LETTERS

Kepone: Hazard to Aquatic Organisms

Rudolph J. Jaeger (Letters, 9 July, p. 94) reports the chronology of mammalian toxicity tests with Kepone (chlordecone) and the exposures of workers at the Life Science Products Company of Hopewell, Virginia. Initial concern has properly been focused on the results of toxicity and carcinogenicity tests on rats, rabbits, dogs, and mice and on the disease that Kepone produced in exposed workers. We would like to document our concern about the hazard of Kepone to aquatic organisms in the James River and the Chesapeake Bay.

On-site tests of organisms taken from the James River showed significantly high Kepone concentrations. These tests, conducted at the Virginia Institute of Marine Science and funded by the Environmental Protection Agency (EPA), revealed that concentrations in edible tissues of most fresh and estuarine fin- and shellfish commonly ranged from 0.1 to more than 1 microgram per gram. These concentrations exceeded allowable health limits for commercial and sport fisheries and forced closure of the river to some commercial and sport fishing. This year Kepone concentrations have increased in anadromous fishes as they spent more time in the river.

Further, after laboratory exposures at the EPA laboratory in Gulf Breeze, Florida, we found that Kepone, like other chlorinated insecticides, is highly bioaccumulative and persists in estuarine organisms. Oysters, grass shrimp, and fishes have bioconcentrated Kepone from 425 to 20,000 times the concentration in the surrounding water. Therefore, action levels for edible seafoods now in force might be reached by as little as 5 parts of Kepone per trillion parts of water (nanograms per liter). In Kepone-free water, oysters can depurate about 90 percent of the accumulated Kepone in 4 days, but fish may require more than 3 weeks to lose 30 to 50 percent. Five weeks after fertilization of sheepshead minnow eggs containing Kepone, the juvenile fish retained as much as 46 percent of the Kepone present in the eggs. Kepone can be accumulated by fish to concentrations that exceed those in their food.

Kepone is acutely toxic to estuarine organisms, but long-term bioassays reveal that the hazard to these organisms is greatly underestimated by the 96-hour tests. The concentrations in micrograms per liter, estimated to be lethal to 50 percent of the test animals in 96 hours (LC_{50}), were 6.6 for spot, 70 for sheepshead minnows, 10 for an estuarine mysid, 121 for grass shrimp, and more than 210 for blue crabs. Kepone was lethal to adult sheepshead minnows exposed to 0.8 microgram per liter for 28 days. A significant number of embryos from adults exposed to 1.8 micrograms per liter were abnormal and died. When embryos were exposed to 0.08 microgram of Kepone per liter of water, 36 days later, resulting juvenile fish were shorter than control fish and some exhibited scoliosis. Mysid shrimp exposed for 20 days to about 0.2 microgram per liter produced fewer progeny; with greater concentrations, their growth and survival were reduced. We are concerned because all concentrations tested thus far in long-term exposures of sheepshead minnows and mysids have reduced survival, reproduction, or growth.

The threat of an even greater impact of Kepone to aquatic organisms in the James River and expansion of this impact into the Chesapeake Bay, therefore, is real and it may continue for some years to come. It is essential that we use knowledge now available to attempt to make decisions that may minimize the future impact of this insecticide on the aquatic environment.

DAVID J. HANSEN, ALFRED J. WILSON
DELWAYNE R. NIMMO
STEVEN C. SCHIMMEL
LOWELL H. BAHNER
Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida 32561
ROBERT HUGGETT
Virginia Institute of Marine Science, Gloucester Point 23062

Structured Water or Pumps?

In her reporting of the ion-water controversy (Research News, 18 June, p. 1220), Gina Bari Kolata is to be commended for her attempt to achieve a balanced presentation of opposing views on a hot argument. All the same, rebuttals to some lines of evidence disputed by the ion "pump" enthusiasts were incorrectly represented, while other telling data, rarely considered, did not surface.

According to the *Science* article, critics claiming that pumps are still feasible despite the thermodynamic conflict, continue to cling to the argument that Minkoff and I have not accounted for the radioactive labeling of the cellular phosphate pool even though we have shown that labeling of this pool occurs instantaneously (*1*). That the "pool would limit the rate at which the labeled phosphate would be

taken up by the cells," as stated in the article, just simply isn't so.

In an important sense, the *Science* article is somewhat unbalanced. Structured water and the sodium pump are thoroughly treated, but the theory that provides the energy conserving alternative to the pump and the theoretical framework for water structuring is somehow lost in the shuffle. With that theory omitted, the reader might incorrectly assume that structured water is intended to substitute for the pump in the opposition view.

Missing from the article is mention of the crucial notion of the ionic "gradient," the high intracellular concentrations of certain ions that cells pile up relative to their exterior. It was to account for these "gradients" that the concept of pumps was invented in the first place. They acquired other duties, such as the cell potential, along the way.

The alternative to the pump theory is the ion exchanger resin model, which I created in 1968 (*2*) and introduced in comprehensive form in 1971 (*3, 4*). Similar in some respects to the antecedent association-induction hypothesis of Ling (*5*), but different in others, it provided a physical model in which a single idea accounted for selective ion accumulation and cell potentials.

The working elements of the ion exchange resin model are the charge groups of the structural polymers of the cell—the nucleic acids, proteins, and phospholipids. The cellular resin is a fabric of these giant molecules. They are ribbed with ionic groups, or "fixed charges," that must be electrically neutralized by ions of the opposite charge.

The ionic groups, chiefly side-chain carboxyls and phospholipid and nucleic acid phosphates, are abundant in biological matter and exist in the cell solution in high concentration. Large concentration differences for a given ion—the ionic "gradients"—develop between the interior and exterior of the cell as mobile ions from the external media accumulate within the cell to electrically neutralize the "fixed charges" anchored to the nonmobile polymers. In the ion exchanger resin model, the "gradients" are established by utilizing the basic electric composition of biological matter, without resorting to pumps.

Water structuring, because of its influence on the parameter that governs the force of attraction between two ions, namely the dielectric constant, regulates the capacity of the fixed charge to select one counterion over another (*6*). The role that Kolata attributes to structured water—excluding sodium (Na) from the cell interior because of low solubility—was only of minor importance.

The origination of the ion exchange resin model was motivated by a number of difficulties with the classical pump theory, only one of which was the energy discrepancy mentioned by Kolata. At least as important was a little noted discrepancy that proved to be a most obstinate impediment to attempts to construct self-consistent potassium (K) pump theories to account for K transport in *Escherichia coli* (*4*).

One salient feature of normal bacteria cultivated in various growth media was the constancy of cell K regardless of the extracellular concentration. The intracellular value of cell K^+ remained fixed at very nearly 200 millimolar, whether the external concentration was 20 micromolar or 200 m*M*. Designing a "pump" that could be positioned on the cell membrane between two aqueous pools of potassium and could sense the internal and external concentrations and speed up or slow down to compensate for 10,000-fold variations in external concentration proved impossible. Pursuit of the problem along the classical lines of enzyme kinetics led directly to a contradiction of the Michaelis theory (*4*).

I have since encountered convoluted enzyme schemes purporting to circumvent the difficulty much as Ptolemaic epicycles claimed to surmount the regressions of the inferior planets in an Earth-centered solar system. None have the appealing simplicity, however, of an internal K concentration, held constant, regardless of external concentration, because the number of fixed charge sites in a cell is a constant.

Kolata's statement that there is now "strong evidence for the existence of pumps" seems ingenuous. The evidence cited is not very convincing and has been experimentally discredited by Ling (*7*). Furthermore, it seems that if all the criteria of a bad theory could be enumerated, the pump theory would satisfy each one. It is a theory of infinite tractability (pumps themselves being quite arbitrary structures that can be altered at will so as to adapt to different technical tasks). At the same time it is sufficiently lacking in definition to be of much use. How much longer can scientists endure a theory in the interpretation of their experiments that leads nowhere? When there is a theory at hand that easily interprets old experiments and forecasts new ones, such as the "nuclear resonance effect in cancer" (*8*), with prophetic accuracy, it does not seem as though the end of the pump theory can be far off. Good scientists do not knowingly toil in vain.

Even should the end of the pump theory come, all is not lost for its supporters. It will not pass unceremoniously into obscurity. It has made its mark in history as a concept that ranks with the geocentric solar system and the ether medium of light transmission as a type of latter-day phlogiston.

RAYMOND DAMADIAN
Biophysical Laboratory, Downstate Medical Center, State University of New York, Brooklyn 11203

References

1. L. Minkoff and R. Damadian, *Biophys. J.* **14**, 69 (1974).
2. R. Damadian, *J. Bacteriol.* **95**, 113 (1968).
3. ———, *Biophys. J.* **11**, 739 (1971); ———, M. Goldsmith, K. S. Zaner, *ibid.*, p. 761; R. Damadian, *ibid.*, p. 773; *Ann. N.Y. Acad. Sci.* **204**, 211 (1973).
4. R. Damadian, *Crit. Rev. Microbiol.* **377**, 386 (1973).
5. G. N. Ling, *A Physical Theory of the Living State* (Blaisdell, New York, 1960).
6. L. Minkoff and R. Damadian, *J. Bacteriol.* **125**, 353 (1976).
7. G. N. Ling, *Physiol. Chem. Phys.* **5**, 295 (1973).
8. R. Damadian, *Science* **171**, 1151 (1971); in *The Nuclear Resonance Effect in Cancer*, R. Damadian, Ed. (Pacific Press, New York, in press).

Kolata, in bringing attention to an important but hitherto largely ignored issue in cell physiology, deserves praise, but unfortunately her article suffers from several misleading omissions.

1) The excessive energy demand of the Na pump, experimentally demonstrated, cannot be dismissed in a cavalier manner merely by speculating, without proof, on a relocation of Na^+ in the cell. Experimental data available at the time when this speculation was made (*1*) as well as that published subsequently (*2*) have shown that this speculation has no validity. Kolata's article makes no reference to these facts.

2) Proponents of the pump theory must reconcile the energy available to the cell with the needs of not just one pump but of an ever-lengthening list of other pumps as well. At the last count, made in 1968, more than 20 pumps had been proposed (*3*). This crucial issue, long ignored by proponents of the pump theory, is also left out of the article.

3) Electron microscopy has shown that Freedman's claim that "the red cell ghosts have almost no proteins" is incorrect. These ghosts are, in fact, filled with cytoplasmic proteins (*4*). This key finding is omitted from Kolata's article. In the case of giant squid axons, preparing a functionally intact membrane sheath without cytoplasm has long been routine. Yet repeated attempts have failed to demonstrate active transport of K^+ and Na^+ across this membrane (*5*).

4) Similar claims regarding the hollowness of bacterial vesicles have been rendered highly doubtful by the high solid contents of these vesicles [30 percent, according to Kaback (*6*)]. This and the red cell ghost story should serve as a warning that electron microscopic veri-

fication must precede speculation about the membrane pump functions of the vesicles and other structures of this type. On theoretical grounds alone, one finds it hard to understand how a fundamentally *symmetrical* lipid bilayer membrane system with adenosine triphosphatase inserted can recognize direction, pumping K^+ in one, and Na^+ in the other.

5) Left out of Kolata's article is the key experimental demonstration that muscle cells *without functioning membrane pumps* do nevertheless retain the capacity for (ouabain sensitive) selective K^+ accumulation and Na^+ exclusion (*3*, p. 31; *7*).

These omissions have helped to create an impression that the Na pump theory is still tenable, while in truth, it no longer is.

GILBERT N. LING
Department of Molecular Biology, Pennsylvania Hospital for the Sick and Injured, Philadelphia 19107

References

1. G. N. Ling, in J. C. Freedman, thesis, University of Pennsylvania (1973), addendum.
2. G. N. Ling and C. L. Walton, *Physiol. Chem. Phys.* **7**, 501 (1975).
3. G. N. Ling *et al.*, *N.Y. Acad. Sci.* **204**, 9 (1973).
4. G. N. Ling and M. Balter, *Physiol. Chem. Phys.* **7**, 295 (1973).
5. G. N. Ling, *Perspect. Biol. Med.* **9**, 87 (1965).
6. H. R. Kaback, communication at the Gordon Research Conference on "Transport phenomena in synthetic and biological membranes," New London, N.H., 14–19 July 1975.
7. G. N. Ling, *Physiol. Chem. Phys.* **5**, 295 (1973).

As I have previously pointed out (*1*), the pump view evolves directly from dilute solution theory. In Kolata's article, we are told that "many investigators, including advocates of pumps, agree, however, that cell water may have some ordered structure that makes it different from liquid water." If this is so, then the proponents of pumps are stating that the fundamental premise (that is, dilute solution theory) that gave rise to their concept is incorrect. Such contradictions between fundamental assumptions and experimental findings should provoke considerable doubt as to the validity of the hypothesis.

Damadian's original observation that nuclear magnetic resonance relaxation times of water protons are longer than normal in cancer cells (*2*) is reported by Kolata to be challenged by opponents who argue that this is simply due to an increase in cancer cell water content. The reader is not informed about other work which demonstrated that the observed relaxation times of water in cancer cells involve much more than just a change in water content (*3*). More important, Kolata does not mention that we have found that relaxation processes of water protons can be independent of the cellular hydration phenomena (*4*).

The two opposing views of the living cell propose theories to explain cellular function which are based on completely different fundamental assumptions, and technology now exists that permits the evaluation of the correctness of these assumptions. Indeed, the fundamental assumptions of the classical view are challenged and the tradition of the scientific method requires a response to that challenge in the scientific forum.

CARLTON F. HAZLEWOOD
Departments of Pediatrics and Physiology, Baylor College of Medicine, Texas Medical Center, Houston 77025

References

1. C. F. Hazlewood, *Science* **177**, 815 (1972); *Cardiovasc. Dis. Bull. Tex. Heart Inst.* **2**, 83 (1975).
2. R. Damadian, *Science* **171**, 1151 (1971).
3. C. F. Hazlewood, *J. Natl. Cancer Inst.* **52**, 625 (1974); ———, G. Cleveland, D. Medina, *ibid.*, p. 1849; D. C. Chang and C. F. Hazlewood, *J. Magn. Reson.* **18**, 550 (1975).
4. P. T. Beall, C. F. Hazlewood, P. N. Rao, *Science* **192**, 904 (1976).

While the conventional view of cell salt and water is widely known, the new picture is not, and only fragments were presented in Kolata's discussion. Therefore, I should like to describe the new picture in a brief but unified way, to emphasize that cell water structuring and cation association are not separate hypotheses but are two parts of a single integrated concept.

We regard the cell as an ion exchange granule with negatively charged sites with which Na^+ and K^+ associate. The sites markedly prefer to associate with K^+, which is what maintains the observed high intracellular concentration of K^+. In the interstices of the cell ion exchange granule, the water is structured (not as much as ice but more than liquid water) so that cation solubility is low. The Na^+ solubility in cell water is therefore low, and little Na^+ is associated with charged sites because of their preference for K^+, so that total cell Na^+ concentration is low. As with an ion exchange resin, cations in the cell exchange freely from association sites to water. Thus cation concentration gradients are maintained and cation exchange occurs without cation pumps and without energy consumption, which is consistent with the findings of Ling (*1*) and of Minkoff and Damadian (*2*) that the cell does not produce enough energy to operate cation pumps.

The above describes the cell at rest. During excitation, configurational changes of cell proteins occur, causing changes in the association preferences for Na^+ and K^+ and in the water structuring (*3*). Energy in the form of adenosine triphosphate is needed to reestablish the resting state (*3*). The development of a detailed understanding of excitability in terms of the new picture, to replace the Hodgkin-Huxley theory, has only just begun.

The new picture leads immediately and obviously to predictions regarding the nuclear magnetic resonance (NMR) behavior of liquid water, Na^+, and K^+ in cells. Short NMR relaxation times of hydrogen and deuterium expected for structured cell water are observed (*4*), and short NMR relaxation times expected for cell Na^+ and K^+ approaching those observed for Na^+ and K^+ on ion exchange resins are also observed (*5*). Proponents of the conventional picture have generated complex, additional, unproven hypotheses to try to make the NMR observations consistent with the conventional picture. These intellectual exercises are unnecessary, because all NMR findings are the obvious expectations based on the new picture.

FREEMAN W. COPE
Biochemistry Laboratory, Naval Air Development Center, Warminster, Pennsylvania 18974

References

1. G. N. Ling, *Am. J. Phys. Med.* **4**, 89 (1955); *Int. Rev. Cytol.* **26**, 1 (1969).
2. L. Minkoff and R. Damadian, *Biophys. J.* **13**, 167 (1973).
3. G. N. Ling, *A Physical Theory of the Living State* (Blaisdell, New York, 1962).
4. F. W. Cope, *Biophys. J.* **9**, 303 (1969); C. F. Hazlewood, B. L. Nichols, N. F. Chamberlain, *Nature (London)* **222**, 747 (1969); F. W. Cope, *Nature (London) New Biol.* **237**, 215 (1972); P. S. Belton, R. R. Jackson, K. J. Packer, *Biochim. Biophys. Acta* **286**, 16 (1972); C. F. Hazlewood, D. C. Chang, B. L. Nichols, D. E. Woessner, *Biophys. J.* **14**, 583 (1974).
5. F. W. Cope, *Biophys. J.* **10**, 843 (1970); R. Damadian and F. W. Cope, *Physiol. Chem. Phys.* **5**, 511 (1973); F. W. Cope and R. Damadian, *ibid.* **6**, 17 (1974); R. Damadian and F. W. Cope, *ibid.*, p. 309.

Kolata's article omits the strongest evidence against the contention that structured water is the sole or major basis for the exclusion of Na ions from and accumulation of K ions in cells: If Na is excluded because of its low solubility in structured water in cells, then it is distributed at thermodynamic equilibrium; the free energy difference between Na ions outside and inside is zero. The situation is reversed for K ions, which are supposed to be more soluble in the cell's structured water and hence are accumulated in the cell at an equilibrium concentration. Equilibrium distributions of these ions are not compatible with the compelling evidence that the upstroke of the action potential in excitable cells (including muscle) is generated by an inflow of Na ions moving down their electrochemical gradient and that repolarization is brought about by an outflow of K ions moving down their electrochemical gradient. This was first shown by Hodgkin and Huxley (*1*) and has been repeatedly confirmed. These flows of Na and K ions

(Continued on page 608)

LETTERS

(Continued from page 532)

exist and are always passive (that is, down the electrochemical gradient). If there is no electrochemical driving force, there is no net Na or K flux.

The most direct evidence (*2*) comes from experiments on giant axons of the squid (up to 1 mm in diameter). These axons can be cannulated, and about 95 percent of the cell plasm replaced by solutions of known composition. Normal action potentials are generated when the internal solution has a composition similar to that of the axoplasm. The fluxes of Na and K ions vary with the composition of the internal and external solutions and with transmembrane voltage (voltage difference between inside and outside solutions) in the direction predicted from the electrochemical gradients for these ions. A perfused axon can generate 300,000 action potentials. It is difficult to attribute these results to the residual cell plasm, and one is forced to a membrane theory of action potentials powered by Na and K gradients across the membrane. This and much other evidence firmly establish that excitable cells have Na and K ion activities which are respectively lower and higher than these activities in the solution bathing the cells, that is, Na and K ions are *not* in thermodynamic equilibrium across the cell surface. The energy for generating action potentials comes from the concentration differences of Na and K ions and these differences are maintained throughout life. Thus the necessity for (but not the existence of) ion pumps in excitable cells is established.

Those who contend that the properties of structured water are the complete or major explanation of Na and K ion distributions between cells and bathing media must not only "prove" that ion pumps are energetically impossible in normal excitable cells but must also provide an alternative equilibrium explanation of how excitable cells can produce net flows of Na and of K across their surfaces in the absence of electrochemical gradients of these ions. The latter is difficult, since the second law of thermodynamics apparently has to be violated.

J. WALTER WOODBURY
Department of Physiology, University of Utah Medical Center, Salt Lake City 84132

References

1. A. L. Hodgkin and A. F. Huxley, *J. Physiol. (London)* **117**, 500 (1952); *Proc. R. Soc. London Ser. B* **18**, 1 (1958); *Science* **145**, 1148 (1964); A. F. Huxley, *ibid.*, p. 1154.
2. P. F. Baker, A. L. Hodgkin, T. I. Shaw, *Nature (London)* **190**, 885 (1961), *J. Physiol. (London)* **164**, 330 (1962); *ibid.*, p. 355; W. K. Chandler and H. Meves, *ibid.* **180**, 788 (1965).

RESEARCH NEWS

(Continued from page 566)

contact the neuroblasts temporarily wrap themselves around the fiber before undergoing differentiation and the migratory movements needed for the development of the mature *Daphnia* optic system. Levinthal thinks that the contact between the lead fiber and the neuroblasts is somehow involved in signaling the neuroblast to differentiate.

He says that these observations would not have been possible without three-dimensional reconstructions of the developing neurons at different stages of development. For example, the investigators would not have been able to distinguish in electron micrographs the stage in which the neuroblast wraps around the nerve fiber from ordinary nerve fibers with myelin sheaths. These sheaths are also composed of cells wrapped around nerve fibers.

Sydney Brenner and his colleagues at the MRC Laboratory of Molecular Biology in Cambridge, England, are now using computers to study the anatomy of the nervous system of a simple roundworm. The investigators have produced genetic mutants of the worms that have unusual behavioral patterns. They hope to determine how the neuronal anatomy of the mutants differs from that of normal animals and to unravel how genes control neuronal structure and function.

The techniques of computer reconstruction of neuronal structure are not limited to cells from simple invertebrate animals, however. Several investigators are using them to study the nervous systems of a wide variety of vertebrates including the frog, cat, rat, and even the human. One goal is to determine how environment affects the development of cells in various areas of the brain. For example, the visual responses of cats raised in a striped environment differ from those of normal cats. How these behavioral changes are related to neuronal structure is a major question that computer techniques may help answer.

Once a body of information about the structure of nerve cells and their interconnections has been acquired, whether from computer or other studies, it can be fed into an appropriately programmed computer in order to build models of whole areas of the brain. Llinas and A. Pellionisz of Semmelweis University Medical School in Budapest, Hungary, are using this approach to study the function of the frog cerebellum.

A great deal is known about the structure and activities of the different types of neurons that make up this portion of