously dominance is the primary factor responsible for the low diversity indices developed, as is common in algal blooms. Further details on the principles and practice of the microcosm algal assay procedure are given in the reference originally cited (*1*), which in turn refers to still further details (*2*). The numbers of genera and their individual populations shown there are typical of those encountered in the detergent study. Whether or not the Shannon diversity index is the ideal way of describing algal communities, it has been very useful for comparing these systems with each other and thus for estimating the effects of the variables tested. The 98 and 95 percent confidence levels quoted for the differences in the results in the detergent work came from a statistical analysis by Russell and Mitchell (*3*).

I regret any seeming attribution of my own statements regarding eutrophication to Wilhm and Dorris. I was well aware that their paper deals with the diversity of the invertebrate community. However, their discussion of the use of biological parameters for assessing water quality is the clearest explanation for the general scientific audience that I have seen. For that reason I included it as a reference rather than others which also support the interrelationship between reduced diversity and eutrophication (*4*).

Although Godfrey chooses to discount the pertinence of the diversity index to environmental research, many others have found it to be extremely useful. Likewise, the microcosm is certainly not an exact model of a real lake, yet it does offer a means of comparing the effects of variables of interest while others are kept more or less under control. This approach is much closer to reality than a study of unialgal cultures in synthetic media. Further improvements are greatly to be desired, and the opportunity is open to all.

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3. Our procedure (R. A. Russell and D. Mitchell, unpublished data) was to first fit the data (Shannon's diversity index versus time) to a first-order decay model

$$Y = ae^{-bt} + c$$

where Y is the index number and t is the time in weeks. A nonlinear regression technique was used to determine the coefficients (a, b, and c) by least-squares analysis. A t-test was then used to compare the corresponding coefficients for the control microcosms with each of the other treatments. The test for t was

$$t = \frac{a_1 - a_2}{(S^2a_1 + S^2a_2)^{\frac{1}{2}}}$$

where a is the coefficient and Sa is the standard error of the coefficient. The degrees of freedom were taken to be the sum of the degrees of freedom for the control set (a_1 and Sa_1) plus the degrees of freedom for the set of treatment data (a_2 and Sa_2). Similar t-tests were of course also carried out for the coefficients b and c. The results from these tests were then confirmed by paired t-tests applied to the data on weeks 6 through 14. This procedure showed detergent 2 to be significantly worse than the others for a low diversity of means. Further confirmation of the t-tests on the coefficients was obtained by applying Duncan's multiple range test [D. B. Duncan, *Biometrics* **11**, 1 (1955)] on the means for weeks 6 through 14.
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