Energy, structure and carbon dioxide:

A realistic view of the organism

by **Ray Peat, PhD**

"But the philosophy of Causes & Consequences misled Lavater as it has all his Contemporaries. Each thing is its own cause & its own effect." *W. Blake, c. 1788*

What could be more important to understand than biological energy? Thought, growth, movement, every philosophical and practical issue involves the nature of biological energy.

The question of biological energy is usually handled in the manner of the cosmologist who explained that the earth rests on the back of an elephant; when asked what the elephant stood on, the cosmologist replied that "it's elephants all the way down." Several decades ago, it was discovered that ATP mediates many processes in the energized cell, but there is still fundamental disagreement on the question of how ATP is synthesized, and how its energy is used to produce movement, to control the movement of water in cells and organs and to regulate the ionic balance of cells and fluids, and even why its absence produces rigor mortis.

When people actually try to examine the question of how the "high energy bond" of ATP can be transformed into usable energy, they sometimes find that it is easier to propose fundamental changes in the laws of physics than to find an explanation within ordinary physics and chemistry. (For example, Physiologie 1986 Jan-Mar;23(1):65-8, "The non-conservation of parity in the domain of elementary particles and a possible mechanism for the delivery of energy from the ATP molecule," Portelli, C.) More often, biologists simply prefer not to go beyond the first or second elephant.

However, there is a way of looking at the nature of life that doesn't involve mythical beings. The writing of history, in science as in politics, is often an ad hoc convenience, usually done to achieve maximum self-justification. When Harry Truman announced that he had dropped an atomic bomb on "Hiroshima, a military base," he was revising, for a moment, the history and geography of Japan. Usually, fictional histories created by powerful institutions become part of a general culture, and are relatively permanent. "Scientific revolutions" and "paradigm shifts" are just tardy acknowledgments of the silliness of the ruling fiction. In the process of accepting a slightly more rational way of doing things, those who have reluctantly given up the old doctrine will look for ways to show that they were on the right track all the time, that all of the nonsense was necessary. They want to maintain the illusion that scientists are intelligent and rational, for the same reason politicians want to create the illusion that they are just and wise. Television networks and newspapers agree that genocide is exactly where the president says it is, because they and the president have common interests; likewise, science journals and textbooks are there to protect the orthodox beliefs of institutionalized science, much more than to search for truth. For historical doctrinaire reasons, Aristotle's ideas and the culture that had been built up around them were practically eliminated from Anglo-American culture a few hundred years ago. In Aristotle's "formative principle," nature itself was creative and purposive, "teleological." His ideas, and the people who held similar ideas, were suppressed because of the dangerously democratic implications seen in them by the ruling classes. Since Ilya Prigogine's Nobel Prize, a false cultural history of "emergence" has been formulated, to derive the idea of the sudden appearance of order out of disorder,

from the official anti-teleological (platonistic) rationalism that had seen change as a matter of random fluctuations in a time-reversible system, in which numbers are real and substance is unreal. In this new version of history, cybernetics is blended with neodarwinism, to explain order as something external to matter, and dependent on chance rather than purpose.

Since I was following N.A. Kozyrev's work (on stellar and planetary energy) from the late fifties through the sixties, I thought of volcanism as a process essentially equivalent to solar energy. Then, in 1968, I read Sidney Fox's experiments with heated amino acids, and saw that volcanism was a more appropriate energy than sunlight for driving the origin of life. In some of his experiments, Fox put nearly dry amino acids onto hot volcanic rocks, and when he added a little water, the amino acids polymerized spontaneously, and nonrandomly, into peptide chains; when these were put into water, they spontaneously formed microspheres, that looked like, and behaved very much like, bacteria. Fox saw his work as a validation of the principle that nature itself created higher order spontaneously.

Deep in the volcanic earth, or deep in the ocean, life would be protected from destructive ultraviolet radiation, whether or not the atmosphere contained enough oxygen/ozone to screen out that radiation. And thinking about volcanoes, I questioned the idea that life had to originate in the "reducing atmosphere" that was dogmatically required by the conventional protobiologists. Volcanoes emit water, carbon dioxide, and a variety of strong oxidants. I think it is possible that atmospheric oxygen preceded green plants.

If Sidney Fox's spontaneously formed proteins and microspheres are similar to the original living cells, these forms of life appearing in volcanic seeps would have originated in an environment rich in carbon dioxide, and I have gradually come to imagine that the present ordinary respiration based on oxygen might have originated as an adaptation to an environment deficient in carbon dioxide, as life spread out from its volcanic origins.

The history linking volcanic life to contemporary sun-based life is still in doubt, but in outline we can think of the sun as a present energy source, and the chemistry of the earth as, relatively, an energy sink. Electrons activated by light energy from the sun give up that energy, as they move through various steps until they combine with oxygen. Energy flow, in this sense, is somewhat like the energy flow between the negative and the positive poles of a battery; ultimately, it produces heat, but in the process, it can produce work.

Life interposes itself between the "poles" of energy flow, and the flowing energy creates organization and structure, as it is dissipated into heat. Structures store some of the energy, and tend to increase in complexity, taking advantage of the flow of energy to create phase differences with expanded internal surfaces, like a finely mixed emulsion. Like a finely divided emulsion, the more highly energized the organism is, the stabler it is. It adapts to the available energy; energy is used in adaptation; the structures built with the energy are adaptive structures.

This idea of the development of organismic complexity as a response to conditions that are "far from equilibrium" was first clearly stated by V.I. Vernadsky, about 80 years ago, but now the idea is associated with Ilya Prigogine. The only difference is that Prigogine has inserted an element of indeterminacy, which seems to have ideological appeal for much of the academic world.

In Fox's production of the proteinoid microspheres, the ordered growth is a consequence of the properties of the substance in a permissive environment. The order is not imposed from the outside onto passive matter. When Darwin was a university student, he accepted Paley's

doctrine of “Natural Theology,” in which a watchmaker god inserted design into the material world. As he matured, he allowed for a certain Lamarckian intrinsic process of ordering, but he emphasized the role of natural selection, in which the design was imposed by the environment, in the same way that animal or plant breeders use artificial selection to impose the traits they want. The neodarwinist movement “corrected” Darwin, putting all of the responsibility for the “design” of organisms onto the natural selection of strictly random variations. The cybernetic culture is having a strong influence on neodarwinism, and some of the new histories of science that are being written are trying to place the design process on the abstract mathematical level, rather than looking for it in the nature of substances. Devotees of “chaos theory” would entirely displace the designing principle from the material world. The meaning, and the effects, of this process of mathematizing neodarwinism are antagonistic to the facts demonstrated by Sidney Fox’s experiments, and are tending to move research farther away from the creative nature of life.

Despite the present emphasis in “nonlinear dynamics” on random fluctuations and instabilities, the fact is that complex organisms, and finely mixed emulsions, are very stable, and the direction of their development is essentially determinate. Vernadsky described this fact as a law of evolution, that organisms and systems would tend toward the production of a high metabolic rate and large size. This means that evolution tends toward a maximum of energy use, a maximum of adaptive structures. The brain is the dominant organ of adaptation, and the evolutionary tendency toward “cephalization” is an illustration of Vernadsky’s law.

The stability of the fine emulsion, or of the evolved organism (a person has greater homeostatic powers than a rat), involves the fact that, within a given range of available energy, the very complex structure has dissipated the energy within itself to a high degree. Every point of the system has come very close to being in equilibrium. It’s a situation analogous to that of a road that climbs a mountain with a nearly infinite number of switchbacks—as the number of switchbacks tends toward a maximum, the slope of the road at any point tends toward a minimum.

Mammalian cells are smaller than frog cells; we are like a well homogenized emulsion, compared to animals with lower rates of metabolism. An unstimulated cell is practically in equilibrium with its environment. This is the “high energy resting state.” Activity generates structure, but when a cell is inactive, it is stable and doesn’t have to expend energy. This is exactly contrary to the doctrine in which a “cell membrane” maintains the cell’s organization by a constant expenditure of energy, running “pumps” to maintain differences in the ions and dissolved substances on the opposite sides of the membrane. In that doctrine, each cell, even at rest, is far from equilibrium; life is a struggle, and the cell must spend energy even to stay as it is. Gilbert Ling showed that the concept of membrane pumps to preserve the cell’s order is both unnecessary and impossible. In the real organism, energy is spent to grow, to adapt, and to evolve, but not to merely persist.

If we understand Sidney Fox’s spontaneously formed microspheres, I think we will get some insights into our own cells. For example, the microspheres have a remarkable uniformity of size, which they preserve even during growth, by dividing instead of simply enlarging. They tend to assemble into orderly chains, without coalescing with each other. They are stable in warm water, but dissolve in cold water. This indicates that the hydrophobic, “fatty” quality of the proteins, causes them to be expelled from the bulk water, forcing them into association with each other. Cold water has greater tolerance for fatty substances. The proteins, however, also contain regions that are water soluble, and when the proteins assemble into droplets, they continue to associate with a certain amount of water. This water is now “dissolved in the protein,” in the sense that the properties of the protein are relatively

dominant. (Bungenberg de Jong's studies of "complex coacervates" are still the best introduction to this subject.)

The modern practice of biochemists has been to extract soluble substances from cells, and to study them in dilute watery solutions, and then to believe that the things they observe in the test tube are the real properties of cells, of the "dilute solutions enclosed in a lipid membrane." If I hadn't had the experience of talking to dozens of biochemists who believed that no other kind of biochemistry was conceivable, I would find it hard to imagine that something like this could exist in a culture that defines itself as "scientific."

Small particles have a large surface area in proportion to their mass. The balance, within the proteins, between hydrophilic and hydrophobic groups, will determine the proportion of surface area in contact with the bulk solvent water, relative to the mass of the microsphere droplet. More hydrophilic proteins will form smaller droplets, and at a certain point of hydrophilicity, will no longer form droplets. The temperature, by altering the structure of the water, interacts with the hydrophilicity/hydrophobicity of the protein. Structures are generated as complex physical equilibria are achieved.

In our own cells, the microtubules, which are a part of the cell framework involved in cell division and movement, are dissolved at low temperatures, and are reformed when the temperature is raised. Some enzymes have this same temperature sensitivity. Since the water which is "dissolved in the proteins" of the cell is largely dominated by the proteins, its actions on microtubules and enzymes and other proteins will reflect both temperature and the influences of proteins and a variety of dissolved substances. Estrogen, for example, promotes the formation of microtubules, at a given temperature, as if it had made the water "wetter," or warmer.

When cells are stimulated, they adapt, with substance flowing into complexification until an approximate, appropriate equilibrium is reached. Stimulation is a need, and an opportunity, for adaptation and differentiation. If there is a need for adaptation, without the necessary substance and energy, the cell or organism will either deteriorate or withdraw.

Polyunsaturated fats with inappropriate structure interfere with these adaptive flows of energy and substance in all of the known systems of cellular response. These exogenous substances suppress the respiratory energy system, the intercellular communication systems, and the intracellular response systems. Immunodeficiency, autoimmunity, inflammatory diseases, aging, cancer, heart disease, nervous diseases, and hormonal imbalances are produced when these fats interfere with the spontaneous self-regulatory processes of the organism.

When respiration is suppressed, the cell's production of carbon dioxide is suppressed. If we start with the best known example of carbon dioxide's effect on a protein, the Haldane-Bohr effect on hemoglobin, we will have a model for visualizing what happens to organisms in an environment that is poor in carbon dioxide, but rich in vegetable-derived unsaturated fats. Carbon dioxide associates with protein in a variety of ways, but the best understood association is its reaction with an amino group, to form a carbamino group. In the presence of a large amount of carbon dioxide, the hemoglobin molecule changes its shape slightly, along with its electronic balance, in a way that favors the release of oxygen. The opposite happens in the presence of a high concentration of oxygen and a lower concentration of carbon dioxide. Other factors can modify the effects of these gases on hemoglobin's shape, electronic properties, and its binding affinities. Wherever there is lysine or other free amino group (practically every protein and peptide), carbon dioxide can be expected to react with it

to some degree, which will depend on other things in the environment. Lysine also reacts with sugars, so there is a competition between CO2 and glucose. In aging and diabetes, many proteins are altered by the inappropriate binding to sugars. There are enzymes which can remove sugars that have altered proteins, but these enzymes are inhibited by the presence of small fragments of starch molecules.

The absence of carbon dioxide bound to a protein is likely to have an effect on the protein's structure and function, but the presence of a relatively large sugar molecule, in a site normally occupied by carbon dioxide, will have drastic effects on the protein, including tending to solublize it, and to cause it to associate with its environment in other abnormal ways. In general, the presence or absence of carbon dioxide involves relatively quick and subtle changes in structure and function, analogous to the phosphorylation of proteins, but possibly competitive with it, while the presence and absence of sugars, as glycated or glycosylated proteins, tends to be relatively permanent, and to require enzymes to restore the original state. Carbon dioxide's regulatory effects have been studied in only a few enzymes and hormones, but there is enough evidence to show that its reactions with proteins and peptides constitute a major regulatory system.

The formation of carbon dioxide itself, from organic materials, has recently been demonstrated to provide the energy for synthesizing ATP. (Arch Microbiol 1998 Aug;170(2):69-77, "Energy conservation in the decarboxylation of dicarboxylic acids by fermenting bacteria," Dimroth P, Schink B.)

Around 1970, someone used a new technique that etched away the surface of a red blood cell, revealing an interior that was obviously highly structured, partitioned into orderly segments, but when I talked to biology professors, they still believed that a red blood cell was "just a bag of hemoglobin, enclosed in a lipid membrane." One of my biochemistry professors, who was smart enough to have opinions of his own, in private sarcastically referred to the "lipid bilayer membrane" as "the fat sandwich theory." But it would be several years before it became socially acceptable to talk about the cell's internal framework. Early in the century, before electron microscopes existed, a biologist had inserted tiny particles of carbon into cells under the microscope, and described their movement as they fell through the cytoplasm as resembling the movement of a pebble falling through a brush pile; it was obvious that the clear cytoplasm was highly structured. The same biologist also rearranged the organelles within the cell, and demonstrated that they spontaneously returned to their normal positions. The cytoplasm can flow like a liquid, but it has some of the properties of a highly organized solid.

When I moved a microelectrode through a cell, using an apparatus that could move it forward or backward in very small increments, I found that the voltage fluctuated with the location in the cell, and that withdrawing or advancing the electrode, each location would show the same voltage as before, when the electrode returned. This meant that, even electrically, the cytoplasm was behaving as a solid, not as a liquid. According to the "membrane theory" of the cell, the liquid part of the cytoplasm has to have the same voltage in all of its parts.

In that doctrine of a cell as "a drop of water containing dissolved molecules enclosed by a membrane," biochemists were required to think that enzyme-catalyzed reactions are governed by random collisions of the substances reacting with the enzymes, and that only a few properties of the solution, such as temperature, pH, and ionic strength, would have any influence on the behavior of the enzyme. Their doctrine seemed tenable to them, at the beginning of the 1970s, only because they had an essentially unscientific attitude that refused to consider the evidence, on the basis that valid evidence couldn't disagree with their position. In the case of hemoglobin, the idea that substances bound to the protein molecule

could change its chemical and physical properties was accepted, and by analogy with that, additional “allosteric” (shape-changing) enzymes were beiing studied.

But, because of the commitments made to the “membrane enclosed cytoplasm” theory, the structural proteins were for a long time treated according to the rules established for enzyme chemistry—only local, random interactions were considered to govern their behavior.

In the 1950s, Gilbert Ling introduced a model of the cytoplasm that took account of its observable features. He called it the “Association-Induction” hypothesis. He proposed that substances such as ATP, hormones, and ions participated in cell physiology according to the ways that they associated with proteins and water, and that a powerfully adsorbed molecule, such as ATP, would influence the structural proteins in the cytoplasm as “cardinal adsorbants,” altering the proteins’ affinity for other adsorbed substances, such as potassium and sodium. The behavior of hemoglobin was a model for the behavior of the cytoplasm and its components. Unfortunately, most biologists didn’t even understand the role of adsorbants in hemoglobin’s function, so practically no one bothered reading his work. The well-accepted fact of “backbone chemical shift” that results from something as simple as calcium binding to a protein is just another way of talking about the principle of association-induction. The actual chemical structure of the cytoplasmic framework in most types of cell had hardly been studied, and Ling concentrated on studies of the physiology of cells, treating the cytoplasm as an ensemble. Now that many cytoplasmic proteins are being studied in detail, the significance of his cell physiology can be seen more easily.

The “membrane” people like to talk about “ion channels” and “channel proteins,” but they are simply describing fragmentary examples of the adsorption-induction process, in which strongly bound substances change the affinity of a protein for small ions and other associated substances. One of the effects of the membrane theory, and of studying enzymes dissolved in water, is that many biochemists got into the habit of thinking of proteins as water-loving materials; otherwise, why would they have to be enclosed by an oily membrane? But, in fact, proteins have a great affinity for fats. Fats are powerful regulatory substances. In excess, the wrong kind of fat associates with the cell framework, and alters that regulatory system, at the same time that it poisons enzymes and other functions. Insoluble proteins tended to be discarded; sometimes they were called “membrane proteins”; when it turned out that the insoluble structural proteins often had “ATPase” functions, this enzyme came to be thought of as the “membrane pump.” Even under ordinary assumptions about the way cells use ATP in their energy economy, Gilbert Ling showed that cells don’t have the energetic capability of maintaining all of their gradients by “pumping” ions and other dissolved substances. But, the common idea that the phosphate bond in ATP is a very “high energy bond,” with 14 kcal of energy, is an unfounded belief; in 1959, for example, Sidney Bernhard showed that a more realistic figure was around 4 kcal. But under relatively water free conditions, the bond forms spontaneously. One of the implications of this fact is that the control of water, the presence or absence of water, and the state of the water, is itself a matter of high-energy interactions. ATP does have a remarkably high energy of adsorption or binding to proteins, and this binding energy allows it to influence the protein’s interactions with water. A very thin layer of water between two objects can bind them together very tightly. The structures and movements in cells exist because of very specific interactions between large molecules, especially proteins, and the water which binds them and separates them. Both the water and the proteins are modified by the presence of carbon dioxide.

Two kinds of experiment show that the standard ideas about ATP and pumps have to be reconsidered. When muscles are stretched, they synthesize ATP (Experientia 1971 Jan 15;27(1):45-6, “Stretch induced formation of ATP-32P in glycerinated fibres of insect flight muscle,” Ulbrich M, Ruegg JC); this strongly suggests that its synthesis is a physical

process, occurring in an environment in which water is inactive, allowing the reaction to be close to equilibrium. (In the heart, stretching has an anabolic effect.) In another experimental setup, the temperature is measured near the surface of a nerve; when the nerve is stimulated, the temperature rises momentarily above the starting temperature, but as the nerve recovers and repolarizes, the temperature falls below the ambient temperature. This "refrigeration," or heat absorption, isn't compatible with the activation of chemically powered "pumps" to restore the initial arrangement of ions, and it suggests something physically closer to the way that heat is emitted and absorbed by a rubber band when it is stretched and then relaxed. When heat production in a myelinated nerve is measured, the membrane theory would require that the heat production, like the electrical potential, should progress in a saltatory manner, jumping from one node to another, but the measurements showed that the heat production moves continuously along the nerve. This supports the idea that the bulk of the cytoplasm is undergoing a progressive phase transition.

Physically, all of these observations (which make no sense in the membrane theory) are compatible with a view of the cytoplasm as a cooperative molecular ensemble that is poised so that its alternative states are close to equilibrium, allowing it to spontaneously revert to its original state following a stimulus that changes its state slightly, or to cause systematic changes in chemical cycles which produce the substances, such as carbon dioxide and ATP, which tend to restore the original state. Nerve conduction, muscle contraction, and secretion are now recognized to involve the factors that cause "allosteric" shifts in molecular structure, association, and affinities. It is the myth of the cell as a "dilute solution organized by a membrane" that prevents the recognition that cell physiology consists primarily of such processes, coordinated into cooperative phase transitions. The recent discovery that cell filaments form responsive systems extending from the cell's surface to the chromosomes makes it possible to see the process of genetic expression as an extension of this organized and unified system.

The standard doctrine about the structure of the membrane is that it is a lipid bilayer, meaning that an outer layer of fat (phospholipid) is arranged with its acidic water-soluble end turned outward toward the watery environment, and its fatty water-repellent tail turned inward, against the fatty tail of another layer of molecules, which has its acidic end turned inward, toward the supposedly watery cytoplasm. In support of this arrangement, an "oil loving" stain is applied to hardened cells (otherwise no membrane can be seen under the electron microscope), and a double line appears near the cell's surface. This is called the "lipid bilayer." However, since the theory says that the fatty parts of the two layers are pressed against each other, there is in the theory a continuous band of fat, separating two layers made up of the acidic heads of the molecules, and the theoretical structure of the "lipid bilayer" has no resemblance to the double line that is created by the stain. The material generally used to produce the image of a bilayer membrane is osmic acid, an oxidant; it wouldn't be expected to stain the layers of acidic heads of fat molecules. This might seem to be an embarrassing inconsistency, but apparently not to most scientists. After the electron microscope began making pictures of cells, it took some time to find the stain that would produce any membrane at all, and then it took about thirty years to learn to produce a "membrane" image that had a thickness that seemed appropriate for the theory. Considering the great effort required to produce a "membrane" image of the right size in the right location, they are willing to overlook the fact that the fat-loving stain hasn't quite found its way to the single band of fat between the acidic layers which their theory describes. Gilbert Ling described the boundary at the cell surface as a phase bouundary, of the sort that exists where two different materials meet, for example at an oil-water interface. When the two substances have different electrical-chemical properties, the forces between the phases move electrons and/or molecules near the surface into what is called an electric double-layer. Since stains have their own electrical and chemical properties, the stain molecules would be affected by the fields that produce an electric double-layer. Osmic acid would be

expected to stain certain protein groups, including sulfhydryls and amines, which could be exposed in such an area of strong fields. (Brain tissue that is deprived of oxygen stains diffusely with these “membrane” stains, suggesting that proteins are changing shape sufficiently to expose groups of this sort.) The forces between fat molecules, that allow them to form “hydrophobic bonds,” are actually so weak that they should hardly be called “bonds,” at least at normal temperatures. Fatty surfaces seem to seek each other out in a watery environment because water molecules bind so powerfully to each other that they tend to force out anything that doesn’t bind to them. So, if we even consider the association between fat molecules as a “bond,” it is the weakest bond that exists between any biological molecules. When a cell is attached to a surface, it can be torn to bits in trying to move it, without breaking its attachment to the surface. Obviously, it isn’t attached to the surface by its “lipid bilayer membrane.” The strength of a lipid bilayer would be limited by the extremely weak affinity of fat for fat; if you step on a sticky floor wearing tissue-paper slippers, your foot won’t be ripped from your leg. A lipid bilayer has no more strength than the rainbow that forms on a puddle of water when a microscopic film of oil spreads over its surface. And the rainbow on the puddle is something that really exists.

Even though a cell’s substance can flow, it has a cohesiveness that can greatly exceed that of ordinary watery solutions. The toughness of a steak isn’t affected just by the extracellular connective tissue, as was once believed; the intracellular filamentous materials contribute greatly to its resistance.

Protein filaments can bind cells firmly to the materials that surround them, including other cells. Red blood cells normally float freely in a watery environment, but under some conditions they stack up into a rouleau, roll of coins, formation. The membrane theorists like to explain this pathological association in terms of ionic surface bonds, but experimentalists have pried the cells apart under the microscope, and photographed long extensible, apparently elastic, strands binding them together. The condition appears when the cells’ energy is depleted, suggesting that the strands result from an alteration in the cells’ internal framework. This kind of process would have practical application in the formation of a clot, producing strength and continuity that would be inconceivable if the red cell were “bags of hemoglobin enclosed in a lipid membrane.”

If the cell’s cytoplasm can be mechanically continuous with its environment, then the principle of allosterism, the conditionally responsive change of shape and affinities that is recognized in hemoglobin and some enzymes, has the potential for explaining the cell’s ability to respond to its environment, and to alter that environment in a controlled way. Filamentous, or other space-encompassing structures in effect are carriers and transmitters of fields of various kinds. A cooperative phase change (cooperativity means that a change which is slow to start will proceed quickly to completion once it gets started, because of interactions of its parts) can occur in a structure which has fluidity, so the signal transmitting function needn’t be tied to mechanically fixed filaments. An ensemble of molecules can behave in a coherent manner resembling the behavior of hemoglobin. In fact, hemoglobin is a molecular ensemble which behaves cooperatively, as a functional unit, so there is nothing essentially novel in thinking about larger molecular ensembles making up the cytoplasm.

Ions such as calcium are bound to oppositely charged ions, counter-ions, which are abundant on proteins. As the cell’s state changes, calcium (and other) ions can be liberated from the binding proteins, and the momentarily high concentration of ions can serve to transmit an excited and activated state to other molecules, promoting enzyme activity, muscle contraction, nervous transmission, or other cell function. Not long ago, these movements of ions within the cell were explained in terms of membrane pumps and organelle membranes. Now, calcium-binding proteins and “channel proteins” have been

identified; the term “channel” derives from the idea that the impermeable membrane had to have pores for the entry and exit of ions and other substances. Supposedly “leakage” through those pores required pumps to compensate by moving substances in the opposite direction. At present, publications on ion channels are more than ten times as frequent as publications on their associated “membrane pumps.” Many years ago, it was discovered that large numbers of sulfhydryl groups (a hydrogen bonded to a sulfur atom, which is often in the cysteine group of a protein) appeared during cell division. This represents a rapid and massive change in cell chemistry. The sulfhydryl group is ionizable, but in the late sixties and early seventies when the sulfhydryl shift still seemed important to biologists, there was no support for the idea that these groups could be involved in ion regulation, as part of Gilbert Ling’s association-induction model of the cell. However, recently it has been found that a “calcium channel protein” contains a cysteine group that ionizes during the molecule’s change of state. (Am J Physiol 1997 Jul;273(1 Pt 1):C230-8, “Possible thiol group involvement in intracellular pH effect on low-conductance Ca(2+)-dependent K+ channels,”Riquelme G, Diaz M, Sepulveda FV.)

Gradually, the idea of allosteric regulatory molecules that are altered by the reversible binding of regulatory substances has gained common acceptance, but the tendency is still to look for these signal receptors at the cell membrane and in association with the control of gene expression. But the cell filaments that make up the cytoskeleton are now known to form continuous systems from the cell surface, through the nuclear membrane, and into the vicinity of the chromosomes. These various filaments have “membrane-like” properties, allowing them to act at, and across, phase boundaries, but also making them sensitive to subtle changes in their environment, such as temperature, ionic balance, and the presence of fatty materials and materials combining various degrees of polarity in their structure; for example, the extremely toxic bacterial endotoxins are lipopolysaccharides, that derive their unique toxicity from the combination of fat and sugar in the same molecule.

For many years, the enzymes of glycolysis were the paradigm for the idea of random interactions between enzymes and their substrates, the materials they catalyze. They were thought to be the most random elements in a randomly organized system. Although it has been over ten years since Sidney Bernhard showed that these enzymes don’t wait for their substrates to randomly diffuse into their active sites, this important fact is still generally ignored. (Others, from 1940 to 1998, have reported evidence that the enzymes of glycolysis are “bound to the cell framework.”) The ordered behavior Bernhard demonstrated for these “most random” enzymes should be taken as a clue to the nature of other components of the cell.

Rather than having to transmit randomly received signals through random movements into the nucleus, the model of the cell that is implied by the work of Sidney Fox and Gilbert Ling is one in which “receptors” and “effectors” are distibuted throughout the cell substance. Rather than “feedback” of signals along channels of communication to processing centers, the processes of perception and response are distributed throughout a cooperative system, with the possiblity of response governing the process of judgment. There is intelligence in the system at every level, there is no coercion of stupid slave molecules. Fields, forms, associations, and movements all interact in a sensitive and responsive unity. At least they do in health.

In the process of an organism’s development, the cell’s form precedes its mature chemical functioning. The form depends on the internal framework, and that depends on the cell’s contact with a specific kind of extracellular material. The matrix governs the basic pattern of gene expression, acting through the structural elements. In aging and stress, the matrix tends to deteriorate progressively. The matrix, being outside the cell, isn’t constantly being

renewed as the cell itself is, but it can be enzymically repaired, if the enzymes are not inhibited. Being located between the bloodstream and the metabolizing cells, it is necessarily exposed to all circulating environmental toxins.

There is a functional continuity between the extracellular matrix and the expression of genes. (Weaver and Bissell, 1996; Pienta, et al., 1992.) This has been recognized for several decades by many researchers, but the doctrine of the cell membrane enclosing a watery solution has obstructed progress in this direction.

SOME IMPLICATIONS

There is a chemical continuum from volcanic conditions to nerve cell structures and functions. The chemical precursors of life, ammonia, acetic acid, pyruvic acid, and amino acids are formed under volcanic conditions of high temperature and pressure. (Gunter Wachtershauser, H. J. Morowitz, R. M. Hazen, and J. A. Brandes.) Under slightly milder conditions, amino acids spontaneously form proteins and self-replicating bacteria-like structures. (Sidney Fox, in the 1960s; in the July 31, 1998 issue of Science, Wachtershauser reported the synthesis of peptides, in a weirdly feeble parody of the work Fox did more than 30 years earlier.) Fox's spontaneously formed proteins improve nerve cell function–memory (in mice), and in vitro growth, survival (increased 250%) and function, suggesting their functional similarity to ancient natural cellular proteins. Natural proteins become modified during development and stress, and these new primitive proteins might be interpreted by the cell as embryonic proteins, temporarily refreshing some cellular processes. The spontaneously formed protein structures are stable in warm water, and dissolve in cold water; microtubules and other fundamental cell components also "dissolve" when cooled. Formed in a hot environment, the synthetic proteins are biologically very compatible materials.

In this perspective, there is no point in which one has to insert an "assumption of randomness" into the process of cell formation or functioning. The old idea of randomly arranged material, being ordered by the accumulation of random changes, was an idea that derived from the old concept of a watchmaker god inserting order into formless matter. In this more realistic perspective, the significant issue is what happens when disorder is introduced into the ordered cellular system. The introduction of disorder is a stimulus, a challenge to respond and to adapt. Excitation and assimilation, or excitotoxicity and degeneration, are two kinds of response to the introduction of disorder. Although the creation of order is a spontaneous tendency of the molecules, the introduction of disorder causes energetic changes that lead to the creation of a new order. The achievement of a new order builds on the old, emerges from the old, but contains the old order implicitly. The implicit presence of old structures accounts for the phenomena of memory, imprinting, transgenerational influences, and the recapitulation of phylogeny in development.

In the Randle effect (it's called the "Randle cycle," but there is no cycle), increasing the amount of fat in the bloodstream decreases the ability of cells to metabolize glucose; glucose tolerance decreases, as in diabetes, except that the response to fat is instantaneous. Respiration decreases, mitochondria retain calcium, which tends to accumulate until it destroys the mitochondria. The calcium, when it is released from the mitochondria, causes excitation to increase. Stimulation without efficient energy production leads to proteolysis and apoptosis or other forms of cell death. Sugars replace carbon dioxide and acetate on lysines. This process is involved in diabetes, Alzheimer's disease, arthritis, and other degenerative diseases, probably including osteoporosis. Mitochondrial damage tends to increase the

production of lactic acid instead of carbon dioxide, and lactic acid can stimulate the inappropriate overgrowth of blood vessels, as occurs in the eyes in diabetes. During stress and aging, free fatty acids appear in the bloodstream in large quantities.

Besides their chemical effects, which lead indirectly to chronic disruption of signalling systems, the unsaturated fats have direct and immediate effects on regulatory processes, water uptake, intercellular communication, and excitation. Cell proteins have an affinity for fats, and their hydrophobic surfaces tend to adsorb them. Unsaturated fats have a greater affinity for water than saturated fats do, and the location of the unsaturated bonds along the fat's carbon chain will affect the ways proteins interact with water. The fact that animal cells synthesize only fatty acids with a chain of eight fully saturated carbon atoms in their tails undoubtedly has something to do with the toxic effects of other unsaturated fats on the respiratory apparatus.

The unsaturated fats that are so systematically disruptive to warm-blooded animals are characteristically produced in plants at relatively low temperatures. In organisms that live at low temperatures, they probably serve a function (among others) that is analogous to the function of estrogen in warm animals, namely, raising the "structural temperature" of water, modifying chemical activity by liberating water to some extent from the domination of the cellular proteins.

One of the old theories of aging was that something (they called it metaplasm) accumulated in cells as a result of metabolism, the way ashes accumulate in a stove. Lipofuscin, or age-pigment, is related to the oxidation of unsaturated fats, and has been proposed to be such a material, that progressively limits a cell's adaptive capacity because of its physical and chemical properties. Amyloid, a clear mass of protein deposited in and around cells, is another such age or stress-related material, that is currently being studied in Alzheimer's disease and other degenerative diseases. Glycation, the attachment of sugars to groups that otherwise could be occupied by carbon dioxide, seems to be a crucial factor in the formation of amyloid. (The term "amyloid," in fact means "starch-like.") Changes in the extracellular matrix, for example the cross-linking of collagen molecules, have been thought to cause some of the characteristic changes of aging, and again, glycation is the major mechanism in the formation of cross-links.

In Alzheimer's disease, the commonly recognized features are tangles, amyloid deposits, hypometabolism, and evidence of inflammatory processes. Cells related to inflammation can produce amyloid, as well as remove it. Glycation, the attachment of sugar molecules to proteins, can happen quickly, and can occur either with or without enzyme catalysis. The failure of glucose consumption and of carbon dioxide production in Alzheimer's disease predisposes to glycation.

Glycation imitates mutated forms of proteins, for example normal transthyretin behaves like the prion protein, forming amyloid. Transthyretin, the protein that carries thyroid hormone and vitamin A, is normally taken up along with cholesterol under the influence of thyroid hormone. Abnormal cholesterol metabolism is one of the traits associated with Alzheimer's disease. In the absence of thyroid-supported respiration, carbon dioxide and other respiration-associated molecules (e.g., acetate) are replaced by lactate and unused sugar, causing abnormal modifications of proteins such as tau, which regulates microtubule assembly. Glycation of collagen in the extracellular matrix alters the properties of the matrix. The glycated matrix would become a preferred site for glycated prion-like proteins.

It is possible that the altered transthyretin makes vitamin A less available to cells. Vitamin A

deficiency creates major disruption of the framework proteins. Fragments of starch molecules inhibit the enzymes that remove inappropriately bound sugar molecules from proteins, and the inability to metabolize sugar into carbon dioxide increases that binding. Starches and unsaturated fats cooperate in this process of inappropriate sugar binding, while thyroid hormone, and the carbon dioxide it produces, tend to prevent the binding.

Considering the universal importance of carbon dioxide to life, the ways it interacts with all of the important substances that make up organisms, that it is involved closely with ATP synthesis and other “energy-related” processes, that it participates intimately in the regulation of water and ions, that it is therapeutic in a range of conditions including angina pectoris, hypoxia, epilepsy, inflammation, shock, lipid peroxidation, pneumonia, and asthma, I think we can at least conclude that it is a largely overlooked mediator between chemical energy and life processes. In many cases, its movements and reactions constitute the actual motive force that so many fantasy theories have failed to explain. In other situations, it fills out the context for understanding the energy-mediating actions of ATP, calcium, and hormones.

In the special arrangement of matter that is the living state, in which the most common events involve processes that are so close to equilibrium that some of them can be thought of as oscillations in an elastic system, carbon dioxide participates in both enzymic and nonenzymic reactions that produce, conserve, transfer, and transform energy. In its quickly reversible binding to protein amino groups, for example, it alters the protein’s electrical charge, its folding, and its manner of associating with water and other substances. Its availablility to occupy these groups protects them from attack by substances that would degrade the organism’s energy and structure. If the protein, water, ionic system is thought of as energized matter, like a wound-up watch spring, it is the formation of carbon dioxide which has energized it and stabilized it.

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Ukr Biokhim Zh 1995 May-Jun;67(3):84-92, [Role of low molecular weight metabolites as natural regulators of metabolism]. Mel’nychuk DO, Mykhailovs’kyi VO.

The paper presents results of scientific activity of the Department of Metabolism Regulation. The main sections are: carbamates formation and their role in metabolism regulation; metabolic system of acid-base homeostasis in animals; polyamines metabolism in the extremal states; mechanisms of metabolic adaptation in mammals. Experimental data are presented which evidence for the fact that tissue proteins in vivo are subjected to nonenzymic carboxylation with formation of carbominic groups. In this case a charge variation in definite sites of protein molecule is observed, which specifies variation of the protein conformation and biological properties. Basic regularities of protein carbamate formation reactions are revealed with factors affecting their intensity. It is shown that the presence of carbonic acid in the medium increases the rate of reactions catalyzed with lactate dehydrogenase from the rabbit liver, glucose-6-phosphate dehydrogenase from yeast and trypsin. Under the same conditions the reaction velocity rate catalyzed with glucose-6-phosphate dehydrogenase from the rabbit liver and with ATP-citrate (pro-35)-liase is considerably decreased. Changes in the concentration of carbonic acid within the physiological limits are found to have no effect on lactate dehydrogenase from the cattle heart and chymotrypsin. The rate of the reaction catalyzed by NAD-dependent malate denydrogenase was studied as affected by carbon dioxide. It is shown that acceleration of

the catalysis in these systems depends on the presence of both a bicarbonate anion and soluble carbon dioxide. IR spectra of NAD-dependent malate dehydrogenase in the deuterium oxide solutions were studied in the CO2-free solutions and solutions saturated with it.

Formation of peptides from amino acids by single or multiple additions of ATP to suspensions of nucleoproteinoid microparticles. Nakashima T; Fox SW, Biossystems, 1981, 14:2, 151-61.

“When lysine-rich proteinoid, which catalyzes the formation of peptides from amino acids and ATP, is complexed with acidic proteinoid to form microspheres of mixed constitution, the normal synthesis by basic proteinoid alone is multiplied several-fold. The product consists not only of small peptides but also of a high-molecular-weight fraction of substituted proteinoid. Suspensions of particles of lysine-rich proteinoid complexed with polyadenylic acid catalyze the synthesis of peptides from each of the amino acids tested with ATP.”

Compartmentalization in proteinoid microspheres. Brooke S; Fox SW. Biosystems, 1977 Jun, 9:1, 1-22.

Interactions between diverse proteinoids and microspheres in simulation of primordial evolution. Hsu LL; Fox SW. Biosystems, 1976 Jul, 8:2, 89-101.

Experiments demonstrating an incorporation of different enzymelike activities into a single preparation of proteinoid microspheres provide a conceptual basis for the primitive lengthening of protometabolic pathways. An enhancement of one enzymelike activity by another proteinoid in the same microsphere has been found. This effect, plus the pathway-lengthening propensity of combinations of microspheres, indicates selective advantages contributing to adaptive protoselection. Data reported in this paper also bring into purview the concept of internally controlled variation. Inferences are derived for the origin of protosexuality in protocells. When allowance is made for a closer relationship to the environment than that needed in contemporary selection, the fundamental mechanistic requirements of protoevolution are regarded as met by the proteinoid microsphere.

Q Rev Biol 1991 Jun;66(2):181-5. Synthesis of life in the lab? Defining a protoliving system. Fox, S.W. Department of Plant Biology, Southern Illinois University, Carbondale 62901-6509.

“The synthesis of a living system in the lab has been judged by a number of critics as partly attained by the proteinoid microsphere because of its primitive properties of metabolism, growth, and reproduction. These same critics, however, judge the organism as not alive, or as being 50 to 75 percent alive (Baltscheffsky and Jurka, 1984), owing to the absence of a nucleic acid genetic coding mechanism. The experiments in retracing evolution suggest, however, that the self-sequencing of amino acids was the evolutionary precursor of modern nucleic acid templating; the genetic memory is the molecule. The proteinoid microsphere is not a modern living system, but does represent at least a protoliving system (Fox and Dose, 1972). Berra (1990, p. 75) has commented on other difficulties in defining a protoliving system. In Berra’s opinion, metabolism, reproduction, responsiveness to stimuli, and cellularity constitute or describe aliveness. These properties characterize proteinoid microspheres.”

Brain Res 1991 Feb 15;541(2):273-83. Promotion of neuronal survival in vitro by thermal proteins and poly(dicarboxylic)amino acids. Hefti F, Junard EO, Knusel B,

Strauss WL, Strang PF, Przybylski A, Vaughan G, Fox SW. Andrus Gerontology Center, University of Southern California, Los Angeles 90089.

Evaluating molecules for their ability to promote survival and growth of neurons, we tested thermal proteins on cultures of dissociated fetal rat forebrain neurons. (Thermal proteins are polyamino acids formed when mixtures of amino acids with minimal proportions of glutamic or aspartic acid are heated.) Thermal proteins, added to low-density cultures in serum-free medium, stimulated neurite outgrowth and induced the formation of neuronal networks which survived for 6-10 days. Neurons in control cultures failed to grow and degenerated completely within 2-4 days. Effective concentrations (EC50) of thermal proteins ranged from 3 to 100 micrograms/ml. They were equally effective when present in the medium during the culture time or after precoating of the culture dishes. A single preparation which contained only aspartic and glutamic acid was effective, and similar survival promoting actions were then found for polyglutamic acid and mixed polyamino acids containing glutamic or aspartic acid. Thermal proteins and polyglutamic acid acted in a specific manner since, under the same experimental conditions, many control peptides, proteins and growth hormones failed to promote survival of neurons. Furthermore, their effects were antagonized by heparin, but not heparan sulfate nor chondroitin sulfate. These findings suggest that sequences of successive dicarboxylic amino acid residues are able to promote survival and neurite elongation of cultured neurons and that such sequences are responsible for the survival promoting action of thermal proteins. They invite the speculation that sequences of successive dicarboxylic amino acids, while occur in many proteins and show a high degree of evolutionary conservation, may have functional role in molecular recognition processes during neuronal development.

Proteinoid microspheres more stable in hot than in cold water. Syren RM; Sanjur A; Fox SW Biosystems, 1985, 17:4, 275-80.

“Experimental examination of the question of whether some proteinoid microspheres might be stable in hot water has revealed proteinoids that are soluble in cold water but precipitate on heating.”

From proteinoid microsphere to contemporary cell: formation of internucleotide and peptide bonds by proteinoid particles. Fox SW; Jungck JR; Nakashima T. Orig Life, 1974 Jan-Apr, 5:1, 227-37.

A model for the origin of stable protocells in a primitive alkaline ocean. Snyder WD; Foxm S.W. Biosystems, 1975 Oct, 7:2, 222-9.

“When a mixture of the eighteen proteinous amino acids are suitably heated in the dry state with seawater salts, a copolyamino acid results.” “When one fraction of the seawater proteinoid is dissolved in hot water, and the solution is cooled, proteinoid microspheres result.” “These processes thus constitute a simple model for the origin of a protocell stable in a primitive alkaline ocean.”

Membrane, action, and oscillatory potentials in simulated protocells. Przybylski AT; Stratten WP; Syren RM; Fox, S.W. Naturwissenschaften, 1982 Dec, 69:12, 561-3.

“Electrical membrane potentials, oscillations, and action potentials are observed in proteinoid microspheres impaled with (3 M KC1) microelectrodes. Although effects are of greater magnitude when the vesicles contain glycerol and natural or synthetic lecithin, the results in the purely synthetic thermal protein structures are substantial, attaining 20 mV amplitude in some cases. The results add the property of electrical potential to the other known properties of proteinoid microspheres, in their role as models for protocells.”

Synthesis of peptides from amino acids and ATP with lysine-rich proteinoid. Nakashima T; Fox, S.W. J Mol Evol, 1980 May, 15:2, 161-8.

“Lysine-rich proteinoids in aqueous solution catalyze the formation of peptides from free amino acids and ATP.”

Self-sequencing of amino acids and origins of polyfunctional protocells. Fox, S.W. Orig Life, 1984, 14:1-4, 485-8.

The primal role of the origins of proteins in molecular evolution is discussed. On the basis of this premise, the significance of the experimentally established self-sequencing of amino acids under simulated geological conditions is explained as due to the fact that the products are highly nonrandom and accordingly contain many kinds of information. When such thermal proteins are aggregated into laboratory protocells, an action that occurs readily, the resultant protocells also contain many kinds of information. Residue-by-residue order, enzymic activities, and lipid quality accordingly occur within each preparation of proteinoid (thermal protein). In this paper are reviewed briefly the phenomenon of self-sequencing of amino acids, its relationship to evolutionary processes, other significance of such self-ordering, and the experimental evidence for original polyfunctional protocells.

The evolutionary significance of phase-separated microsystems. Fox SW Orig Life, 1976 Jan, 7:1, 49-68.

The source, preparation, and properties of phase-separated systems such as lipid layers, coacervate droplets, sulphobes, and proteinoid microspheres are reviewed. These microsystems are of interest as partial models for the cell and as partial or total models for the protocell. Conceptual benefits from study of such models are: clues to experiments on origins, insights into principles of action and, in some instances, presumable models of the origin of the protocell. The benefits to evolution of organized chemical units are many, and can in part be analyzed. Ease of formation suggests that such units would have arisen early in primondiae organic evolution. Integration of these various concepts and the results of consequent experiments have contributed to the developing theory of the origins of primordial and of contemporary life.

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