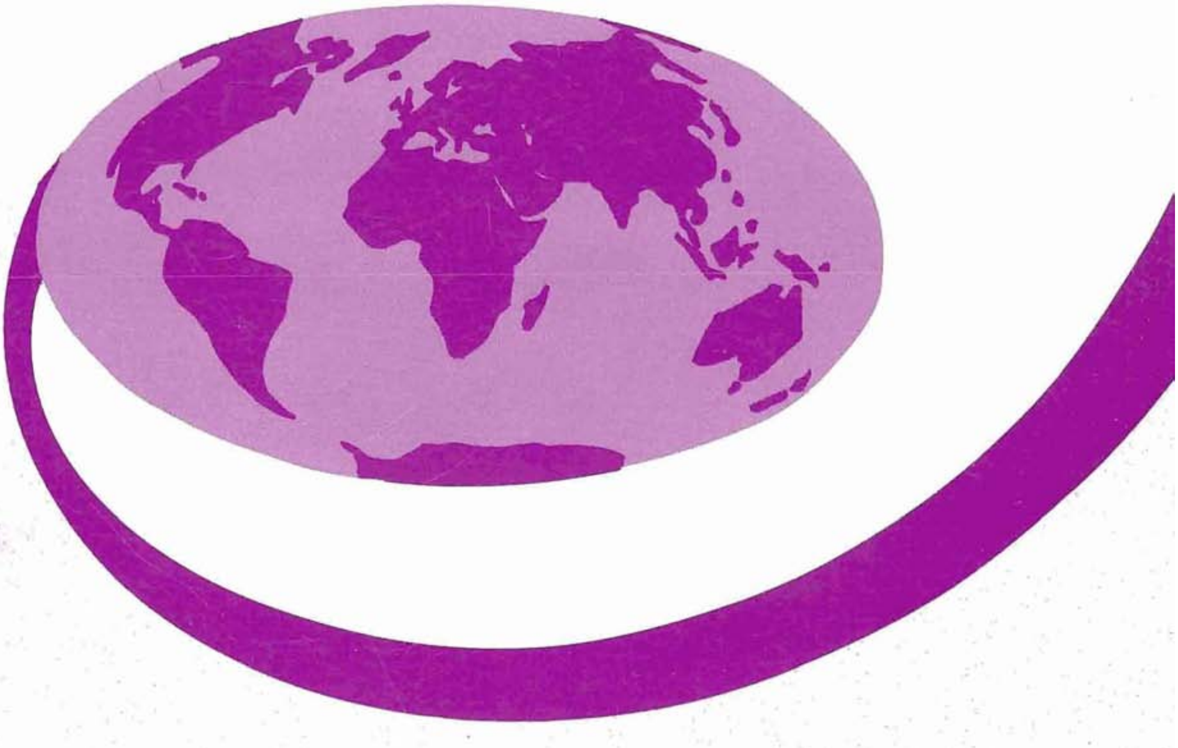
IUBS

BIOLOGY AND COMPLEXITY
Some Physical Facets

Edited by
G. CARERI
and
G. NICOLIS



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BIOLOGY AND COMPLEXITY

Some Physical Facets

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BIOLOGY AND COMPLEXITY: SOME PHYSICAL FACETS

On 3 September, 1985, a Symposium on the theme "Biology and Complexity" was held in Budapest on the occasion of the 22nd General Assembly of the International Union of Biological Sciences. Its purpose was to bring together physical and life scientists interested in the possibilities afforded by physical approaches and mathematical modelling in biology. Upon request of the Editors, a report featuring two papers presented at this Symposium, both based on similar yet complementary approaches, is presented here.

In our view, an essential feature of the complexity underlying biological organization is the ability to regulate and correlate the various activities in both space and time according to the system's past history and to the environmental conditions; to store past experience as well as to generate or exchange information; and to evolve toward new forms and functions.

Our main objective in the two following complementary papers is to approach the problem of biological complexity from the standpoint of the Physical Sciences. More specifically we would like to show that the ideas and methods of Thermodynamics and Statistical Physics on the one side, and of the Mathematical Theory of Dynamical Systems on the other side, can provide new insights in understanding some of the multiple facets of biological complexity, be it at the molecular or at the macroscopic level.

At this point the reader might rightly demand a definition of Complexity. We believe that Complexity is one of these elusive notions which are better understood after analyzing a wide-series of concrete situations. We can however, immediately warn the reader not to identify Complexity with the "complication limit" arising from the practical impossibility to carry on a full description when an excessively large number of degrees of freedom is involved. Rather, the reader will become aware that organized complexity of a system is intimately related with the possibility to display a rich space-time correlation spectrum superimposed to noise, in much the same way as, say, sound waves are superimposed to random molecular motion. The ability to extract these correlations implies that statistical laws are at work, and it is for this reason that an approach based on the concepts and tools of Statistical Physics and Thermodynamics is appropriate. Moreover, an important feature underlying organized complexity is the possibility to use these correlations to build up new, unexpected modes of behavior associated with abrupt transitions.

One of the principal points that we hope this analysis will convey to the reader is a deep unity in the physical concepts common in an enormously diverse array of complex situations. Still, this does not imply that we reduce biological organization to a simple set of physical rules. On the contrary, different levels of description appear to be necessary in order to capture its essential aspects. As a matter of fact, it is our feeling that the approach to complex phenomena advocated in the two following papers brings a serious challenge to the reductionist view in Biology.

TOWARDS A COMMON LANGUAGE FROM MOLECULAR BIOLOGY TO BIOSPHERIC ECOLOGY?

Francesco di Castri

It is a usual frustration to come back from a large congress where a variety of scientific themes have been covered, with the feeling of having been affected by a "Tower of Babel syndrome". Hopefully, one can be in the lucky position of having well understood something of about a quarter of the contributions.

This is particularly true in the field of biology, whose expansion has experienced a centrifugal movement with a number of disciplines that have become more and more segregated, to such an extent that they have almost completely lost their intercommunicating power.

No doubt, this problem is of particular concern for an International Union of Biological Sciences (IUBS). How to keep a unitary logic between and among such diverse commissions and societies as those covered by IUBS' umbrella?

Even within a single biological discipline, for instance, zoology, botany or ecology, this problem is so acute that different solutions have had to be envisaged: the simple discontinuation of the Congresses of Zoology, the subdivision of the Congresses of Botany into numerous specialized workshops held in different locations, or the holding of dozens of simultaneous workshops, as for the Congress of Ecology held in Syracuse (USA) last year.

It was therefore a most pleasant feeling -during the last General Assembly of IUBS in Budapest- to have listened to a group of distinguished physicists debating precisely on complexities and to have easily understood their lucid presentations, being free to any possible extent of the all too usual disciplinary scientific jargon. It was even more gratifying to realize how many convergent points are apparent in the evolution of their thinking when compared with what is actually happening in a number of our own biological fields.

It was not a real surprise sensu stricto, since the works of Prigogine, Careri, Nicolis and others are well known to most biologists. The excitement was, rather, in recognizing how important it would be for the "Unity of Biology" -so eloquently advocated by Prof. Straub (1985) at the same Assembly- to establish more regular and more in-depth relations on these topics between biologists and physicists.

Complexity is obviously inherent to the very essence of life. Indeed, we -the ecologists- claim that we deal with the highest levels of complexity, which may be true ad litteram when approaching the study of an ecosystem with hundreds of species and several orders of magnitude of individuals, genes, molecules, including furthermore the physical environment; even more, at present, when we cautiously explore unknown fields for understanding the functioning of the overall biosphere.

As a benchmark in realizing how many of the statements in the articles of Careri and Nicolis could be interpreted as representing a real epistemological convergence or at least good analogies with similar aspects at biological levels of integration, I will provocatively take ecology, perhaps the most complex, more likely the most theoretically confused branch of biology.

It has to be underlined that ecology in many countries needs -probably more than any other biological discipline- a kind of unitary logic to conciliate its numerous subdisciplines with a rather conflicting behavior, and -even more- needs to be strongly inserted in, and well recognized by, the molecular biology and the underpinning chemical and physical sciences.

Figure 1 and its long legend attempts to familiarize those involved in lower levels of integration with the main branches of ecology. Rebuilding an ecology shows still a main bifurcation between an energy-budget ecology, where flows of energy and cycling of matter are expressed at different scales of space, and an evolutionary ecology, approaching changes dealing with genetic information at increasing time scales. This unfortunate bifurcation is often a source of sterile and fratricide fights of schools, while much closer interactions at every level are needed towards the goal of providing ecology with a coherent theory.

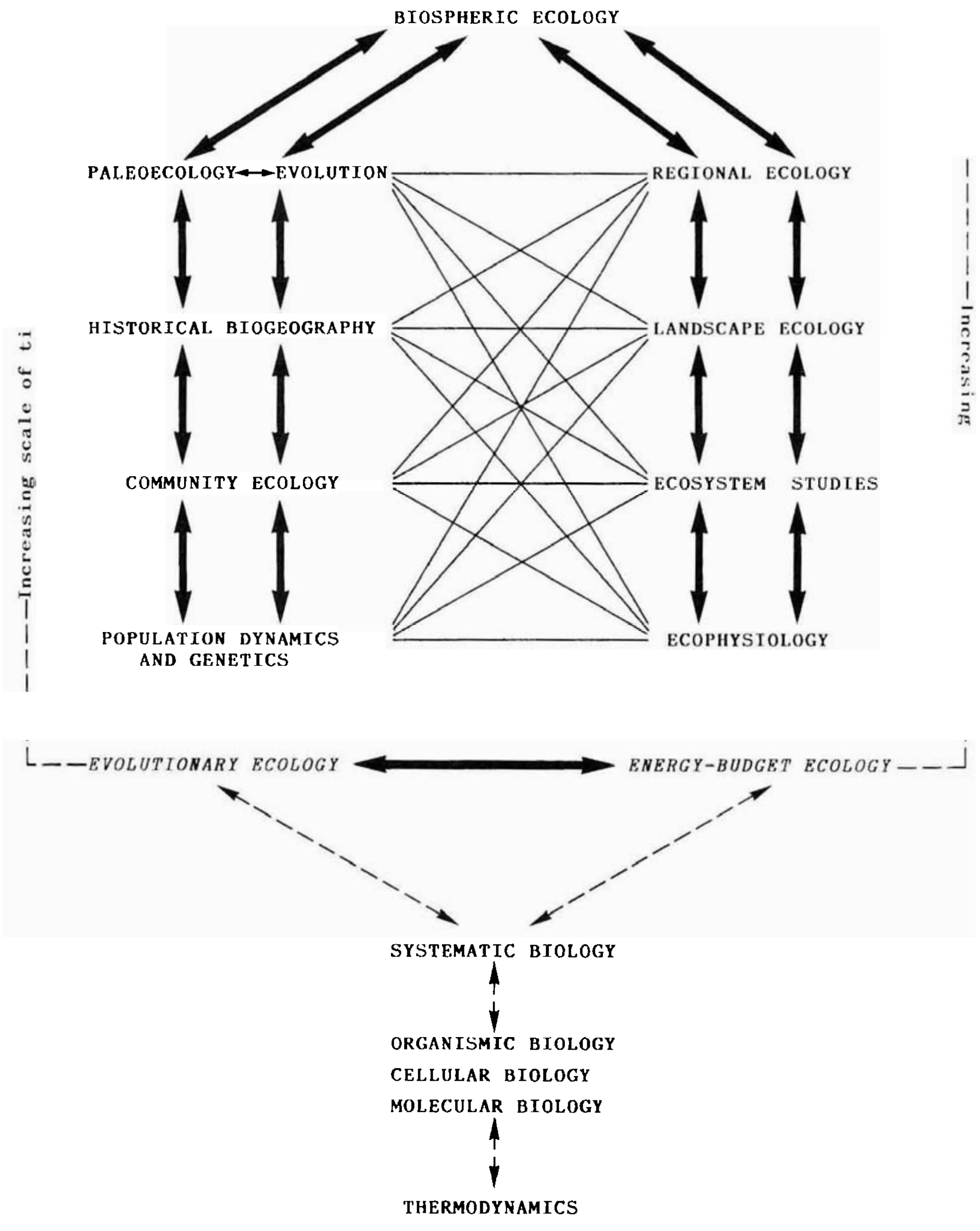
In any event, the left hierarchical axis covers in particular problems of biological diversity, and the right one those of biological productivity; they are likely to be the two key issues for a self-supporting biosphere. Again, there is here a great gap in our knowledge: to what extent the biological diversity is supportive of the productivity of the biosphere, and to what extent changes in productivity undermine biological diversity?

In order to avoid being involved in a quarrel on the relative importance of each one of these ecological subdisciplines, I want to stress again that they are linked by hierarchical scales of time and space (see also Salthe, 1985); there is not a special merit or scientific value to be placed at the top or bottom of the axes. A kind of "zooming up and down" effect is needed to perceive the relevance and constraints of a given problem at higher levels, and to search for explanatory mechanisms down at lower levels (see for instance Jarvis and McNaughton, 1986).

After this long ecological "intermezzo", let us go back to the original question on the convergences or analogies between the two articles of Careri and Nicolis based on the methods of Thermodynamics and Statistical Physics and of the Mathematical Theory of Dynamical Systems, and some of the recent trends of what may be perceived as the most faraway discipline or biological level of integration: ecology and the biosphere.

For this comparison, I am quoting almost textually -as a florilegium- some of the statements of Careri and Nicolis that could apply to both thermodynamics and ecology. First of all, in both cases time and history should be considered as an integral part shaping the functional and structural attributes of the system under study; a system is a historical object, and time is a correlator of events. Secondly, all events are interlinked along scales of space and time, so that emergent properties (or unexpected behavior) from one to another level become a kind of supervenience. Thirdly, stochasticism or chaos and determinism are intermingled features that depend on the initial stage, and on different scales of space and time; for instance, a chaotic behavior may have a deterministic origin (see also Dubois _et al._, 1987), or a long-range connectivity may have developed stochastically. Finally, given the non-linearity of the functions and the existence of chaotic attractors, there will be multiplicity of "solutions" and limited predictability.

The aspects related to the "attractors", either as "a universal point of equilibrium" or as chaotic and non-equilibrium attractors, also show many analogies with ecology, when concepts such as those of resilience, succession and climax, pest outbreaks, ecotone and patch dynamics, disturbance regimes are evoked. In addition, the paramount importance at given time scales of rare (episodic) events seems to be equally important in thermodynamics and ecology, at least when an evolutionary approach is adopted.


BIOSPHERIC ECOLOGY
PALEOECOLOGY
EVOLUTION
REGIONAL ECOLOGY
HISTORICAL BIOGEOGRAPHY
LANDSCAPE ECOLOGY
COMMUNITY ECOLOGY
ECOSYSTEM STUDIES
POPULATION DYNAMICS AND GENETICS
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EVOLUTIONARY ECOLOGY
ENERGY-BUDGET ECOLOGY
SYSTEMATIC BIOLOGY
ORGANISMIC BIOLOGY
CELLULAR BIOLOGY
MOLECULAR BIOLOGY
THERMODYNAMICS

FIGURE 1. A world of ecological subdisciplines (the "house-like" middle-upper part of the figure) put concomitantly into action by strengthening their multilevel interactions.

During the last 25 years or so, a previous almost unitary ecology -still not theoretically settled- has been disrupted and segregated into multiple subdisciplinary parcels. They have often been kept fighting among themselves, and in too many cases, been losing ground regarding their foundations on a good systematic biology, as well as on the flourishing biologies placed at lower integration levels and thermodynamics.

Splitting up has occurred either by level of integration (e.g., a population and an ecosystem approach claiming the uniqueness of their flag: reductionism for the first, holism for the latter), either in accordance with their main goals (e.g., the exchange of genetic information on the one side, of energy and matter on the other).

As a result, ecology as a whole has lost scientific coherence and credibility. It has become marginalized in some countries (di Castri, 1984), or is drifting towards the conditions of a soft science -closer to the social sciences realm (di Castri and Hadley, 1985); a strong but usually badly formulated social demand -followed uncritically- is partly responsible for this situation (di Castri and Hadley, 1986).

The above statements are admittedly overgeneralizations. There have been several unifying attempts based on principles taken from the Information Theory, energetics, analogical extrapolations of economic laws, the General System Theory, and -operationally- system analysis and modelling. Each attempt has had only a partial success as "unifyer"; too often the "followers" have undertaken an isolated "escalation" away from the original unifying purposes.

Conversely, there seems to be at present a centripetal movement of coalescence along a few hierarchical axes, both in ecology (O'Neill et al., 1986) and in the so-related evolution (Eldredge, 1986).

The "construction" of Figure 1 is still an arbitrary one; some subdisciplines, particularly ecophysiology and landscape ecology, could stand as well at the left axis. The desirable trend is towards the fusion in only one hierarchical axis, unifyed by a progressive strength of linkages and interactions. At that time, hopefully, a coherent theory will be born for ecology, which would acquire the status of a hard science, indeed not so distant from the highly refreshing "old" natural history.

It is certainly not possible to be involved here in a theoretical debate on whether and to what extent these aspects are really epistemologically convergent or only analogically appealing. The important point is that there is, in my view, a clear euristic value in pursuing these comparisons on the evolution of convergent concepts in physics and in different disciplines of the biological world. At least, some of the obnoxious "religious wars" because of concepts and approaches that are erroneously considered as antagonistic -but are often only opposite faces of the same medal- will lose their "raison d'être". This would be the case of some traditional ecological controversies of stochasticism (and chaos) *versus* determinism, reductionism *vs* holism, non-linearity *vs* linearity, non-equilibrium *vs* equilibrium, etc. I also fully agree with Careri and Nicolis on their statement that simplicity and complexity do not increase -necessarily- along successive integration levels, and that the gap between both may become very narrow.

I am not optimistic enough to believe that totally unifying principles will ever be found. However, one can perceive the need and the ways to reach at least a "low-denominator common language", which may be able to make the fundamental principles of each other's thinking in interdisciplinary endeavors understandable (something like the present days' scientific congresses, where skeletal English may be good enough to communicate the essence of the worldwide research).

A rudimentary common language is, at the very least, badly needed, when biological diversity from the molecular to the biospheric level may be a problem of survival, and where some predictability on the effects of man-induced global changes can only be achieved through methods and techniques scaling down and up from remote sensing to physiology, from tropospheric chemistry to elementary processes of photosynthesis and decomposition at the organismic level (as in the "International Geosphere and Biosphere Programme" on Global Change, newly launched by ICSU); at a moment too, when some understanding of long-term future trends can only be grasped by looking deeper and deeper into the remote geological and evolutionary past.

Accordingly, I sincerely hope that publishing this Special Issue of *Biology International* will not remain an isolated initiative, and that IUBS will take a strong lead in fostering these exchanges among disciplines concerned with "complexity" issues at different hierarchical levels. This would not only be a most stimulating avenue towards the "necessary utopia" of a common language, but a unity of biology. After all, an integrating focus of biology -at least as "an attractor"- is also the "raison d'être" of a scientific union like IUBS.

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BIOLOGY AND COMPLEXITY:

SOME PHYSICAL FACETS AT THE MOLECULAR SCALE

Giorgio Careri

Introduction

In this paper I shall consider one biological macromolecule in its bath in the autonomous description level of statistical physics(1). Biologists agree that the molecular level is the lowest integration level of the biological ladder (2); consequently we should expect this level to be the simplest for an approach from the viewpoint of physics. As we shall see, a biological macromolecule is such a small body that spontaneous fluctuations are to be expected near thermodynamical equilibrium. But the relevant biological question to answer will be whether fluctuations can achieve a role in the functional order of the living cell. To this end, we shall first explore the possibility of cross-correlating the fluctuations of different parts of the same macromolecule, but the intrinsic complication of real biological macromolecules will rule out further analysis in this direction. However, the study of a simple model system, such as hydrated enzyme powders, where subsequent events can be conveniently separated, will show the presence of an abrupt and unexpected onset of the catalytic activity. This relevant biochemical event will be described as a cooperative transition in the frame of the percolation model, a physical model where one focuses on the connectivity process between parts of the same complex system, thus bypassing the complications arising from macromolecular structure. Generalizing this result, we shall outline a physical approach where statistical space/time events and their correlations are the relevant entities to be considered for a better description of the structure/function binomium.

SPONTANEOUS FLUCTUATIONS AND CORRELATED EVENTS

Although globular proteins have long been considered "floppy bodies", meaning systems capable of displaying a large set of conformational fluctuations, the description of these fluctuations and of their biological significance is a comparatively recent acquistion. Individual globular proteins are small systems that consist of relatively few atoms, and physics can offer quite general expressions for the mean square fluctuations of a small system (the macromolecule) in thermal equilibrium with its bath (the solvent). Because of the small size of a single macromolecule, transient fluctuations are inevitable, even at thermodynamic equilibrium. This is not a unique property of globular proteins, but here the occurrence of these large fluctuations can have pertinent implications upon their functions, for instance as enzymes. Thus the fitting procedure is to offer a detailed description of how the macromolecular structure can use these random processes for a specific catalytic action. To this end, we must first consider the time scale of these spontaneous processes.

For any single process, statistical physics show that the self-correlation function is an exponential law with the same time constant as the relaxation response of the system. Therefore, each of the relaxation processes observed in a macromolecule is representative of one class of spontaneous fluctuations around equilibrium, their amplitude being distributed along a Gaussian curve and their decay being governed by the same time constant. To identify one such class of fluctuations without necessarily specifying the molecular process involved, we shall use the term "statistical time event" throughout this paper, because the only requirement for its operational definition is the measurement of time (3). In other words, we mean that there is a class of spontaneous fluctuations which persist inside this time interval, so that different fluctuations can interact and eventually become cross-correlated within this time. In Table 1, we list a number of time events observed in globular proteins and enzymes.

Table 1. Time events in globular proteins and enzymes (from Ref. 3)

Determinants	Time (sec)
Protein surface	
Bound water relaxation	10^{-9}
Side-chains rotational correlation	10^{-10}
Proton transfer reaction of ionizable side chains	10^{-7}–10^{-9}
Protein conformation	
Local motion	10^{-8}–10^{-9}
Isomerization process	10^{-2}–10^{-7}
Folding-unfolding transition	10^{+2}–1
Enzyme substrate complex in solution	
Encounter rate	diffusion controlled
Estimated lifetime of the transition state in covalent reactions	10^{-10}
Change in metal ion coordination sphere in metalloenzymes	10^{-6}–10^{-9}
Enzyme-substrate local conformational motion	10^{-9}
Covalent enzyme-substrate intermediate lifetime	10^{-2}–10^{-4}
Enzyme-substrate complex conformational isomerization	10^{-2}–10^{-4}
Enzyme-substrate complex unfolding transition	10^{+2}–1

The first evidence that rapid structural fluctuations are present in a large number of globular proteins and enzymes comes from experiments on fluorescence-quenching, performed in 1973 by Lakowicz and Weber (4). Almost contemporaneously I suggested (5) that the ability to time-correlate these fluctuations could be an essential kinetic property of the macromolecule if it is to work as an enzyme. Since then, the notion of the enzyme as a fluctuating unit has gained credit; its experimental and theoretical basis has been progressively strengthened (6) (7).

In order to proceed in our physical description of biomacromolecules near equilibrium in their thermal bath, we shall first take a closer look at the interconnection between three major physical concepts, namely "events", "statistical macrovariables", and "statistical correlations" (1). Let us begin with a proper definition of a statistical macrovariable Y. By this we mean a macroscopic statistical variable that is necessary for describing function at a molecular level. Examples of these macro-variables are the average charge on specific residues at the active site of an enzyme; the concentration of a particular ligand; and so on. Clearly, Y quantities are themselves functions of several microscopic quantities: for instance, the average charge on an active-site residue is a function of the charges and distances of nearby atoms, and so forth. Note that these Y macro-variables are of a truly statistical nature, because the small size of the system necessarily implies a certain number of fluctuations.

Events can now be defined as the measurable change Δ Y of the Y macro-variables. Of course such a measurement is in practice possible only if amplitude and duration can be calculated using available experimental techniques; this means that the energy must be well above average thermal energy. One such typical event is the transition over a free-energy barrier, the measurable quantity being the rate of chemical species produced during the barrier transition. As a rule, only rare large amplitude fluctuations can be considered as measurable events.

Once a set of relevant macro-variables is identified, their cross-correlation can be mathematically expressed as the non-vanishing average of these quantities over a duration time. It stands to reason that in order to be cross-correlated, macrovariables must display close values of self-correlation time, so that some form of coupling can become effective among them. The notion of "correlated events" then emerges in a simple and direct way. As a matter of fact, large amplitude fluctuations of the macro-variables can be described as relevant spontaneous events that can become cross-correlated if their duration is similar and if their coupling is sufficiently strong. Thus it becomes quite evident that when too many such events become cross-correlated, the complexity limit within this description level may be reached.

Other descriptions may be necessary when a biological macromolecule is only a part of a larger body, for instance when it is a subunit of a larger macromolecule, or else when several macromolecules are imbedded in a common matrix, as in membranes. It is often found that these large systems exhibit unexpected behavior that cannot be directly extrapolated from the description of smaller systems. The coherence observed in dissipative cycles out of equilibrium, as in glycolysis, is a good example of this state of affairs. It will be considered in the companion paper by Nicolis.

THE FLUCTUATING ENZYME

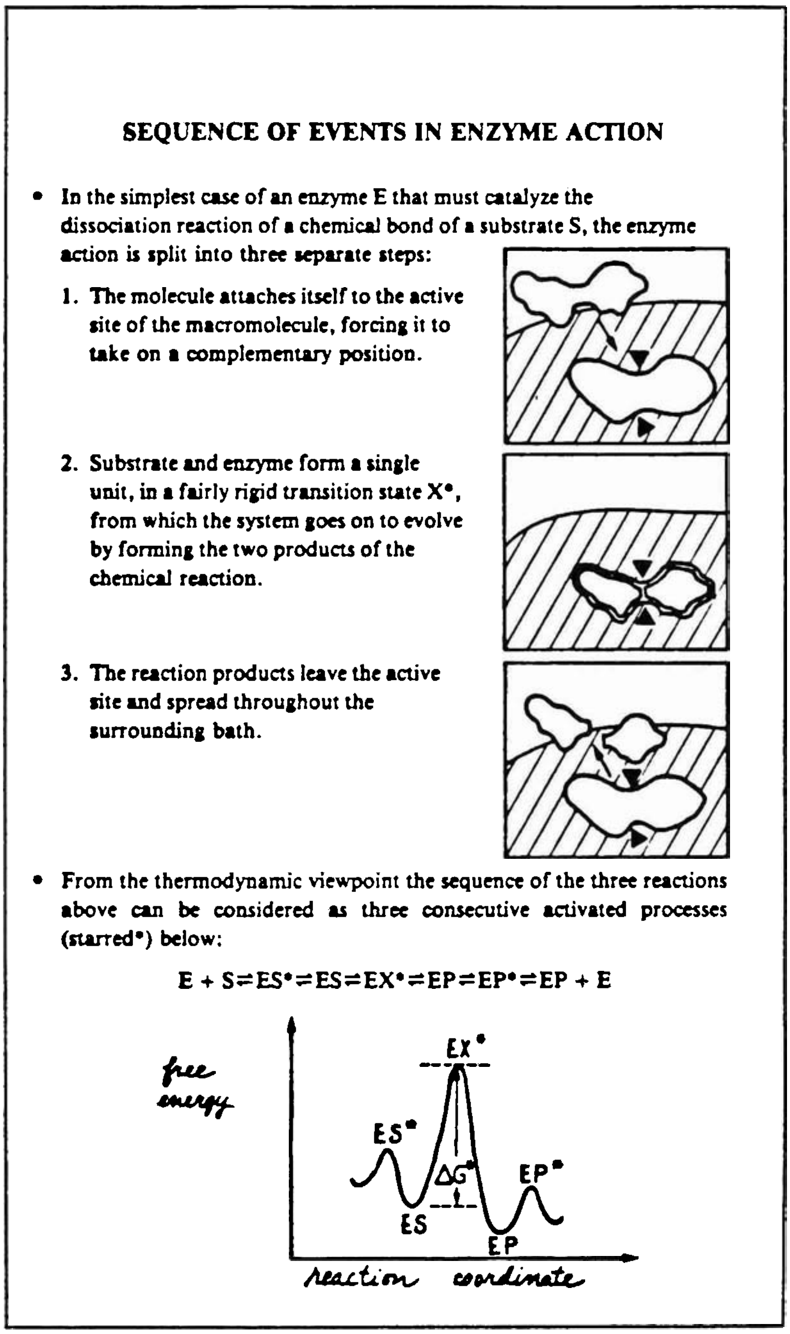
Nature has solved the problem of rapidly and faithfully generating significant biochemical events in a thermal bath, thanks to the flexibility of a few globular proteins, the enzymes. These are composed of chains of stratigically arranged atoms, which permit the best sequence of certain internal events. For our purposes here, we shall call "internal events" those relevant occurences that take place inside the macromolecule, while we shall call "global event" the final and permanent change observable in the reaction products. The enzyme is essentially a catalyst; to an observer it appears as a generator of events, because almost no events would be observed in the same time scale without it.

Biochemists describe enzyme action as a sequence of steps that compose the reaction pathway which transforms the substrate. As shown in Fig. 1, the first internal event is to link the substrate to a steric convenient site; next, certain changes in the configuration of the macromolecule must occur to optimize the active site where the transition state must be built and then overcome. This optimization implies the correct orientation of the active groups and the formation of steric barriers to create the transition state of lowest free energy. The flexible nature of the protein structure under thermal agitation allows the temporal sequence of internal events, whereas the cooperative nature of the interactions gives rise to that particular order of atoms that characterizes the transition state.

Some aspects of enzyme action are truly general; they will be the focus of our concern hereafter. For instance, physics aim to describe how free energy is exchanged from the thermal bath to the active site in order to overcome free-energy barriers, and since such barriers operate in a time sequence, physics hope to understand how this planned sequence of internal events can be achieved in a thermal bath. This implies that an enzyme should be described not only by its spatial but also by its temporal structure, the two merging together in the space-timé fluctuation spectrum of the macromolecule (8). This ambitious programme has not been achieved as yet. Since the structural aspects of enzymes have been widely considered in literature, only some of the temporal aspects will be considered below.

Let us start by trying to increase the precision of our notion regarding the statistical time event relevant for catalysis (6). According to the picture of the fluctuating enzyme, the reaction coordinate ought to be a function of only a few statistical macrovariables, the high-amplitude spontaneous fluctuations which should create biological function. Therefore, we must first consider the relationship between the time scale of the rare event active towards catalysis versus the time scale of the spontaneous fluctuations of the macrovariables involved,

Fig. 1. Sequence of Events in Enzyme Action (from Ref.8)



SEQUENCE OF EVENTS IN ENZYME ACTION

- In the simplest case of an enzyme E that must catalyze the dissociation reaction of a chemical bond of a substrate S, the enzyme action is split into three separate steps:

1. The molecule attaches itself to the active site of the macromolecule, forcing it to take on a complementary position.
2. Substrate and enzyme form a single unit, in a fairly rigid transition state X*, from which the system goes on to evolve by forming the two products of the chemical reaction.
3. The reaction products leave the active site and spread throughout the surrounding bath.

- From the thermodynamic viewpoint the sequence of the three reactions above can be considered as three consecutive activated processes (starred*) below:

$$E + S \rightleftharpoons ES^* \rightleftharpoons ES \rightleftharpoons EX^* \rightleftharpoons EP \rightleftharpoons EP^* \rightleftharpoons EP + E$$

and accordingly we must identify the main classes of these relevant macrovariables as a function of the time-data considered above. If this class is relevant for catalysis it must provide the active fluctuation; namely, the high-amplitude fluctuation that occurs rarely and well away from the mean value of the Gaussian curve. In our picture, this active fluctuation is the bottleneck for catalysis. We believe that a free-energy increase of one order of magnitude above the average thermal energy (say, up to 5 Kcal/mole) should be considered sufficient, because this increase must be lower than the net free energy of structure stabilization of a globular protein, which is the order of 10 Kcal/mole. Therefore, active fluctuations towards catalysis must occur with a probability factor around 10^{-5}, and if we take 10^{-3} as a representative value for the enzyme turnover, we anticipate that some active fluctuations must originate from that part of the frequency spectrum which is centered around 10^{-8}s. In this estimate we have used the canonical ensemble expression which relates the probability P of a free-energy change Δ G between one subsystem and its bath associated with a fluctuation

$$P \simeq \exp(-\Delta G/KT)$$

where T is the temperature of the thermal bath, and K is the Baltzman constant. Incidentally, in statistical physics, from this general expression one can easily derive the Gaussian distribution we have mentioned above.

At this stage we should combine the two main points: on the one hand, the free-energy transducing property of the enzyme between the thermal bath and the active site and, on the other, the value of the common-time constant of the spontaneous fluctuations involved in the transducing processes. This will help us identify the specific classes of fluctuating macrovariables which are relevant for catalysis. Expressed in different and more concrete terms, we should identify some specific process occurring on the macromolecule surface and others occurring at the active site. There is reason to assume these processes can couple, because both have a time constant close to 10^{-8} sec. This search was performed some time ago(3), and among suitable candidates which could account for processes occurring at the protein surface, we have already pointed out the proton-transfer reactions on bound water, the charge fluctuations of the ionic medium and the side-chain motions.

Once this set of relevant macrovariables is identified, we should proceed to calculate their statistical cross-correlations, in order to derive the natural laws which describe the behavior of this macromolecule. Such is the final aim of this description level, and it is obviously quite remote because of the non-linear coupling which probably exists among the macrovariables themselves. At this point, different coupling regimes may occur, according to the degree of displacement of the system from thermodynamic equilibrium. In practice, the enzyme will use the regime that fits best in the overall course of the functional order. There is no preconceived scheme according to which every enzyme must work; rather, in each instance, nature has evolved the best way to achieve its goal, based upon the laws of statistical physics.

Close to equilibrium we say that the Y macrovariables are cross-correlated if non-vanishing static coupling terms are present when the entropy is expanded, so that the probability distribution is no longer the product of two independent Gaussian curves. We may expect this type of static coupling to be widespread in nature, as in allosteric enzymes where the free energy for ligand A-binding is a direct function of ligand B-binding. This phenomenon can be formally expressed by introducing an appropriate coupling term: $\Delta\ G_{AB}$. The cooperative effects arising from multiple binding at equilibrium are well known, and we do not need to dwell upon them here.

For small displacements from equilibrium, true kinetic coupling may develop, and it may be treated in the frame of linear irreversible thermodynamics by means of the Onsager phenomenological theory. In this approach, we can express the linear coupling between the time derivatives of the statistical macrovariables (fluxes y) and the generalized forces X, thanks to the Fourier components of the time fluctuations of the macrovariables; the pertinent expressions have already been quoted (5), so we shall not repeat them here.

Let us stress again that in the case of enzyme catalysis, this also implies a coupling between the time changes of the appropriate macrovariables, creating a new time property that may well characterize enzymes. Notice also that this theory operates in the realm of detailed balance principle (see the paper by Nicolis in this issue), an assumption which is likely to be fulfilled for enzymes that operate reversibly around equilibrium. For all these reasons, I have suggested that the ability to time-correlate the fluctuations of certain relevant conformational variables could be the **characteristic kinetic property** which allows a macromolecule to work as an enzyme. This property may be given an elegant analytical expression in terms of the inverse Onsager matrix (5)(7), but for the time being its experimental detection possibilities are severely limited by the difficulty of measuring the relevant variables in a suitable manner. This is because in a real macromolecule these relevant variables are so tightly coupled among themselves that the macromolecule appears to the observer as a unit. In other words, the above considerations show that the complication limit is soon reached at this description level.

A MODEL SYSTEM: HYDRATED LYSOZYME POWDERS

To partially circumvent the complication limit problem encountered at the end of the previous section, we shall hereafter consider a real experimental system where the environmental conditions can be controlled in order to separate relevant biochemical events of increasing complexity.

Over the past years we have studied in detail several thermodynamic and dynamic properties of the hydration of Lysozyme powders, because here hydration can be varied by adding water to dry macromolecules until a dilute solution is obtained. The advantage of this system is that several measurements can be made at all system compositions, using different techniques. This makes it possible to study the subsequent events induced by hydration in a series of increasing complexity.

Lysozyme is a comparatively simple enzyme. Today, almost everything is known about its hydrated powders, thanks to I.R. spectroscopy, E.P.R. relaxation, heat capacity and other thermodynamic and dynamic properties, and especially the enzyme activity towards appropriate substrates (9). We may summarize these data by saying that the hydration-stepwise process consists of three well-defined stages: 1) from 0 to about 60 H_2O molecules/macromolecule, dominated by the interaction of water with the charged group of the protein; 2) from 60 to about 220 H_2O molecules/macromolecule, where some major changes in surface water arrangements take place; 3) from 220 to about 300 or more H_2O molecules/macromolecule, where the enzymatic activity starts and grows with increasing hydration, together with the condensation of water molecules onto weakly interacting unfilled patches of surface where the molecules are in rapid motion. It is important to note that no structural transitions in the absorbed water or in the protein itself have been detected in the range from 60 to 220 H_2O molecules/macromolecules (or hydration h included between 0.07 and 0.25 g H_2O/g dry weight).

Since the water molecules carry a strong dipole moment, we have recently investigated the dehydration process of Lysozyme powders, using a dielectric technique at Megahertz frequencies (10). When the dielectric losses were measured in H_2O- or D_2O- isopiestic hydrated samples, the relaxation showed a strong isotope effect, indicating that the inferred conductivity was mainly protonic. Moreover, the combination of pH dependence and an effect produced by complexation with a substrate indicates the existence of a proton conduction that involves ionizable side-chain groups of the protein surface, particularly of the active site of the enzyme.

More recently, the hydration dependence of the capacitance was analyzed (11) in the low hydration limit region, to carefully detect the onset of dielectric properties in order to describe this process in the framework of the percolation model. This general physical model has been proven applicable to a broad range of processes where spatially random processes and topological disorder are of vital importance, particularly to study the electrical conductivity of a network of conducting and non-conducting elements (12)(13). One of the most appealing aspects of the percolation transition is the presence of a threshold, where a long-range connectivity among the elements of the system suddenly appears at a critical concentration of carriers.

In native Lysozyme powders the dielectric capaticance displayed a sharp increase at a water content threshold h_c = 0.150 ± 0.016 g/g, followed by saturation at increased hydration. Since the hydration of one macrolayer is h_m = 0.38± 10% g/g, the experimental volume ration for surface percolation is 0.40 10%, a value very close to the 0.45 ± 0.03 predicted by theory (12). Notice that for three-dimensional networks, regardless of their structure, the conduction threshold predicted by theory is 0.16 ± 0.02, and this rules out connectivity through the protein interior, where water molecules are known to be very sparse. Moreover, the threshold h_c was found to be constant from pH 3 to pH 8, indicating that the local geography of water clusters about ionizable sites of the protein surface is not of primary importance. Thus only the number of water molecules acting as interconnected conductivity sites is relevant; and as a matter of fact, the same threshold is found for both H_2O- and D_2O- hydrated samples.

In other words, we picture the percolative path as a proton transfer along a random but continuous thread of hydrogen-bonded water molecules absorbed on the protein surface, the water molecules acting as valves in the proton flow along these fluctuating threads. The long statistical path of interconnected water molecules thus acts as a "short-cut" to bypass the local complex details of the protein surface. Finally, since the percolation theory is size-independent, we may expect that the percolation threshold will be similar for all proteins which display similar adsorption isotherms.

For lysozyme-saccharide complexes a higher value of the percolation threshold, near 0.25 g/g, has been found, suggesting that the presence of a "foreign body", where the water bridges may not be favorable for proton transfer, must affect the long-range conductivity on the protein surface. This hydration level, H_c = 0.25, is so close to the critical level for the onset of enzymatic activity in Lysozyme powders(9) that it suggests protonic percolation is involved in Lysozyme catalysis. At least three concurrent factors are required for Lysozyme catalysis, that is to say, Asp 52 and Gln 35 residues must dissociate with one H_2O molecule. Coupling among these processes seems to be assisted by a percolation of protons along random threads of water molecules on the surface of protein. We can see in this concrete example how a new description level, the one offered by the percolation model, can be more useful than the one used in the Onsager regime near equilibrium, where no threshold can be predicted for the enzymatic activity and no microscopic picture can be offered for kinetic coupling among the macrovariables, because of the complication limits soon reached in this system.

LONG-RANGE CONNECTIVITY IN PROTEINS AND IN MEMBRANES

The relevance of structural transitions for biological function is well known and needs not be recalled here. The percolative transition we considered above reflects a change in a process, the onset of a long-range connectivity developed stochastically in a randomly-structured system of many elements. In the particular case of Lysozyme powders considered above, a 2% change in the surface water content shifts the system from a non-conductive to a proton-conductive mode. The threshold for this process, the proton conduction, is discontinuous, whereas the physical or chemical structure of the system is not. The event which suddenly occurs is a large-scale one, because it requires a long-path proton movement over a non-uniform surface. We may wonder if percolative transitions can be relevant in other biological systems at a higher integration level than the one of a simple enzyme. To this end, let us consider the possible importance of percolative conduction in membrane transport(14).

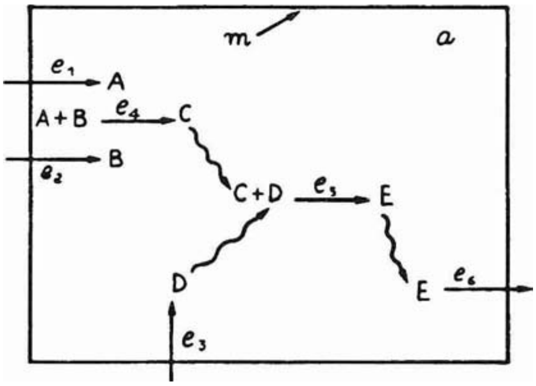
Percolation theory suggests three points that are relevant for mechanisms of membrane conduction(11). A) Conduction is possible when channels are filled only partly with water of conducting elements (for a three-dimensional channel, even 16% may suffice); B) Conduction can be turned on or off by adding or subtracting a few conducting elements, with no need for change in protein or membrane conformation. Thus simple chemical events such as the ionization of a surface group or binding of a ligand should be able to switch conduction state and serve as gates, without involving a conformational change. C) Percolation theory focuses on the randomness of the arrangement of conducting elements. A membrane channel could incorporate this aspect of percolation, to bypass the need for a high level of structure extending over the full thickness of the membrane on its hydrocarbon core. The requirement for unique structure would in this view be limited to the gate region, and the dimension of the active site would be reduced to one more common in other biochemical processes, such as enzyme reactions.

In our opinion, we may extend the above considerations to other biological processes where a large-scale process can occur if we perform the appropriate correlation of many smaller-scale events (8). For instance, we may describe the conductive regime of hydrated Lysozyme powders as a series of correlated single-proton transfers along a random thread of water molecules. Since each single-proton transfer can be considered a small-scale event caused by a local fluctuation, the large-scale event results from the correlation of a great many of these small-scale events. Actually, a similar process is observed in laser physics, where several microscopic events (the transitions among atomic states) become cooperatively time-correlated to produce one macroscopic event (the building of the electromagnetic field).

CONCLUDING REMARKS

Life exists only insofar as it evolves in time, as a never-resting stream of events. Thus it may be appropriate to approach organized complexity by the serial order of events which are relevant to the organism. From this viewpoint, for instance, even a biological membrane can be described as an appropriate correlator of events, as shown in Fig. 2. In addition, the presence of several hierarchically-superimposed levels of integration in the organism must require that some events occurring at one level be correlated among themselves, to produce one event significant at the next higher level of integration. This is the molecular physicists's outlook regarding the phenomenon of biological emergence (15), once the functional order of a living cell is identified in the ensemble of correlations among the significant biophysical events.

Fig. 2. Correlation of Events in Biological Membranes (Ref. 8)



A schematic example of events e, correlated in a compartment a enclosed within a biological membrane m. The input of the molecules A, B, D, E is supported by active transport through the membrane and can be represented as the simple events e_1, e_2, e_3, e_6. Because of the local spatial correlation among these primary events, secondary events are next formed: the e_4 type directly on the membrane helped by the enzymes attached there, or the e_5 type in the internal liquid phase. The final product E then leaves the compartment, whereas the intermediary product C cannot get out.

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SELF ORGANIZATION IN NONEQUILIBRIUM SYSTEMS AND BIOLOGICAL COMPLEXITY

G. Nicolis

INTRODUCTION

In their vast majority, natural objects are composed of a large number of strongly interacting subunits. Until not long ago "elementary" particles were thought to be the ultimate building blocks of the universe. They are now known to have a rich internal structure and to be subjected to a variety of dynamical processes, including processes responsible for a finite life time and ultimate decay. Closer to the preoccupations of this essay and of the companion paper by G. Careri, the genetic material, the immune system, the mammalian brain or the entire biosphere for that matter, owe their specificity to the fact that their behavior transcends the individual subunit and presents, under appropriate conditions, a concerted character encompassing the system as a whole.

At the basis of the specificity and coherence of such composite systems is the fact that a given object at a certain level of organization is an extremely limited sample of the tremendous number of possibilities afforded by simple combinations of its constitutive parts. Moreover, at each level of organization new collective properties are generally appearing and impose new constraints, conditioning the **future course of events.** This remarkable selectivity, which is perceived by the observer as organized **complexity**, implies in turn that **time** and **history** are integral parts of the system's structure and function. In his beautiful book Le jeu des possibles (1) François Jacob expresses this idea in a particularly forceful manner, by stating that the genetic system and the immune system are two forms of memories registering respectively the past of the species and of the individual, while the nervous system invents and shapes the future.

In this essay we show that complexity, concerted behavior and the acquisition of historical dimension can also be typical properties of a wide class of quite ordinary physico-chemical systems. We will see that for such systems the gap between "simple" and "complex" is very narrow and can be bridged under appropriate environmental conditions. We hope that this realization will provide useful insights in some of the multiple facets of biological order.

The level of description at which the phenomena that we will analyze are manifested in supermolecular. In other words, we will see that as a result of cooperativity at the molecular level on the one side (see paper by G. Careri), and intermolecular communication on the other side, supermolecular assemblies may exhibit a variety of new dynamical behaviors extending over macroscopic space and time scales.

OPEN SYSTEMS

We consider a system embedded in an environment with which it communicates through the exchange of certain properties, which we call **fluxes** (Fig. 1). As a result of these exchanges the variables describing the instantaneous state $\{X_i\}$, vary in time and attain values which are typically, different from those characterizing the state of the environment $\{X_{i}, e\}$. In a physico-chemical system $\{X_i\}$ may denote temperature, flow velocity, chemical composition, electric polarization, and so forth. In a biological system they may describe the concentration of various effectors such as substrates or products of an enzymatic reaction pathway, the density of cells in a nutrient medium or the electric potential across the membrane of a neuron. One may likewise apply the picture of Fig. 1 to problems arising outside the domain of strict applicability of Physical Sciences. Thus, in an animal or human society $\{X_i\}$ may represent the populations of individuals exerting different kinds of activities, the amount of food available, and so forth.

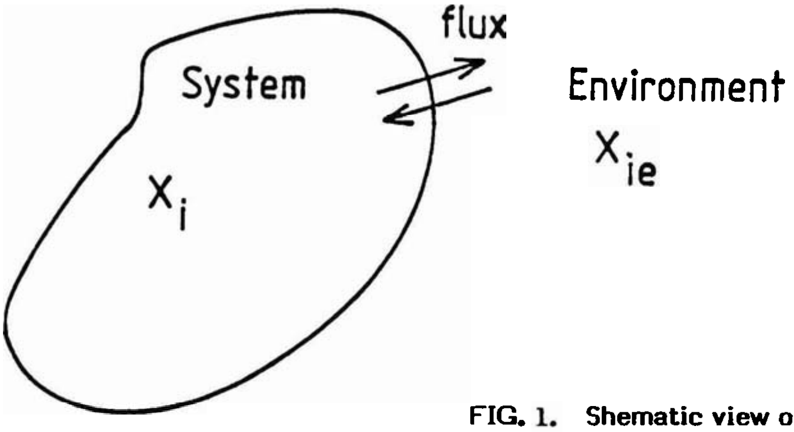


FIG. 1. Shematic view of an open system.

Whatever the detailed interpretation of X_i might be, the evolution can often be cast in the following general form (2)

$$\frac{dX_i}{dt} = F_i(X_1, \dots, X_n ; \lambda_1, \dots, \lambda_m) \quad (i = 1, \dots, n) \qquad (1)$$

in which F_i denotes the rate law and $\lambda_1, \ldots, \lambda_m$ a set of parameters present in the problem, which can be modified by the external world. We call these quantities **control parameters.**

Now, a very characteristic feature of the vast majority of systems encountered in nature is that the F's are complicated **nonlinear functions** of X's. In chemical reactions or in biology this has to do with the ability of certain kinds of molecules to perform autocatalytic and other regulatory functions. And in an animal or human population, nonlinearity may reflect the processes of communication, competition, growth, or information exchange. In short, the equations of evolution of all these systems should admit under certain conditions several solutions, since multiplicity of solutions is the most typical feature of a non-linear equation. Our basic working assumption will be that these solutions represent different modes of behavior of the underlying system. Their study constitutes the principal goal of the **theory of dynamical systems,** which today is one of the most promising branches of mathematical and physical sciences.

Before we proceed to the classification of these states let us discuss briefly the particular regime in which properties X_i attain a constant value throughout the system and equal to environmental value X_{i}, e. This state, which is referred to as **thermodynamic equilibrium**, is characterized by the property of detailed balance, that is to say,

Probability of a "direct" process = Probability of a "reverse" process.

We can easily understand that in such a state any attempt at a diversification will be smeared out immediately. Equilibrium is therefore a state of full homeostasis characterized both by uniqueness and by strong stability properties, and for this reason it cannot serve as a model for understanding complexity.

FIG. 2. Three views of the state of equilibrium.
(a) Monotonic approach of state variable X_i towards its final value as $t \to \infty$
(b) The state of equilibrium $X_{i,eq}$ is the extremum of the thermodynamic potential F
(c) Equilibrium is a universal point attractor in the system's phase space.

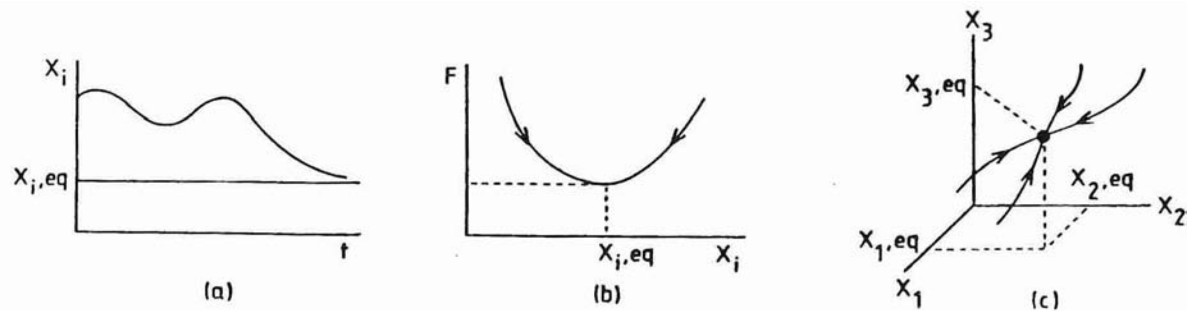


Fig. 2 represents three alternative views of equilibrium. In (a) a state variable X_i initially far from its equilibrium level X_i, tends to it monotonously, performing at most a few undulations before the final decay. In the mathematical literature this property is known as **asymptotic stability.** Its physical origin is to be sought in the celebrated second law of thermodynamics, according to which equilibrium is attained in an irreversible fashion and corresponds to the extremum of a quantity known as state function or thermodynamic potential: maximum of entropy S in a system at constant energy, or minimum of free energy F (Fig. 2b) in a system at constant temperature. Experiment has shown that all physical systems containing a large number of degrees of freedom obey to this law. We shall refer to them subsequently as **dissipative dynamical systems.** On the other hand certain simple systems encountered in mechanics like the pendulum or the two body problem remain invariant under time reversal and as a result they cannot enjoy the property of asymptotic stability. The connection between these **conservative systems** and the dissipative dynamical systems is a deep problem of physics which is outside the scope of the present paper (3) (4).

Fig. 2c represents a still different view of equilibrium, which will turn out to be the most useful one for our subsequent discussion. We embed the evolution of the system in a space spanned by the state variables which we call the **phase space.** An instantaneous state is represented in this space by a point (and vice-versa), whereas a succession of such states defines a curve, the phase space trajectory. As time grows the trajectory of a dissipative dynamical system will tend to an object representative of the regime reached by this system when all transients will die out. We call this regime the **attractor.** According to our previous discussion the attractor representing equilibrium will have to be unique and to describe a time-independent situation. Obviously this will give in phase space a point toward which all possible histories have to converge monotonously. In other words, the state of equilibrium is a **universal point attractor.**

We are now in the position to define more sharply the goal of our approach, as the search for new attractors arising when a system is driven away from its state of equilibrium. We proceed to this point shortly. We will close our present discussion by a simple example showing how nonequililbrium can indeed affect deeply the behavior of a system.

Consider the two coupled chemical reactions

$$
\begin{aligned}
A + 2X \; &\underset{k_2}{\overset{k_1}{\rightleftharpoons}} \; 3X \\
X \; &\underset{k_4}{\overset{k_3}{\rightleftharpoons}} \; B
\end{aligned}
\qquad (2)
$$

Here the concentration of product X is taken to be the only state variable, being understood that A and B are continuously supplied from or removed to the outside thus maintaining fixed concentrations. At equilibrium, detailed balance implies that

$k_1 \; a \; x^2 = k_2 \; x^3$ (equilibrium of forward and backward steps in the first reaction)

$k_3 \; x = k_4 \; b$ (equilibrium of second reaction)

These relations fix x_{eq} uniquely and impose, in addition, a condition on the concentrations a and b of constituents A and B:

$$x_e = \frac{k_4 b_e}{k_3} = \frac{k_1 a_e}{k_2}$$

$$\left(\frac{b}{a}\right)_e = \frac{k_1 k_3}{k_2 k_4} \qquad (3)$$

On the other hand, in a stationary state far from equilibrium it is sufficient to cancel the overall effect of the two forward reactions by that of the backward reactions, yielding

$$-k_2 x_s^3 + k_1 \alpha x_s^2 - k_3 x_s + k_4 b = 0 \qquad (4)$$

This is a cubic equation for x and can have up to three solutions for certain values of a and b, unlike to what happens in the case of equilibrium. We can therefore say that nonequilibrium reveals the potentialities hidden in the nonlinearities, which remain "dormant" at or near equilibrium.

The monotonic character of the approach to the state of equilibrium stressed earlier in this Section implies that the evolution laws, eq. (1), should obey in the neighborhood of equilibrium to some very particular conditions.
Introducing the deviations of X_i from the equilibrium values, $X_{i,e}$

$$x_i = X_i - X_{i,e} \qquad (5)$$

one can indeed show that the evolution of $\{ x_i \}$ can be cast in the form

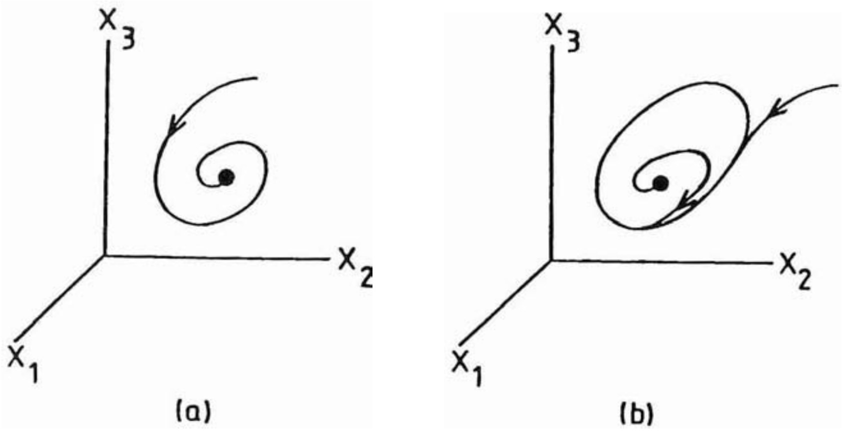
$$\frac{dx_i}{dt} = - \sum_j \Gamma_{ij} \left(\frac{\alpha F}{\alpha x_j} \right) \qquad (6)$$

where F is a thermodynamic potential taking its minimum at equilibrium and $\{\Gamma_{ij}\}$ a symmetric, positive-definite matrix. As shown by Onsager(5) this symmetry can be traced back to the property of detailed balance or, alternatively, to the invariance of the equilibrium state under time reversal. This remarkable result can be extended to the evolution around nonequilibrium steady states maintained close to equilibrium, thanks to the theorem of minimum entropy production due to Prigogine(6).

DYNAMICAL SYSTEMS, PHASE SPACE, ATTRACTORS

The search for a generalized thermodynamic potential in the nonlinear range has attracted a great deal of attention, but these efforts finally failed. Typically therefore, beyond the **linear domain** of irreversible processes eqs. (6) are expected to break down. A first consequence is that the steady state (point) attractor extrapolating the state of equilibrium as the distance from equilibrium is increased, can now be approached through damped oscillations (Fig. 3a).

FIG. 3. As the distance from equilibrium is increased a system can (a) evolve toward a steady state in a non-monotonic fashion or, (b) evolve toward a periodic attractor represented by a closed curve in phase space.



This behavior heralds a still more interesting possibility, depicted in Fig. 3b, in which the oscillation becomes eventually sustained. Topologically, this implies the emergence of a new, **1-dimensional attractor** in phase space, known as **limit cycle.** Such attractors should therefore constitute the natural models of the rhythmic behavior observed in nature, the most striking example of which are, undoubtedly, biological rhythms (7).

It is remarkable that biological rhythmic phenomena can nowadays be realized in the laboratory. A beautiful example is provided by glycolytic oscillations in yeast cell-free extracts (8). There is general agreement that the allosteric nature of phosphofructokinase, one of the enzymes participating in the reaction chain, and the autocatalytic control of this enzyme by the product ADP and by AMP are responsible for the oscillations (9). We see here quite clearly how the collective properties at the macromolecular level (stressed in G. Careri's contribution) and the nonlinearities at the supermolecular level (stressed in the present paper) are both necessary for the emergence of coherent behavior.

By allowing the intrinsic nonlinearities to be manifested beyond the regime of detailed balance, nonequilibrium can also lead to the coexistence of multiple attractors. This immediately leads to a topological problem, namely, how to delimit the relative **basins of attraction**, that is to say, the set of initial states in phase space that will evolve to either of the attractors. The solution of this problem depicted in Fig. 4, involves necessarily an intermediate unstable state as well as a family or orbits remaining invariant under the evolution known as **separatrices.**

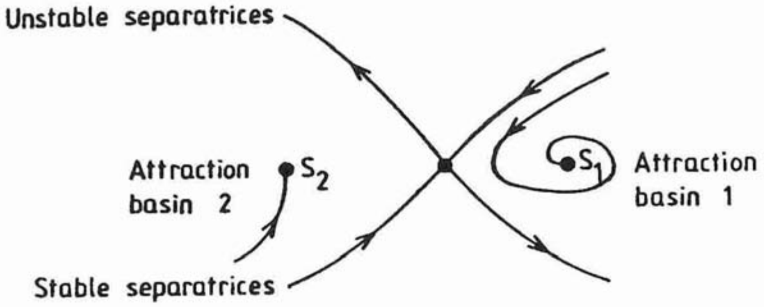


FIG. 4. Coexistence of multiple attractors in a dissipative system far from equilibrium. The set of initial conditions attracted by each of the stable fixed points S_1 and S_2 is determined by the configurations of two pairs of invariant orbits emanating from or merging to an unstable fixed point, known as separatrices.

Clearly, the coexistence of multiple attractors constitutes the natural model of systems capable of showing adaptive behavior and of performing regulatory tasks. It is therefore at the basis of the understanding of such important phenomena as genetic regulation in connection with induction and repression processes (10), or the activity of the immune system (11).

The existence of 1-dimensional attractors suggests the possibility of higher-dimensional attracting objects whose cross section along different phase space coordinates would give a limit cycle. Such objects can indeed be shown to exist and to have the topology of a **torus.** They model multiperiodic behavior which is observed under appropriate experimental conditions. We do not discuss these attractors in detail. Instead, we jump directly to what is undoubtedly the most complex and challenging attractive object known to date, namely a **chaotic attractor** (12). An example constructed from a model involving three variables is shown in Fig. 5.

We observe two opposing trends. On the one side (see horizontal arrow in Fig. 5) an instability of the motion tending to remove the phase space trajectory away from the steady state solution, which turns our to be the state $x = y = z = 0$; and on the other side (see vertical arrow) the bending of the outgoing trajectories followed by their reinjection back to the vicinity of the steady state. Two ingenious tricks of nature allow the reconciliation of these contradictory tendencies.

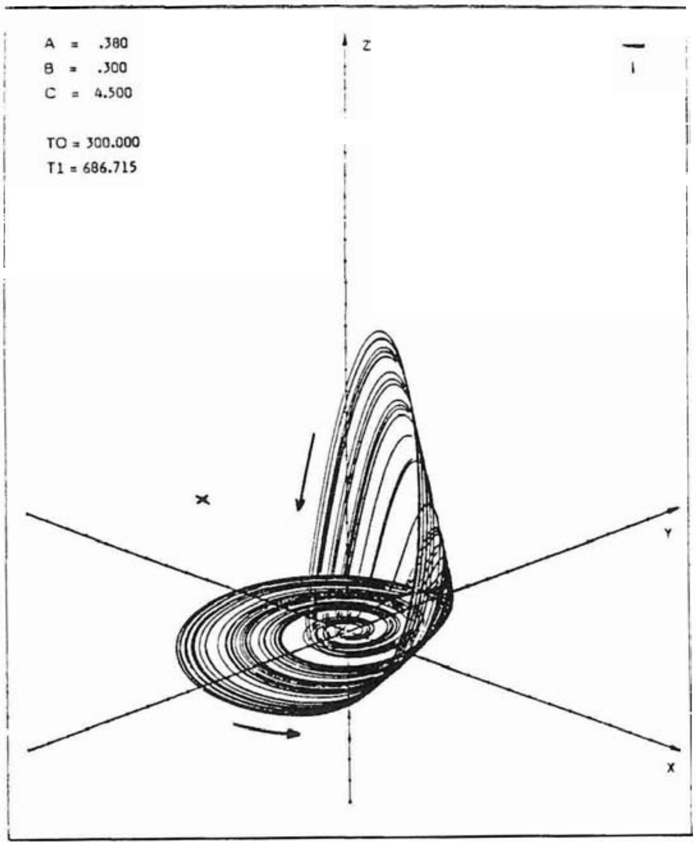


FIG. 5. Chaotic attractor constructed from a model system involving three variables and a single nonlinearity of second degree. The horizontal arrow indicates the instability of the motion of the trajectories spiraling away from the fixed point at the origin. The vertical arrow indicates the reinjection of the trajectories back to the vicinity of the origin.

One is the adoption of a **fractal geometry,** that is to say, the existence of attracting objects of non-integer dimensionality, d_F, which are intermediate between a surface and a volume. And the other is the sensitivity of the trajectories on the attractor to small changes in the initial conditions, as a result of which two nearby initial states can diverge, momentarily, in an exponential fashion. Quantitatively this divergence is measured by the **Lyapounov exponents,** σ_L. To be specific, let d_0 be an initial (small) separation of two trajectories in phase space. Under the action of the dynamics this distance will take a value d_t at time t. If d_t/d_0 varies exponentially with time during this interval the expression $\sigma = (1/t)\ln(d_t/d_0)$ will exist and be finite. More significant is the limit of this expression for large t and small d_0, as it provides the **mean** rate of this exponential variation. By definition, the Lyapounov exponents σ_L are the values taken by this limiting σ when d_t viewed now as a vector in phase space, is projected successively along the unit vectors of a coordinate system. There are, therefore, as many Lyapounov exponents as phase space dimensions. If the motion is unstable and presents sensitivity to initial conditions at least one of them will be positive, indicating an exponential rate of divergence along some direction in phase space. This will be so, in particular, for chaotic attractors. Clearly then, chaotic attractors provide the archetype of natural phenomena characterized by a **limited predictability.** Some implications of chaotic dynamics in biology are discussed later on in this paper.

BIFURCATION AND THE ORIGIN OF COMPLEXITY

One might be tempted to deduce from the above discussion, that a given system can only be modelled by a particular type of attractor. This is not so, however. As a matter of fact, the most exciting aspect of nonequilibrium phenomena is that the same physical system can show a great variety of behaviors, each corresponding to a different attractor. The mechanism which is at the origin of this diversification is the **instability** of a "reference" state and the subsequent **bifurcation** of new branches of states as the parameters $\lambda_1, ..., \lambda_m$ built in the system are varied.

The simplest bifurcation is depicted in Fig. 6. We represent in a graph the way a state variable of the system X is affected by a single control parameter λ. We obtain in this way a **bifurcation diagram.**

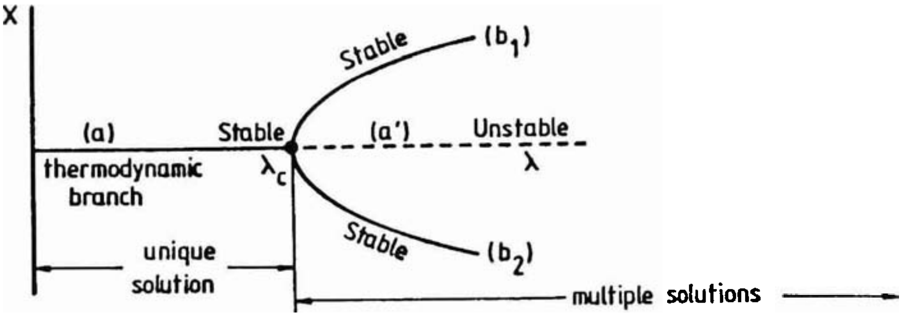


FIG. 6. Bifurcation diagram showing how a state variable X is affected when the control parameter varies. A unique solution (a), the thermodynamic branch, loses its stability at λ_c. At this value of the control parameter new branches of solutions (b_1, b_2) which are stable in the example chosen, are generated.

For small values of λ only one solution is accessible. It is the direct extrapolation of equilibrium and shares with it the important property of asymptotic stability, since in this range the system is capable of damping internal fluctuations or external disturbances. For this reason we call this branch of states the **thermodynamic branch.** But beyond a critical value, denoted by λ_c in Fig. 6, we find that the states on this branch become unstable: the effect of fluctuations or of small external perturbations is no longer dampened. The system acts like an amplifier, moves away from the reference state and evolves to a new regime. The two regimes coalesce at $= {}_{c}$, but are differentiated for $\neq {}_{c}$. This is the phenomenon of **bifurcation.** We can easily understand why this phenomenon should be associated with catastrophical changes and conflicts. Indeed at the crucial moment of transition (vicinity of $= {}_{c}$) the system has to perform a critical choice (Fig. 6, branches (b1) or (b2)). Nothing in the description permits to the observer to assign beforehand the state that will be chosen. Only chance will decide, through the dynamics of fluctuations. The system will scan the "ground", will make a few attempts, perhaps unsuccessful at the beginning, and finally a particular fluctuation will take over. By stabilizing it, the system will become a **historical object** in the sense that its subsequent evolution will depend on this critical choice. We have thus succeeded in formulating, in abstract terms, the remarkable interplay between chance and constraint, between fluctuations and irreversibility, presiding over the birth of complexity and self-organization.

Note the similarity between these ideas and the notions of mutation and selection familiar from biological evolution. As a matter of fact, one can say that fluctuations are the physical counterpart of mutation, whereas the search for stability plays the role of selection. Even the very structure of a bifurcation diagram is reminiscent of the phylogenetic trees employed abundantly in biology!

Fig. 7 represents a mechanical analogy of the phenomenon. A ball moves in a valley (Fig. 6 branch (a)) which at a particular point becomes branched and leads to either of two new valleys (Fig. 6, branches b1 and b2) separated by a hill.

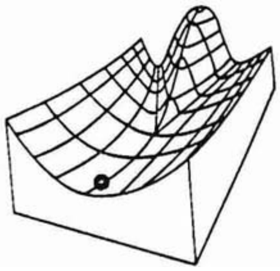


FIG. 7. Mechanical illustration of the phenomenon of bifurication.

Although it is always perilous to draw analogies and extrapolations, it is nevertheless thought-provoking to imagine for a moment that instead of the ball in Fig. 7 one could have a dinosaur sitting there prior to the end of the secondary era, or a group of our ancestors about to settle on either the ideographic or the symbolic writing!

BIFURCATIONS AND CHAOTIC DYNAMICS IN BIOLOGY

The modelling of biological regulatory phenomena by multiple point attractors or limit cycles in connection respectively, with all-or-none transitions or rhythmic behavior is, by now, a classical subject which has been covered extensively by many authors (2, 7, 9). In this Section we focus therefore our attention on the role of more complex dynamical phenomena, notably those giving rise to chaotic attractors, in the understanding of the multiple facets of biological complexity.

In as much as a system undergoing chaotic dynamics is unable to maintain sharp and reproductible correlations between the values of its state variables as time grows, chaos should constitute the natural model for the understanding of states of biological disorder. This viewpoint has received considerable attention recently. Impressive evidence has been produced that pathological situations related, for instance, to respiratory diseases or to arrhythmias of the cardiac muscle can be analyzed from a novel point of view by appealing to chaotic dynamics (13). This allows one to understand the role of some key parameters in the passage from normal to pathological behavior and to develop in this way new "dynamical" tools of diagnosis.

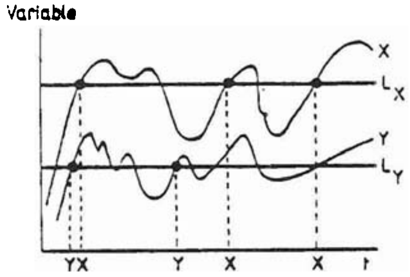
The idea we want to develop in the present Section is different. We want to stress that, in addition to being involved in pathological activities, chaotic dynamics is likely to play a **constructive role** of the utmost importance in biology (4, 14, 15). We illustrate this possibility by two selected examples.

A. Complexity and Information

In the Introductory part of the present essay we suggested that one of the characteristic features of complexity and concerted behavior is that objects at a certain level of organization are not random agregates of their constitutive parts but correspond, instead, to privileged realizations of an underlying selection process. In biology, a particularly dramatic manifestation of this phenomenon is the genetic code. On the one side, the code is essentially unpredictable since the global structure of the DNA cannot be inferred with certainty from the knowledge of one of its parts, however large. In fact, it is this unpredictability that allows us to speak of **information.** But on the other side with all its probabilistic character, the code is still expressed in terms of a limited number of variables, far less numerous than all possible combinations of atoms or elementary particles that one could think of a priori. This **compression** of the universe, as it were, in a single molecule, enables one to express biological complexity in the symbolic language of information theory, in other words, it brings out a new level of description. In this subsection we show that in certain classes of physico-chemical systems it is likewise possible to evolve spontaneously to states in which such concepts as symbols, codes, and information become the natural mode of description.

The need for a symbolic representation arises when one seeks to describe the outcome of a natural phenomenon in a way which is sufficiently compact and general to allow for a unified description of whole classes of other phenomena similar to the observed one. In some cases this decription turns out to be straightforward. For instance, suppose that we deal with a periodic motion. A characteristic feature of this type of behavior is that two variables, say X and Y, keep a fixed phase lag between them.

FIG. 8. Symbolic description of a dynamical system constructed by monitoring the successive crossings of prescribed thresholds L_X, L_Y, ... by the values of the state variables X, Y ...



If we therefore monitor the times of intersection (with prescribed slope), of their trajectories with preassigned levels L_X, L_Y, (Fig. 8) we will find the repetitive sequence

XY XY .... XY ... (a)

which may subsequently be regarded as the symbolic description of all periodic motions. This description brings out clearly two further properties: the full predictability of the behavior as well as the independence of the content on the direction of reading.

Let us now consider another extreme case, namely random (white) noise. This time the level crossings of X and Y would be distributed randomly and one would be led to a sequence like

XXYXXXY ... YYXYYX ... (b)

which could be generated equally well by a coin tossing. Although this sequence remains independent of the direction of reading, the full predictability of the preceding case has now been replaced by full unpredictability, in the following sense: if we monitor another pair of white noise variables we will find a sequence which, typically, will have nothing to do with (b) or better, a sequence which could in no way be inferred from (b). Two elegant (and related) mathematical concepts allowing to express this feature in a compact way are **algorithmic complexity** (16) and **information** (17). Algorithmic complexity measures the length of the shortest description of a given (finite) sequence. In this sense therefore, sequence (b) has the maximum possible algorithmic complexity, essentially equal to its length, whereas sequence (a) has the minimum possible algorithmic complexity since a single instruction (the initial sate XY) suffices to reproduce it fully. Similarly, information is considered to be maximum in case (b), since the realization of one particular sequence out of the enormous number of random sequences amounts to localizing the system very sharply in the state space. It is zero in case (a), since the outcome could be predicted on the basis of the initial state (XY).

It should be clear from the above discussion that the complexity of natural objects should lie somewhere between cases (a) and (b). Consider once again the genetic code. As Schrödinger put it so lucidly, the DNA is an "aperiodic crystal": it can therefore not be of the form (a). If, on the other hand, all random sequences of the four naturally occurring nucleotides (whose number is 4^N for a nucleic acid long of N nucleotides) could be equally good candidates for the genetic material, life would amount to selecting one unique event out of a tremendously large number of possibilities. The a priori probability of such a selection would be completely negligible. What is needed therefore, is a process capable of producing with high probability a complex, information-rich aperiodic sequence of states. Moreover, we want this edifice to be stable (in the sense of reproducibility) and asymmetric (in the sense of a well-defined direction of reading, as observed in present day DNA). A similar reasoning clearly holds for brain activity, the structure of a language and, most probably, for music and other forms of art.

Now, the self-organized states of matter allowed by non-equilibrium physics and arising through the mechanism of bifurcation reviewed in the previous Sections provide us with models of precisely this sort of complexity. Most important among these states ranks, for our present purposes, chaotic dynamics. Indeed, the instability of motion associated with chaos allows the system to explore continuously its state space thereby creating information and complexity. On the other hand, being the result of a physical mechanism, these states are produced with probability one: the selection of a particular sequence out of a very large number of a priori equiprobable ones simply does not arise. We suggest therefore that dynamical systems generating chaos act as efficient selectors rejecting the vast majority of random sequences and keeping only those compatible with the underlying rate laws. Equally important perhaps, the irreversibility incorporated in these laws gives rise to a preferred direction of reading and allows for the existence of attractors enjoying asymptotic stability and thus reproducibility. A more detailed presentation of these ideas can be found in Ref. 19.

In conclusion, the insertion of the symbolic concepts of Complexity and Information into Physics achieved by chaotic dynamics establishes a new link between Physical Science on the one side, and Biological and Cognitive Sciences on the other side.

B. Reconstruction of the Dynamics of the Electrical Activity of the Brain

We now turn to a more concrete example. Specifically, we show that nonequilibrium physics and dynamical systems theory allow us to literally resurrect the dynamics underlying the highest levels of biological organization from experimental data independent of any modelling.

The quantitative study of a natural system is based on the observation of a variable, or of a limited set of variables, during a sufficiently long period of time.

Figs. 9 and 10 provide some important examples of such **time series** pertaining to the electrical activity of the brain. In 9 of the EEG of a normal human subject in the stage of deep sleep is described whereas Fig. 10 corresponds to the EEG of a patient during an epileptic seizure. Both records show an irregular succession of peaks, although 10 looks definitely more "coherent" than 9. Is it possible to characterize this coherence more sharply? Do we deal with a well-defined dynamics or, rather, with a random course of events impossible to control? Mathematical modelling alone cannot settle the issue, since for both alternatives one can produce equations fitting the record in a reasonably satisfactory way. A new argument is therefore needed. Below, we show how nonlinear physics can provide us with clues to these important questions (20).

The first step is to embed the dynamics of the system under study in phase space. This means, in particular, that one should go beyond the "one-dimensional" view afforded by a time series of a single variable, $X_0(t)$. It can be shown that a phase space satisfying all requirements of dynamical systems theory is the phase space generated by $X_0(t)$ and its successive lags $X_0(t + \tau)$, ..., $X_0(t + (n-1)\tau)$. It suffices to choose τ in such a way that these different functions are linearly independent.

Next, in each of the above defined phase spaces (that is to say, for each choice of value of n) one tries to identify the nature of the set of data points, viewed as a geometrical object in an n-dimensional space. Again, dynamical systems theory provides algorithms for accomplishing this. One particular quantity which can be identified in this way is the dimensionality, $d_F(n)$, of our data set. Once $d_F(n)$ is determined one can also obtain information on dynamical properties like, for instance, the largest positive Lyapounov exponent σ_L, if any.

Finally, $d_F(n)$ and σ_L are plotted against n for increasing values of the embedding dimensionality. If these dependencies are saturated beyond some relatively small n, the system represented by the time series should be a deterministic dynamical system possessing an attractor. The saturation values of d_F and σ_L will be the dimensionality and the largest Lyapounov exponent of the attractor. The value of n beyond which saturation is observed provides the minimum number of variables necessary to model the behavior represented by the attractor. If on the other hand there is no saturation trend, the conclusion will be that the system described by the time series evolves in a random way.

Applied to Figs. 9 and 10 the procedure leads to two strikingly different predictions (21, 22). In both cases a chaotic attractor seems to exist. Figs. 11 and 12 depict their projections in a two-dimensional phase space. As it turns out, for Fig. 11 the dimensionality is slightly above 4 whereas for Fig. 12 it drops to a value slightly above 2. This provides us with a quantitative measure of the

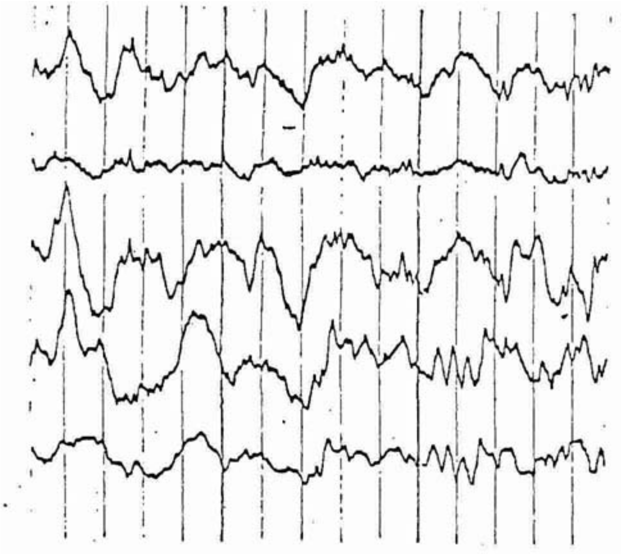


FIG. 9. EEG record during the deep sleep stage of a normal human subject.

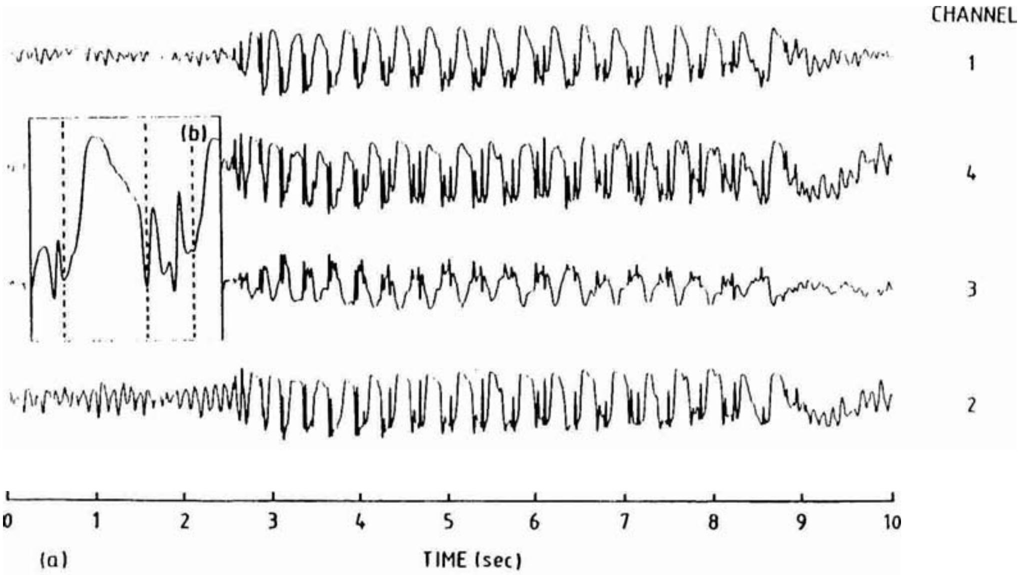


FIG. 10. EEC record during a 5 second epileptic seizure of a human subject.

excessive coherence associated to the pathological state of the brain. Moreover, as pointed out at the beginning of the present section, chaotic attractors are potential information-generating devices. We are thus led to a tantalizing picture of how information, one of the most conspicuous attributes of the human brain, can be linked to, and even emerge from its dynamical activity.

CONCLUDING REMARKS

The principal idea that we developed in the present essay is that the physics of nonequilibrium phenomena and the mathematical theory of dynamical systems provide us with the concepts and tools necessary to handle complexity. We have seen that nonequilibrium attractors constitute the natural achetypes for a variety of phenomena which are conspicuously present in biological organization, such as regulation, rhythmic activity, and information generation or processing. Similar ideas are currently being applied to the analysis of other complex phenomena encountered in our natural environment, such as atmospheric dynamics (23), climatic variability (24, 25), or economic activities (26).

It is the author's strong conviction that the existence of physical archetypes of phenomena that usually one tends to ascribe exclusively to life will have a lasting influence in the attempt to comprehend the multiple facets of biological complexity.

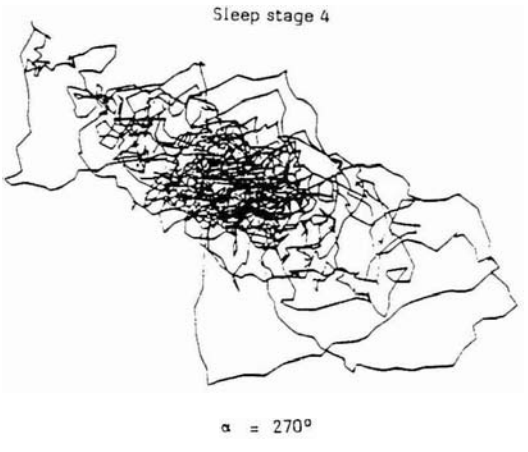


FIG. 11. Phase portrait of EEG recorderfrom human deep sleep stage (2000 equidistant points sampled at 100 H_z and $\tau = 10\Delta t$).

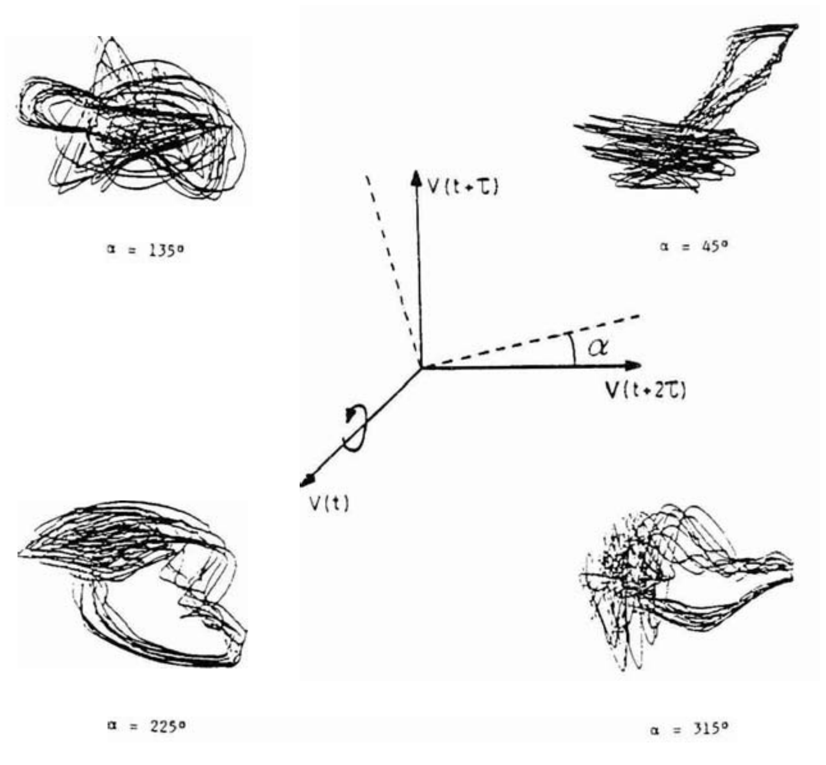


FIG. 12. Phase portrait of human epileptic seizure (5000 equidistant points and $\tau = 19\Delta t$).

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