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The Fröhlich Model and DNA Studies.

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DISSIPATIVITY AND ORGANIZATION IN LIVING MATTER: A QUANTUM FIELD THEORY APPROACH

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Introduction

For a long time there has been the widespread belief that the second principle of thermodynamics would have posed a major barrier to the spontaneous appearance of ordered structures in matter. In particular, thermodynamics would have prevented the natural evolution from prebiotic matter to living matter. This point of view, however, has been shaken when far from equilibrium (non linear) processes have been taken into account and thermodynamics of irreversible processes has began to be formulated. It has been recognized that open systems kept far from thermodynamic equilibrium by an external flow of energy can organize themselves spontaneously, exhibiting definite ordered patterns in space or in space and time.

Ilya Prigogine and the Brussels school investigated a number of general conditions which ensure the possibility of self-organization in matter.(1) This line of investigation, however, aims to outline the very general structures which are mathematically possible in the framework of a general theory of dynamical systems. A complementary approach investigates the microscopic mechanisms responsible for such ordering processes pointing out how atoms, molecules and their interactions cooperate to build up ordered structures out of the initial random molecular distribution.(2,3).

Atoms, molecules and their interactions are successfully described by quantum theory. Furthermore, quantum field theory has proven to be the most effective tool for describing systems with so many degrees of freedom that they can be phenomenologically understood only in terms of a few microscopic parameters obtained through averages on a number of microscopic variables.

Quantum field theory (Q.F.T.) describes the interactions among the elementary constituents by "quasi-particles". These quasi particles represent the collective dynamics responsible for the difference between the initial set of unordered elementary objects (atoms, molecules) and the resulting ordered structures. Examples of such quasi-particles are phonons in crystals, plasmons and so on. Furthermore, it should be noticed that a collective structure appears as less symmetric than the unordered set of its elementary constituents. For instance, a gas of atoms interacting according to the fundamental laws of electromagnetism exhibits a rotational invariance. However, when a collective interaction among the atoms produces an (ordered) crystal, the original rotational invariance usually disappears and is replaced by some discrete symmetry. Order has been produced at expense of symmetry.

Quantum Field Theory actually provides a possible dynamical scheme for this complementarity between symmetry and order (4). The spontaneous loss of symmetry implies the appearance of boson massless modes or "quasi-particles" which act as the messengers necessary to establish the correlation among the elementary constituents. In this way they are the building blocks of a collective structure.

It would be interesting to provide a microscopic analysis of living matter along the lines elucidated above. Living matter is basically a dielectrics, namely a set of electric dipoles, with peculiar properties since the large number of elementary components gives rise to high inhomogeneities in the dielectric parameters. Our aim is to extract as much information as possible from general symmetry principles without going into details, yet almost unknown, of the dynamic equations. The mathematical treatment is presented in Ref.5. Here we limit ourselves to a purely qualitative approach.

Electric polarization waves as a result of the dynamical rearrangement of symmetry

A living system can be schematized as a set of many macromolecular electric dipoles embedded into water, i.e. into another set of electric dipoles. We consider water as the ground state ("vacuum") of our system and we describe the excitations of the macromolecular dipoles by Heisenberg field operators Ψ_i's. The macromolecular dipole interactions will be described by a Lagrangian whose explicit form cannot be specified, but can be expected to be rotationally invariant too, the system will be a conservative one. Suppose on the contrary that our vacuum, namely water, depend upon a preferred direction, breaking the initial rotational symmetry. This could occur for instance, in the presence of a water electret; a net electric polarization P has been actually observed around almost all biomolecules and measurements on the hydration of the molecules allow to attribute it to water surrounding the biomolecules. (6).

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The existence of a rotationally non invariant vacuum induces via Goldstone theorem a dynamical rearrangement of the symmetry of the system. Let us recall the Goldstone theorem. (7) Suppose a system be described by a Lagrangian dependent upon a certain set of fields ϕ_i, the Lagrangian be invariant under a certain group of transformations G. Suppose now that the ground state (vacuum) of the system be non invariant under the same group; that means that there exists some fields ϕ_i which does not annihilate the vacuum or in other words whose average value on the vacuum is nonzero.

$$\langle 0|\phi_i|0\rangle \neq 0$$

The Goldstone theorem states that in such a case the physical spectrum of the system must include states degenerate with the vacuum (zero mass states) whose quantum numbers correspond to the physical quantity non conserved by the vacuum. The flow of quantum numbers carried by these Goldstone bosons take in care the seeming nonconservation of the same quantum number by the original system. Notice that for the validity of the theorem gauge field must be absent.

In order to take care of the original dipole rotational invariance, which is broken when the electret state arises, massless boson modes must appear which carry the quantum number corresponding to the broken symmetry. In our case, the rotational tridimensional symmetry is reduced to a rotational symmetry around the electret axis so that two massless bosons describing electric dipole waves would appear. Moreover, by general theorems the symmetry dynamical rearrangement gives rise to an "observable symmetry" of the same dimension of the original one. (8) In our case the two broken rotations transform themselves into translations in the space of fields (i.e. $B \to B + c$). This invariance implies that an extended homogeneous condensation of the boson Goldstone quanta takes place.

The above considerations hold in the case of an infinite volume. In that case the electric dipole waves would appear spontaneously, namely without expense of energy. But a real living system has a finite size, so that the above symmetry transformation is not global but local, occurring inside a finite region, whereas the symmetry is preserved outside. In this case the boson dipole quanta are confined inside the finite region and this is in contrast with a zero mass. The quanta acquire a mass inversely proportional to the size of the involved region. Consequently the appearance of the dipole waves requires some initial expense of energy, i.e. a threshold equal to the boson mass. It is interesting to point out that the smaller the system, the higher the threshold. If these bosons are the correlating agents which bring about coherence into the system, the above result shows that an energy supply is required. Moreover the non invariance of the vacuum ensures that the quantum number carried by such quanta is not conserved by the original system. We can conclude that flow of such quantum number, and consequently the flow of energy carried by the corresponding bosons, must traverse the system. In conclusion the macroscopic property of thermodynamic dissipativity at a microscopic level appears as the consequence of a spontaneous symmetry breakdown. The rotational invariance of the set of macromolecule electric dipoles is broken by a perturbation that introduces a preferred direction in the dynamics of the system. The fact that the living matter is built up by electric dipoles, i.e. living matter is a dielectric, implies that the energy flow required by the dissipativity is realized by giant electric polarization waves, corresponding to extended condensations of boson quanta. The existence of such waves has been predicted in 1968 by Herbert Fröhlich. (9) The argument discussed here shows that these waves are a very general feature of living matter and thus are not dependent upon the specific form of the dynamic equations.

Solitons and symmetry breaking

We have seen in the best section that electric waves appear in a dissipative electric dipoles set when an energy threshold is overcome, since the symmetry breaking element requires an expense of energy corresponding to that of the threshold. This energy is inversely proportional to the size of the involved region.

In the case of a metabolically active cell we can describe its life cycle as an interplay of a charge and a discharge regime. In the charge regime energy can be uptaken from the environment by specific biomolecular chains at definite localized sites as discussed by Davydov for muscle cells.(10,12) The energy produced by chemical reactions of small metabolites, which occur in proximity of the cell biopolymers, can be stored in form of a vibrational soliton on certain onedimensional chains of the system. Davydov has studied in detail the soliton dynamics on the -helix proteins. However, it seems likely that Davydov solitons can exist on a number of onedimensional molecular chains when a positive anharmonicity characterizes the dynamics of the molecular groups along the chain. (11) Spectroscopic evidence suggests that this is the case for α-helix proteins (12) as well as for DNA in B form (13,14) and other polymers.(15,16)

Energy stored in form of vibrational solitons can travel along molecular chains and gives rise to a number of biologically relevant implications. First of all it can be a very efficient carrier of energy at long distance in the

cell, overcoming the problem of bioenergetics as pointed out by Davydov. Moreover, while travelling, this soliton induces conformational changes in the chain that can be relevant for its biological function (possibly B→A transition of DNA) (13) . Such conformational modifications together with associated electronic changes in the system could in turn be responsible for the observed variations in the water surroundings the chain (for instance, changes in the hydration accompanying DNA conformational transition B→A) (13). Furthermore it is important to note that an electret state has been reported for a large number of biomolecules (proteins and nucleic acids) (6),i.e. a net electric polarization lasting for a long time, say some hours. It has been observed that this electret state, which is mainly due to the water molecules surrounding the biomolecule, requires an activation energy of approximately 0.3 eV per site. This value is of the same order of magnitude of the energy that can be trapped in a soliton form on α -helixes. Moreover, Davydov has shown (17) that a soliton travelling on a chain behaves as a moving potential well for loosely bound electrons. Consequently, a soliton would produce an electric current on the chain without further expense of energy. This current could induce an orientational ordering of the water which in the proximity of the chain is known to exhibit ordered structures, spatial order for bound water and yet a less defined order (possibly dynamical order) for "vicinal" water. (18) The interaction between the soliton induced current on the chain and the surrounding water could then be responsible for the appearance of a net electric polarization and of an electret state. The numerical equivalence between the energy trapped in soliton form and the activation energy of the electret supports the above hypothesis: no further expense of energy is required for the electret formation once the soliton and the associated electric current is produced in the chain. In cells the charge regime then transforms the metabolic energy output into a water electret, via the solitons on the chain. When enough energy has been stored in such an electret to reach the threshold for the onset of electric polarization waves, a different dynamic regime is established, where energy is discharged all through the system in form of the electric waves described in the last section.

We will sketch a possible role of such waves in the cell organization in the next section. Before completing this section we stress that the one presented here is one of the possible mechanism for getting the break-down of the rotational symmetry necessary for the appearance of the electric waves. A process leading to an electric polarization directed along some direction would be an useful starting point. The corresponding threshold is fixed

according to the size of the involved region. In non biological conditions ordering occurring in a system with macroscopic size would require a much small[illegible] energy threshold than in cells. In the former case, small fluctuations therefore could be at the onset of the ordering process provided that the fluctuation be along a preferred direction.

Organization in living systems as a result of the propagation of coherent electric waves.

A relevant aspect of dissipativity is the production of organization. In this section we like to examine at a molecular level the organization that is brought up in a biological system by the dissipative dynamics discussed above which leads to the appearance of coherent electric waves. It is important to investigate the propagation of such waves in the biological media, namely cell cytoplasm and intercellular media in the case of tissues. A model system for these media is a water solution of proper molecules: actin and tubulin in the cytoplasm, plasma proteins such as fibrinogen and albumin in the suspending medium of red blood cells. Notice that the biological media are non linear dielectrics. It has been found that very high electric fields are present in living cells up to 10^8 V/m .(3) For comparison it is interesting to note that atomic electric fields in semiconductors are about 10^9V/m and in dielectrics about 10^{11}V/m. Non linear effects should be therefore taken into consideration when biological systems are studied.

We investigate here the propagation of an electric coherent wave

$$E = \tfrac{1}{2}\vec{A}(r,t)\exp i(\omega_o t - \vec{k}_o.\vec{r}) + c.c. \qquad (1)$$

in an homogeneous aqueous solution of macromolecules taken as a model for the biological dielectrics. It will be seen that the propagation of the wave will induce inhomogeneities in this medium and possible formation of molecular structures. The medium, initially homogeneous, can be described by its refractive index n, which is a function of electric field E

$$n = n_o + n_2|A|^2 + n_4|A| + \ldots. \qquad (2)$$

n_o is the linear index of refraction. Therefore the refractive index of the medium will be different in presence or in absence of electric fields. If we consider only the lowest term in (2), it can be seen that the region of the medium traversed by the electric wave is optically denser when $n_2>0$.

Electric nonlinearities in the index of refraction of a system can be de-

tected at the lowest order ($\propto E^2$) by the Kerr effect, where birefringence is induced by an applied electric field. Positive birefringence corresponds to $n_2>0$, whereas negative birifringence to $n_2<0$. It is interesting to recall that in Kerr liquids with $n_2>0$ such as CS_2, CCl_4, nitrobenzene non linear effects in the propagation of electric waves have been observed such as self-focussing and self-trapping (19). The same behaviour is therefore expected for these biological media characterized by positive Kerr birefringence. Indeed this is the case for a number of relevant biomolecules. Positive birefringence has been observed at values of the applied electric field of the order of 10^4 V/m for aqueous solutions of G-actin and F-actin of short polymer length ($\lesssim 1.5\mu$m) (20). Actin accounts for the 20% of the total proteins contents in the cell and for the largest component in cell cytoplasm, and can therefore be considered responsible for non linearities of the cell refractive index. For intercellular suspending media of red blood erithrocytes, it has been found that fibrinogen, one of the most abundant blood proteins, also display positive birifringence with high Kerr constant. (21)

We consider now the consequences of the propagation of an electric coherent wave in a biological medium having a non linear refraction index with $n_2>0$. When the power of the electric field exceeds a critical value self-focusing of the beam can take place, namely the beam shrinks to a limiting diameter after a path in the medium of critical length

$$R_{nl} = \frac{a}{2}\sqrt{\frac{n_o}{n_2 |A|^2}} \qquad (3)$$

which is called effective self-focusing length (a is the diameter of the incoming beam). If non linear refraction compensates for the diffraction spreading the wave is self-trapped within a thin waveguide ("filament") where the medium is optically denser since $n_2>0$. (22-25)

It has been shown (22) that these self effects, self-focusing and self-trapping can take place only if the power of the electric field of the wave exceed a critical value given by

$$P_{cr} = \frac{(1.22)^2 \lambda^2 c}{256\, n_2} \qquad (4)$$

A typical critical power for self focusing in water is approximately 1 MW, in Kerr liquids such as CS_2 and benzene respectively 0.2 MW and 0.25 MW.

The penetration depth of the electric wave is greatly enhanced by self-focusing and self-trapping because the beam energy become concentrated on the small cross section of the "filament". A further enhancement of the penetration depth arises from the formation of a waveguide around the filament according to the following mechanism. The inhomogeneity in the dielectric permissivity induced by the beam self-focusing creates a force acting across the filament boundary on molecules present in the solution. It is known from the general theory of dielectrics that an electric field E and a magnetic field H acting in a medium with electric polarization P produces a force per unit volume:

$$\vec{F} = \rho\vec{E} + (\vec{P}.\vec{\nabla})\vec{E} + \frac{1}{c}\frac{\partial \vec{P}}{\partial t} \times \vec{H} \qquad (5)$$

Under very general assumption, which hold in the case considered here, the second term can be rewritten in order to show the explicit dependence upon the frequency of the wave and the typical vibrational frequencies of the molecules present in solution: (22)

$$(\vec{P}.\vec{\nabla})\vec{E} = \text{const.}\ \Sigma_k \left(\frac{\omega_{ok}^2 - \omega^2}{(\omega_{ok}^2-\omega^2)^2+\Gamma_k^2}\right) \nabla E^2 \qquad (6)$$

One realizes that in the non linear regime for very high values of E, the second term in eq.(5) is the dominant one because ∇E^2 becomes very high at the filament boundary. The coefficient of E^2 displays a typical resonant structure; when the frequency of the propagating electric wave matches one of the frequency of the vibrational spectrum of the molecules, the gradient force becomes very high. Such a force is positive when $\omega<\omega_{ok}$ and negative in the opposite case; small changes of ω can reverse the sign of the force.

A molecule with the appropriate frequency present in the solution, can be then strongly attracted toward the filament wave and a condensation of these molecules builds up at its boundary. Moreover, because of the magnetic field associated to the transverse part of the electric field E, the third term of eq.(5) introduces a longitudinal force acting on the concentrated molecules in the direction parallel to the filament axis.

The final result is that identical molecules tied on the filament surface are squeezed one against the other and this preassociation process is an important prerequisite for polymerization. If such a process actually occurs, the filament of energy produced by the propagating electric wave gets surrounded by a material filament constructed as a long chain polymer of the monomer molecule that has vibrational frequencies almost resonant with the one of the beam. This material filament behaves as a wave guide respect to the electric signal and further enhances its penetration depth.(26)

The above mechanism is a necessary consequence of the propagation of coherent electric polarization waves in a non linear medium.

Notice that these forces have been observed in many experiments in the framework of laser physics. A well collimated monocromatic laser beam has been observed to produce along its path condensation or rarefaction of molecules (isotope separation), or small sized bodies (laser induced levitation. (27,28)

Modern investigation on cell cytoplasm, on the other hand, has produced some evidence that the "optical mechanics" sketched above could be at work in living matter also. The cytoplasm of a living cell appears as a multiphase system with three separate networks of material filaments, along which many relevant biomolecules are concentrated and water circulating in the interstices.(29,30)Each network is built up by a peculiar protein: actin in the case of microfilaments, tubulins for microtubules. These filaments are not static structures but seem to be in a dynamical equilibrium, changing shape and size according to the general state of the cell. Length and thickness of the filaments are controlled by a polymerization-depolimerization mechanism maintained by a flow of monomers from the solution to the filament and vice-versa. Moreover, transport of material occurs along these filaments (31,32) both the monomers and other molecules involved in the cell biochemistry. Actually, cytoplasm microstructures seem to play the role of transporting belts in the cell chemical plant.(18) Furthermore all this cell architecture is disrupted when the cell dyes, hinting that it is maintained by the dynamical processes underlying the living states.

It is apparent the qualitative agreement of the observed behaviour of the cytoplasm microstructures with the "optical mechanical" consequences of the electric waves propagating in the cell medium as derived above. (26,35)

Additional evidence on the filamentation of the basic biological interaction mediated by the electric waves can be found also in the case of metabolically active human erythrocytes as recently reported. (34) Electron micrographs show that contractile filaments connect erythrocytes when they are aligned in aggregate forms called rouleaux. The appearance of material filaments has been found to depend upon the presence in the cell suspending medium of fibrinogen, a blood protein for which high positive Kerr birigringence has been observed. (21,35)

Structures and behaviour of the same kind can be observed also in the axon of neural cells. (32)

The reported evidence shows that coherent electric waves could play a key role in the cell biochemical organization. Coherent electric waves on the other hand have been shown to be the necessary consequence at a microscopic level of the macroscopic properties of dissipativities. These waves appear then the dynamic microscopic engine which transforms dissipativity into biochemical order.

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"IN VIVO" ORDERED STRUCTURES AS SEEN BY LASER RAMAN SPECTROSCOPY

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Abstract

Laser Raman Spectroscopy has been proposed and used to explore the inner dynamics of living matter. Sydney J. Webb has performed an extensive investigation of the Raman response of metabolically active cells. The striking result is that the pattern of Raman lines changes with time and a spectrum dependence upon the different phases of the metabolic cycle is observed. A number of difficulties in obtaining reproducibility of the spectra have been encountered. However, an analysis of the Raman lines in the high frequency range 300-3000 cm^{-1} has pointed out that a regular and common pattern is exhibited in all the observed spectra. All the lines are integer multiples, sums, or differences of a few fundamentals which appear in the first period of the life cycle. The actual values of the fundamental frequencies change from one cell type to another and seems to depend upon the supplied nutrients. This reproducible pattern which has been proposed to arise from a collective time dependent dynamic process, suggests that there is an underlying order to the observed Raman spectra.

A possible "in vivo" underlying order has been also recently suggested by the analysis of the Raman lines of E. coli in the low 5-200 cm^{-1} frequency region. A striking correspondence is observed between the low frequency lines of the cell and the Raman lines of the aminoacids that have been found attached to the E. coli DNA (mainly aspartic and glutammic acids). Since the strength of the Raman response of these aminoacids in microcrystalline form has been found to depend upon the degree of order of the sample, the analysis suggests that a time dependent ordering mechanism is at work in the cell, able to produce "in vivo" space-time ordered structures involving aminoacids.

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As in-vitro biochemical knowledge has grown regarding the structure and function of its large macromolecules which go to make up the anatomy of living cells so it has become apparent that, "in-vivo", these macromolecules form a specific somatic architecture in which (a) all molecular entities occupy specific positions in space, relative to one another; (b) function as complexes not as individuals and (c) at rates which far exceed those possible by random diffusion and collision mechanisms. In addition, each metabolic event occurs at a specific time in the lifetime of a cell and these form into time ordered series leading to the synthesis of proteins, nucleic acids and polysaccharides by the sequencial placement at a specific time of a specific aminoacid, nucleotide or sugar to a growing chain. This process, now known as the cell or metabolic time clock, is well established but its mechanism is not understood. To produce such order from a random assortment of "nutrient" molecules requires not only the use of energy but its use at a specific place at a specific time, i.e. some form of directional energy flow, and to affect the rapid rates of syntheses with the accuracy performed by a cell necessitates. moreover, an equally rapid method of transit for this energy. Since all the thermal functions of a cell may be severely altered by a small increase as from 4 to 5°C in temperature above an optimum of 37°C, "in vivo" physiological processes they appear to be isothermal ones driven by the accumulation of electrical charges present in the vibrational modes of the chemical groups of large macromolecules and complexes of them and the directional transit of this combined charge to vital sites in the cell at an appropriate time.

For many years Fröhlich[1] has studied theoretically the possible condensations of the oscillatory modes of large molecules and their importance in matters biological and thus has postulated that resonances of about 10^{11} Hz should exist. It has been shown[2] that such waves characterize the living dynamics under very general conditions in a quantum framework. In separate experimental science it has now been established, without reasonable doubt, that external millimeter microwave fields of between 40 and 150 GHz (i.e. 4×10^{10} to 1.5×10^{11} Hz) are able to alter "in-vivo" metabolic activities and the cell time clock [3,4,5,6] by a process that does not involve an elevation in temperature above 0.2°C; i.e. by some nonthermal process. Such experiments have shown, moreover, the biological activity of millimeter microwaves to be strongly dependent on their frequency with any given active frequency able to produce its effect only at a certain time, or times, during the lifetime of the cell. These findings suggest first: a resonance-like interaction occurs between the electromagnetic waves and the cell and second, the cell produces, and makes use of, specific vibrations only at particular times during its development. On the other hand a similar non thermal resonant effect has been recently observed[7] in the visible interval at the red frequency of 6328 Å. Just as in the microwave case the effect appears only when a certain (very low) intensity threshold is overcome.

Oscillations at $\sim2\times10^{11}$ Hz and above can, in principle, be observed by Raman spectroscopy and an extensive study of this possibility has been made by Webb and his colleagues over the past decade or so.[8] This study was not made originally to check any particular theoretical model on living matter, but to explore the physical nature of the oscillatory modes of macromolecules "in-vivo" since such studies had provided valuable new information regarding the structure of such molecules isolated from the cell. An excellent review of these biological works along with appropriate easily understood Raman theory has been published recently by Tu[9] who has spent many years researching the use of Raman spectroscopy to study the bioactivity of macromolecules.
Webb undertook the task of trying to adapt these findings and methods to intact living cells, mainly those of Bacillus megaterium and Escherichia coli, but some avian and mammalian cells also were studied. Unexpectedly with whole cells in a resting state, suspended in water or isotonic saline solutions, no Raman spectra were recorded; only when the cells were activated by the addition of an oxidizable nutrient which set their metabolism in operation did a spectrum appear. The implications from these observations were that a) in alive but not metabolizing cells an organization existed among cell molecules "in vivo" which rendered the whole cell Raman inactive and b) metabolism required and produced dinamically ordered structures responsible for the observed strength of the Raman response.

In his review of these works Webb[8] reported that a large number of mechanical technical problems involving the power and wavelength of the laser, scanning rates, and the exposure time of the cells to the laser had to be overcome before any spectrum could be observed but more important still was the preparation of the cells and control over their biological activities. Of greatest significance were the use of (a) cell populations in which their metabolic activities had been synchronized; (b) a master shaken culture of cells from which small 0.1 to 0.5 ml aliquots could be removed at appropriate times for a 3 to 5 minute exposure to the laser during a Raman scan; (c) a constant temperature control between the master culture's environment and that in the Raman sample chamber; (d) cell populations of not more than 1×10^{8} ml to avoid

cell deaths and lysis as the latter process produces a mixture of free macromolecules in the culture medium all of which are Raman active; (e) a culture medium of colourless inorganic salts containing a single oxidizable nutrient such as glucose and (f) a continuous monitoring of the total cell numbers and their size by Rayleigh scattering or the use of a Coulter counter. The latter is important since the total elastic scatter from any particulate suspension is a function of the number of particles/ml, their size and shape and their refractive index. Any suspension in which all or any of these parameters changes rapidly with time will produce violent changes in elastic scatter and equally violent erratic ups and downs near the laser line and on the Rayleigh wings of a Raman spectrum taken from particles in an aqueous suspension. It is unfortunate that in one particular reported research study,[10] due possibly to lack of experience, the authors ignored all of these warnings. They used an unwashed powder of a commercial preparation of dried yeast cells, made a slurry of them in H_2O at 100 mg/ml and made a 1 in 10 dilution of this slurry by placing a small aliquot of it on the top of a 0.45 ml Raman cuvette filled with H_2O and then measured immediately the elastic scatter and Raman scatter. Not surprisingly they observed violent changes in elastic scatter over a 3 or 4 min. period after the cells had been placed in the Raman cuvette and recorded a Raman spectrum which they claimed was due simply to the changing elastic scatter. As a result they concluded that coherence of the Fröhlich type did not exist and all of Webb's spectra were due to elastic scatter changes.

A 100 mg/ml of a dried yeast powder would, in H_2O, become 500 mg/ml wet weight of cells, i.e. it would be 50% cells, many of which would be dead, many would lyse and die, most would be in large clumps, and a large amount of culture nutrients and cell lytic debris would be released into the suspension. In such a system the debris and nutrients would yield a Raman spectrum and the rapid settling and break-up of clumps would produce erratic changes in elastic scatter, in addition some cells, using the lytic debris as nutrients would begin to grow and this may provide something to the Raman spectrum observed. With such error, therefore, in the microbiology alone that was employed no reasonable correlation could be made between the elastic and Raman scatter observed, certainly the whole concepts of coherence, nonlinear phenomena and so on, observed and calculated by many physical-biological researchers, could not be thrown out on the basis of this work as the authors say they should.[10]

The above work,[10] is discussed here because it is felt important that alternate or differing opinions must always be considered; for the time being, however, due to the lack of the proper procedures used in it, little value can be placed on it, except as an example of the possible artifacts that can occur

when whole cells are used. With this in mind, it is noteworthy that many researchers have failed to observe a Raman spectrum from resting or non-synchronized populations of cells, while others have recorded spectra, similar to those of Webb, from active E. coli[11] although their cell time scale was much longer. Moreover, analogous to the lack of Raman activity from resting bacterial cells, the strong Raman spectral lines of isolated chlorophyll have been shown to be absent from the spectra of intact algal cells even though these cells possess a large quantity of this material[12] suggesting, once again, that the Raman activity of "in-vivo" molecular complexes is different from that of isolated ones.

One of the main features of Webb's work was the discovery of the time dependence of the Raman spectra obtained from active cells. With accurate control over the cells lifetime from one culture to another, he found that a given spectrum appeared at a given time in the cell cycle and although some of these lines appeared at other times, the in-toto spectrum changed as the cells progressed through a single lifetime. These changes, however, were not random but proceeded in a definite repeatable pattern, which could be severely altered by respiratory inhibitors such as carbon monoxide or azides and indeed, by certain frequencies of millimeter microwaves. These findings alone reveal the spectra observed to be derived from metabolic activity and not via the elastic scatter from the surface of cells because this does not change significantly with time in cultures in which the cell numbers remain constant and no clumping or settling occurs. With this in mind the significant features of Webb's spectra are the following:

1) Two well separated groups of Raman lines could be recognized, one including frequencies higher than 300 cm^{-1}, the other frequencies lower than 200 cm^{-1}.
2) The lines of the first group have fixed frequencies, but their intensity is time dependent. Lines up to 900 cm^{-1} appeared between the 10th and the 25th minute of the cell life cycle; lines from 900 to 1500 cm^{-1} between the 25th and the 40th minute, lines above 1500 cm^{-1} in the final part of the life cycle.
3) The low frequency group of lines arises just after the appearance of some of the first high frequencies lines, i.e. after approximately 15 minutes of incubation time.

   Although the low frequency lines appeared to show a gradual move to lower frequencies with increasing time, the major effect was again a strong time dependence of their intensity.
4) Antistokes counterparts of the low frequency lines with essentially the same

intensity and the same time dependence have been observed. The corresponding intensity ratio between AntiStokes and Stokes lines was higher than the value expected at thermal equilibrium, well above the experimental uncertainties.

An analysis of the Raman lines in the frequency range 300-3000 cm^{-1} has revealed that a regular pattern exists in all observed spectra with all lines being integer multiples, sums or differences of a few fundamental frequencies which appear in the early part of the first period of the cells life cycle.[13] Moreover, these fundamentals vary from cell type to cell type and depend on the nature of the supplied nutrients, particularly the oxidizable carbon source. This reproducible pattern, which has been suggested to arise from a collective time dependent dynamic process[14] indicates an underlying order to the observed Raman spectra. A possible underlying order recently has been suggested also for the lines between 5 and 200 cm^{-1} as some of the low frequency lines seen in bacterial cell spectra have been found at essentially the same wave numbers in the spectra of aminoacids and proteins in crystalline and solid states although absent in the spectra of solutions of these macromolecules. Moreover, they are not present in spectra of highly disordered crystals so it seems, therefore, that these lines are connected with the existence of some kind of ordering[15,17] of molecules in the intact metabolically active cells.

To explore the nature of this ordering system it is perhaps pertinent to examine some of the general features of Raman scattering in systems with varying degrees of order as outlined in a previous paper.[18]

Raman effect is a very sensitive tool to look at the inner dynamics of a system through the detection of its vibrational states and of the corresponding populations. If the system has for instance two levels whose energy difference is $E_2-E_1=h\nu_o$, an impinging photon with an energy $E=h\nu$ ($\nu>>\nu_o$) may scatter elastically (Rayleigh scattering) or inelastically (Raman scattering) by inducing a transition between the levels. A transition $1\rightarrow2$ gives rise to a Stokes line with a scattering frequency $\nu-\nu_o$, while the opposite transition $2\rightarrow1$ gives rise to an Antistokes line with a frequency $\nu+\nu_o$. It is apparent that the Antistokes/Stokes intensities ratio measures the ratio between the population of the two levels. Moreover if the incoming radiation (Laser Raman Spectroscopy) is coherent, the degree of coherence of the Raman scattered radiation is just a measurement of the degree of coherence among the scatterers in the target. In the extreme case of a completely amorphous material the negative interference among the wavelets emitted by scatterers uncorrelated both spatially and dynamically produces a spectrum with very broad bands around the expected shift frequencies. A sharp peak spectrum on the contrary is got when the wavelets are allowed to add up constructively, as for instance in the case of a perfectly ordered crystal whose Raman spectrum coincides with the spectrum of its molecular constituents. A sharp line spectrum can be got also in a completely different case where no spatial order exists at all, but a part of the constituents oscillates coherently in phase because of some collective dynamics.[1]

It has been often pointed out that a living system does not exhibit spatial order only as a crystal does, but since it is a dissipative system kept far from thermodynamic equilibrium by the flow of metabolic energy, it can organize itself by building up a dynamical order.[10,20] The reported Raman spectra of bacterials fits completely into this theoretical framework. As a matter of fact they exhibit:

a) a sharp line spectrum which means that some kind of order must exist in a metabolically active system.

b) A spectrum dependence upon time, with a reproducible law. This result means that the above mentioned order is time dependent and follows the evolution of metabolism , nutrition and external stimuli with time. By the way, the time dependence of the spectra of synchronized cultures of bacterials (including an empty spectrum in the first ten minutes of incubation time) would make indeed very troublesome an explanation of these results by means of Rayleigh scattering again: actually clusters or debris possibly present in the target would give indeed an average spectrum throughout all the incubation time without any reproducible time dependence.

c) The observed value of the Antistokes/Stokes intensities ratio indicates a population ratio higher than the one expected in the thermal equilibrium case. This is a proof that the observed living system is out of the thermodynamic equilibrium.

As a last point we will show now that at least the low frequency group of the lines can be linked to a time-dependent ordering of some aminoacids of the cell.

Comparison of the low frequency Raman spectra of E. coli and pure aminoacids

In accordance with the above considerations, the low frequency Raman spectra of E. coli and of aminoacids have been examined to try to connect each with the metabolic activity of the cells in the minimal salts medium used to culture the cells for Raman experiments. The early synthesis of aminoacids and their

distribution has been widely investigated by Webb[21] following reports by several workers that many aminoacids become attached to the cell's DNA which cannot be removed by DNA purification methods only by the hydrolysis of DNA.[22-24]

By the use of ^{14}C labelled glucose, Webb was able to show that (a) these aminoacids were newly synthesized ones (b) they were produced during the first 15 minutes of the cell cycle and (c) they attached to the DNA as individual aminoacids not as proteins. Since almost a ten fold increase in the quantity of these aminoacids attached to the DNA of cells grown in a minimal medium where all genes are in operation, as opposed to the quantity associated with the DNA of cells grown in a rich infusion medium where many genes are repressed Webb concluded that the association of these particular aminoacids with DNA played some role in the transcription of the DNA genetic code, not its repression as was once thought and were perhaps responsible for the enhanced stability of minimal medium grown cells to physical stresses from dehydration and various types of radiations. The composition and quantities of these aminoacids for E. coli DNA and the effect of nutrition and radiations on them is documented fully in Ref.20.

As mentioned above aminoacids in crystalline form have been surveyed in the region between 20 and 200 cm^{-1}.[15,17] The purity of the specimen has been varied by adding controlled small amounts of impurities in order to check the dependence of the Raman spectra upon the (spatial) order existing in the crystal. The results agree with the above scheme. First of all a strong dependence of the peaks upon the order has been found.[17] Small impurities induce large broadening of the lines. Moreover the intensities of these low frequency peaks are greater than the intensities of the other peaks in the spectrum.[17]

A comparison between the low frequency Raman lines of the crystalline aminoacids and the lines of E. coli is presented in Table 1 of Ref.(18). All of the low frequency lines of E. coli could be assigned to the lines of crystalline aminoacids and those found attached to the DNA of E. coli. On the other hand we know that aminoacids in the cell are not in the crystalline form. In the E. coli spectra a similar dependence of these lines upon the integrity and metabolisme of the cell is found as in crystal experiments. Therefore we have to conclude that a different ordering mechanism is at work in the cell that at definite time selects definite subsets of aminoacids enhancing their Raman spectra and making them observable. Actually the above discussion on the relationship between sharp Raman lines spectra and order suggests that the mere appearance of a Raman spectrum indicates the existence of some kind of order.

Moreover, the E. coli strong lines at 45, 85 and 90 cm^{-1} correspond to Aspartic and Glutamic Acid which happen to be the most abundant of the aminoacids found attached to E. coli DNA.[21] It is known that Glutamic and Aspartic acids are the first formed aminoacids which are then metabolized into other aminoacids. Thus if the above interpretation of the low frequency Raman spectrum of E. coli is correct, one would expect to observe first the lines of Aspartic and Glutamic acids. But, as the cell progresses through its lifetime, the dominant lines might become those of the aminoacids known to be biochemically produced from Aspartic and Glutamic acids. Low frequency Raman spectra of synchronized cells cultures would allow the elucidation of the discussed mechanism.

In conclusion, the emergence through the Raman Spectroscopy of a dynamical order existing in the cell could allow to get a glimpse on the correlated order of the biochemical reactions, responsible for the cell material structures. These structures could involve the DNA and thus the message transcribed from it at a given time.

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OPEN PROBLEMS

- Existence and biological role of solitons** on DNA chains. Role of water

-- Order parameters and macroscopic variables in Fröhlich's model (nonequilibrium phase transition: order-disorder and order-order transitions)

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- Coherent excitation of a single polar mode
  Excitation of a metastable highly polar state
  Vibrations arising from more complex processes and giving rise to limit cycles a la Lotka-Volterra oscillations.

- Electric and electromagnetic wave interaction with biological systems
  ⇒ nonthermal biological and medical effects

- Electric and electromagnetic wave emission from biological systems

- Soliton decay and interaction with phonons. Possible cooperative effects in proteins