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Metal Binding in Biology

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Dr. Christensen has very effectively described the main experimental technique we have used in our metal binding studies. I now propose to outline some of the work we have been doing in this field. A major objective of our research effort is to understand on a molecular level the role metals play in three key activities in living organisms. These activities are: first, how metals operate in enzyme catalysis; second, the role of metals in the normal functioning of RNA and DNA which are substances determining the hereditary characteristics of living organisms as well as being involved in protein synthesis; and third, the role of metals in the transfer of information within the organism. I will discuss only the last of these, namely information transfer. This choice should be a good one since most of us are involved daily in information transfer in our chosen field of education, and all of us are, or have been, involved in learning processes which require considerable thought and practice. The fact, of which most of us are probably not aware, is that information transfer of all kinds in our bodies including learning, thinking, muscle action and related processes is possible only because the metals sodium and potassium move across cell membranes in response to particular stimuli. We can find counterparts in our own bodies for the most modern communication systems and usually our counterpart is much less likely to break down or to need repair. Just as it is necessary for a mechanic to know an automobile thoroughly in order to keep it functioning, so a complete understanding of our nervous system is desirable

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in order to maintain it at top efficiency and treat or prevent the diseases which sometimes affect its operation.

The research in which we are involved doesn't make use of any living material except ourselves and our students. You might reasonably ask how one can learn about living systems without using them in the study. Scientists accomplish this by studying what are called "model" systems. "Model" systems are used when for one reason or another the scientist cannot investigate the actual system. This is the way many research scientists work and the basis for much of the material which is taught in chemistry, physics and biology courses in secondary schools and universities. Since I plan in a few minutes to describe a model for one aspect of nerve action which we use in our study I would like to take just a moment to illustrate the use of models with an example which will be familiar to many of you. If someone were to ask any of us the question, "What is an atom?" we would probably have some stock answer which would likely satisfy the questioner, such as, "an atom is a very small particle which consists of electrons orbiting a nucleus which contains protons and neutrons." Actually, what I have just stated is a crude description of one model of an atom. We will probably never know exactly what an atom is since its very small size prevents any direct observation and we must rely on the answers our instruments provide to the questions that we are clever enough to ask. From these answers we formulate models of what the atom must be in order to have the properties our experiments tell us it has.

These models, of course, are subject to change as our experiments improve and our knowledge increases. The first modern model of an atom was that proposed by John Dalton in 1803.[1] This model, based on the experimental work to that date, proposed that an atom could be likened to a hard ball as shown in Figure 1. Scientists took that concept and began asking which known properties of matter could and

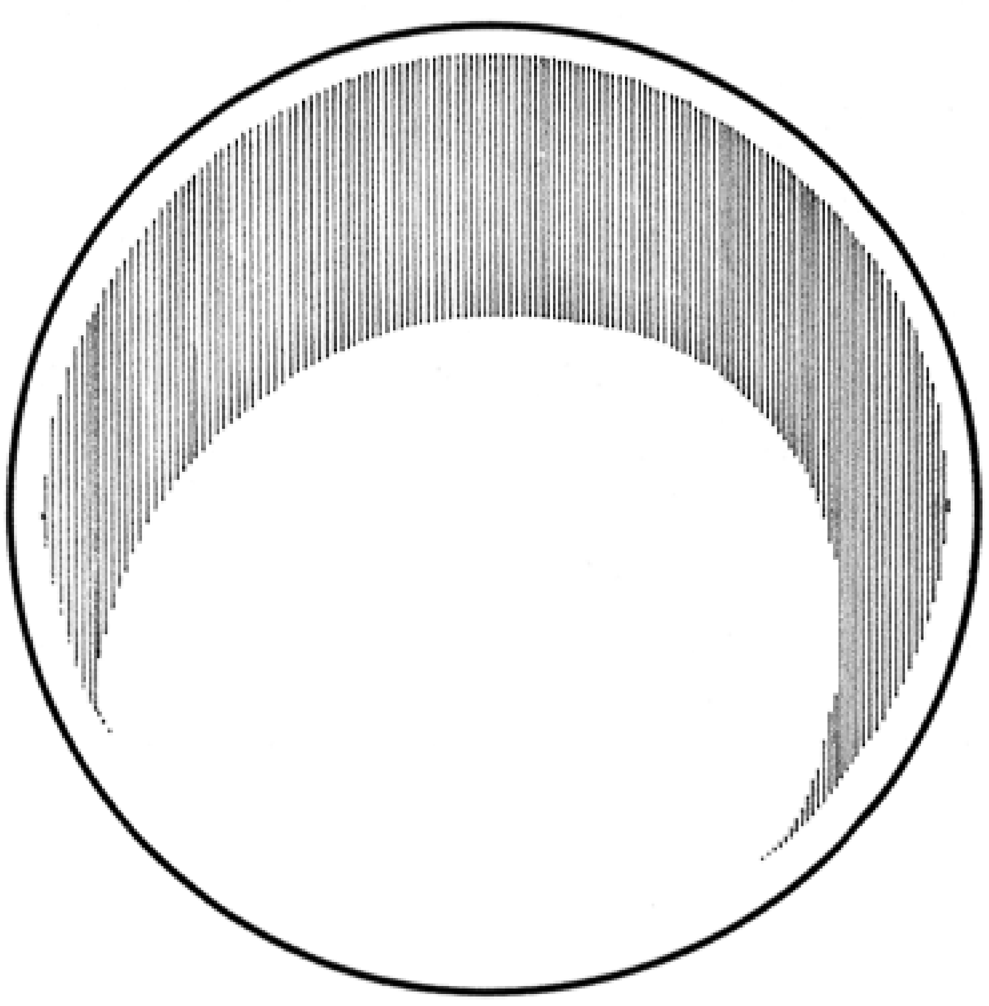


Figure 1

which could not be understood if atoms were considered to be like hard balls. The discovery of radioactivity nearly one hundred years later necessitated a sudden and drastic revision of the hard ball model. Actually, this revision was overdue anyway because it had been learned in the mid-19th century by Michael Faraday and others that matter and electrical charge were in some way related. The model shown in Figure 2 was proposed about 1900. In this model there were positive and negative charges embedded in the hard ball matrix; some called it the rasin-in-the-bun model. However, within about a decade experiments had been carried out that could only be rationalized by removing the electron from the rest of the atom and postulating a very tiny nucleus containing all of the positive charge and nearly all of the mass of the atom.

Figure 2

Now it is obvious to all of us at this point that our model making had gotten us into a real dilemma. Our experience is that positive and negative charges attract each other, yet we have in this model positive and negative charges separated from each other with no reason why they should not combine. We have abundant evidence, including our own existence, that this recombination does not occur so the model had to be revised to agree more nearly with the known laws governing the behavior of electrical charges. The first change in the model is shown in Figure 3. This involved setting the electron in rotation around the nucleus with a velocity just sufficient to exactly counteract the pull of the positive charge, much like a satellite in orbit around the earth. Even though this model was very appealing and until

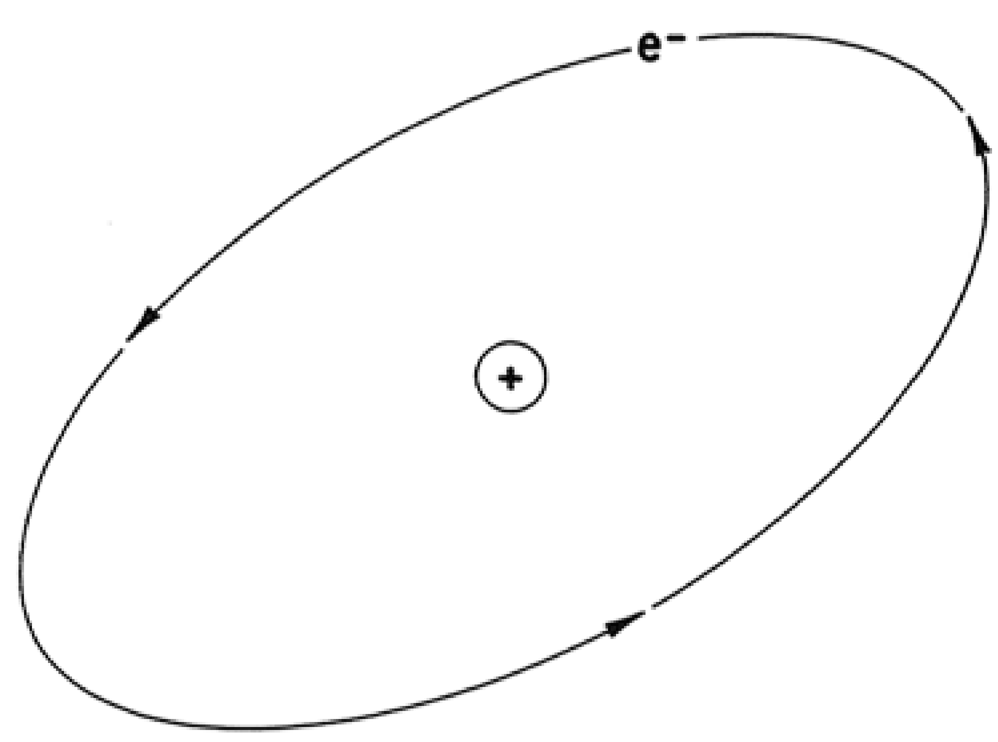


Figure 3

just a few years ago was taught in nearly every high school chemistry course, it was early shown to be incorrect in many significant details and has undergone several important modifications since it was proposed in 1913. One representation of the hydrogen atom as we view it in 1970 is shown in Figure 4.[2] If you can't see why one of these models is any better than any other you might profit from a semester of freshman chemistry! This process of refinement has resulted in a much better understanding of what an atom is or, in other words, a model of atomic structure which is much more consistent with known facts about matter than were previous models. In a similar way our knowledge of many natural phenomena has been greatly increased. Taken cumulatively, the work of many people during the past 150 years, each contributing certain knowledge or ideas, has provided the basis for our remarkable scientific and technological progress.

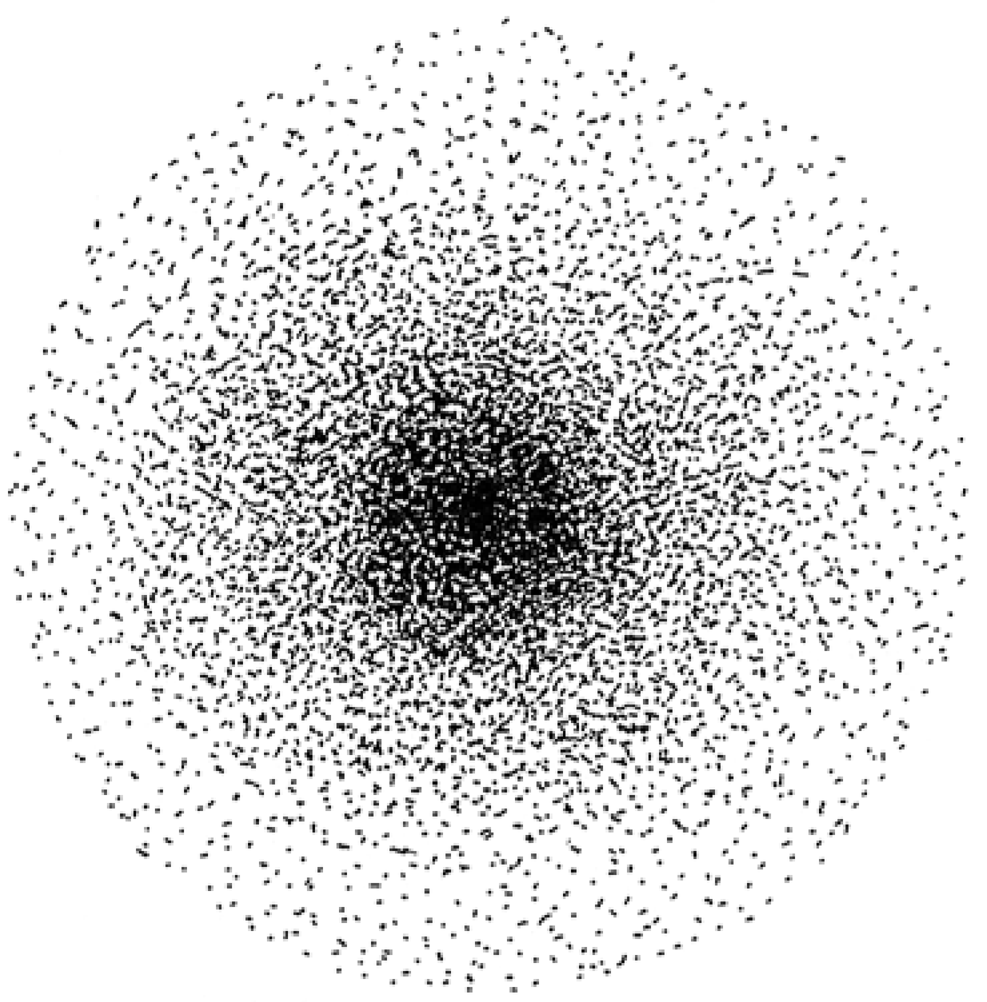


Figure 4

I will now describe what I mean by information transfer since this is undoubtedly one of the most important processes which takes place in our body. I will also provide some background information so you can better appreciate why we feel that our research in this area may lead to results of some significance. We are all familiar with the rapid transfer of information from the affected part to our brain when we have a toothache or touch a hot object. We also realize that this transfer can be stopped by the use of a local anesthetic such as novocaine. The transfer of this information takes place through our nervous system which plays an extremely important role in our welfare. Two of the most significant factors in our survival are our ability to respond to stimuli from external sources and to regulate our own internal environment. These abilities are all the more remarkable because they are largely automatic and require little or no conscious effort on our part. We possess sense organs which are specialized to literally tune in on certain information about the environment.[3] The essential elements of these sense organs are receptor cells, which respond to physical and chemical disturbances and transmit information about them to the central nervous system, thereby allowing either us or our bodies to react as necessary.

Each type of receptor is in general very specific in the stimulus to which it responds. I have listed a number of receptors and stimuli in Table I. There are different receptor cells which gain information about the external environment and the internal environment. Among the external type are photo receptors located in the retina which are sensitive to light, chemoreceptors which receive taste and smell stimuli located on the tongue and nose, mechanoreceptors which

TABLE 1

Receptors	Stimuli
Photoreceptors	Light
Chemoreceptors	Taste, Smell
	Oxygen Pressure
	pH
Mechanoreceptors	Sound, Pain
	Balance, Position
Thermoreceptors	Heat, Cold

receive sound, pain, and touch stimuli and thermoreceptors which respond to heat and cold. Among the internal receptors there are cells located in the walls of our arteries to respond to blood oxygen pressure and in the respiratory center of the brain stem to respond to blood carbon dioxide pressure; mechanoreceptors such as those in the inner ear which record movement and position; and even a receptor to keep track of the pH changes in the body fluids which cannot be allowed to vary without serious consequences to our health. Furthermore the exterior receptor cells can often give a great deal of information concerning the object in contact with them with respect to weight, temperature and patterns of movement as in the case of a bug walking across our hand.

Nerve fibre terminals are scattered over the whole surface as well as throughout the interior of the body, and the stimuli from the receptor cells are received either by free nerve endings or by specialized sensory endings. All receptor cells have an important function in common. They are transducers, that is they convert one form of energy into another. For example, the sense organs in the skin convert mechanical and thermal energy into the electrical energy which is necessary to trigger nerve impulses through the nerve endings with which they are in contact. Of course, the process is very complex, but several specific steps are involved. First, a stimulus is received by internal or external receptor cells. Second, the energy received from its original mechanical, thermal, chemical or other receptor is changed to electrical energy and third, when the stimulus reaches a critical level, the electrical impulse is moved suddenly and rapidly to the appropriate part of the central nervous system so that the necessary conscious or unconscious action can be taken. A surprisingly large number of our body functions which we take for granted are triggered by the electrical energy produced within our nervous system. This system is involved either consciously or unconsciously on our part in heart action, sensations of pleasure and pain, memory, learning of all kinds, limb movements, etc.[3-5] It is especially interesting how by our conscious effort we can refine this control mechanism to produce marvelous effects both mental and physical such as are seen in the coordinated efforts of the practiced athlete, or musician, the knowledge and ability of the scholar, etc. The following passage, often quoted by Heber J. Grant,[6] describes rather well the effect practice has on accomplishment: "That which we persist in doing becomes easier for us to do; not that the nature of the thing itself is changed, but that our power to do is increased." Perhaps the nervous system reaches the ultimate in marvelous perfection in our own brain.

Paul Weiss, professor emeritus at Rockefeller University has given us an interesting view of the human brain.[7] He points out that our brain contains more than ten billion nerve cells, each of which averages about ten thousand complex macromolecules, not only in constant agitation but being renewed about ten thousand times in a lifespan. Thus, looking at it from the worm's-eye view of the macromolecule, brain action must deal in a lifetime with at least 10^{22} or 10,000 billion billion macromolecular entities in various degrees of instability and impermanence. Also, as Dr. Weiss notes, there is a fact that the individual molecule, of course, cannot know but which our integral brain cannot help but ponder and this is that throughout all that churning and changing of a population of molecules, which is ten thousand billion times as large as the human population on earth, we retain intact our sense of individual unity and identity, our habits and our memories. The next time you are required to recall some bit of information, consider how like a computer your brain is as it searches through its stored information to find the answer to the question.

I will next discuss how metals are involved in the functioning of the nervous system and how their action can be understood.[3-5,8] I have already indicated that the nervous impulse is electrical in nature. The role of metals is to generate this impulse, and the metals involved are sodium and potassium in the ionic form. Normally, the nerve cell contains much more potassium and much less sodium than the cell fluid in which it is located. This is shown in Figure 5 where in the upper part we see a representation of the movement of sodium represented by Na^+ and potassium represented by K^+ across a red blood cell membrane. Studies of the giant axon of the squid which is merely a very large nerve fiber, being about 1 mm (or 0.02 inch) in diameter show that in the intact nerve fiber there is an electrical potential of about 50 millivolts. When appropriately stimulated the cell wall of this fiber suddenly becomes very permeable. Sodium ions rush into the cell as seen in the lower part of Figure 5, the electrical potential drops and this triggers a cascading effect in the direction of the brain center. Over a period of time much longer than the impulse but relatively short in seconds the sodium is pumped back out of the nerve cell, the electrical potential is restored and the nerve cell is ready for another impulse. Since these impulses cannot be generated continuously without loss of sensitivity the nerve can become deadened by repeated impulses. In this connection, we are all familiar with the fact that we can become numb to pain. Now the origin of the electrical current lies in the fact that the sodium and potassium exist as ions or charged particles in the fluid, so when the sodium is pumped out of the cell after a nerve impulse this is equivalent to charging a battery and energy is

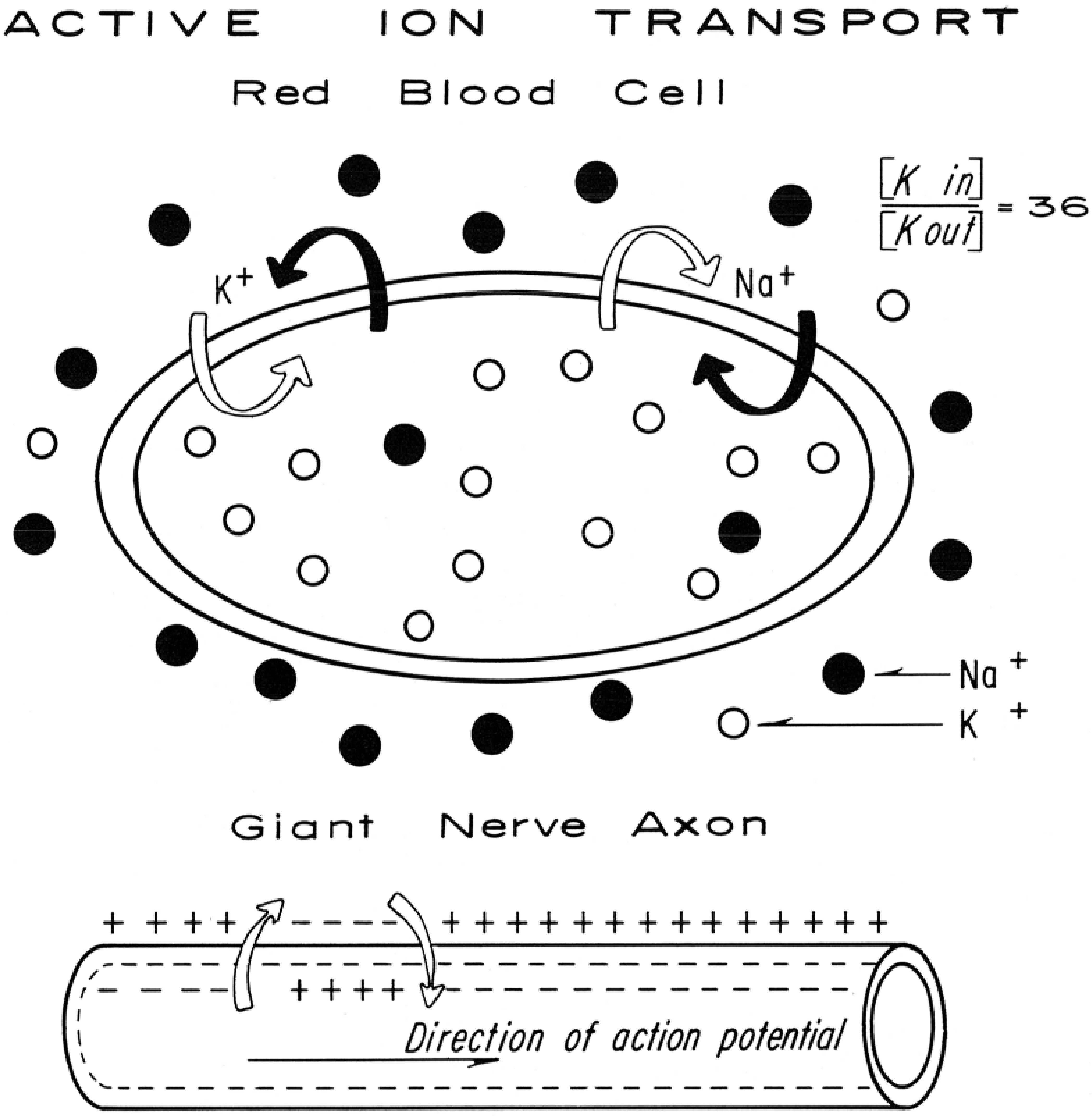


Figure 5

required. The process of pumping the sodium out of the cell has been likened to cocking a gun in readiness for firing it. Receipt of the pain, pleasure or whatever signal from the receptor is then like pulling the trigger with an immediate result in both the gun and the nerve. Here now is the problem for which we are seeking answers—what is the mechanism by which the sodium is pumped out of the cell and why is potassium relatively unaffected by the pumping procedure?

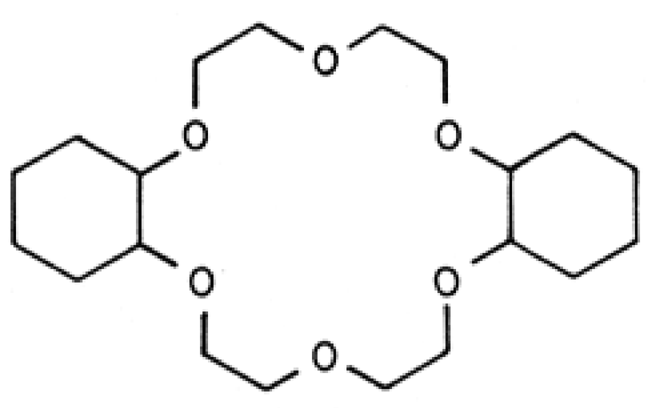
Before answering these questions let me digress to relate an interesting true story involving a naturally occurring substance which has the interesting property of interfering with the conduction of the nervous impulse in a dramatic and often fatal way.[9] On September 8, 1774, His Majesty's sloop *Resolution* under command of Captain James Cook lay at anchor off the South Pacific island of New Caledonia discovered by Cook only a few days earlier. That afternoon the ship's clerk traded with a native for a fish. Captain Cook asked to have the fish prepared for a supper he was to share with the expedition's two naturalists, J. R. Forster and his son Georg. Later Cook recorded in his journal:

> "The operation of describing and drawing took up so much time till it was too late so that only the Liver and Roe was dressed of which the two Mr. Forsters and myself did but taste. About 3 or 4 o'clock in the morning we were seized with an extraordinary weakness in all our limbs attended with a numbness or sensation like to that caused by exposeing ones hands or feet to a fire after having been pinched by frost. I had almost lost the source of feeling nor could I distinguish between light and heavy bodies, a quart pot full of water and a feather was the same in my hand. . . . In (the morning) one of the pigs which had eat(en) the entrails was found dead."

These events were a mystery to Captain Cook, but we now know that the puffer fish which he received from the natives contains a chemical substance, tetradotoxin, which in extremely small amounts is deadly because it blocks the nervous impulse pathway. This chemical is also found in the California Newt which is a type of salamander. It is about as potent as rattlesnake venom and in lethal amounts it is reputed to stop the heart between beats. Actually, the puffer fish is a delicacy in Japan where if proper care is taken to dispose of the liver where the majority of the tetradotoxin is found it can be safely eaten. It may be some reflection on the quality of the cooks in that country that in 1957, 176 cases of puffer fish poisoning were reported, 90 of them being fatal.

Now, back to the question: How is the sodium ion pumped out of the cell? At the present time the structure of the cell membrane is open to considerable question and the nature of the substance which transports the sodium out of the cell is completely unknown. The thickness of the cell membrane is less than 10^{-6} cm. This is approximately 100 times too small to be seen by our best light microscopes, but it is still 100 times larger than the sodium or potassium which moves across it. The membrane contains fat and protein macromolecules which are very large and whose composition is only imperfectly understood. It is probable that some component of these large molecules is the sodium carrier. However, for our experiments it seemed impractical to attempt to use the natural material. Therefore, we have resorted to a study of model compounds which mimic the behavior of the actual biological system in which we are interested. The two specific properties of the biological system which we are investigating are: first, the ability to carry metals across membranes (especially sodium and/or potassium) and second, the ability to interact with sodium or potassium, but not with the other. Once we have such compounds we would extend the study to other metals in an attempt to learn why the compounds have the unusual properties they show.

Through the study of others[10] we have learned that certain members of a class of compounds called cyclicpolyethers show different affinities for sodium and potassium. An example of a cyclicpolyether is shown in Figure 6. Actually, these compounds are unusual in several ways and are presently being actively studied by workers in many laboratories throughout the world. Certain of these cyclicpolyethers have been found by others to transport potassium, but not sodium across artificial membranes under the influence of an electric current.[11] We have found in our calorimetric determinations that in water solutions one of them interacts very strongly with potassium and not at all with sodium.[12,13] Thus they fill two criteria, they are selective toward metal ions and they do transport ions across membranes. Unfortunately, they don't have the property of reacting specifically with sodium as the actual carrier molecule does, but the behavior we have observed lead us to some further experiments. These cyclicpolyethers are unusual in having a large central cavity ringed with oxygen atoms which are well known for their ability to bind metals. In Figure 7 is shown a model of a cyclic polyether-metal complex. The red balls represent six oxygen atoms and the ball in the center represents a metal ion. You can see that the fit is quite good. Some of the cyclic polyethers we have studied are very unusual among



2,5,8,15,18,21-Hexaoxatricyclo $(20.4.0.0^{9,14})$-hexacosane

(Dibenzo-18-Crown 6)

Figure 6

chemical compounds in interacting very strongly with potassium, but not at all with sodium.

Having learned these things, we now have two further objectives in mind: first, we would like to find a compound of this general type which reacts strongly with sodium, but not at all with potassium; and second, we would like to understand why these compounds are selective toward sodium and potassium in the first place. Looking at the compound in Figure 7 it is tempting to say that it is just a matter

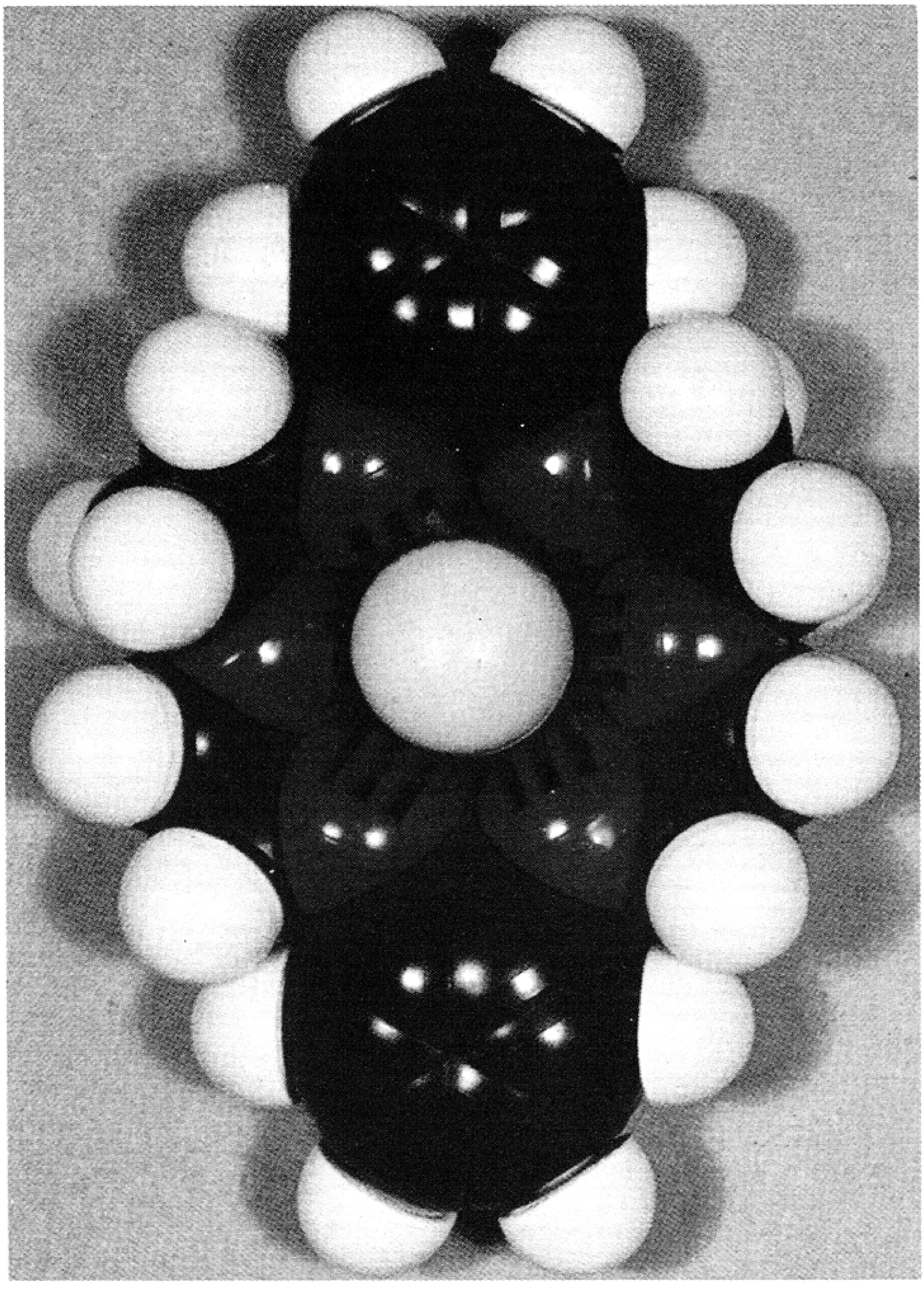


Figure 7. Model of Dibenzo-18-crown-6 shows complex with metal ion

of size—potassium will fit and sodium which is much smaller will not. We have learned, however, that this simple idea is only partly true. There are a number of other factors which must be considered. I have shown in Figure 8 several compounds which illustrate some of these factors. We are presently studying the effect on metal selectivity of replacing part or all of the oxygen atoms (represented by 0) by sulfur atoms (represented by S) as seen in the structures on the lower right. We are also studying the effect of using a different size ring and of placing other atoms or groups at various places on the molecule, or of making the calorimetric measurements in solvents other than water. In addition we are cooperating with Dr. Kent Dalley of the Chemistry Department in an X-ray study designed to learn where the metal is located relative to the ring atoms in the solid metal complex. We have learned one interesting thing so far in this study. An X-ray study made in Great Britain showed that rubidium was in the center of, but slightly above, the plane of ring atoms.[14] We have looked at the potassium compound and found the potassium to be in the center of the plane of atoms which is consistent with the fact that potassium is a somewhat smaller atom than rubidium and also with our calorimetric determination of the extent of binding of the two with the cyclicpolyether, potassium being bound about twice as tightly as rubidium.[12, 13]

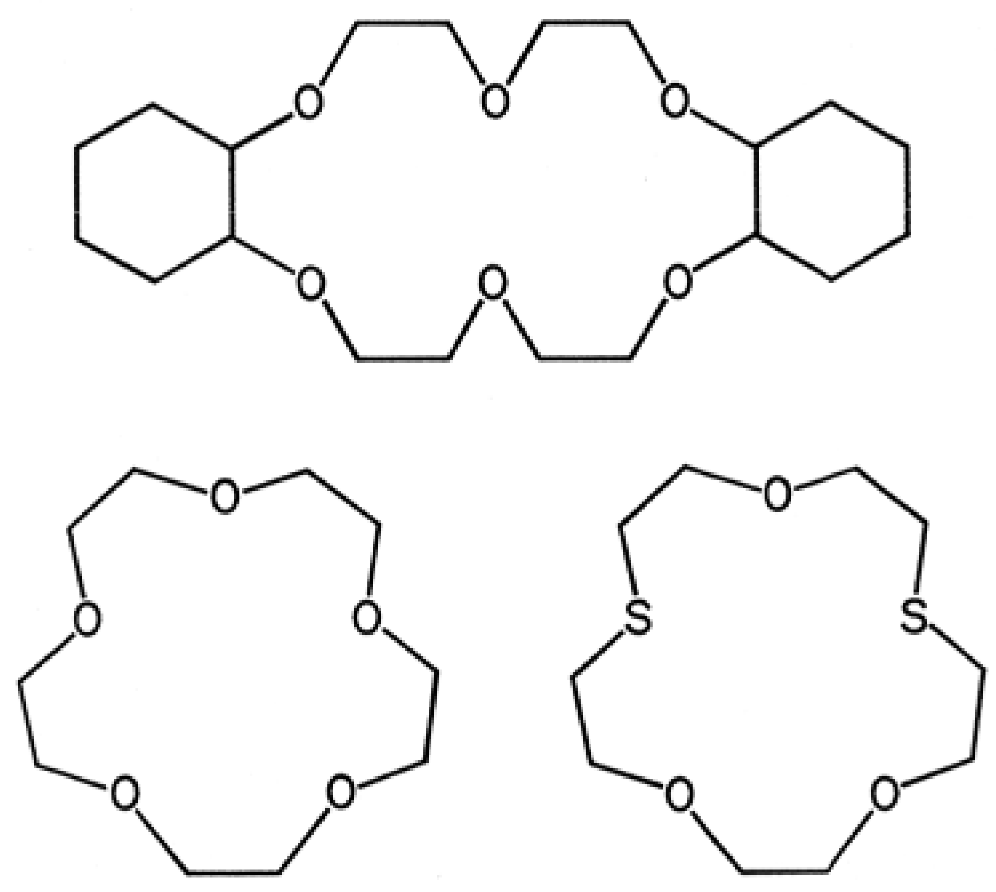


Figure 8

We suspect that there are structural similarities between our model compounds and the actual substances which carry sodium across the membrane, and that the ring structure is a very important part of the natural carrier molecule. We have found that exactly similar compounds which do not have the ends connected to form a ring do not have any affinity for either sodium or potassium. The formation of the ring is necessary, at least in water solutions, for the metal complex to exist. These cyclicpolyether compounds resemble structurally several classes of cyclic antibiotics which show the same sodium potassium differentiation and also transport metals across membranes under the influence of an electric current. Examples of these antibiotics as given in Figure 9 are valinomycin and nonactin, which have ring structures and inwardly directed oxygen atoms. As I indicated earlier, proteins are important structural constituents of membranes. It is not difficult to visualize how protein molecules or certain parts of them could behave toward sodium and potassium in a way similar to that we see in the cyclic polyethers and antibiotics.

There are several areas in which we anticipate that our experiments will have some value. First, since we are working with compounds which behave in a similar way to the as yet unknown carrier substances in membranes, we hope to be able by varying the experimental conditions to find specific compounds which exactly mimic these substances. We can then by judicious experimentation learn the factors which cause these cyclicpolyether compounds to have their observed selectivities and hopefully suggest reasons why this selectivity is shown in the intact nerve cell. Second, the results of our experiments will provide us and others with information which could lead to improved theories of cell membrane structure and give a better idea of what to look for in the way of carrier molecules. Third, we hope to learn more about the general process of transport across cell membranes. This transport is not limited to metals or to nerve cells. All materials used by living organisms in their life processes pass through membranes since this is the only way they or their breakdown products can enter the

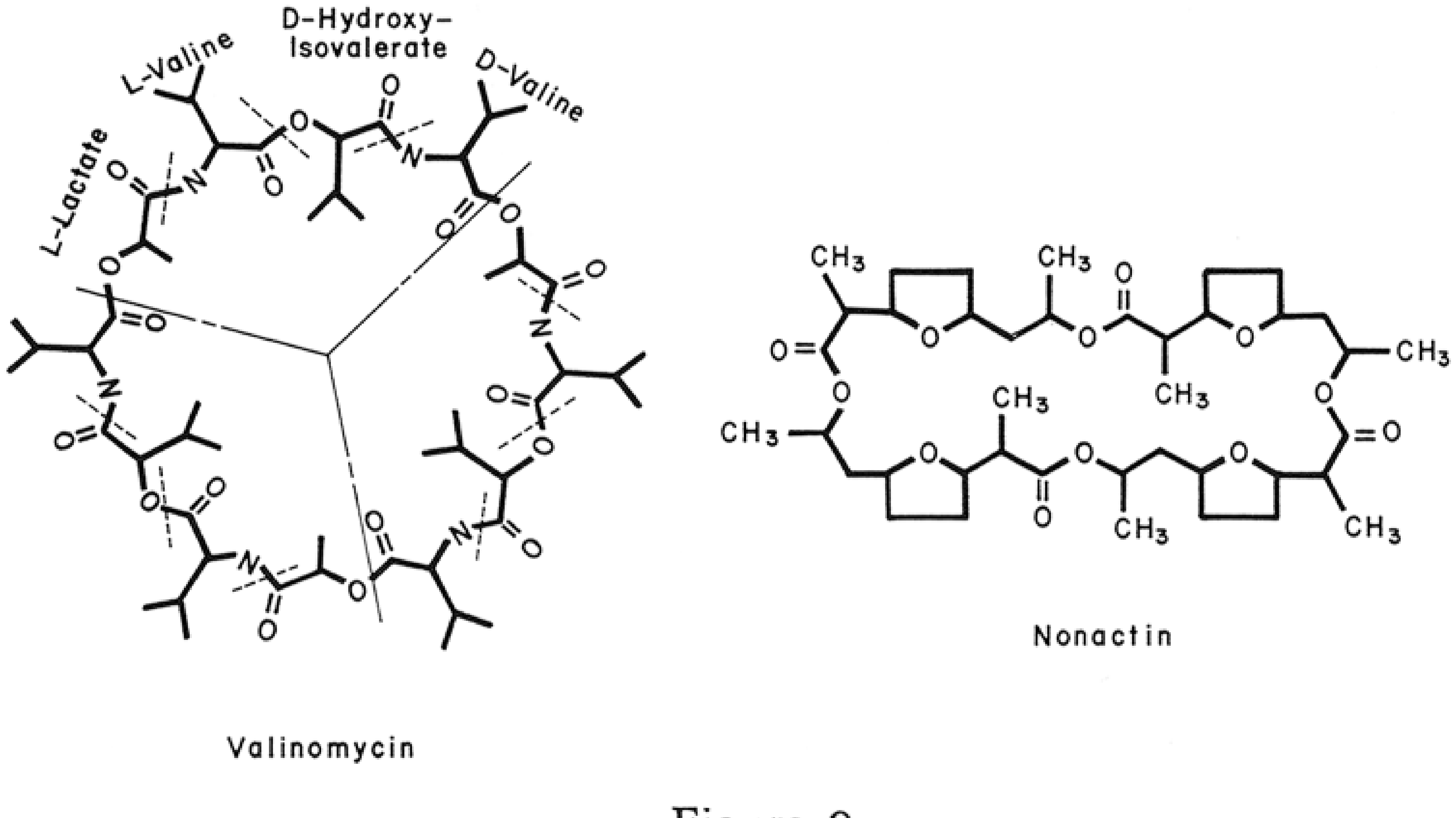


Figure 9

cell where transformation into materials which can be used by the organism takes place.

In addition, many cells have the remarkable property of being able to concentrate certain metal ions or other substances. This concentration process amounts to a transport of the metal involved against a concentration gradient, that is, the metal moves from a region of low concentration to one of high concentration. The driving force for this movement is not well understood. One example of this is the transport of sodium out of the cell after a nerve impulse. Another example is the concentration of the element chromium by a nucleoprotein in beef liver by a factor of 20,000 over the amount in the surrounding medium. A third example is to be found in the normally highly acid condition of our stomach. The amount of acid in the stomach is maintained approximately one million times higher than the amount in the fluid from which it comes, which is just on the other side of the stomach membrane. We would hope that the results of our research will shed light on how the cells in living organisms can do these remarkable things.

Fourth, there are many potential industrial applications for effective ion separation procedures. Our work could lead to the design of effective artificial membranes or ion exchange materials with the property of distinguishing between very similar metals, such as sodium and potassium or calcium and magnesium. This type of membrane or ion exchange material would be welcome news to many industries such as those involved in the petroleum and chemical businesses where unwanted metals frequently contaminate the main product or where it is desired to recover the metals economically. Another possible application of this type would be in saline water conversion where it is desired to effectively remove metals such as sodium, calcium and magnesium to make water suitable for drinking or for industrial processes.

Last, many serious diseases involve the central nervous system or other parts of the nerve network. Increased understanding of the normal and abnormal operation of this system is therefore of obvious importance in the treatment or prevention of such diseases. We would hope that the basic information obtained in our study concerning the reaction of metals with cyclicpolyethers will provide this increased understanding of this most important life process. Recently at the annual meeting of the 12th Annual Science Writers Seminar of the American Cancer Society a report was made that the division of body cells is controlled precisely by the amounts of sodium and potassium on either side of the cell membrane.[15, 16] Mr. Clarence D. Cone, Jr., head of NASA's molecular biophysics laboratory at Langley Field, Virginia, reported that cells having large negative membrane voltages seldom if ever divide, whereas cells with small negative electrical potentials divide at maximum rates. Since malignancy is related to uncontrolled cell growth, the interest in this idea is obvious.

In summary, then, our research program involves primarily the application of calorimetry to the measurement of those energy changes which occur when metals interact with substances of biological interest. Specifically, we are interested in understanding better the processes by which living organisms transport metals in nerve conduction and how they are able to remove and concentrate metals from their environment. Knowledge obtained about these processes should be useful in a wide variety of applications. Again, Dr. Christensen and I express our appreciation for this honor. Thank you.

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