

Water Structure and Bioenergetics

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Introductory Remarks by Chairman

Strange as it may seem to the soils engineer, soil science is usually listed as belonging to the biological sciences. Even if we do not completely subscribe to the propriety of such classification, natural soil systems represent a meeting ground par excellence between animate and inanimate nature. In addition, the non-living as well as the living components of soil normally contain an appreciable percentage of water and owe their properties in a large degree to the interaction of their well-developed internal surfaces with the water substance. Dr. Albert Szent-Györgyi, an eminent physiologist and Nobel Laureate, has kindly prepared the contribution on "Water Structure and Bioenergetics." Since no unique "life principles" are invoked in this treatise, the facts and conclusions presented hold true for non-living mineral soil systems the same way as they do for organic living or dead systems. They are, therefore, of greatest pertinence to the soil scientist and engineer that really wants to understand what goes on between soil and water.

● LIFE WAS born in water and is carrying on in water, 80 percent of our own body being water. Water molecules have unique properties, and it seems most unlikely that life should not have made use of them. Bio-chemistry has recognized the central role played by water in bioenergetics. Water is the fuel of life. What the sunshine (which is the ultimate source of our energies) does, is to separate the elements of water. The gradual approach of these elements, H and O, and their eventual union to H₂O with the gradual decrease in free energy is what is moving life. While admitting the role of water in energetics, biology failed to concede any role to water in the machinery of life and looked upon water as a more or less inert matter filling the space between the single parts of that machinery. This outlook on water had two main-springs: the nature of coulombic forces and macroscopic experience. Electric forces from charged points decrease with the square of the distance, and so the orienting effect of charged points on the water dipoles could reduce the mobility of water molecules only in layers of no more than a few molecules deep. Our every-day macroscopic experience is that water, at room- or body-temperature, is a random fluid, unable to build structures. The fallacy of this impression is born out by the fact that a decrease of its kinetic energy by no more than 10 percent, a cooling from room temperature to - 1 C, i. e. from 293 K to 272 deg, suffices to turn water into a rigid solid with a most regular crystalline structure. This freezing is permanently going on, also at room temperature, only the crystal lattice thus formed is smashed up by heat agitation and so the size of the crystals formed does not exceed molecular dimensions. However, the balance between heat agitation and the trend to unite to solid and regular structure needs to be shifted but slightly to lead to extensive lattice formation. This lattice formation is due to the H-bonds established between the H-s and the non-bonded ("lone pairs" of) electrons of the different water molecules. So if a solid surface binds and immobilizes one layer of water molecules, this layer will protect its neighbors both parallel and normal to the surface from the heat agitation present in the bulk. This will stabilize the H-bonds. This mutual protection from heat agitation will be cooperative and thus can be expected to lead to the formation of extensive lattices, reaching under favorable conditions thousands of molecules deep into the fluid, and if another parallel surface is found within this distance, the water can be expected to freeze up altogether between the two surfaces. So, in closely packed biological structures where the distance

between the various macromolecules is of the order of 100 \AA , we can expect to find "ice", lattice-ordered water, even if this ice does not share the rigidity of common ice. Even common ice is deformable, its bonds being easily broken and rebuilt, as can be demonstrated by the "dropping" of a steel ball through ice. This property, deformability, will be present in increased degree in the "ice" of our body, which will offer no considerable resistance to deformation or the diffusion of smaller ions.

A distinction must be made between two water structures. The one corresponds to the idea of the "bound water" which has occupied many a research worker in the past. This bound water is due to the attraction of charged points of the surface on the water dipoles. Coulombic forces fading out rapidly with the distance, these layers can be but very thin. Proteins, for instance, can appreciably immobilize, as a rule, a layer of water but one molecule deep. However, this immobilization of the water in the immediate vicinity of the surface may upset the subtle balance between the ordering influence of the inherent qualities of the water molecule and the disordering action of heat agitation, and may lead through a cooperative action to the formation of lattices reaching deep into the fluid.

This ice-formation belongs in no way in the realm of speculation, and it is strange that biology should have taken no notice of it. Henniker and MacBain collected, already ten years ago, 175 references to papers dealing with multi-layers around surfaces (5). Frank and Evans were led by entropy calculations to the conclusions that even dissolved molecules may be surrounded by "icebergs" (4). Bernal and Fowler took X-ray pictures of water a quarter of a century ago, introducing the idea of "structure temperature", by which they meant the temperature at which common water would have the same degree of structuredness as has the water around the surface in question (2). In this scale, most of the intracellular water is probably around 0 C .

In the last twenty years, the author was interested in the working muscle and could demonstrate that the contractile matter is a complex protein, actomyosin, which can be extracted, given the shape of a muscle fiber and made to contract by ions and ATP (adenosine triphosphate). Though mammalian muscle contains 10 percent actomyosin, owing to the great hydrophilicity of this protein, it is difficult to prepare actomyosin solution containing less than 97 percent water. Such an actomyosin solution, at physiological ionic concentration, forms a rigid gel. It is difficult to explain this strong binding of water in any other way than by the formation of "icebergs", water lattices formed around the fibrous actomyosin particles. On addition of ATP, this water structure collapses, and the actomyosin becomes a practically anhydrous material. This collapse of the water structure declares itself to the observer as a contraction of the actomyosin thread. If, within this thread, the actomyosin filaments had a random distribution, then the contraction takes place in every direction, and what we see is an extensive shrinking. If the actomyosin filaments were ordered parallel to the axis of the thread, then this latter becomes shorter only, behaving very similarly to contracting muscle. Essentially, this change observed in an actomyosin thread is identical with muscular contraction which is intimately connected with the collapse of a water structure, while the relaxation of the contracted muscle has to be connected with the rebuilding of the collapsed water lattices. The structures induced in the surrounding water by the actomyosin particles are thus just as much a part of the contractile machinery as the protein itself; the two make one single system. What is true of the generation of motion, one of the oldest and most basic signs of life, is probably true, also, for the other biological functions.

The assumption of water structures makes many biological phenomena appear in a new light. One of the most basic phenomena of biology, for instance, is semi-permeability. This was hitherto explained by holes in the membrane, or by the presence of "careers" within the membrane. It seems likely that the membrane, with its pores, is covered by a sheet of "ice" while the substance which it has to let through is surrounded by an "iceberg". The latter's transmission through the membrane will thus depend less on the relative size of the hole and the permeating molecule or ion than on the nature of the two ices, the one covering the membrane and the one surrounding the dissolved particle. If the two ices are similar in structure and can fuse, then only will the substance be able to penetrate. The ice inside the cell will also resist the admission of

ions which disturb its structure, and it has been shown that the specific permeability of cells to potassium, as compared to Na, might be also explained by the lack of disordering effect in K^+ , and the disordering action of Na^+ (1).

If a $10^{-4}M$ watery solution of Rhodamin B is frozen in dry ice, it shows practically no light emission, if observed in near ultraviolet light. The situation is not altered by the presence of 0.1 M KCL, but in presence of 0.1 M NaCl, an intense light emission is observed, coming from a triplet excitation of the dimer of the dye. The presence of Na-ions thus makes an electronic transition probable which was improbable in its absence, the transition from the excited triplet state into the ground state under emission of photons being "forbidden" by the selection rules. This perturbation is, in all probability, due to the disorder-producing effect of Na^+ within the thin sheet of lattice ordered water surrounding the water crystals. This simple experiment suggests an intimate relation between the excited state, transition probabilities and the lattice ordered nature of water, and it is not impossible that these water lattices are also instrumental in the energy transmission in biological processes. They may also make "proton conduction" possible, holding the water molecules in an orientation favorable for the transmission of H^+ from one molecule to the other.

Lattice ordered multilayers are also found in systems where no coulombic forces can be involved in the immobilization of the fluid around the surface in question. This is the case, for instance, when the liquid phase consists of a non-polar substance, as is the case with lubricants. We can expect that any solid surface will protect the adjacent liquid layer from heat agitation and thus immobilize it. That this is the case is shown by the fact that in streaming fluids, the layers adjacent to the solid surface are stationary. So we can also expect that around protein structures the water will be immobilized even at points where the protein has no charges. As shown by Rodenbush and Buswell at this symposium, the lattices, thus formed around non-polar groups will be different from the lattices formed around polar groups or formed in the bulk of water on cooling. This complexity of the situation, with its very subtle equilibria, makes conditions most colorful and begins to resemble the subtle complexity which characterizes life.

REFERENCES

1. S. L. Baird, G. Karreman, H. Mueller and A. Szent-Györgyi, Proc., National Academy of Sciences, 43, 705, (1957).
2. J. D. Bernal and R. H. Fowler, J. Chem. Phys. 1, 515, (1933).
3. W. H. Rodenbush and A. M. Buswell. This symposium, Meeting in Ottawa, Canada, on 9 Oct. 1957.
4. H. S. Frank and M. W. Evans, J. Chem. Phys. 13, 507, (1945).
5. J. C. Henniker and J. W. MacBain, "The Depth of a Surface Zone of a Liquid". Technical Report N6 ori 154 T. O. 11, Stanford Research Institute, Stanford, Calif., (1948).