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HOMEOPATHY: A FRONTIER IN MEDICAL SCIENCE
EXPERIMENTAL STUDIES AND THEORETICAL FOUNDATIONS

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TEXT without figures

Chapter 5

5. COMPLEXITY, INFORMATION, AND INTEGRATION

The increase in biomedical knowledge which has come about over the past two decades as a result of the molecular approach has led to a further upsurge in our awareness of the extreme complexity of living systems. Human beings possess roughly 100,000 different genes and thus, theoretically, may be regarded as constructions made of a vast number of bricks of about 100,000 different types (considering proteins alone). In actual fact, these bricks are not arranged at random, but are assembled in a highly coordinated manner and, what is more, are constantly being rearranged by interaction within the system and with the environment. To this we should add our heightened awareness of the existence of numerous biological differences between individuals of the same species which make it difficult, if not impossible, to establish normal values and predict the outcome of external regulatory interventions.

In other words, the increase in knowledge of the subcomponents of the living system is accompanied by increasing difficulty in describing the unitary behavior of the system itself in scientifically precise terms and, as a consequence in the biomedical field, by an increasing awareness of the limitations of therapies based on a reductionist approach. The basic reason for this lies in the fact that the physician invariably finds himself having to apply general biological notions to a particular, unique and unrepeatable case, who reacts as a unitary being in a context with which he or she has countless interrelations.

In this connection, it is worth quoting what the Nobel Prize winner for Medicine, Alexis Carrel, one of the few top-ranking scientists to have an open mind on the study of nonconventional forms of medicine (he even edited a book on the subject), has to say: “The future of medicine is subordinate to its concept of the human being. Its greatness depends on the wealth of this concept. Rather than limit the human being to certain of his aspects, it must be all-embracing, fusing body and spirit in their essential oneness. (...) Any human individual is at the same time complexity and simplicity, oneness and multiplicity. Every individual presents a different story from all the others. He or she is a unique aspect of the universe. (...) To date we have studied ourselves only in such a way as to procure fragmentary concepts of what we are. Our analysis began right from the outset by severing the continuity of the human being and his cosmic and social environment. Then it separated the soul from the body. The body was divided into organs, cells, and fluids. And in this process of dissection, the spirit has vanished. Thus, there are many sciences, each dealing with an isolated aspect. We call them sociology, history, pedagogy, physiology, and so on. But a human being is

much more than the sum of these analytical data. He should thus be considered both in his parts and as a whole, inasmuch as he reacts in the cosmic, economic, and psychological environment as a single entity and not as a multiplicity of entities" [Carrel, 1950, pp. 9-10].

Medicine may provide evidence to the effect that "a human being is much more than the sum of these analytical data," by opening its doors to the study of complexity, totality, individuality, systemic interrelation phenomena, and ecology. In this context, forms of complementary medicine such as homeopathy, acupuncture, and that vast and as yet poorly defined area known as "natural medicine" may have a role to play as a stimulus or a challenge to scientifically conceived medicine.

The need to address these problems in relation to homeopathy and its possible mode of action derives from three series of considerations:

a) Health and disease are basically determined by states of order or disorder of the body, which are an expression of the complexity of living beings.

b) The homeopathic method presents itself as an all-embracing, integrated approach to health and disease: it takes account of all the possible factors of a biological and psychological nature which characterize a given individual, seeking to grasp the essence of their interrelations. These interrelations can only be understood within a logical framework which takes due account of complexity.

c) Homeopathy claims that information is contained in the aqueous or water-alcohol solution in a "meta-molecular" form. If this is true, then it follows that such solutions are characterized by some sort of order and memory (information store). The problem of the hypothetical transfer of information from the solute to the solvent falls within the sphere of the complex behavior of liquids, and is investigated in that context. In a later section we shall see that physicists are constructing models of water in the liquid state and positing ways in which these may hypothetically store information.

To better clarify the importance of these issues for the formulation of hypotheses regarding homeopathy, we intend to examine here below a number of sectors of modern medicine in which complexity has come to play a prominent role, precisely on the basis of present-day biological and molecular knowledge.

5.1 Complexity of diseases

In an initial approach to the question of complexity we can take as our starting point a reflection, which is only apparently theoretical, on the nature of disease in general. These considerations are theoretical only in appearance because *it is inevitable that the diagnostic and therapeutic approach to disease depends on the concept one has of it* in general, on the theoretical and philosophical plane, rather than in its individual details. This is all the more true for those who seek to reason and act within a holistic rather than a specialistic conceptual frame of reference.

Disease, in essence, is a disorder of structures and/or functions, with characteristic abnormalities at the cellular and molecular levels. The definition of disease inevitably depends on the standpoint (meaning not the personal opinion, but the perspective from which the matter is viewed) of those attempting to define it [Laplantine, 1986]. Today, the standpoint of modern medicine, as conceived scientifically, is represented by molecular pathology because the astonishing progress which has come about as a result of the introduction of molecular biology techniques, particularly in the analysis of proteins and nucleic acids, has led to an enormous increase in our knowledge of the molecular abnormalities (both quantitative and qualitative) present in many diseases, whether hereditary or acquired. This new molecular-based knowledge of many diseases is beginning to have significant positive repercussions in terms of diagnosis and a more rational utilization of drugs. It has been the task of the molecular approach to clarify the biological basis of disease, and this will continue to be the case for many years to come. In view of the variety of possible pathological situations at this level, it is a task which is several degrees of magnitude greater than that performed in the past by pathological anatomy at organ or cell level.

The problem of an exhaustive and satisfactory definition of the concept of disease cannot be solved merely on the basis of a knowledge of molecular biology, however detailed that may be. The great increase in the *extent* of our knowledge is not enough to guarantee an *intensive* understanding of the deeper meaning which the abnormalities observed have in the dynamics of the onset and development of a disease process. Any satisfactory description of the nature of disease necessarily entails a search for the causes (etiology) and the mechanisms (pathogenesis), or, in other words, the "why" and "how" the disease process sets in and develops. The search for causes will be successful when the causes are precise and usually confined to one or

only a few physical, chemical or biological damaging factors, but encounters often insurmountable obstacles when the causes are multiple or when the disease originates from a series of causes in succession, each dependent upon the previous one. Establishing the pathogenesis requires the largest possible number of notions on the objective changes (whether anatomical, biochemical, molecular or electrophysiological), but also calls for identification of the cause-and-effect relationships and for the hierarchical ordering of phenomena in terms of space and time. Our attempt here is to outline an approach to the definition of disease based not so much and not exclusively on the molecular paradigm, but on a way of reasoning which takes account of new epistemological horizons.

Life is an expression of the complex behavior of nature. It is essentially the property of an open system in which information governs matter and energy, but without completely suppressing, and indeed often benefiting from, the chaotic element (the concepts of information and chaos will be dealt with in Chapter 5, Sections 5 and 7, respectively). Life is a meta-stable state: it maintains and reproduces itself as a thermodynamically far-from-equilibrium event, thanks to the exchange of energy and matter taking place between the living system and the environment [Guerritore, 1987; Guidotti, 1990]. The fact that homeostatic biological systems exist which maintain certain parameters within suitable oscillation limits does not mean that the body or its subsystems are in a state of "equilibrium," but merely that the body is well organized and knows how to channel the *flow* of matter in a manner which is productive for life itself.

For instance, it is well known that there are very substantial differences in the concentrations of ions (sodium, potassium, hydrogen, calcium, magnesium) between the various cell compartments separated by biological membranes. The cells actually make use of these differences and asymmetries to generate signals, information, and even energy. Thus, the transmission of the nerve impulse depends on the imbalance between sodium and potassium across the fiber membrane; cell division requires a transfer of hydrogen ions from the intra- to the extracellular space (alkalinization of the cytoplasm); cell movement involves an unstable alternating process of assembly and disassembly of multimeric structural proteins. The maintenance of life and health therefore consists in controlling a dysequilibrium ("controlled dysequilibrium") [Guidotti, 1990].

Clearly, the state of good health cannot be maintained indefinitely and ageing is inevitable. This problem, too, has no simple explanation, entirely attributable to molecular parameters. As the neuropharmacologist M. Trabucchi says: "The latest biomedical research has attempted to clarify the ways whereby life leaves its traces on biological structure. Some of these ways are predictable because they obey objective (or scientifically parametrized) laws, while others are unpredictable. To the former group belong the experiments, mainly conducted in animals, on the basis of which a number of environmental characteristics are reflected in simple parameters of neuronal functioning (dendritic arborization, number of synapses, etc.); to the second group belong those series of events which can be characterized by the theme of complexity, whereby enormously variable external stimuli are interpreted via various man-environment interfaces and translated into highly differentiated biological and personal realities" [Trabucchi, 1992, p. 137].

At the opposite extreme to the organization of life is death, which therefore represents the maximum disorder, dissipation of information, and increase in entropy, tending towards thermodynamic equilibrium (the concept of *entropy* in living systems will be discussed in Chapter 5, Section 8.1). Disease lies somewhere between the two, consisting in partial disorder of systems of information, energy, and matter, localized in space and time.

When reflecting upon the question of disease, one problem which immediately springs to mind is understanding which of the various events observed are primary and which secondary: not everything in the disease process is pathological, in the sense that it is damaging. Disease is disorder, but it nevertheless obeys certain laws, and thus embodies some measure of order, though this is conditioned by chance events. The homeostatic biological systems which govern health are the same that cause most pathological phenomena, when activated inadequately, excessively or unsuitably in relation to the circumstances. On the other hand, it is also true to say that many phenomena that are called pathological are biologically useful (even if they cause pain), representing a stage of transition to a state of greater vitality, energy, and resistance to pathogens (= information gain). For instance, we need only mention inflammation and immunity, both of which are pathophysiological processes which, though carrying a certain price to be paid in terms of subjective symptoms and possible organ damage, in actual fact serve the purposes of repairing, defending, and inducing a state of enhanced resistance. This enhanced resistance derives from the biological memory of past experience.

These considerations lead us to our first conclusion: judging what is useful and what is damaging, on every occasion and in every aspect of disease, is by no means easy, in that it presupposes a knowledge of the "logic" of disease and normality, a knowledge of the language of complex systems (some of these systems are inflammation, immunity, neuroendocrine organizations, subtle metabolic regulatory mechanisms) rather than of the language of molecules. Disease is a problem of molecules, but also, in a different dimension, it is a problem of cells, of physiological systems and of the human being as a whole: if the molecular disorder is not compensated for by supramolecular systems, it is the latter that are responsible for the disease, and not the molecule. Disease is a problem of the individual, but it is also a problem of the environment: the individual is often the victim of a disease greater than him- or herself (e.g. violence, pollution, epidemics, misinformation by the mass media, social alienation, loneliness), and whoever reflects upon the real nature of diseases can hardly be satisfied with a reductionist explanation which fails to go beyond the latest biochemical consequences of these problems.

Disease is thus essentially an information disorder. Genetic diseases are the most striking examples of this: the *order* of the genetic code sequence is changed, and the disorder lies in the very information store itself. Genetic disease can also be caused by a very minor transcription error in the basic cell library. Even acquired diseases, or diseases in which genetic and environmental factors are mixed (these are by far the majority) are disorders of information at a more complex level: what is altered is not just the molecular order of the DNA, but also the information order governing the supramolecular systems. In most diseases we can identify an imbalance of the homeostatic biological systems at various levels. The molecular, cellular, tissue, organic, and neuroimmunohematological systems, tend in themselves to function according to deterministically correct parameters.

For example, in inflammation, thrombosis, atherosclerosis, hyperplasias, and endocrine disorders, it often proves possible to identify not a primary defect of the system itself, but a defect in its regulation. The platelet, when it causes a thrombus, is doing its job, as are thrombin and fibrin. The macrophage, when it engulfs oxidized lipoproteins, is doing its job (scavenging), even if this then causes an accumulation of foam cells in the tunica intima of the artery. It is true that a particular genetic defect may cause the pathological event (e.g. a lack of C/S proteins in thrombosis or a lack of LDL receptors in atherosclerosis), but more often than not, in practice, such genetic defects are neither marked nor decisive. Furthermore, every disease, even if primarily genetic, depends to a large extent in its clinical course on the occurrence of regulatory imbalances, inasmuch as the system would tend to counteract every defect with an adequate compensation mechanism.

The concept of the host and its importance in pathology come back into the reckoning with a reappraisal in more updated terms, so that today we could talk about a neuroimmunoendocrine system, but the substance of the matter does not change: every disease has a strong component related to endogenous reactivity. It is, in fact, only after a long time and after continuous perturbations of the homeostatic biological systems that multifactorial diseases manifest themselves clinically, when the homeostatic systems have departed from the norm, adapting to a chronic situation of abnormal pathological symptoms. In practice, a substantial proportion of the diseases have as their basic mechanism an upward or downward shift in the activity of various homeostatic systems, with all the possible range of symptomatological sequelae and permanent structural abnormalities which in turn then come to form the basis for new imbalances.

A simplified block diagram of the pathophysiological events occurring in the course of a typical disease is shown in Figure 2. This diagram represents a general frame of reference, which is useful above all for illustrating the interrelationships between various steps in the disease process which integrate one another dynamically. It can be seen that if etiological agents of various kinds (chemical, physical, biological, and deficiency-related) overcome the first set of defense systems, they cause structural and/or functional biochemical damage. This damage triggers reactions on the part of the systems responsible for the conservation and restoration of biological integrity, also called *homeostatic biological systems*. These systems thus occupy a central position in the dynamic evolution of a disease: good functioning leads to repair and healing, but the homeostatic systems themselves can cause further damage, giving rise to a kind of positive pathological feedback. Obviously, if the damage, be it direct or indirect, is severe or irreversible, a no-return situation is created which may lead to death or to permanent disability (pathological state).

Most of the *signs and symptoms* of the disease stem not so much from the direct damage caused by the etiological agent as from the reactions of the body.

Figure 2. Diagram of a typical pathophysiological frame of reference representing the possible events involved in a disease. For an explanation, see text.

The block diagram in Figure 2 also shows another possible evolution of the typical pathophysiological pattern, namely *adaptation*. In a certain sense, this is an intermediate course midway between healing and the pathological state, since it represents a new state of normality adapted to the changed circumstances. For example, if there has been lung damage which has reduced the alveolar-capillary exchange surface, the homeostatic system controlling the oxygenation level will react with production of a greater number of red blood cells (polycythemia). Polycythemia is not normal in subjects who do not live at high altitude, but at the same time it cannot be regarded as a pathological state, despite constituting a long-term modification. If, for the sake of hypothesis, we succeeded in bringing about a regression of the pulmonary picture, the polycythemia would disappear. Other examples of adaptation may include cardiac hypertrophy and changes in kidney function in the course of hypertension, lymphadenomegaly in children exposed to continuous immunological stimulation, hyperinsulinemia in obese subjects, steatosis or xanthomas in certain serum lipid disorders, and many others.

If disease is an information disorder of complex systems, to get to the heart of the matter the molecular approach, which analyzes only one aspect of information, is necessary, but not enough. New approaches, new models, and new concepts are beginning to be introduced in biology in order to overcome this hurdle. It is not enough to understand the individual elements and try to put them together according to a computer-aided or cybernetic model: no-one believes any longer that the formulation of a precise, predictive model, capable of taking account of all the variables involved in a single cell, is remotely feasible, not to mention the even unlikelier construction of exact models for the functioning of organs or systems.

Faced with this plain fact, one might be tempted to conclude that we can never describe a disease exactly and consequently that it is impossible to come up with a therapeutic measure which is completely rational and correctly oriented towards curing the disease. If this were the case, we would therefore have to settle for understanding only certain aspects of the disease (something which is certainly possible today) and opt for a therapy based on these aspects (e.g. antiinflammatory, analgesic, replacement therapy, etc.). This does not mean that these latter therapies are not useful and efficacious in many cases, or that they cannot favor definitive healing processes set in motion by the body itself. What it means is being realistically aware of the type of measure being applied and thus also understanding the reason why in many cases the current therapies are not enough to solve the problem.

If we want to capture in a single image the whole crux of the problem of information regulation in vital processes, and thus also of its pathological aspects, it may be illuminating to refer to the model of an orchestra. The orchestra is the body, and the music is its life. In the orchestra, there is a material, “molecular” part, composed of instruments with a precise structure and of musicians with their receptive, elaborative, and motor capabilities. What matters most, however, is that the orchestra plays in harmony according to a program provided by the score and at a pace dictated by the conductor.

A performance may prove unsuccessful because one of its material parts breaks (e.g. the strings of a violin, or the stool of one of the musicians), but it may also fail because the various musicians are in disaccord. The quality of the music the orchestra produces depends on conditions such as the quality of the instruments, the quality of the score, the skill of the conductor, and, above all else, the degree of unison and harmony between the members. If there should be interference due to some outside noise or disturbance, or if an orchestra is tired or distracted, there is a risk that the orchestra will play out of tune, and this risk is all the more serious, the less able the conductor is to keep the orchestra under control. If the music is seriously out of tune, or the conductor is weak, the flaw may involve the entire orchestra to the distinct detriment of the work as a whole.

This example shows us that there does not necessarily need to be a primary structural abnormality for the overall effect to be a disaster. An information disorder can also arise as a result of subtle and not immediately perceivable deviations from the norm, which are then amplified and/or stabilized by adaptation and positive feedback mechanisms. In the healthy body this orchestra plays continuously in a coordinated manner. It is difficult to say whether there is a “conductor” because all the parts, including the brain, function properly, influencing one another reciprocally. It is undeniable, however, that there is a hierarchy, whereby some systems have greater control functions and therefore may be regarded as more important from the regulatory point of view.

Apart from the example provided here, is there any way of tackling the problem of complexity rationally? Given that there is no possibility of constructing exact models of the various phenomena endowed with predictability, we have to see whether at least certain "groundrules" of behavior of complex systems can be identified, which can be used in possible attempts at modulation. As mentioned above, the study of complexity is a frontier area for science, where mathematicians, physicians, and biologists seek to break new ground. It is very likely that these studies will yield appreciable windfall gains in the biomedical area, too. One can easily imagine that substantial links will emerge between these concepts, which refer to the behavior of complex systems, and the interpretation of the mechanisms whereby complex biological systems may deviate from the state of normal homeostasis (see also Chapter 5, Sections 7 and 8).

Any approach to the definition of disease will reflect reality more closely, the more *integrated* it is, in the sense of its taking account of all the possible levels at which the pathological information disorder manifests, perpetuates, and communicates itself. What is emerging with increasing clarity and with a growing body of corroborative evidence is that order in the biological sphere is produced by an interactive network of molecules, cells, organs, and systems, made up of both *horizontal* (e.g. molecule to molecule) and *vertical* messages between systems with different levels of complexity (e.g. molecule to cell, but also cell to body, and body to environment). No biological system exists in isolation; it could never survive, since it would be overwhelmed by entropy. Thus, neither can disease be an isolated, self-sufficient event; disease is not simply an error of nature, but rather one of its "ways of being," the deeper significance of which remains largely inaccessible.

5.2. Example of a complex biological system: inflammation

Inflammation is the response of living tissue to damage, which may be of a physical, chemical or biological nature. We are in the presence, then, of a phenomenon which is at the same time physiological and pathological and which is in some way implicated in all diseases; its comprehension on the scientific plane and its modulation on the pharmacological plane represent one of the greatest challenges to medical science.

Starting from a whole variety of endogenous and exogenous causes of noxious stimulation, a process is set in motion in which various molecular components (plasma proteins, lipids, prostaglandins, hormones, peptides, ions) and cellular components (leukocytes, platelets, macrophages, endothelial cells, neurons) take part. Though inflammation is usually a local tissue alteration, it would appear increasingly clear that various organs or systems take part directly or indirectly in its regulation.

While the defensive function of inflammation as it has developed in the course of evolution is beyond doubt, an increasing amount of attention is being devoted today to diseases due to excessive activation of this pathophysiological mechanism and to the secondary damage it causes. The effector systems and the regulation mechanisms themselves turn on the host, causing a series of increasingly widespread diseases due not so much to outside causes as to malfunctioning of the inflammation and immune systems. As these same mechanisms are capable of acting both defensively and offensively, the *interpretation* of the language of inflammation (i.e. of the various messages which the systems involved exchange) is of fundamental importance for its possible control and modulation. The *language* which the inflammation systems speak tends today to become coherent through a *cybernetic model* and contains words such as "signals," "mediators," "targets," "activation," "regulation," "message" (inter- and intracellular), "priming," "desensitization," and "memory."

The inflammatory process is vital for the survival of all complex organisms and is involved in many aspects of health and disease. There can be no doubt that the inflammatory process has evolved as a basic mechanism for protecting the integrity of the organism. This protection is exerted both against possible invasion by pathogenic microorganisms present in the environment (the fight against infection) and against pathological modifications of the normal constituents of the body: for example, an inflammatory process intervenes when necrotic processes set in due to tissue anoxia, trauma or burns, when there is vascular damage and hemorrhage, when a tumor develops, when a transplant is inserted, and in tissue affected by ionizing radiation. In all these cases, the basic defensive, reparatory meaning of the inflammatory process is self-evident. This is proved by the fact that certain functional deficits or a reduction in the number of inflammatory cells easily lead to a state of high susceptibility to infection.

Since living organisms are constantly subject to stress and to aggression of various kinds, the development of more or less marked inflammatory processes is inevitable and, in a certain sense, may be

regarded as a positive factor, since it contributes towards augmenting the natural defenses themselves. There are gross and painful manifestations of the inflammatory process which can quite easily be interpreted as the inevitable price to be paid if elimination of pathogens is to be achieved. These include, for instance, most of the symptoms accompanying acute infectious diseases (fever, asthenia, anorexia, pain in the infected area, exanthema). On the other hand, there are inflammatory phenomena which are frankly unjustified and thus largely damaging to the body; they include, for example, those related to autoimmunity, or to rare defects of the inflammation-inhibiting systems (e.g. hereditary angioedema), or to transplant rejection.

Midway between these two extremes lie a whole series of diseases in which the inflammation initially occurs for defensive and/or reparatory purposes, but later, for various reasons, becomes a pathogenetic mechanism which conditions - possibly to a decisive extent - the course and outcome of the disease itself. In these cases, the processes triggered off hover in a state of constant imbalance between damaging attack and defense. The inflammatory process does not succeed in fulfilling its reparatory purpose and is involved in a general *organizational disorder* of the body, with the result that its original aim is lost. It is well known that a substantial proportion of the most common diseases today can be traced back to disorders of the inflammatory process, due essentially to two mechanisms, namely either to failure to recognize its own components which are regarded as foreign, or to abnormal control of an inflammatory process, which would otherwise be suitable in terms of the molecular target (excessive amplification, nonsuppression of the process, and spread beyond the area anatomically necessary).

The basic problem is thus understanding when and how the inflammatory process strays into pathological territory. The question applies to both acute and chronic inflammation. Obviously, this is a major problem both from the conceptual and, consequently, from the clinical and therapeutic points of view. Understanding the dynamics of inflammatory processes, of the biochemical mechanisms involved in them, of the functions of the various cells involved, and of the regulatory centers responsible for centralized control constitutes one of the greatest challenges to medicine and the basic prerequisite for effective therapy.

We deal here fairly extensively with the inflammatory process because it is the main reaction and repair system for damage of any kind and must therefore occupy a central position within the framework of any hypothesis with regard to the mechanism of action of homeopathy. It has been seen, in fact, that this type of medicine has always claimed, ever since its inception, to aim at activating the endogenous reactive capability of the body ("vital force"). Some parts of this section have been taken from a previous review of the same topic [Bellavite, 1990b].

5.2.1. Basic characteristics of the inflammatory process

Inflammation (also called phlogosis) was recognized by the ancient Roman Cornelius Celsus as the process causing "*rubor, tumor, cum calore et dolore*" (redness, swelling, with heat and pain). In modern terms, it can be defined as an *integrated response of tissue and even of the entire body (when the process is sufficiently extensive) to damage caused by external or internal agents*. This series of responses consist in changes in blood vessels, circulating plasma, and cells, especially leukocytes. On a general scale, many other phenomena occur caused by remote repercussions of the originally localized process.

The view of the inflammatory reaction as a phase in a continuous process going from damage to healing or to further damage is in tune with the concepts of "*progressive vicariation*" and "*pathological metastasis*" (in the broadest sense of the term as used in conventional medicine) traditionally propounded by homotoxicology and homeopathy to suggest that there is a profound, consequential link between the various phases making up the *disease history* in a patient. The field of inflammation is an ideal subject of study for homotoxicology and natural medicine in general. Indeed, the very aim of this type of therapeutic approach is to try to use systems of care which cooperate with the natural healing process, exploiting its great intrinsic potential.

The terrain where most of the inflammatory process takes place is the connective tissue, consisting of cells of mesenchymal origin, leukocytes, afferent and efferent nerve endings, hematic and lymphatic vascular networks, fibers, and basic substance. The network of capillaries in a tissue is composed of endothelial cells resting on a thin basal membrane. The blood flow in the capillaries is determined above all by the degree of patency of the arterioles and their terminal branches which are endowed with smooth muscle with sphincter functions. At this level, the control exerted is nervous, hormonal, and also dependent upon partial oxygen pressure and pH. When a traumatic event occurs in this territory, or bacteria arrive, or toxins or irritating

chemical substances are present, many biological phenomena come into operation, the most important of which are:

a) After initial contraction, the smooth muscle cells of the arterial terminal branches relax, thus allowing the entry of much more blood, which circulates in the capillary network, first rapidly and then more and more sluggishly, engorging the entire tissue (hence the old descriptors "calor" and "rubor"). An important role in this phase is played by the endothelial cells themselves, which, when activated by physicochemical changes in the surrounding environment, give rise to a series of molecules which go on to mediate further events.

b) The mast cells present in the connective tissue release their granules containing histamine and other substances, thereby causing the opening up of spaces between the endothelial cells with leakage of the fluid portion of the blood (plasma) and formation of exudate (the old "tumor," in the sense of edema, or swelling).

c) The exudate may dilute and remove microbes and toxic substances, mainly via the lymphatic network, thus contributing to activation of the immune response. The exudate may form a fibrin layer, which also constitutes a barrier to the spread of infectious pathogens. It also contains many substances which are active as mediators of the further development of the inflammation and as mediators of the amplification of the reaction. These substances include components of complement which stimulate the mast cells to release histamine (anaphylotoxins) and others which play a direct role in the killing of bacteria. Some of the mediators also stimulate sensory nerve endings, causing pain (the old "dolor") and, as discovered only fairly recently, the release by these nerve endings of neuropeptides, which in turn increase the inflammatory response.

d) We then have the action of the white blood cells in the inflammation focus, primarily granulocytes, which, in response to perceived changes in the endothelium and tissue fluids, emerge from the vessels under the influence of the bacteria themselves, cellular debris, endotoxins, fibrin fragments, activated complement, and specific cytokines such as interleukin-8.

e) In the later phases of the reaction, we also find lymphocytes, monocytes, and macrophages (chronic inflammation). Fibroblasts, as is well known, come into play in the processes of wound repair and scar formation. Among the possible consequences of inflammation there is the development of sclerosis; we need only mention the healing of wounds by second intention, cheloids, cirrhosis of the liver, pulmonary fibrosis, and atherosclerosis itself, many of whose pathogenetic elements constitute a "*response to injury*."

We are in the presence therefore of a complex series of integrated phenomena, in which the granulocytes intervene as the most active cells in the production of toxic oxygen radicals, but also as cells capable of producing a series of mediators acting as signals for other cells. We can do no more here than merely outline some of the aspects of the biochemical regulation of the inflammatory process, this being a vast and much debated topic.

The granulocytes arising in the bone marrow remain in the bloodstream only a few hours and then adhere to the endothelium and pass into the tissues to prepare the defense against possible foreign agents. The molecular mechanisms whereby cells attach themselves to one another are well known; for instance, we know that, in the vicinity of the inflammation focus, granulocytes express anchoring proteins on the outer membrane (these are of various types, including the so-called integrins) which attach themselves to specific receptors produced and expressed by the endothelium, called intercellular adhesion molecules.

A great deal is also known today about cell orientation and motility systems, which underlie the phenomena of chemotaxis and phagocytosis. Receptors have been described for dozens of different substances to which granulocytes are sensitive and which stimulate them to move and migrate in an oriented manner. It is also known that the mechanical apparatus which the cell needs in order to perform these functions is based essentially on phenomena of continuous polymerization and depolymerization of proteins of the cytoskeleton, which form organized filaments in the cytoplasm. Among the granulocyte cytotoxic and bactericidal systems a key role is played by the production of toxic oxygen radicals.

This particular type of oxygen metabolism is activated during phagocytosis or as a result of other soluble stimuli and is based on the fact that oxygen is not used to produce energy, as in all the other cells, but to produce electronically activated derivatives such as free O_2^- radicals (superoxide), OH· (hydroxyl radical), oxygenated water, and other highly reactive molecules such as singlet oxygen and hypochlorous acid. Involved in the production of these latter derivatives is the enzyme myeloperoxidase. Oxygen metabolism, as described here, implies the consumption of NADPH as a donor of electrons and consumption of glucose-6-phosphate, which is necessary for the production of new NADPH [Rossi, 1986; Bellavite, 1988]. In addition to the NADPH oxidase system, the inflammation cells also produce *nitric oxide*, which has bactericidal and

parasiticidal functions, as well as regulatory functions in the inflammatory process. Nitric oxide, in fact, can cause vasodilation and can also interfere with superoxide and inactivate it.

The effects of free radicals at molecular level are essentially changes in proteins (oxidation, aggregation), polysaccharides (depolymerization), lipids (peroxidation), and nucleic acids (strand break, mutations). To what extent such effects may be useful or damaging depends on the situation in which these changes occur. In fact, if the release of toxic radicals occurs in the context of a destructive action against invading pathogens or against toxins (endogenous or exogenous) or tumor cells, it may undoubtedly be regarded as useful. On the other hand, however, the same biochemical changes may be of prevalently pathological significance in other contexts such as atherosclerosis phenomena, postischemic tissue damage, pulmonary emphysema, Parkinson's disease, multiple sclerosis, shock, burns, rheumatoid arthritis, respiratory distress syndrome, and many others. Clearly, in such situations, what determines the end result of a biochemical reaction is not so much the molecule itself, or its quantity, as the *regulation* of the inflammatory process in the broadest sense of the term.

One of the most recent fields of study of the function of leukocytes in the context of inflammatory processes has to do with the regulation of their different levels of activation, which are multiple and varied. This is demonstrated both in systems in culture and in *ex vivo* cells, extracted from healthy subjects and patients suffering from various different diseases. These types of tests are not simple laboratory artefacts, but allow us to reproduce a situation which occurs *in vivo*, i.e. where the cells in a patient presenting inflammatory manifestations are *different* from the cells in a healthy person. It is also known that if we take leukocytes from a patient during a bacterial infection of a certain degree of severity, we find not only that they have grown in number, but also that they are functionally more efficient [Bass *et al.*, 1986]. In other pathological conditions, such as, for instance, in the course of viral infections or after severe burns, blood leukocytes are in a state of desensitization, and therefore present activity deficits. There may also be functional changes in leukocytes in different areas of the body in the same patient. We ourselves and other investigators have shown that leukocytes extracted from an inflammatory focus are more active in response to particular factors compared to leukocytes extracted from the bloodstream of the same subject [Briheim *et al.*, 1988; Biasi *et al.*, 1993]. The fact that the disease conditions cause changes in receptor and transduction system sensitivity in various cells of the body is well known in many fields of medicine [Brodde and Michel, 1989].

Whereas up until not so very long ago it was thought that the states of activation were essentially only two, namely, cells at rest and activated cells (e.g. in the case addressed here, cells activated to produce free radicals), today at least five different levels are known to exist:

a) Cells at rest, or inactive cells, such as, for example, young leukocytes, just produced by the bone marrow and circulating in the blood of a healthy subject.

b) Activated cells, such as young leukocytes a few seconds after coming into contact with the surface of a bacterium or of a cancer or virus-infected cell, bearing specific antibodies and complement on its surface.

c) Inactivated, or spent cells, in which the entire enzymatic and metabolic machinery has been consumed in the attack on a preponderant amount of foreign agents, with the result that it can no longer be activated by any kind of stimulus.

d) Cells specifically desensitized in relation to a number of molecules, whereas they continue to function normally or even above normal in response to the action of other stimuli.

e) Hyperresponsive or primed cells, meaning cells which in themselves are not active, i.e. do not produce free radicals, but which, once placed in contact with low doses of a stimulant, present a metabolic response which is much greater than that of normal activated cells.

Changes in sensitivity and in response intensity are the ways in which the inflammatory response regulation system intervenes at cell level (Figure 3). Cell priming is due to subtle molecular modifications, probably related to receptors and enzymes, but whose mechanism is still largely unknown, and which are induced by previous contact with small doses of chemotactic substances, cytokines, or bacterial products. Desensitization, on the other hand, is basically related to receptor dynamics, which may lead to loss of affinity, to down-regulation (internalization), or to uncoupling of specific receptors as a result of their excessive or prolonged occupancy. By no means extraneous to both priming and desensitization are other biological events occurring at post-receptor level, such as intracellular levels of cyclic AMP, calcium ions, sodium ions, and protons, the phosphorylation-dephosphorylation of specific proteins, the state of assembly of macromolecules, and the constitution of membrane lipids, which are in a state of constant rearrangement (cf. Chapter 5, Section 6.3).

Figure 3. Various states of activation of a phagocytic cell. The same stimulus at the same dose (X) evokes quantitatively very different responses in cells in different sensitivity conditions.

Table 2 lists the substances which have regulatory effects at the above-mentioned levels.

Table 2. *Agents capable of regulating the production of free radicals by neutrophilic granulocytes*

a. Agents causing priming

1. Most cytokines, at low doses
2. Chemotactic factors, at low doses
3. Components of complement (e.g. C5a)
4. Ionophores
5. Lipids and derivatives (arachidonic acid, platelet activating factor, diacylglycerol, leukotriene B_4)
6. Bacterial endotoxins
7. Neuropeptides and tuftsin
8. Adenosine triphosphate
9. Muramyl peptide
10. Fibronectin
11. Growth hormone and insulin-like growth factor
12. Endothelin-1
13. Cytotoxic agents at very low doses

b. Agents which desensitize or inhibit

1. Repetition of medium-high doses of agonists
2. Beta-adrenergic agonists
3. Adenosine
4. Prostaglandins E_1, I_2
5. Factors produced by tumors
6. Corticosteroids
7. Bacterial toxins (e.g. pertussis)
8. Opioids
9. Contact with healthy endothelium
10. Various anesthetic and antiinflammatory drugs
11. C-reactive protein
12. Platelet-derived growth factor

The distinction between *positive* effect (priming) and agents with a *negative* effect (desensitization or inhibition) should not be made too schematically; from studies conducted in this field, the concept is emerging that the leukocytes are involved in the cybernetic information networks of inflammation in a highly sophisticated and complex way. The intensity of the response depends, for instance, on:

a) The previous condition of the cell (biochemical “memory”).

b) The doses of regulatory agents, with the possibility of inverse effects: at low doses a substance acts as an activator, while at high doses it acts as an inhibitor [cf. Naum *et al.*, 1991; Bellavite *et al.*, 1993b], or, conversely, it inhibits at low doses and stimulates at high doses [Bellavite *et al.*, 1993c; Bellavite *et al.*, 1994] (see also Chapter 5, Section 6.2).

c) The coexistence of several active and antagonistic compounds (synergisms and antagonisms at receptor and transducer system level).

d) The individual's general state of health (neuro-immuno-endocrine equilibrium).

The cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor (TNF), are examples of molecules which are active in a large number of cells and have endogenous inhibitors which moderate their effects. These inhibitors, which are practically *soluble* receptors for the molecules themselves or antagonists which bind competitively to their receptors, were first extracted from the urine of patients with inflammatory

diseases and are actively studied today with a view to their possible use as natural antiinflammatory drugs. There are diseases (myeloid leukemia, autoimmunity) in which an *imbalance* between the active molecule and its endogenous inhibitor can be detected [see review by Dinarello, 1991].

The free radicals produced by activated phagocytes can become a mechanism whereby molecules of the cell environment, neurohumoral mediators and bacterial products are altered, usually in the sense of oxidative inactivation. The alteration of proteins by oxygen radicals is very easy if these proteins contain methionine, a sulphurated amino acid which is particularly susceptible to oxidation. If this structural alteration involves proteins with intracellular signalling functions, there may be repercussions in terms of information: a signal protein may become inactive or may even be transformed into an antagonist of the original protein (negative feedback effect on the course of the inflammation). While, however, a number of substances have a useful effect, their oxidation may cause pathogenic effects, as is the case, for instance, with low density lipoproteins and with protease inhibitors such as alpha-1 antitrypsin and the plasminogen inhibitor.

The complexity of the regulation of the inflammatory system is multiplied several fold if we consider the immune aspect in the strict sense of the term, where an inflammatory process with a potentially damaging action is activated by an information disorder of antigen, antibody, or some form of lymphocyte activity. Autoimmune diseases, for example, have a pathogenesis attributable to disorder in the organization of the *network* regulating the interactions between various lymphocyte subpopulations. A possible schematic representation of this network is proposed in Figure 4, which illustrates the intricate interplay between activation and inhibition, the use of various molecular messengers and the interfaces with other control systems or with systems interacting in some way with this network.

Figure 4. Example of an interleukocyte communications network responsible for the immune response. Depending upon the genetic or environmental factors intervening, the same network may give rise to a normal defensive response or to autoimmune disease. Solid lines: activation or transformation; dashed lines: inhibition. Based on a review from Immunology Today [Shoenfeld and Isenberg, 1989].

5.2.2. Relationships between the inflammation focus and the rest of the body

The inflammation focus is intimately linked to the rest of the body in various ways. The relationships are bidirectional, i.e., on the one hand, the localized inflammation is capable of influencing the entire body, and, conversely, the body influences the inflammation. The systemic effects of inflammation are currently thought to be due mainly to the production, on the part of cells involved in whatever way in the inflammation focus, of biochemical messages consisting largely of cytokines, but also of hormones such as ACTH and TSH, or of endorphins.

Table 3, which lists only a limited number of the molecules with such effects, clearly shows what a broad spectrum of changes are brought about by inflammation in the entire body. It should also be recalled that most cytokines have pleiotropic effects, i.e. they influence many types of cells, triggering a whole variety of different responses.

The other direction in which links are observed between inflammation and the body as a whole has to do with the effects of the neuroendocrine system on inflammation [Goetzl and Sreedharan, 1992]. This is a vast topic which proves difficult to tackle, in that it ranges from biochemistry to immunology, neurology, and psychology, i.e. fields which are clearly very distant one from another and can hardly be mastered by any single researcher or even by a single research team or institute. It had been known for some time that immune reactions can be conditioned like other physiological reactions in the classic Pavlovian manner, and that an involution of the thymus and depression of immunity occur during stress. It is now known that immune responses can be increased or suppressed by numerous situations of mental or psychological stress such as the loss of a spouse, depression, the psychological pressure of examinations or competitions, and even an infant child entrusted to a day care nursery.

The pathogenesis and clinical course of diseases ranging from the common cold to juvenile diabetes, arthritis, and hyperthyroidism are profoundly influenced by psychological stress [Vandvik *et al.*, 1989; Khansari *et al.*, 1990; Cohen *et al.*, 1991; Haggloff *et al.*, 1991; Winsa *et al.*, 1991]. Cerebral lesions in specific areas of the CNS can cause abnormal immune responses.

For a long time, however, the only link discerned between the nervous system and immunity was the pituitary-adrenocortical axis and the production of glucocorticoids. Stress induces the release of ACTH from the anterior hypophysis, which stimulates the adrenal gland to release corticosteroids, which, in turn, act as immunosuppressive agents. This is certainly true, but other mechanisms are probably involved, both because it was clearly demonstrated that even animals subjected to stress and adrenalectomized are functionally immunosuppressed, and because increasing numbers of receptors have been identified for neurotransmitters on peripheral cells as well as increasing numbers of molecules produced by the neuroendocrine system which interact with the immune system and the inflammation cells. A list of molecules of neuroendocrine origin with effects on leukocytes is given in Table 4.

Table 3. *Molecules produced by leukocytes which mediate the systemic effects of inflammation*

Molecule	**Effects or systems controlled**
Interleukin-1	Fever Sleep T lymphocyte activation Liver protein synthesis
Tumor necrosis factor	Anti-tumor defenses Cachexia Hypotension, shock
Interleukin-2	B/T lymphocyte activation ACTH production
α,β-interferon	Antiviral activity Antiproliferative activity Fever Analgesia Adrenal activation
Colony-stimulating factors	Hematopoiesis
Endorphins	Analgesia
ACTH	Adrenal activation
TSH	Thyroid activation
Complement components	Biological defenses
Clotting factors	Fluidity of the blood and fibrinolysis

Table 4. *Neuroendocrine molecules that regulate leukocyte function*

Molecule	**Effects or systems controlled**
Corticotropin releasing factor (CRF)	ACTH production
Thyrotropin releasing factor (TRF)	TSH production
Vasopressin	γ-interferon production
Vasoactive intestinal peptide (VIP)	Function inhibition
Endorphins	Function inhibition
Growth hormone	Increased proliferation Priming
Insulin-like growth factor	Priming
Substance P	Function stimulation
Angiotensin II	Proliferation inhibition
Glucocorticoids	Function inhibition Growth inhibition
Prolactin	Increased metabolism

As regards stress in particular, we should point out that this is an ever-present condition in human life and that, though the tendency usually is to highlight the negative aspects, it has a positive component. In fact, as emphasized by Selye (the "discoverer" of stress), there is also a constructive form of stress called "eustress" which activates the physiological responses, preparing the body to react effectively to environmental stimuli [Farné, 1990]. Figure 5 clearly illustrates this concept, showing that efficiency and state of health diminish both when stress levels are too high and when they are too low.

Figure 5. Trend of effects of state of health on stress as a function of the degree of stimulation. Diagram taken, with modifications, from a review of the topic [Farné, 1990].

5.2.3. Difficulties in controlling inflammation

There is a great deal of activity today in the field of studies on the molecular mechanisms of inflammation and immunity regulation. Deciphering the signals which are exchanged by cells and which are transmitted within cells for regulatory purposes goes hand in hand with the possibility of implementing specific modulation of a biological type. For example, after clarifying the important role played by interleukin-1 in activating various inflammatory phenomena, it was also discovered that there are endogenous inhibitors of interleukin-1 which have recently been proposed as a new class of antiinflammatory agents, based on a

completely different action principle from the classic chemical inhibitors [Arend and Dayer, 1990; Dinarello, 1994]. Potentially, most of the cytokines known to date may eventually find therapeutic applications.

The difficulties envisaged with regard to the application of the new insights into molecular biology in medicine stem mainly from the fact that both inflammation in general and the production of toxic oxygen derivatives in particular present contradictory and apparently paradoxical aspects, in that they can be viewed either as defensive or as offensive phenomena. *At molecular level, it is by no means easy to distinguish between what is* "*defensive*" *and what is* "*offensive*": the same biochemical mechanisms (receptors, signal molecules, enzymes such as adenylate cyclase, cyclo-oxygenase, kinase, ion pumps, oxygen radicals) are used, according to circumstances, with two different outcomes. The "defensive" or "offensive" nature of such mechanisms, like the terms "normal" and "pathological" in the more general sense, take on a clearer meaning if we abandon the molecular level and go over to considering the system at a higher level of organization (tissue, organ, body), i.e. from a broader, more all-embracing and outcome-oriented standpoint, capable of assessing the health of the individual carrier of a given disease as a whole.

On account of the substantial ambivalence of the biochemical mechanisms of inflammation, any attempt to interfere with the inflammation system at purely biochemical level so as to direct it towards the desired objective, e.g. by increasing inflammatory activity in immunodepressed or neoplastic patients, or by reducing inflammation in individuals suffering from hypersensitivity or autoimmune diseases, will also prove ambivalent.

Both in the field of immunostimulation (specific and nonspecific) and in that of immunosuppression, there is no lack of pharmacological instruments (the pharmaceutical industry today can synthesize virtually any type of molecule, or produce it with genetic engineering techniques), but often they cannot be used successfully in clinical practice. Though there are sometimes "simple" cases in terms of therapeutic strategy, where it is fairly easy to understand whether it is necessary to activate or suppress certain phenomena (e.g. in cases in which the pain symptom is predominant, or in cases of transplant rejection, or in cases in which there is a precise molecular deficiency that can be corrected by replacement therapy), on many other occasions the pathophysiological picture is so complex that the outcome of some form of external modulatory intervention is utterly unpredictable. To give just a single example, it is becoming increasingly clear today that in AIDS cases we have the coexistence of immunodeficiency phenomena (T4 lymphocyte depletion) and autoimmune phenomena (attack of cytotoxic lymphocytes, even on healthy leukocytes). This coexistence theoretically precludes the possibility of utilizing both immunostimulants and immunosuppressors and prompts a search for other forms of modulation which are more specific.

We are still far from being able to construct an exact model, not only as regards inflammation control systems, but also even for a single cell. This situation should not lead to a sort of lack of trust or confidence in scientific medicine, but should spur researchers to construct new investigation approaches better geared to the problems they have to deal with. There is a conviction today that, whereas the activity aimed at investigating biological systems at molecular level (leading over the past two decades to an enormous amount of progress in theoretical and practical terms) continues unabated, we also have to start considering the importance of integration of the various new cognitive advances in a single overall picture. This can be done by studying the interrelations, or better the exchanges of information, between subsystems on the same level of complexity (e.g. information exchanges between molecules, cells, organs, individuals) and between subsystems on different levels (e.g. relationships between molecules and cells, cells and organs, organs and the central control system, individuals and society). Effectively speaking, in the field of inflammation and immunity, whereas, on the one hand, we have an immense series of publications on sequences of genes coding for various receptors, antigens, and proteins of the different types of leukocytes, on the other, we are witnessing the birth of a new discipline, namely neuroimmunoendocrinology, which embraces the tendencies to integrate knowledge in fields previously left to the various specialists. The two tendencies - analytical and synthetic - are not antithetical, but are simply jointly essential for an understanding of reality.

The neuroimmunoendocrine system is a typical complex of homeostatic biological systems which at various levels attempt to maintain the most suitable equilibrium for the survival of the body. As in all homeostatic systems, it is well known that each subsystem has its own feedback mechanisms. There are endogenous inhibitors for every major activator system, ranging from clotting to cytokines, growth factors, membrane ion flow control mechanisms, second intracellular messengers, receptor sensitivity, etc. These feedback systems are one of the decisive elements in the dynamics of the pathological process.

In diseases with a predominantly inflammatory component, a chain of changes and adaptations is generated, in which one is rarely able to clearly perceive any single defect that can be defined as decisive, i.e.

the correction of which would allow the disease to be resolved. The fact is that in most diseases, if we exclude single-gene defects, the pathogenesis is multifactorial and more often than not dynamic, i.e. it continues to change as the disease progresses. We encounter external and endogenous factors, equilibria shifted in a positively reactive sense, unshifted equilibria, or pathological adaptations to situations of abnormality. Within this framework, while it is true to say that diseases heal or are cured only when the cause is removed (or when one succeeds in interfering correctly with the pathogenesis), current immunostimulatory and immunosuppressive measures are still too far from coming to grips with the basic etiological level, or even with the pathogenetic mechanism, going no further than barely touching upon the final - or almost the final - effector level.

The fact that the system tends to be self-regulating and that it is hard to distinguish between the defensive and offensive functions of inflammation makes any attempt at intervening pharmacologically a difficult task. Theoretically, the ideal drug should simultaneously activate the defensive, reparatory functions and inhibit the destructive, pain-inducing functions. Obviously, no molecule possesses such a specific integrated action, not least because the various functions are often triggered by the same biochemical mechanisms. From this viewpoint, it even appears reasonable to doubt whether the use of antiinflammatory agents (steroidal and nonsteroidal), though regarded as necessary in many situations to suppress the clinical signs and symptoms, is always scientifically justified.

One major line of pharmacological research has recently focused on the use of substances with a scavenger effect on oxygen radicals and antioxidants in the hope of being able to use them to prevent or cure toxic effects. All this research has substantially confirmed and underlined the great importance of tocopherols, carotenes, ascorbate, ceruloplasmin, cysteine, selenium, metal chelating agents, and superoxide dismutase as protective systems against free radicals. However, it is also true that, while there can be no doubt as to their biochemical and physiological functions, there is no general consensus of agreement as to the therapeutic efficacy of administration of high doses of these agents in human diseases [Dormandy, 1983; Halliwell, 1987; Southorn, 1988: Halliwell *et al.*, 1992]. This may be due to the fact that the diet normally contains a fair amount of these substances and that in any event it is difficult to demonstrate the effects of dietary supplementation in complex diseases such as those mentioned above (Chapter 5, Section 2.1). Moreover, precisely on account of the ambivalent significance of the changes induced by free radicals, any therapeutic intervention against these molecules will inevitably be of a very nonspecific type.

What we have said obviously does not mean that the current therapies in the field of inflammation and free radicals are devoid of any kind of rationale or justification, since there are, in actual fact, a vast series of molecules which have demonstrated a certain degree of efficacy “in the field,” at least when it comes to resolving problems of a symptomatological nature. We merely wish to stress the need to seek more specific measures based on regulatory mechanisms located at “higher” levels which take account of the complexity of the system.

Genetic engineering has recently made available large quantities of cytokines to medical practitioners. The hope, of course, is that we shall be able to use them to stimulate the biological defenses both in immunodeficiency syndromes and in immunological cancer therapy, thus attempting to activate phagocytes, NK cells, and cytotoxic lymphocytes with interferons or interleukins (cf., for instance, Rosenberg's therapy with LAK cells). Attempts have also been made to use *tumor necrosis factor* (TNF) as a cytotoxic agent against cancer cells, and *colony stimulating factors* (CSFs) in those cases in which the production of white blood cells by the bone marrow is deficient for various reasons.

Without denying the undoubted value of, and need for, such research, it should be said, however, that the success rate to date has been below expectations and confined to very few types of tumors, particularly because these molecules have no specific effect on the target one is aiming at, but interfere with the entire network described above. Thus, we inevitably witness the occurrence of side effects, such as fever, hypotension, oliguria, weight gain, liver abnormalities, nausea, vomiting, and shock, which will be all the more severe, the higher the doses.

The problem of the side effects of drugs is clearly not confined to this sector, but in this case the risk:efficacy ratio appears highly critical. Medical practitioners faced with this problem in clinical practice are gradually coming to the conclusion that to achieve more effective use of cytokines we shall have to experiment with doses and treatment regimens, subtly exploiting synergistic effects, e.g. by administering two or more different cytokines at low doses, which in themselves do not produce side effects, but which together bring about the desired effect on a common target.

In complex systems such as inflammation and immunity, it thus proves very difficult to transpose the vast body of knowledge of cellular and molecular biology concerning the individual mechanisms involved onto the therapeutic plane. This is particularly evident in the therapy of autoimmune diseases and, generally speaking, in any type of immunomodulatory therapy [Bach, 1988; Wybran, 1988]. Faced with this situation, it is hard to see why there cannot be a reappraisal of the empirical approach as a pathway affording scope for further investigation, starting from the traditional use of phytotherapeutic and organotherapeutic preparations, as well as from homeopathic and homotoxicological experience itself (bacterial extracts, nosodes).

Complex natural preparations may prove useful precisely because they contain various different biologically active compounds which act together in a coordinated manner. The clinical and laboratory study of these compounds as possible means of modulating inflammatory phenomena is problematical, particularly on account of the difficulties encountered in standardizing and characterizing the preparations used. Despite this, the availability of highly sensitive analytical methods and the possibility of testing various biological effects in cell cultures mean that studies of this type are steadily gaining in terms of scientific rigor and are proving increasingly promising.

The possible importance of the homeopathic approach in this sector, however, goes far beyond the identification of natural preparations with a regulatory action. Homeopathy, in fact, offers itself above all as a methodology for identifying the specific remedy for each individual patient on the basis of analysis of the symptoms. Chapter 6 will show us how this methodology has adapted to face the challenge of diseases in which complexity predominates.

5.3. Another example: cancer

Cancer consists in the more or less uncontrolled growth of cells as a result of serious disorders of information contained in the cells and of information which the cells exchange with one another and with the environment. It is therefore a complex pathological phenomenon, as are the cells themselves and their regulatory mechanisms, or the human body in its entirety and in its relations with the environment.

We cannot and do not wish here to attempt a complete survey of such an enormously broad-ranging topic, but will confine ourselves to a review of the most recent advances in this field and the main lines of research, along with a number of reflections on the relationships between phenomena at molecular level and phenomena regarding the body as a whole. Cancer is one of the greatest challenges facing modern medicine, and what often happens, in practice, is that patients tend to seek the aid of “alternative” therapies as a last resort, when conventional treatments have failed to prove effective. For this reason, there is a real risk that the “alternative” therapies are applied incorrectly and to the detriment (economically and in terms of health) of the patient.

Within the framework of an extensive overview of the possible scientific basis of homeopathy, the problem of the relationship between homeopathy and tumors cannot be neglected. To get the problem into proper perspective and clear the field of common misunderstandings, it is advisable first to present a preliminary outline of our current knowledge of neoplastic disease, before going on to expound a number of considerations on the role of homeopathy. It is only in this way that we can indicate and reasonably define and delimit the fields of application of the various therapies.

The basic problem in neoplastic growth is that it is due to loss of normal control of cell proliferation and differentiation.

Each cell in the course of its life cycle, which varies in length according to the type of cell, engages in many activities which are useful both to itself and to the body, but essentially it finds itself faced with a basic choice of behavior: to undergo mitosis or to differentiate, or, in other words, to replicate or to mature. Schematically, we can go along with the simplification whereby these two possibilities are alternatives, i.e. they are mutually exclusive. This choice of behavior is often repeated several times in the life cycle of the cell and of the clone deriving from it. The decision to replicate or proliferate is typical of less specialized cells in the line of evolution both in the individual (embryo) and in the tissue (e.g. basal cells of the epidermis, bone marrow blast cells). When the result of the choice is division, the result will be two daughter cells identical to the mother cell, i.e. two fairly immature cells. When the choice is to differentiate, the cell progressively takes on the morphology and properties of a greater degree of maturity in the line of evolution of the specific tissue.

In a given tissue, then, we find cells in a state of replication and cells which progressively mature and then gradually age and die. As is well known, the mature cells of some tissues (e.g. striated muscle, nervous system) have practically no proliferative or irretrievable activity, whereas other cells conserve varying degrees of activity according to the functional demands and environmental stimuli. Forms of regulation of proliferative activity are particularly evident in endocrine-regulated glands and tissues. A number of cell clones in rapidly proliferating tissues (hemopoietic marrow, mucosa) conserve very substantial proliferative activity and poor differentiation, representing the germinative pool which constantly supplies the tissue with large amounts of cells.

For instance, in a population of myeloid cells such as those of normal bone marrow, there are cells (blast cells) in the proliferative phase alongside cells in the differentiation phase versus the various types of leukocytes. Even the very mature cells in a state of rapid proliferation are under strict surveillance, however, so that their activity is always in equilibrium with the disappearance rate of the mature cells and with the needs of the body in general. This control is exerted primarily by other neighboring or distant (by the endocrine route) cells by means of growth factors and differentiation factors, as well as by cell-to-cell and cell-to-matrix contacts. These factors are fairly specific for each tissue and are often produced by the tissue cells themselves as they mature.

In tumor development this fine control is lost, and it is therefore at this level that cellular and molecular biologists have begun to understand which control mechanisms have gone missing. Both the study of the normal cell cycle and the study, more recently, of neoplastic cell genetics are rapidly revealing a picture of how the proliferative activity is regulated and what the basic differences are between normal cells and cancer cells. For instance, in a population of leukemic cells, many more cells will be found in the proliferative state and fewer (or no) differentiated, mature cells. As we are well aware, by and large, the more immature the cells are, the more malignant the tumor will be. A number of the possible reasons for this imbalance are related to the fact that leukemic cells have very little need of growth factors, or they produce them themselves in amounts in excess of their needs, or they do not produce differentiation factors. The cells in almost all tumors show a similar behavior pattern.

5.3.1. Biochemical control of cell proliferation

What are the lesions or biochemical defects leading to this type of abnormal behavior in cancer cells? The lesions clearly have to do with disorders in the cell division mechanisms and in the extracellular and intracellular signals pertaining to its control. It is therefore useful to briefly analyze the molecular and functional characteristics of these mechanisms, firstly in the normal cell, and then in the tumor cell, in order to pinpoint the essential differences.

Figure 6 illustrates a number of cellular biochemical events summarized very schematically for reasons of brevity and clarity. We may take as our starting point in this analysis the *extracellular signals* which trigger off the proliferation. In the first place, we are familiar with many growth factors, i.e. molecules mainly of a protein nature, with more or less marked tissue specificity. Many of these growth factors have been cloned and are also produced today by genetic engineering; these have added themselves to the series of traditional hormones with effects stimulating tissue growth, such as the pituitary, thyroid, and gonadal hormones. The extracellular signals can reach their intracellular targets either via membrane receptors or, after crossing the membrane, via cytoplasmic or nuclear receptors which directly regulate gene expression.

Figure 6. Mechanisms of transmission of the proliferative signal from the extracellular to the intracellular space. For an explanation, see text. Abbreviations: G-P: guanosine triphosphate binding protein; D.G.: diacyl-glycerol; IP_3: inositol 1,4,5 triphosphate; cAMP: cyclic adenosine monophosphate; ATP: adenosine triphosphate; A.P.: activator proteins, proteins which induce mRNA transcription by binding to DNA; mRNA: messenger RNA; Polymer.: polymerases, enzymes which replicate DNA or copy DNA in RNA.

Another major line of research has highlighted the *membrane and intracellular phenomena* activated by hormones and growth factors (Figure 6). Many receptors for growth factors have the carboxy terminal portion of the intracytoplasmic protein chain, endowed with protein-kinase activity. This means that one of

the first events to occur in the cell which is starting to divide is the phosphorylation of proteins, i.e. the incorporation of phosphorus groups on particular amino acids (usually, though not exclusively, the amino acid tyrosine is involved in events related to cell proliferation and cancerogenesis).

What is the significance of protein phosphorylation? the fact is that the incorporation of phosphate in specific sites changes the electrical charges, the structure and thus the activity of the target protein, in the sense that it makes it capable of activating some kind of replication mechanism at the cytoplasmic level (e.g. movement of the cytoskeleton, protein synthesis) or at the nuclear level (e.g. gene expression, assembly/disassembly of the nuclear membrane and chromosomes during mitosis). Via other biochemical pathways, phosphorylation may also involve particular phospholipids of the cell membrane, such as phosphatidyl inositol, which play an important role in the stimulus-response coupling process.

In view of their importance, a great deal of attention has been focused upon phosphorylation processes in recent years, particularly since it was demonstrated that tumor cells contain large amounts of phosphorylated tyrosine. There are many types of protein kinases, associated directly with the receptor or activated indirectly via a series of cascade reactions schematically represented in Figure 6. These cascades are produced by G-proteins, activation of enzymes such as adenylate cyclase and phospholipase, and generation of second intracellular messengers, Among these second messengers a role of primary importance is played by cyclic adenosine monophosphate (cAMP), both because it is an activator of (type A) protein kinases and because it is itself a signal captured by proteins of the nucleus closely associated with DNA synthesis (cAMP-responsive elements).

Other important events related to activation of cell replication are Ca^{++} elevation, depolarization of the membrane, and activation of the Na^{+}/H^{+} antiport, with a consequent increase in intracellular Na^{+}, alkalinization of the cytoplasm, and acidification of the external medium. It would thus appear that the acid/base equilibrium of the cell is important for regulating cell proliferation and thus for tumor growth.

A small minority of receptors, i.e. those for small hydrophobic molecules such as steroid or thyroid hormones, do not need these transduction mechanisms, since they are already inside the cell or even in the cell nucleus.

There are a whole variety of other biochemical modifications related to cell activation, including the translocation of proteins from one compartment to another, acetylation or ADP-ribosylation of proteins, formation of a broad spectrum of lipid derivatives, partial scission of macromolecules, and metabolic variations of the Krebs cycle. A number of the modifications are transient and easily reversible, while others are longer-lasting, or even permanent, thus constituting a sort of *memory* of the biological history of the cell.

The purpose of the phenomena described is to constitute a transmission system, or more precisely a transduction system, a *signal network* going from external growth factors towards the interior of the cell, i.e. towards the inside of the control center, consisting in the cell nucleus. Not all receptors activate all the intracellular signals, in that there are *preferential pathways* according to the type of receptor involved. The *network* of membrane and cytoplasmic biochemical modifications, corresponding essentially to the laws of cybernetics, receives not only activator, but also inhibitor inputs, and serves the purpose of amplifying or modulating the signal. The modification of any single element in the system has far-reaching repercussions on all the other elements [Egan and Weinberg, 1993].

At this level, the synergisms and antagonisms between factors mean that the transmission of the signal is never a drastic on/off, all or nothing matter, but rather denotes the setting up of dynamic modes of being, of conditions more or less favorable to growth, which evolve continually as the stimulation proceeds and interacts with the genetic and metabolic conditions of the cell. This interpretation accounts both for the multiplicity and variety of the biochemical mechanisms described and for the synergisms between the proliferative responses triggered by the classic growth factors and other mediators active in other systems such as inflammation and metabolism (bradykinin, thrombin, insulin, and others) [cf., for example, Pandiella *et al.*, 1989].

The membrane and cytoplasmic information network, which is overwhelmed in the case of neoplasia, carries signals *at nucleus level*, where they interact with specific transcriptional factors (activator proteins [A.P.], and repressor proteins [R.P.]) which in turn bind with (A.P.) or detach themselves (R.P.) from specific sequences of the DNA in a position upstream of the promotor, i.e. of the starting point of RNA polymerase. The transcription of the genes involved is thus activated, and RNA transcripts are formed, which after suitable maturation become the messengers governing the new synthesis of proteins, which, in turn, serve in various ways for cell division (DNA synthesis, formation of the mitotic spindle, etc.). Among the regulatory proteins, a place of primary importance has recently been accorded to the cyclines [Murray and

Kirschner, 1991], which, as the name itself suggests, are thought to be the key factors acting as *pace-makers* for the cell cycle.

5.3.2. Oncogenes and proto-oncogenes

It has been common knowledge for some time now that tumor cells present abnormalities at both genetic level (mutations) and epigenetic level (biochemical and functional disorders of the gene control systems). However, it is only in the last decade that we have witnessed dramatic progress in the interpretation of the genetic and epigenetic abnormalities of cancer cells, due essentially to molecular biology techniques, which have led to the discovery of oncogenes and their products.

In point of fact, from the generic notion that cancerogenic agents cause damage to the DNA we have now homed in on the precise targets of such damage and their characterization; in practice, the defective genes in tumors have been identified. Out of all the genes present in our genome, only a few dozen can be directly involved in cancerogenesis, hence the term *oncogenes* [Hunter, 1984; Nishimura and Sekiya, 1987; Frati, 1989; Varmus, 1989].

The neoplastic cell contains one or more oncogenes in active form, i.e. expressing the information content. Generally speaking, they have been given 3-letter names deriving from the tumors in which they were initially identified.

The history of these discoveries owes a great deal to virology because it was thanks to the oncogenic viruses that investigators obtained the first evidence that particular DNA (or RNA) sequences may represent the decisive element in neoplastic transformation. In actual fact, the importance of viral oncogenes was underestimated, particularly on the strength of the argument that very few human tumors are definitely known to be of viral origin.

An enormous step forward of a conceptual nature was the demonstration, obtained by means of nucleic acid hybridization techniques, that viral oncogenes also presented homologous sequences in normal cells. The viral oncogenes (*v-onc*) made it possible to "unmask" the cellular oncogenes (*c-onc*). This means that the oncogenes present in most human tumors are not totally abnormal genes and are not genes introduced, for example, by viruses into the cell, which would thus be the victim, as it were, of a molecular parasitism, but are genes which have normal counterparts in all cells.

That is to say, oncogenes stem from the transformation of genes which are important in the functioning of all cells. These normal genes have thus been termed *proto-oncogenes*, which is perhaps something of a misnomer. The term "proto-oncogenes" is not particularly apt because it has been seen that they are beneficial, being active at various times in the cell cycle and essential for its activity and for its replication. These are genes which have been well conserved in evolution and are present practically in all eukaryote cells.

Two basic queries arise from what we have said:

a) *How does a proto-oncogene become an oncogene?*

b) *How does the oncogene cause the transformation of a normal cell into a cancer cell?*

The transformation of a proto-oncogene into an oncogene is the basic event, the *sine qua non* for the onset of cancer. Whereas it was already known on the basis of epidemiological and experimental evidence that this event is related to mutation of the genetic code induced by cancerogenic agents of various kinds, the oncogene theory has shed light on a number of aspects relating to how this can come about. One concept is very clear: the proto-oncogene becomes an oncogene as a result of the action of the cancerogenic agents. This is the *transformation* event at genetic level, which only needs to occur once to be transmitted then to all the daughter cells. For this reason, it is also called *initiation*.

The main cancerogenic agents are, as is well known, chemical substances contained in cigarette smoke, polluted air, foodstuffs, ionizing and excitatory radiation, radio-isotopes, genotoxic drugs, and viruses. Obviously, we have considered here only the general categories of cancerogenic agents, inasmuch as the molecules with cancerogenic and/or mutagenic activity amount to several hundred. The manifestation or otherwise of the transforming action of a cancerogenic agent depends on both the dose and the duration of exposure to it, as well as on factors having to do with the body, such as, for instance, the detoxification capability and the ability to eliminate the carcinogen, and the ability of cell systems to repair the DNA. It should not be forgotten, however, that many exogenous substances become mutagenic and thus cancerogenic following particular metabolic conversions which occur in the body and make them active. The common

feature of cancerogenic substances is that they have a strongly electrophilic molecule (usually around carbon or nitrogen atoms), capable, therefore, of reacting with the nucleophilic centers rich in unshared electrons and present in various positions of the DNA (e.g. position 6 of guanine, the phosphoric group of the sugar-phosphate-sugar bonds).

As far as tumor pathogenesis is concerned, leukocytes may be directly involved in the genetic transformation, since they could metabolize (and thus activate) cancerogenic substances [Trush *et al.*, 1985], but, above all, because they may produce toxic oxygen radicals. It is known, in fact, that DNA mutations may originate, amongst other things, as a result of the effect of free radicals produced by leukocytes [Weitberg *et al.*, 1983; Weitzman *et al.*, 1985; Birnboim, 1986].

In this case, too, we can see the "*two faces*" of a biological phenomenon: radicals can have varying effects depending upon their quantity and upon the existence or otherwise of specific *scavenger* substances or enzymes. At high doses, radicals have a cytotoxic and thus a defensive effect, in that they cooperate in the destruction of tumor cells (particularly if the latter have not developed detoxifying systems); at low doses, oxygen radicals have no effect because they are rapidly degraded; at intermediate doses, they have both genetic effects (mutations) and epigenetic effects (activation of protein kinases and other enzymes including poly-ADP ribosyl-transferase), thus being able to behave both as cancerogenic and promoting agents [Sekkat *et al.*, 1988; Cerutti, 1991].

Lastly, many tumors are known to have defective free radical disposal systems [Casaril *et al.*, 1985; Bannister *et al.*, 1986; Vo *et al.*, 1988]. This might constitute either the basis for more effective action on the part of the anti-tumor cytotoxic surveillance systems, or, however, it may also be the cause of further damage to the genetic endowment of the neoplastic cell, with consequent activation of other oncogenes and an increase in malignancy (tumor progression process).

Thus, in the inflammatory focus (particularly of the chronic type where the histogenetic and proliferative events are more marked) and within the context of the macrophage populations infiltrating the tumor, antitumor events and cancerogenic events may co-exist in a state of unstable equilibrium. A familiar phenomenon, in fact, is the onset of dysplasia, metaplasia, and even neoplasia coming on top of chronic inflammation, particularly at bronchopulmonary, gastrointestinal, and hepatic level, these being sites where cancerogenic agents also tend to be easily localized.

The modification of the genetic information, related to the transformation of a proto-oncogene into an oncogene, may take the form of various eventualities in molecular terms:

a) Classic *point mutation*, with deletion or substitution of one or more base pairs with other erroneous base pairs, thereby obtaining a protein with a different amino acid sequence. This, for example, has been observed in connection with the first oncogenes characterized (as *src*) and in many human tumors with mutations of the *ras* proto-oncogene (some cases of carcinoma of the bladder, 10-20% of acute myeloid leukemias, approximately 30% of colorectal carcinomas, and even the majority of carcinomas of the exocrine pancreas).

b) *Translocation* of the proto-oncogene to a DNA site where it comes under the control of enhancer sequences or viral promoters ("insertional mutagenesis") or of a very active cell promotor and is thus itself activated excessively. This is the case with the *myc* oncogene in 90% of Burkitt's lymphomas and in some cases of T cell lymphomas (translocation from chromosome 8 to 14), as well as with the *bcl* oncogene in many other lymphomas (translocation from chromosome 14 to 18).

c) A particular variant of this problem related to translocation presents itself when, as a result of translocation, the oncogene undergoes *fusion with another gene*, with formation therefore of an abnormal hybrid or chimeric protein (e.g. *bcr/abl* in the classic Philadelphia chromosome of chronic myeloid leukemia, with translocation from chromosome 9 to 22, but it has been seen that this can also occur in 10-20% of cases of acute lymphatic leukemia).

d) An increase in copies of the proto-oncogene due to erroneous duplication of DNA (*amplification*) or to the insertion of various copies of retroviral oncogenes (acutely transforming retroviruses). Amplification of the oncogene *myc* has been observed in sporadic cases of promyelocytic leukemia, lung cancer, and stomach cancer, whereas a particular variant of *myc*, called *N-myc* is seen to be amplified in most neuroblastomas. Amplification of the *erb-B* oncogene has been described in 10-30% of cases of cancer of the breast, and this characteristic is associated with a poor prognosis.

In a nutshell, then, the proto-oncogene becomes an oncogene when the nucleotide sequence is altered, or when its transcription is excessively activated, or when its amount is increased in terms of genetic material. The various possibilities are not mutually exclusive, but can coexist side by side.

The second important issue regarding the molecular pathology of cancer is the role played by oncogenes in the phenotypic and behavioral transformation of the cell. Why are they so dangerous or damaging once activated? To answer this question the fundamental step has been to identify the *products of oncogenes*. It is not enough for us to know the oncogene and its nucleotide substance to know what it does because the role of any given gene is strictly related to the protein encoded by it and to the activities or functions of the protein itself. These are proteins which are localized on the membrane, or in the cytoplasm, or even in the nucleus itself in close contact with the DNA. More interesting even than the location is the function which the products can have in the biochemistry of the cell which produces them: in general, the products of oncogenes merely mimic the factors involved in the control of cell proliferation (see Figure 6).

A few details may be in order here: it has been seen that some proteins are very similar to the receptors for growth factors. For example, the products of the *erb-B*, *fms*, and *kit* oncogenes present homologies with the receptors for epidermal growth factor (EGF), colony-stimulating factor 1 (CSF-1), and stem cell factor (SCF), respectively. These types of receptors, however, are abnormal compared to those of healthy cells: the EGF receptor is truncated, i.e. it lacks the part that binds to the growth factor, and thus appears to be dysregulated; in practice, it transmits proliferative signals even in the absence of the legitimate ligand.

The proliferative signals are related essentially to the tyrosine kinase enzyme activity which these abnormal receptors have maintained and express improperly. Other oncogenes (*src*, *abl*, *fps*, *mos*), too, code for protein kinases, which, however, are not associated with receptors, but whose activity has similar consequences to the previous ones. Other oncogenes produce intracellular receptors, such as, for instance, *erb-A*, whose product presents homology with the receptor for thyroid hormones. The importance of protein kinases is also strongly borne out by the fact that a number of natural inhibitors of these enzymes (such as bryostatin and genistein) can block the growth of tumor cells in suitable experimental systems [Jones *et al.*, 1990; Watanabe *et al.*, 1991]. The subject is obviously extremely broad-ranging, and research in this field is steadily expanding.

There is also a class of oncoproteins (related to the *ras* genes) of a different type, which present strong homology with the G-proteins, regarded above as important intermediaries in the transmission of the transmembrane signal. It is not hard to imagine that the quantitatively or qualitatively abnormal presence of these proteins incorporated in the membrane of a cell may alter the information system to such an extent as to impart thoroughly abnormal proliferative orders.

A further and extremely interesting aspect of oncogenesis came to light when it was seen that a number of oncogenes produced proteins homologous to growth factors (e.g. *sis* has sequences in common with platelet-derived growth factor, PDGF, and *hst* with fibroblast growth factor, FGF). In this case, the cancerogenesis mechanism is explained, at least in part, by the phenomenon of autocrine secretion: the clone of transformed cells renders itself independent of other exogenous growth factors and is self-supporting in growth; indeed, the more the cells grow, the more growth factor is available.

Finally, a different type of oncogene (the *myc*, *myb*, *fos*, *jun* type) have as their products proteins which are located in the cell nucleus, where they play a decisive role in the control of mitosis (e.g. the *fos* oncogene codes for the transcriptional factor A.P.-1). That this is true is also demonstrated by the fact that the respective proto-oncogenes (i.e. the "benign" versions of these nuclear oncogenes) are highly active in the rapidly growing cells of the embryo and in the blast cells of the bone marrow, as well as whenever any normal cell receives a treatment with growth factors.

In view of the fact that research in this field is booming, it is only to be expected that other properties of oncogene proteins will soon come to light. For instance, a very recent discovery indicates that an oncogene involved in lymphomas (*bcl-2*) codes for a protein which inhibits the so-called "programmed cell death" (an ancestral system which limits the cell life span): the tumor cells with this oncogene would thus have a longer life cycle than normal cells, thus explaining the increased number and selective advantage of the transformed cells.

To recapitulate: the neoplastic cell is characterized by having one or more regulation systems which are impaired qualitatively (i.e. there is an abnormal protein, albeit resembling its normal counterpart) and/or quantitatively (i.e. there is a protein overrepresented compared to normal). All this leads to the phenotypic characteristics of cancer cells.

The result could therefore be a large number and considerable variety of cellular atypia, a few examples of which are the following:

a) Abnormal phosphorylation of proteins of the cytoskeleton, producing a collapse of the structures which confer upon the cell its normal morphology.

b) Alterations of the adhesion plates between cells, and between cell and connective tissue matrix, with possible detachment and metastatic spread.

c) Alterations of the surface characteristics with possible triggering of an immune response against the tumor.

d) Alterations of various metabolic pathways, with increased consumption of oxygen and of nutritive substances and reduced production of energy, or reduction of normal defense systems against toxic oxygen radicals and of DNA lesion repair systems, with a possible increased risk of new mutations.

e) Possible reduction and release of substances with effects on the cell itself (autocrine secretion) or at a distance (ectopic production of hormones).

f) As we have already said, an upheaval in the delicate system of relationships between proteins and DNA, which regulates cell division in the nucleus.

Within the framework of the molecular pathology of cancer we should not neglect to mention another major line of research relating to the existence of genes which counteract the development of the tumor. These genes are therefore called *anti-oncogenes*, or tumor-suppressor genes, or recessive oncogenes [Friend *et al.*, 1988; Vile, 1990]. They were discovered as a result of the observation that a number of tumors (retinoblastoma, Wilms' tumor, neuroblastoma, and adenomatosis of the colon, though today it would seem that it also occurs in many other cases) are associated with a lack of genes, sometimes visible as actual deletions of parts of chromosomes. If the lack of particular genes causes cancer, it has been deduced that these genes are important in inhibiting tumors. Direct proof was provided by the fact that if the deficient gene is reintroduced by genetic engineering (or formation of hybrid tumor-healthy cells), the neoplastic phenotype disappears.

Despite the fact that the importance of these genes is enormous, little is yet known about their precise function in the control of cell growth. From the study of the location of suppressor gene products and from analysis of their sequences a highly complex picture is emerging, showing that these products are involved in the control of cell proliferation in many ways [Algrain *et al.*, 1993; Bryant, 1993]. For instance, in retinoblastoma and Wilms' tumor, deficiencies of transcription factors (Rb-1 and WT1, respectively) have been described, while there have been reports of a deficiency of a membrane adhesion protein (N-CAM) in colorectal carcinoma, and of a protein associated with the cytoskeleton (merlin-schwannomin) in type 2 neurofibromatosis. It is interesting that the deletion of the gene for interferon-α has been reported in a number of leukemias [Diaz *et al.*, 1990], which suggests that this cytokine may be the product of a suppressor gene. One of the suppressor genes which has been found to be mutated in a large percentage of human malignancies (colon, breast, lung, and brain) is the p53 gene, encoding a transcriptional regulator of cell proliferation [Hollstein *et al.*, 1991].

It therefore appears that the products of anti-oncogenes are involved in the same *information network* considered above, counteracting the effects of the products of oncogenes (or of proto-oncogenes), for example by means of dephosphorylation of proteins (phosphatase) or degradation of second messengers such as cAMP (phosphodiesterase). As is observed in many other pathophysiological situations, the control of proliferation constitutes a homeostatic equilibrium between two groups of opposite factors which control one another reciprocally, and this equilibrium shifts in one direction or the other due either to an element being in excess or to a deficiency of the element counteracting it.

5.3.3. Promoting factors and neoplastic progression

After examining the mechanism of transformation at oncogene level, we may ask: *is transformation of a proto-oncogene into an oncogene enough to cause cancer?* The answer is certainly negative: the transformation of a normal cell into a neoplastic cell and the subsequent expansion of the mutated clone in the form of an actual neoplasm are hardly ever the result of a single molecular event. The experimental evidence clearly shows that the transformation of a proto-oncogene into an oncogene is not enough to bring about cancer. For a cancer to develop, starting from a transformed cell, other biological events at *epigenetic* level are necessary, related to the action of the so-called *tumor promoters* (promoting factors, also called co-cancerogenic factors).

These factors do not act directly at the level of the genetic endowment, but at the level of the whole series of reactions conditioning the expression of the genes involved in proliferation and the activity of the enzymes involved in the cell division process. To take a metaphorical leaf out of the automobile book, it

could be said that, while it is the cancerogenic factor that starts up the engine (oncogene) and puts the automobile in gear (abnormal protein), it is the co-cancerogenic or promoting factors that put their foot on the accelerator to set the automobile in motion. In the absence of these factors, theoretically the cell could remain at a standstill or even degenerate and thus disappear without giving rise to any progeny. Table 5 lists a series of agents possessing possible promoting actions.

The classic promoting agents include various substances mainly of vegetable origin or resulting from chemical synthesis: phorbol esters and related diterpenes, indole alkaloids such as teleocidine, iodoacetic acid, phenol, cedarwood oil, a number of detergents, n-dodecane, and other substances. Their mechanism of action is the subject of active research and, predictably, has proved very complex and variable from type to type. The most paradigmatic co-cancerogenic factor is TPA (an active ingredient of the oil extracted from the seeds of *Croton tiglium*), which, by virtue of its hydrophobicity and its structural similarity to physiological mediators, acts by binding to protein kinase C, activating it, and then acting in synergy with other activator events considered previously, The treatment of cells with TPA causes the phosphorylation of many proteins and triggers a whole series of different metabolic responses. In itself it does not cause the onset of the neoplastic phenotype in a normal cell, but if the treatment is carried out on experimental cells or animals previously treated with a cancerogenic agent (e.g. benzopyrene, or ionizing radiation), the likelihood of onset of tumor is significantly greater than in control cells.

Table 5. *A number of agents which may act as tumor promoters.*

Various substances:	lectins
	phorbol esters
	mezerein
	okadaic acid
	bacterial exotoxins
	phenol
Hormones:	estrogens
	thyroid hormones
	insulin
Growth factors:	PDGF (platelet-derived growth factor)
	EGF (epidermal growth factor)
	IGF (insulin-like growth factor)
	FGF (fibroblast growth factor)
	TGF (transforming growth factor)
	a number of other cytokines
Neuropeptides:	bombesin
	vasopressin
	bradykinin
	VIP (vasoactive intestinal peptide)
Prostaglandin E	
Low-frequency electromagnetic fields	

Another substance with a promoting action, okadaic acid, probably exerts its effects by means of inhibition of phosphatases, enzymes, which, as we have seen, constitute a sort of homeostatic equilibrium with the kinases. Low-frequency electromagnetic fields can also have a promoting effect, possibly due to interference with the intracellular homeostasis of the calcium ion [Goodman and Shirley-Henderson, 1990; Yost and Liburdy, 1992]. There are indications that exposure to low-frequency electromagnetic fields increases the risk of being affected by leukemia, though this is still subject to considerable debate [Pool, 1990; Galva, 1991].

Though the mechanism of action of various promoting agents is still under investigation, it seems clear that all they do is insert themselves in the activating information network outlined above, mimicking essentially a number of actions of physiological substances (sometimes with more marked and longer-lasting effects). For this reason it would seem logical to include many other additional agents in the table of compounds possessing promoting activity, such as hormones, growth factors, cytokines, and even neuropeptides [Malik and Balkwill, 1991; Rozengurt, 1991]. There is evidence that even mediators with completely different "traditional" functions (catecholamines, serotonin, angiotensin) may, in certain circumstances, act as growth factors [Williams, 1991]. In this case, too, the sensitivity "status" and predisposition of the cell is of great importance, ranging from the presence of suitable receptors to the existence of conditions favoring the expression of particular transduction pathways, due to previous or concomitant conditioning by factors of a pharmacological or hormonal type. For example, a substantial series of agonist and antagonist agents which influence the intracellular level of cAMP or of protein kinase C activity may behave as growth promoters, both in normal cells and, even more so, in transformed cells. The growth of the cell clone is always conditioned to some extent by such factors, though the effects may differ greatly according to the type of cell concerned, the type of oncogene involved, and the type of receptors

present. Agents which act as co-carcinogens in some cells may behave as factors inducing differentiation, and thus in practice as anticancerogenic agents in other cells.

It should be stressed that, if a promoting effect can be exerted by various endogenous substances, one factor whose importance warrants reappraisal is the *medium* in which the cancer develops. It would appear legitimate to ask oneself what *contribution* the host organism makes to the growth of the tumor, i.e. whether it may play a permissive role or even, in certain circumstances, have an incentivating function. Clearly, the tumor receives a certain amount of "help" from the host with the supply of connective tissue, the vascular network, and energy substrates, but it is likely that the help may also consist in particular biochemical mediators such as growth factors and hormones [Lang and Burgess, 1990]. As regards the latter, it has been reported that progressive tumor is associated with an increase in adrenergic tone, sensitivity and reactivity to beta-agonists, and with an accentuated metabolic effect of beta-blockers [Hyltander *et al.*, 1993].

As far as the mechanism of neoplastic promotion is concerned, it should be borne in mind that the inflammatory focus is a good *culture medium* for cells such as leukocytes, endothelial cells, fibroblasts, and even epithelial cells, thanks to the secretion of special growth factors. This is particularly true in those cases in which the inflammation does not proceed rapidly towards healing with *restitutio ad integrum*, reabsorption of exudate, inactivation of mediators due to lysis or oxidation, and cessation of leukocyte chemotaxis. In inflammation, synergisms may easily occur between substances that have demonstrable mitogenic activity, such as epidermal growth factor, insulin, insulin-like growth factor, bradykinin, endothelin, and even neuropeptides, which, as is known, are released in the site of a tissue lesion. It is therefore not unlikely that such growth factors act as accelerators of proliferation not only of normal cells, but also of those cells which form as a result of a previous or concomitant genetic transformation event.

Cancerogenic and co-cancerogenic (or promoting) factors lead to a progressive complication of the biological situation of the tumor. It has been seen that, in actual fact, several oncogenes are activated in the same cell in tumors, and that there is progression of malignancy related to the number of oncogenes activated. New mutations occur at genetic level, facilitated perhaps by a reduction of the defense and repair systems, and new impulses towards cell disorganization appear, perhaps as a result of inappropriate expression of normal genes, or of the reduced capacity of immune cells due to substances released by the tumor itself. If we examine the cells of a tumor, we find multiple biochemical atypia, and it is often difficult to establish whether they are directly related to transformation or whether they are alterations secondary to transformation.

A fundamental role in *neoplastic* progression is played by the fact that subsequent errors generate a certain degree of heterogeneity in the proliferating cell population, to such an extent that a number of clones, with characteristics allowing greater resistance to defense systems and drugs, may gradually gain the upper hand. The malignancy of the tumor thus tends steadily to increase.

Figure 7 schematically summarizes the natural history of a tumor. The oncogene theory provides us with a unitary view of the process of cancerogenesis, where mutagenic agents, viruses, promotor agents, and endogenous regulatory factors interact at the level of receptors, transduction systems, and genetic information. In relation to this model, the growth of the neoplasm does not appear as an "all or nothing" event, but as a progressive accumulation of information errors leading the cells to increasingly pronounced levels of atypia and thus of malignancy. It is true that clinically a tumor may manifest itself suddenly, but its biological history dates back to much earlier. This concept is consistent with the theory of multistage tumor growth, already developed on the basis of solid experimental evidence back in the pre-oncogene era, and now universally accepted.

Figure 7. The three main stages of tumor growth. O = events at *genetic* level (changes in DNA such as mutations, insertions, deletions or translocations of oncogenes); X = events at *epigenetic* level (various biochemical changes accelerating the expression of oncogenes and genes of normal growth).

The situation *in vivo* is also complicated for many other reasons: quite apart from the major problem of immune reactions - a subject which would need to be addressed separately in view of its importance - there are many other local factors (tissue oxygenation, organ mobility, biochemical constitution of the basic substance of the connective tissue, factors relating to the compression or erosion of adjoining organs) and general factors (energy metabolism, biohumoral mediators, cachexia, hormones, drugs) which condition the

progression of the tumor. Psychosocial stress or psychological or neuroendocrine characteristics similar to a "*type C personality*" (early family frustrations, denial of emotional conflicts, reduced communication with the environment, destructive fantasies, and similar) constitute *risk factors* for cancer [Invernizzi and Gala, 1989].

Tumor progression is conditioned by the tumor-host interaction, also in the sense that the tumor itself, as it grows, influences the body in various ways both via metastatic spread and through the release of soluble products, causing direct or indirect alterations to neighboring and remote organs, including the nervous system. Situations of organ damage and biohumoral disorganization of great complexity and variety are thus generated.

Given the heterogeneity of neoplastic disease and the very substantial importance of the host factor in tumor progression, it is not hard to understand why cancer therapy meets with major difficulties in practical terms as well as why treatments with all the theoretical and scientific prerequisites for efficacy often yield encouraging results in single cases, but prove to have only a poorly significant impact in statistical terms. As things stand at present, it would appear to be largely unrealistic to imagine there may be any single cure for cancer, and this goes for both conventional and unconventional therapy. This is due simply to the fact that "cancer" is an abstraction, whereas in actual fact only particular cases exist and these differ one from another. Without wanting to underrate the value of controlled clinical trials on individual treatments, it would therefore seem important to make some attempt to exploit the *individual* approach to neoplastic disease. This individualization should start on the diagnostic plane and then transfer, if possible, to the therapeutic plane.

The problem of cancer therapy and, in particular, the possible relationships between new scientific knowledge and homeopathic medicine, which is the subject matter of this treatise, will be taken up again in Chapter 6, Section 5, after illustrating the rational model we use to explain the law of similars.

5.4. Homeostasis and complexity

The complexity of pathogenetic mechanisms, stressed in the previous sections in relation to diseases in general and to a number of pathological processes (inflammation and cancer) in particular, is deeply rooted in the complexity present also in physiology and biochemistry. To make this important relationship more explicit and provide better documentary evidence of it, we shall devote the following sections to a review of a number of the general aspects of physiological homeostasis and the exchanges of biological information that regulate it. Understanding the complexity of homeostasis is a *sine qua non* for putting the action of homeopathic remedies into proper perspective.

The concept of homeostasis, first introduced by the physiologist W.B. Cannon in 1929, refers to all those activities which tend to keep the variables of a vital system constant, or, to be more precise, within acceptable limits. It may be useful here to take a look at the homeostatic concept in greater detail, using a schematic model of the type illustrated in Figure 8.

Figure 8. Schematic representation of a typical homeostatic system. + = stimulatory effect; - = inhibitory effect.

A homeostatic system, in its essential make-up, consists in a *set of anatomical, biochemical, and functional elements designed to maintain a physiological variable within minimum and maximum oscillation limits*. Let us consider a *variable A-A′*, which is in a state of dysequilibrium and in conditions of reversibility due to the action of two *operator or effector mechanisms*, which may bring *A* to the level or condition *A′* and vice versa. The system, however, cannot function properly without some form of control, which is provided by a *regulation center* which receives information from *A′* in the form of a *signal* "*a*" associated with its condition (for example, an enzyme reaction product proportional to how much of *A′* is present or to how much of *A′* is functioning). In addition to receiving *a′* signals (for which it has specific receptors), the control system somehow compares these signals with a *memory* in which the optimal value of *a′* is established. When this value is exceeded, the regulation system is activated and produces the *signal r*, which then inhibits the *A* --> *A′* mechanism and/or activates the *A′* --> *A* mechanism. Usually, these effector mechanisms (enzymes, membrane pumps and channels, but also antibodies or cells of various types according to the

systems considered) are endowed with incorporated receptor sites for regulatory signals. The homeostatic system thus consists in a negative feedback loop, in which the information on the result of a transformation or an activity oscillation is fed back in revised and corrected form to the entry point of the cycle.

Obviously, the model outlined above is stripped down to essentials, in that if we want a more complete picture, we have to contemplate numerous variants and additions. For instance, we must be aware that there are not just negative feedback loops, but also positive loops, whereby the reaction product contributes towards accelerating the transformation. This is what occurs during the growth of a tissue or when rapid, intense functional modifications (amplification) have to be set in motion.

The model in Figure 8 should also consider the fact that condition *A* has its own control system and, above all, that the homeostatic system itself is in turn related to other systems: the signals *a′* and *r* may have effects on other control systems and on other effector mechanisms, whereas the regulatory system may have receptors for other signals and thus be influenced by these. The integrations (this is a recurrent point) are of the *horizontal* type, as between cells or between organs, or of the *vertical* type, as between molecular and cell systems, between cell systems and organs, or between organs and the body as a whole.

The essential constituents of homeostatic biological systems are therefore the following:

a) *Anatomical or biochemical structures* with adjustable and reversible effector functions. To mention only a few examples, these structures are represented at cell level by enzymes, membranes, contractile proteins, and at body level by the endocrine glands, vessel walls, the cell mass of a certain tissue, etc.

b) *Signal molecules* which enable nearby and remote structures to communicate, such as neurotransmitters, hormones, local chemical mediators, cytokines, physiological inhibitors, and antagonists. A particular complexity feature of the signal molecules is that their message is never wholly specific: the same molecules can be used to communicate between different systems. The same molecules can be produced by many different types of cells. The same molecules can bind to different receptors present on cells in different tissues and organs. There is thus a substantial degree of *redundancy* of biological information, which enables the system to enjoy a considerable measure of flexibility and plasticity, but at the same makes it difficult to achieve any kind of rigid schematization of the events following the production of a certain mediator in given pathophysiological conditions.

c) *Receptors* for signal molecules or for other types of messengers, endowed with specific affinity and capable of transmitting the signal to other elements of the regulation system. There are membrane receptors, intracellular receptors, and even intranuclear receptors. It should be noted that the receptors are highly *plastic*: the cells are capable of increasing (hypersensitivity, priming) or decreasing (desensitization, tolerance, adaptation, down-regulation) the number of receptors according to their needs, as well as of regulating their activity by modifying the affinity for the signal molecule. On occasion, the cells present more than one receptor for the same molecule, but with different affinities and different intracellular effects. A number of receptor properties are addressed in greater detail in Chapter 5, Section 6.3.

d) *Transduction systems*: coupling of receptor activation and production of signals or activation of effector mechanisms; variations of intracellular *second messengers*, covalent and noncovalent modifications of membrane lipids and proteins, and the opening of ion channels. The multiform characteristics of the transduction systems are too vast a topic to be dealt with here. What is beyond doubt, however, is that the level of responsiveness of a certain (control or effector) system is also controlled by such systems in the cell, that they are also modified in the course of disease, and that they are susceptible to pharmacological modulation.

e) *Elements responsible for storage of information* for a given time period: when a system undergoes a change, this may be rapidly and wholly reversible (e.g. the contraction of a muscle), but it may also be a phenomenon which leaves a more or less permanent trace. Usually, though not always, the longer-lasting changes are those which in some way involve the genetic code of the cells.

Some examples of homeostatic systems are:

a) *At cell level*: membrane transport systems, whereby concentrations of Na^+, K^+, Ca^{++}, Cl^-, H^+, are maintained in a state of dynamic dysequilibrium between the intra- and extracellular space; control of metabolism, whereby the availability of nutrients and the presence of metabolites regulate the activity of enzymes and metabolic pathways.

b) *At organ level*: regulation of blood flow in relation to the O_2 requirement; maintenance of constant numbers in cell populations and of the geometric relationships between the various parts; growth and structure of bone in relation to mechanical and gravitational stress; enzymatic induction of the detoxifying functions of the liver following introduction of toxic substances.

c) *At apparatus level*: the vegetative nervous system, for maintaining blood pressure, thermoregulation, bowel function; the endocrine system, for the control of metabolism, growth, the sexual cycle; the cardiovascular and respiratory systems; the immune system, for discriminating between endogenous and exogenous information; the hemostatic system, as an integrated system of cell and humoral mechanisms responsible for the control of the fluidity of the blood.

d) *At superior function level*: mental and emotional functions aimed at bolstering the ego (personality, character, will-power) and at facing up to variable environmental situations (e.g. survival instinct, mature handling of affectivity, decisions and frustrations).

e) *At interindividual and sociocultural level*: population density and forms of economic conditioning on various scales; cultural models, ethical values, ideologies; power systems and deviance control (laws) or incentive systems for behavior patterns accepted by the social group; learning mechanisms for past traditions and development plan forecasts; information control systems.

Of course, the more complex the system, the more complex must be the control networks responsible for guaranteeing effective and specific responses. The nervous system offers a good example of what *vertical* and *horizontal* integration mean. The neurosciences, resting mainly on molecular and cellular biology, have allowed enormous strides to be made in recent decades in our knowledge of neuronal structure, synaptic mechanisms, and signalling and modulation systems (neuromediators). From this point of view, the brain tends to be described as a "container of molecules in action" [Andreoli, 1991]. In this context, horizontal integration consists essentially in understanding the dynamics of functioning on the molecular plane: interactions between signals and receptors, between receptors and proteins of the transduction system, between ion channels and changes in potential, and so on. At the same time, however, there arises a need for vertical integration, which consists in grouping molecules and neurons in nerve *centers*, where the various cells and molecules are in charge of one or more functions. The nerve center has its own architecture and its own "language," which is composed of single molecular or electrical events, but which takes on meaning only as an integrated whole. Many centers are horizontally integrated to form a cerebral area, or a sequence of centers which confer unity upon a more complex function, as may be the case with a visual or auditory function. The nerve centers, however, are also integrated vertically in one hemisphere of the brain, in that particular functions are connected to other areas which add to sensory perception other properties such as conscience, memory, and emotionality. At this level of analysis, then, functions can be carried out which transcend the previous levels, though presupposing them as necessary elements. The organ of hearing and its nerve centers can perceive a sequence of sounds, but for these to be perceived as music a superior integration process must be realized. If we wanted to define how the brain distinguishes between music and a hotch-potch of musical notes, it would be singularly futile to look for differences in the type of enzymes phosphorylating the proteins of the cells of the cochlea, or in the type of neurotransmitters of the auditory pathways, or in the number of cells involved in the auditory area of the brain.

To proceed with this analysis, we should not neglect the fact that the hemispheres of the brain are integrated horizontally via the cerebral commissure, and vertically with the rest of the brain (where, for example, many vegetative functions reside) and then with the entire body. It is therefore essential to consider the various different levels of integration to which the different functions correspond.

Whereas there can be no doubting the complexity of the nervous system, less evident, perhaps, is the complexity at cell level. However much the cell is regarded as the basic unit of the living organism, it is fairly obvious that its organization is highly complex on account of the multiplicity of receptors, transduction routes, effector systems, and genes, all of these being elements which influence one another reciprocally. If to this we add the fact that many elements (particularly the macromolecules) are permanently subject to dynamic oscillations between activation-assembly and dectivation-disassembly, it will be understood that a precise description of what happens *in the cell as a whole* is practically impossible. Since, however, cell theory has dominated pathology for so long, today there are still people who refuse to accept such a conclusion, which mistakenly comes to be seen as conflicting with the molecular point of view.

Others, however, forcefully underline the need to develop the study of cells on a basis which is not exclusively molecular [Albrecht-Buehler, 1990]. The main argument in support of this thesis is that within the cell are concentrated and organized many structures and systems in a state of activity, the properties of which cannot be inferred only on the basis of analysis of molecules purified and studied out of context. In fact, the *proximity relationships* can be transmuted into *forms of collective behavior* of the molecules (including those of water), coherent motions, resonance phenomena, or long-ranging interactions. In other

words, it is precisely the *complexity* of the systems in which many molecules interact that precludes an exhaustive description of cell functions on a purely molecular basis.

Stress has also been laid upon the importance of considering the forces generated by tension of the fibers of the cytoskeleton and of the anchoring systems of cells to the tissue matrix in cell models. These forces would appear to have an information content, in that they regulate functions such as ion transport, protein synthesis, and expression of specific genes [Ingber, 1991].

5.5. Information

In view of the importance the concept of information has taken on in our reasoning, we feel it is worthwhile devoting a special section to this topic.

It is only too clear to everyone today that information counts more than force, more than armies, and more than energy (without wishing to imply that these are not important). Companies today invest and spend more on information than on energy. Wars are won more with satellites and computers than with tanks. This information, as Maxwell's demon* has shown, has a cost in terms of energy; in a certain sense, it could be said that information *is a special type of energy required to establish order*. However, the fact that producing, transmitting and manipulating information has an energy cost does not necessarily mean that information in itself is endowed with energy, or even with mass. There are certain types of information which in themselves have only minimal energy contents, but which give rise to very precise effects in target systems. For instance, information broadcast by radio has a very low energy content, as does the information reflected by the light illuminating a text, or a word reaching our ear. Thus, it is also clear that molecules with extremely selective and specific mechanisms of action (which therefore exploit a high information content) act at very low doses (cf., for example, hormones, or antibodies, or certain toxins).

(Footnote)

*Maxwell's hypothetical demon wanted to violate the second law of thermodynamics by bringing order into a gas, separating the molecules in two vessels by simply opening and closing a small gate (microscopic and friction-free) whenever a molecule approached the hole communicating between the two vessels. After a short time, the demon reasoned, without making the slightest effort I shall have trapped many molecules in one of the vessels, and will thus have pressure to exploit to perform work. He overlooked the fact, however, that, to be able to perform his trick, he needed to have information about the position and movement of the molecules he wanted to capture. It can be calculated that to acquire this information the demon had to consume more energy than he would have been able to recover from the pressure produced by the gas [Harold, 1986].

Information may also be to some extent assessed quantitatively. The information content is measured in *bits*, one bit being the amount of information necessary to be able to make a choice between two alternatives, i.e. a binary choice (yes or no). Needless to say, the more complex the system, the more information it contains and the more information is needed to describe it. For example, if 1 bit is sufficient to code between two numbers (0 and 1), 5 bits will be needed to code a number N among 32 numbers: 32 is divided into two parts and it is decided in which part the number N lies (1 bit is used). The group of 16 remaining numbers containing the number N is divided in two, and another bit is used to reduce the possibilities to 8. Proceeding in this way, we reach a situation in which only two numbers are left, and the fifth bit is used to make the final choice.

Generally speaking, the information content (I) of a system (or an event) is:

$$I = \log_2 1/p$$

where p is the probability that the system will be in this state (or that the event will occur) by chance. Thus, the information is inversely proportional to chance. The larger the number of choices possible, the less likely it is that the event will occur by chance, or that a certain system will be in a certain state by chance.

The degree of order in a given system can be estimated by calculating how many binary choices must be made to specify its structure. For example, during the synthesis of a protein, the choice of amino acid out of 20 possible candidates requires $\log^2$ 20, or 4.3 bits. An entire protein of 300 amino acids requires 300 $\log_2$ 20, i.e. 1,300 bits, while the corresponding DNA sequence (which, for the sake of simplification, consists in 300 nucleotide triplets) requires 900 $\log_2$ 4, i.e. 1,800 bits of information [Harold, 1986]. The genome of a human cell has approximately 3 billion bases, corresponding to ca. 6 x 10^9 bits/cell, whereas if we consider the entire organization of an individual adult we get up to the astronomical figure of 10^{28} bits.

Acquiring information has a cost in terms of energy (at 27°C one bit is equivalent to 3 x 10^{-21} joules [Harold, 1986]). To understand how much it costs to produce information in physiology, we may consider, by way of an example, the formation and action of typical signals, namely the hormones. These molecules, produced with consumption of energy by the endocrine cell, reach the receptors (also produced with energy consumption), from which a message then departs in the form of an increase in calcium which is released from intracellular stores, dissipating the gradient which had been created by consuming energy (Ca^{++} ATPase). In a certain sense, then, the signals convey the energy of the cell, which is dissipated, i.e. they exert a form of control over the dissipation of thermodynamic gradients. This dissipation is obviously short-lasting and is followed by a new accumulation of thermodynamic gradient (at the expense, however, of energy consumption of another type, e.g. metabolic). To the energy cost account of bio-information processes we also have to charge the biosynthesis of DNA, RNA, and biochemical mediators.

The fact that information can be to some extent measured in bits does not entirely solve the problem in all its complexity because the quantity does not in itself comprise the "*meaning*" of the information. The meaning of information resides in the interaction between the information itself and the receiving system and in the result produced by this interaction. Two sequences of DNA, one of which "normal" and the other "pathological" (e.g. coding for a character that causes disease) may contain the same quantity of information, but the result is very different. Thus, a good musical score may contain the same amount of information (in the form of musical notes) as a very bad score. Accordingly, there is necessarily a qualitative element in information which cannot be quantified.

In the biological world, as stressed earlier, communicating information is essential for life: at molecular level, order is expressed in the form of a given, precise association of atoms in molecules (amino acids, proteins, lipids, nucleic acids, etc.); at cell level, order is expressed in the regularity and reproducibility of the cell organization and of biosynthesis, transport and movement processes. To impose order, i.e. to reduce the entropy of living matter, information is necessary. Information can therefore be defined as *the ability to establish order* [Harold, 1986] or, to quote Jacob's famous phrase, "*the power to direct what is done*" [Jacob, 1973].

DNA, as the main data bank of the cell, has the ability to "direct" the cell development, and at the same time to incorporate and remember information: information is stored in the DNA regarding the entire evolutionary history of the species to which the individual belongs. Of course, DNA is an important information-containing material, but it is not the only one: information is contained in *every* organized structure and in every spatiotemporal event that is not casual. While the language of the gene is fairly simple, in that it is written with only a few symbols and in a linear manner, many signals use more complex languages and symbols of various kinds. Events such as changes in transmembrane electrical potential, changes in the ratios of the various phospholipid species, the alkalinization of the cytoplasm, increases in cyclic AMP, elevations of body temperature, blood pressure, the formation of a certain complex of factors controlling the clotting of the blood, and even the emotion experienced as a result of sudden stress are signals which act in the most disparate ways. What is more, the duration of the signal is extremely important; usually signals are short-lived, since there are many control and modulation systems.

A more detailed definition of information might be the following: *information is an intrinsic function of every spatiotemporal structure, capable of being transmitted to another spatiotemporal structure and, thus, of modifying it in a specific manner*. The term *structure* in this context defines a particular configuration of particles, such as atoms, molecules or ions, but there are also structures organized on a temporal scale. A note of music, for instance, is a structure formed by vibrational waves in the air. The terms structure, order, and coherence may be regarded as synonyms.

Apropos of spatiotemporal regulation, it is interesting to note that the pure passage of time is perceived at biological level: the passage of time is signalled by "biological clocks," which induce cells to perform given functions only at certain times of the day or of the period with which the clock is associated.

Information is therefore contained not only in molecules, but also in the "way" molecules relate to the receiver systems. The quantity of the signal is very important, but so is its quality. For example, the receptor system of cells is often capable of distinguishing the *kinetics* whereby the signal is received, namely whether it is a sudden signal or a signal of slow onset, whether the concentration is stable or oscillating, whether the signal is single or accompanied by other concomitant or preceding signals, whether it is the first prompting or a repetition of something *dejà vu*. Thus, information is not merely quantitative, but essentially *spatiotemporal*. It has recently been suggested that one of the most important intracellular signalling systems, the increase in calcium ions, performs its function by means of pulsations, or rather oscillations of concentration, which constitute a kind of "digital code" for the various sensitive systems: for a response process to be activated, what counts is the frequency of the spatiotemporal oscillations (waves) in the calcium concentration rather than the actual amount of calcium present [Berridge and Galione, 1988; Cheek, 1991].

Single-cell measurements have shown that many hormones trigger a series of calcium *spikes* and that these spikes show a rise in frequency with increasing hormone concentrations. It has been suggested that many cell responses are controlled by *frequency-modulated* rather than *amplitude-modulated* signals, analogous to the transmission of information between neurons by changes in frequency of action potentials [Catt and Balla, 1989]. Such digitally encoded signals could more precisely regulate the cell response to changing hormone concentrations.

Calcium waves can also propagate in tissues and organs, providing a long-range signalling system, as observed in ciliated epithelial cells, in vascular endothelial cells, in hepatocytes, and in monolayers of cultured astrocytes. It has been suggested that this mechanism of cell to cell communication may contribute to the synchronization of large cell assemblies [Meyer, 1991].

In vivo, various hormones are secreted with oscillatory rhythms [Matthews, 1991]. In healthy people, insulin is secreted with pulsations that are repeated every 12-15 minutes, controlled by a pancreatic *pacemaker*, probably influenced by the vagus nerve. The insulin secreted in pulsations is metabolically more efficient in maintaining normal glucose levels, and it is significant that irregularity or even the loss of these oscillations is the earliest abnormality detectable in insulin secretion in patients with type 2 diabetes [Polonsky *et al.*, 1988; Holffenbuttel and van Haeften, 1993].

Biological communication is so important that nature has gone out of its way to find the most differentiated forms of communication (languages). To those we have already mentioned may be added others, the foremost and most obvious of which are the sense organs endowed with photoreceptors, chemoreceptors, baroreceptors, and others. This appears only too self-evident, and there is no point in dwelling on this aspect. More pertinent perhaps to our case is the problem of communication via electromagnetic waves. Light is a basic means not only of transmitting energy (sun-earth), but also of communicating; many fish communicate with light messages, and some cells in mammals also produce light (chemiluminescence), while an infinite amount of luminous information is received by the organs of sight; light would also appear to be important for establishing many biorhythms. Light, however, constitutes only a small part of the electromagnetic spectrum, and it may seem strange that nature has not learnt how to handle other types of electromagnetic fields. We shall return to this problem in Chapter 7.

In a nutshell, then, *every biochemical or biophysical system endowed with a certain degree of order acts as a vehicle for information*, which, when suitably decoded by receptor and transduction systems, may have biological consequences. The information molecules *par excellence* are the nucleic acids because they are characterized by a very substantial degree of order (cf. the arrangement of the nucleotides in very long sequences), by a major degree of complexity (cf. all the mechanisms controlling the expression of the genetic code and also its continual transformation), and by great physicochemical stability (given its particular double-helix structure, DNA is one of the most resistant molecules, and, in addition, many systems exist for repairing and remedying possible errors). Many other molecules, however, contain and transmit information: proteins, peptides, sugars, lipids, and even mineral salts and protons (H^+) serve nature as transmitters of information and thus to regulate biological systems. Leaving aside the molecular field, we also find information transmitted by frequencies, such as sound and electromagnetic waves and rhythmic, oscillatory chemical events. The more complex a system is, the more complex will be its communications strategy, which may be made up of many elements arranged in sequences and networks.

The problem of information has a close bearing on the mechanism of action of homeopathic remedies. As we shall see later (Chapter 6), the "secret" of homeopathy, in its classic form, lies precisely in the meticulous collection of information relating, on the one hand, to the remedy (cf. provings in healthy

subjects) and, on the other, to the patient (every physical or mental symptom is assessed with particular reference to its extent, circadian variations, site, mode of onset, duration, association with other symptoms, and constitutional characteristics). It might be said that the bulk of the effort made in the homeopathic method is precisely in the collection and "*repertorization*" of symptoms (i.e. comparison between the patient's symptoms and symptoms caused by remedies as reported in the materia medicas). It is no accident that a major contribution to this work of data analysis and comparison is made by systems of computerized repertorization [see, for example, Van Haselen and Fisher, 1990].

5.6. Doses, target systems, and effects

In this section we shall deal in particular with a problem already raised earlier, when we talked about the difficulties in controlling homeostatic systems and pathogenetic mechanisms by means of exogenous manipulation. This problem has to do with the fact that a given type of manipulation of a biological system is not always followed by an effect proportional to the extent of the intervention implemented. This is of great importance in clinical medicine and in pharmacology, but it is obviously also of vital interest when it comes to interpreting the mechanism of action of drugs which are used at low or ultra-low doses.

The problems to be tackled here are essentially two: the first relates to the fact that the effects of a certain treatment do not always go in the direction that might appear logical, and the second has to do with the nonlinearity and nonunivocal nature of dose-response curves.

5.6.1. Apparently paradoxical effects

The complexity of homeostatic pathophysiological systems means that it is not always possible to predict the outcome of a given intervention aimed at regulating such systems. Psychiatry and the neurosciences in general represent today the main frontier where this type of problem is taken into consideration. In these disciplines, in fact, the molecular approach to pharmacology shows both its validity and its limitations. The effects of psychotropic drugs often vary very strikingly from one subject to another, not only according to whether or not the subject is sick or healthy, but also according to the type of disease and even the characteristics of the subject's personality.

Pyschopharmacology is thus full of examples in which a certain treatment causes paradoxical effects. This concept is well expressed in a recent article dealing with the relationships between madness and biology: "If an exogenous molecule administered to a mad person is capable of modifying his or her behavior, it is legitimate to postulate that the madness itself is related to endogenous molecules with which one thereby interferes. Corroboration of this is provided by the administration of exogenous molecules to healthy subjects with the result that their behavior is modified pathologically. In this case the molecule produces madness. These two circumstances have a long history behind them in psychopharmacology and have given rise to the two important areas known as the "therapy of madness" and as "experimental psychosis" or "drug-induced psychosis" [Andreoli, 1991, p. 52].

The nervous system is *the* complex system *par excellence*. It manifests in paradigmatic form a behavior typical of all complex systems: the effect of pharmacological manipulation depends both on the direct effect of the drug itself on cells and molecules, and on the sensitivity of the receptor or enzymatic structures, as well as on the reactions which set the system itself in motion and on the reactions secondary to endogenous reactions. This in itself does not mean that an effect is, as a rule, unpredictable, but that the *mechanism* whereby an effect is exerted cannot be interpreted merely on the basis of the direct effect on the deterministic molecular plane.

A precise example of what we have said may be the use of tricyclic antidepressants [Goodman Gilman *et al.*, 1992]. The parent molecule of the class, imipramine, was discovered by chance in 1958 in the course of a clinical trial in psychotic patients. It was noted that imipramine was relatively ineffective in calming agitated psychotic patients, but the drug proved capable of affording substantial benefit in depressed patients, who were stimulated by it. Since then these compounds have been widely used in depression. If imipramine (100-200 mg/day) is administered for a sufficiently lengthy time period to a depressed patient, the patient's spirits are raised. In some cases the effect is so pronounced that there is a real danger of generating a maniac-like

excitatory effect. If, however, a dose of 100 mg of imipramine is administered to a healthy subject, the latter feels drowsy and tends to be calmer, undergoing a slight reduction in blood pressure and experiencing a "light-headed" sensation. Unpleasant anticholinergic effects occur and, sometimes, a slight change in pupil diameter. The gait may become unsteady, and the subject may feel tired and clumsy. Deterioration in performance test results may occur. These pharmacological effects are usually perceived as disagreeable and give rise to a feeling of unhappiness and increased anxiety. Repeated administration of imipramine for several days may cause intensification of these symptoms as well as difficulty in concentrating and reasoning.

Other examples of paradoxical effects of drugs can be encountered in systems other than the nervous system, such as the cardiovascular or endocrine systems, or the immune system and inflammation.

Digitalis, which is regarded today as a fully fledged hormone probably produced by the adrenal glands [*Lancet* editorial, 1991] causes depression of cardiac function in healthy subjects when administered at pharmacological doses, whereas it has a positive inotropic effect in heart failure. Conversely, adrenalin has a positive inotropic effect in healthy subjects, whereas in heart failure it has no effect, or a negative effect (when it activates the $beta_2$-adrenergic receptors, and the muscarinic and adenosine receptors are also stimulated) [Braunwald, 1991]. These changes in responsiveness are related to increases and reductions in sensitivity and in the number of specific receptors, as well as in transduction systems such as the G-proteins. Another possible example, among the many that could be mentioned with regard to the vascular system, is the fact that acetylcholine induces a vasoconstrictor effect in arteries affected by atherosclerosis, which is paradoxical, in that the drug normally causes vasodilatation [Ludmer *et al.*, 1986].

An inhibitor of nitric oxide synthase (monomethyl-L-arginine) causes a marked increase in platelet aggregation in healthy controls, presumably because it removes an endogenous feedback inhibitory mechanism. On the contrary, in hypertensive patients the same agent causes only a slight inhibition of aggregation [Cadwgan and Benjamin, 1993]. Therefore, platelets from hypertensive patients show a markedly reduced sensitivity to a drug that is effective in normal humans, suggesting that an imbalance of these control mechanisms contributes to the pathogenesis of essential hypertension.

Serotonin, which causes vasodilatation in normal arteries, induces vasoconstriction in some forms of hypertension and diabetes and in atherosclerotic arteries. This might be an important mechanism in transient ischemic attacks [Ware and Heistad, 1993].

Japanese endocrinologists have conducted a study in 109 patients with Graves' disease (hyperthyroidism caused by autoantibodies), treated first with a conventional antithyroid treatment, and then, when this was discontinued, with thyroid hormones (thyroxine 0.1 mg/day) long term versus placebo [Hashizume *et al.*, 1991]. In the subsequent follow-up, the treated group showed a significant reduction in recurrences of hyperthyroidism, accompanied by a progressive reduction in the autoantibody count. Treatment with thyroid hormones thus reduces activation of the thyroid in Graves' disease patients. The authors themselves found a convincing explanation of this apparent paradox: by means of an endocrine feedback mechanism, thyroxine inhibits TSH-induced thyroid stimulation and the subsequent release of receptors serving as stimuli for the production of autoantibodies.

In the field of autoimmune disease therapy, one form of therapy steadily gaining ground is that based on administration of immunoglobulins [see, for example, Kaveri *et al.*, 1991; Dwyer, 1992], i.e., in practice, of molecules which are already present as pathogenetic agents. It is likely that the efficacy of this therapy depends either on competition at the level of the cell membranes suffering the immune attack or, much more probably, on the blocking action of antibodies against other antibodies (anti-idiotype antibodies) [Dwyer, 1992].

In addition, we can mention here the therapies for autoimmune diseases based on the oral administration of the same protein that caused the autoimmunity, or of particular fragments of the same proteins ("tolerogenic epitopes") [Marx, 1991; Miller *et al.*, 1991a; Miller *et al.*, 1991b: Whitacre *et al.*, 1991; Miller *et al.*, 1992; Engel, 1992; Ku *et al.*, 1993]. It has been discovered that attacks of experimental autoimmune diseases in animals (allergic encephalitis induced by injection of a protein associated with myelin, arthritis induced by collagen, uveitis induced by a retinal protein) can be suppressed by feeding the animals the same proteins causing the attacks. These studies in animals have yielded such promising results that clinical trials have been started on "oral antigen therapy" (or specific oral tolerance induction) in patients with multiple sclerosis, rheumatoid arthritis, and uveitis [for a review see Weiner *et al.*, 1994]. The investigators conducting this research claim that this type of therapy induces specific immunosuppression, based on activation of certain subsets of T lymphocytes located in the gut lymphoid tissue, which are capable of

suppressing the activity of other cells of the immune system. Very recently, it has been demonstrated in animal models (mice) that the mechanism of these phenomena is determined by secretion of different cytokines according to the antigen dosage: low doses (1 mg every other day) of antigen induce active immunosuppression by the release of more transforming growth factor-β and less interleukin-4, while high doses (20 mg/day) induce anergy (lack of response) by the release of more interleukin-4 and less transforming growth factor-β [Friedman and Weiner, 1994].

It is interesting to note that the possibility that oral ingestion of antigen might modify subsequent systemic immune responses was probably recognized in ancient times by South American Indians who ate poison ivy (*Rhus toxicodendron*) leaves in an attempt to prevent contact sensitivity reactions to the plant [reported by R. Dakin, cited by Mowat, 1987].

The immune system never ceases to amaze us with its characteristics of flexibility and complexity, which physicians seek to exploit for therapeutic purposes. Up until not so very long ago, vaccinations were thought to be an excellent means of preventing infectious diseases, but that little could be done for a disease already in progress. This would seem logical, in view of the fact that the individual affected by an infectious agent is already full of antigens, and, after a short time, of specific antibodies and lymphocytes. Today, however, a different picture is beginning to emerge: vaccinations might also have a curative effect [Beardsley, 1991]. It can be postulated that this new approach to therapy by means of vaccinations (still at the experimental stage) can be justified rationally on the basis of the particular modes of functioning of the immune response.

An initial explanation lies in the fact that vaccinations can be given with antigens in a slightly different form from the natural antigen, for instance, in the form of recombinant protein or as a complex with other immunogens: the antigen therefore would be recognized by the system in a different way and would trigger off a different and possibly more effective response to the original infectious agent. Another possibility is based on the fact that different routes can be used to introduce the vaccine (oral, intramuscular, inhalatory). By modifying the administration route, compared to the route taken by the natural agent, other groups of lymph nodes or reactive centers of the immune system can be activated, and, most importantly, the surveillance cells (macrophages) can be reached via an anatomical route that they "do not expect," thereby bypassing possible blockade or adaptation mechanisms of the system which allowed the pathogen to conceal itself or survive.

Considerations of this type can hardly fail to have an impact on the debate regarding homeopathy, particularly as regards validation of the law of similars. In the sector of therapies modulating the immune system, the boundaries between allopathy and homeopathy tend to blur. *Similar substances may have opposite effects according to the doses and to the particular sensitivity of the systems with which they interact*.

We could also mention nonhomeopathic drugs which act according to a mechanism which could be regarded as an application of the law of similars. A significant example is provided by products based on extracts of *Klebsiella pneumoniae*, *Diplococcus pneumoniae*, *Haemophilus influenzae*, and similar substances, for which a substantial body of convincing literature exists [Nespoli *et al.* 1987; Capsoni *et al.*, 1988; Balsano *et al.*, 1988] and which are classed as belonging to the category of immunostimulating substances. These drugs are indicated, at low doses, for the prophylaxis and therapy of respiratory tract infections. It is interesting to note that the administration of these preparations causes, as an unwanted effect, a temporary increase in symptoms following the first few doses, which is a characteristic phenomenon reported in the literature in relation to the action of homeopathic products.

That many molecules act as "double agents" is also well known in the field of inflammation. By way of an example we can take substance P. This is an undecapeptide belonging to the family of the tachykinins and is found in many organs, such as the central nervous system, the lungs, skin, and bowels. It performs various different functions: the first function assigned to it was pain mediation, both as a neurotransmitter among the neurons of the pain pathways, and in so far as it increases the sensitivity of thermal and pressure receptors (probably by inhibiting the potassium channels of the membranes). It was later seen that substance P, even when released peripherally by the sensitive endings of type C unmyelinated fibers, mediates many inflammation phenomena. Tissues which are a prey to inflammation, as in ulcerative colitis, rheumatoid arthritis, and asthma, present a substantially increased number of receptors for substance P. On the other hand, it has also been seen that interleukin-1, one of the inflammation cell products, stimulates production of substance P by the cells of the nerve ganglia. It is in this that its role as a double agent lies: it increases sensitivity to pain, but also promotes healing of wounds by increasing the activity of leukocytes and the

immune system in general. In practice, it makes the phagocytes more efficient in killing bacteria, and the lymphocytes more efficient in producing antibodies. Thus, the same mediator causes pain and heals [Skerret, 1990].

It is well known that one of the mediators of the occurrence of headache and migraine may be serotonin, inasmuch as this molecule causes vasodilatation in certain cases. On the other hand, drugs have recently been introduced which are effective in migraine and are analogues (i.e. "similar") to serotonin, acting not as antagonists, but as agonists, that is to say they have the same action as the endogenous molecule. How does it come about that a molecule similar to the mediator causing pain has a pain-relieving effect? The answer here is to be sought in the complexity of the receptors: there are at least three distinct types of serotonin receptors, and the analogue stimulates only one type, which evidently serves as a negative feedback mechanism for the pain-inducing effects of serotonin itself.

Histamine is a well known inflammatory mediator causing allergic manifestations and anaphylaxis, but histamine may have antianaphylactic action *in vivo* [Blandina *et al.*, 1987] and prevent antigenic release of histamine from cells of allergic donors [Bourne *et al.*, 1971; Lichtenstein and Gillespie, 1973; Lichtenstein and Gillespie, 1975]. These apparently paradoxical results are probably due to the existence of different histamine receptors and to the fact that stimulation of H_2-receptors causes an increase in cyclic AMP and mast cell inhibition [Masini *et al.*, 1982].

High-dose aspirin can cause hyperthermia [Goodman Gilman *et al.*, 1992], which is clearly a paradoxical effect compared to the main indication.

It has been reported, amongst other things, that onicholysis disappears during treatment with benoxaprofene, despite the fact that onicholysis figures among the possible side effects of the drug [cited by Taylor Reilly *et al.*, 1986].

Going over now to another field of research, we see that studies on tumor cells in culture have yielded apparently paradoxical, but also highly indicative results. The differentiation of leukemia cells versus more mature and thus less malignant forms can be obtained in culture not only by means of specific differentiation factors (this, however, only in a number of cell lines, obviously endowed with receptors), but also by means of agents such as lectins, or classic tumor promoters such as TPA, or low doses of cytostatic agents, or low doses of radiation [Sachs, 1986; Sachs, 1989]. Evidently, effects promoting proliferation or effects promoting maturation, and thus the arrest of proliferation, are not properties related only to the molecule used, but properties which depend on dose, receptor sensitivity, types of oncogenes active in a certain cell line, synergism or antagonism with other factors in the culture medium or produced by the cells themselves, the metabolism the substance undergoes in the cell, and other processes.

Given the complexity of the mechanisms involved in the control of cell proliferation (see also Chapter 5, Section 3.1.), it is understandable that a treatment which, according to traditional concepts, should be pro-cancerogenic may turn out to be anti-cancerogenic experimentally on changing the doses or experimental protocol. Many mitogenic agents, for example, if administered to cells in small doses over lengthy periods, desensitize the cells, almost always in a homologous manner (i.e. to themselves), but sometimes they do so in a heterologous manner (i.e. to other agents with a similar action at the transduction mechanism level) [Rozengurt, 1991]. There are also experimental animal models which show that a carcinogen at low doses can combat the onset of the cancer itself [De Gerlache, 1991] (see also Chapter 5, Section 3).

The examples mentioned here are suggestive indications of how the damaging effects of poisonous or toxic substances can transform themselves into specular therapeutic effects (or vice versa, i.e. therapeutic effects can turn into damaging effects) on changing the doses and modes of administration of the substances and the sensitivity of the system treated. This is of primary importance if we are to understand the possible mechanism of action of homeopathic remedies [Grange and Denman, 1993]. Obviously, we should stress, especially in fields such as oncology, that experimental models can illustrate certain biological phenomena which undoubtedly exist, but cannot be regarded as proof of the therapeutic efficacy of given treatments, since they often represent only particular cases in the context of biological complexity.

5.6.2. Doses

In this section we intend to consider the fact that the effects of a given treatment, aimed at modulating a given biological system, are not always proportional to the doses used. In chemistry, biology, and pharmacology, the analysis of dose-response curves is fundamental for studying the characteristics and

mechanisms of action of any active compound. Such analysis provides information about the mechanisms responsible for a certain reaction. In biochemistry, for example, we can evaluate the affinity of an enzyme for a substrate or the type of inhibition of the reaction (competitive, noncompetitive) by a certain compound. In biology, such curves can be used to measure the number and affinity of receptors for a certain hormone, or active cell responses such as muscle cell contraction, enzyme secretion, and many other functions. Considering the versatility of *in vitro* studies, the effects of various doses of a certain toxic substance on the release of cytoplasmic enzymes can be measured as an index of cell mortality, or the increase in cell number as an index of proliferative activity.

In pharmacology, the production of dose-effect curves first in animals and then in man, is used to establish the correct drug dose and the so-called therapeutic interval (the difference between toxic doses and therapeutic doses).

A common conviction among nonexperts is that the higher the dose is, the greater the effect will be. This conviction is obviously wrong, in that usually the effects of a certain active substance on a certain parameter are known not to be directly proportional to the dose. In the classic dose-response curves (Figure 9) we see first of all a noneffect zone (doses below the sensitivity threshold of the system tested or of the measurement method), a certain dose at which we begin to see some effect (minimum effective dose), an exponential growth of the effect in the first part of the curve, and then a progressive slowing of growth until a plateau is reached, where the effect is maximal and further increases in dose are not followed by any quantitative increase in the parameter measured. This trend presents itself in similar forms, whether we are measuring stimulatory or inhibitory effects. The dose causing a 50% effect compared to the maximum is called ED_{50} (median effective dose), or ID_{50} (median inhibitory dose), or LD_{50} (median lethal dose, i.e. lethal for 50% of animals), according to what is being measured. By means of other mathematical operations, we can extract additional significant information from such curves, but we do not intend to discuss these possibilities here.

Figure 9. Classic dose-response curves. The curves represent the typical trend of biochemical or biological activity as a function of increasing doses of an activator compound (or enzyme substrate) (**A**) or of an inhibitor compound (**B**). The curves show an initial zone in the low-dose area where there is no effect, and then an exponential increase in the effect which later slows down until a plateau is reached (maximum activation or inhibition, which remains unchanged with further increases in dose).

These types of measurements and interpretations figure among the cornerstones of scientifically oriented medicine and clearly cannot be called into question. What appears, however, to be increasingly important in recent years is that there are many exceptions to these basic rules, with the result that they can no longer be regarded as valid in all cases. The exceptions in this case do not prove the rule, nor do they disprove it, but they narrow down its field of application to a part (at this stage it is hard to say whether or not it is the majority) of the events occurring in biology and pathology.

In this section we shall give examples of these exceptions, derived from the conventional biological and pharmacological literature and not from studies conducted for the purposes of investigating homeopathy.

Figure 10. Atypical dose-response curves. Curve **A** plots the trend of the immune response to bovine albumin in mice pretreated with increasing doses of antigen. The immune response is depressed (state of *tolerance*) both in animals receiving very low doses and in animals receiving very high doses of antigen. Intermediate doses, however, cause a *greater response* [from Pontieri, 1987, with modifications]. Curve **B** plots the metabolic activity trend of human granulocytes stimulated with high doses of bacterial peptides after pretreatment with increasing doses of peptides. The graph thus shows the response to the second stimulation, which is strongly potentiated by pretreatment with low doses (*priming*) and inhibited by high-dose pretreatment (*desensitization*) [data reported in Bellavite *et al.*, 1991c, and in Bellavite *et al.*, 1993a].

Figure 10 shows two atypical dose-response curves. Curve A plots the trend of the immune response (e.g. quantity of antibodies produced) as a function of amount of antigen. At the lower doses of antigen there

is a decline in the response, i.e. a desensitization, at intermediate doses an increase, and at high doses again a decline. Curve B, on the other hand, plots the metabolic activity of granulocytes stimulated with chemotactic peptides (fMLP) after pretreatment with increasing doses of the same peptides. At the lower doses we observe an increase in the response with the increase in dose, while at the higher doses it can be seen that the cell activity is depressed to the point of reaching levels which are lower than those of cells receiving no treatment at all. As can be seen from the curves, in these cases the concepts of proportionality of dose and effect clearly call for adjustment.

The reasons for these behavior patterns of biological systems are complex, relating, as they do, to the modes whereby cells, tissues, and organs regulate the degree of sensitivity at receptor, biochemical and genetic level. To cut a long story short, we can refer once again to the concepts of "priming" and "desensitization" (or adaptation), to which reference has already been made in the section on the modulation of the functions of inflammatory cells (Chapter 5, Section 2.1.)

What is meant by *priming* is a state of hyperactivation in response to a given stimulant, which characterizes a cell after it has received pretreatment with low doses of the same stimulant (homologous priming) or of other stimulants of a different type (heterologous priming). The priming is due to exposure of new receptors, to activation of the same receptors and/or to a number of changes in the intracellular communication or enzyme systems. It is worth noting that priming has been described not only at the cell level, such as in leukocytes, but also in tissue and organs, such as in the airways of allergic individuals after repeated challenge with allergens [Koh *et al.*, 1994].

What is meant by *desensitization* is a state characterized by lack of cell responsiveness to a given stimulus after the cell has received pretreatment with low, medium or high doses of the same stimulant (homologous desensitization) or of different stimulants (heterologous desensitization). Generally speaking, desensitization (whether homologous or heterologous) may be due to many mechanisms, including consumption or inactivation of receptors, decoupling of receptors from transduction systems, and de-activation of cell effector systems.

Figure 11 presents dose-response curves which document the phenomenon of priming. This event may manifest itself either as increased sensitivity to low doses (leftward shift of the dose-response curve) (Figure 11 B) or as an increase in maximum effect, doses being equal (Figure 11 A).

Figure 11. Dose-response curve illustrating the priming (or hypersensitivity) phenomenon. The solid lines plot the trends of a functional activity parameter in control cells in response to increasing doses of a stimulant. The dashed lines plot the trends for the same parameter as assessed in cells which have received a pretreatment with low doses of another stimulant. ED_{50} = dose causing 50% of maximal activation. Graph A gives an example where the dose-response curve of the pretreated cells reaches a greater height than that of the control cells, but the ED_{50} and peak activity are observed at the same doses in the two types of cells. This may indicate that the priming effect is the result of an increase in the number of receptors or of other mechanisms of post-receptor regulation. Graph B gives an example in which the dose-response curve is shifted to the left towards the low doses in the pretreated cells. This may indicate that the priming effect is the result of an increase in *affinity* (ability to bind progressively lower doses) of the receptors for the activator compound.

Figure 12 illustrates another important phenomenon which definitely plays a role in priming and in the regulation of responses to pharmacological agents in general. On subjecting the cells to increasing doses of a stimulant, effects of one type (e.g. an increase in intracellular calcium) may be recorded with very low doses, whereas effects of another type (e.g. activation of oxidative metabolism) can be obtained only with much higher doses of the same stimulant. This depends on the fact that for the first type of response the occupancy of a very limited number of receptors is sufficient compared to the number necessary for triggering the second type of response. Another possibility is that the cells present more than one type of receptor for the same compound, types of receptors with different affinities (different binding intensity in relation to changes in dose), and mediators of different responses. In general (meaning that every rule in this field admits of exceptions), low doses are capable of bringing about subtle changes in cell biology, such as the assembly of colloidal proteins of the cytoskeleton, the opening of ion channels, or the exposure of a fair number of receptors. These are precisely the changes associated with priming.

Figure 12. Dose-response curves plotting changes in activity of two functional parameters of the same cells as a function of increasing doses of a stimulant. Curve **A** (dashed line) plots the increase in calcium ions in the cytoplasm of activated leukocytes. Curve **B** (solid line) plots the increase in oxidative metabolism in the same cells treated with the same doses of activator (in this example, bacterial peptides). It may be noted that the increase in calcium ions sets in at much lower doses of stimulant than those needed to activate oxidative metabolism. This may indicate either that there are two types of receptors (one type with high affinity which regulates calcium flux and one with low affinity which regulates metabolism) or that only one type of receptor exists, but the occupancy of a smaller number of such receptors is sufficient to activate calcium flux.

Another example of the multiplicity of factors influencing receptor dynamics consists in experiments conducted in leukocytes [De Togni *et al.*, 1985]: the same dose of a stimulant (chemotactic peptide) may give rise to very different effects according to the mode of administration. When the compound is administered all in one go, within the space of a few seconds, a very marked increase in oxygen consumption is obtained. When the compound is administered slowly, over a period of minutes, the response is very poor in terms of oxygen consumption. It has been seen that the difference in effect between the two modes of adding the compound is not due to a difference in the binding of the compound to the receptors, which exhibit the same degree of occupancy in both cases. Evidently, the receptor-transducer system perceives the molecule-to-receptor *association rate*, since, if this *rate* is high, there is no time for the counterreaction to occur which in some way disactivates the response.

In our laboratory, the adhesion of neutrophils to serum-coated surfaces was investigated in primed and normal cells. In normal cells, adhesion occurs only in the presence of suitable stimulants, such as the peptide fMLP at relatively high doses ($10^{-7}/10^{-6}$ M). However, we noted that neutrophils pre-treated with bacterial endotoxins (LPS) showed enhanced adhesion even in the absence of fMLP, and that, on addition of *low doses* of fMLP, the LPS-mediated adhesion was inhibited [Bellavite *et al.*, 1993b; Bellavite *et al.*, 1993c]. In synthesis, the results indicated that:

a) Untreated cells show only minimal adhesion to serum-coated plastic surfaces and this adhesion is not significantly affected by low doses (1-5 x 10^{-9} M) of fMLP.

b) Fully activatory doses (5 x 10^{-7} M) of fMLP induce a significant increase in cell adhesion.

c) Pretreatment of the cells for 1 h with 1 μg/ml endotoxin augments adhesion in the absence of further stimulation.

d) Addition of very low doses of fMLP (3x 10^{-9} M) inhibits the adhesion of endotoxin-treated cells.

e) High fMLP doses are additive to endotoxin in promoting adhesion.

Similar results have been recently obtained using cells which were primed *in vivo* during an inflammatory process [Bellavite *et al.*, 1994]. In conclusion, the chemotactic agent fMLP, which is considered to be an activator of neutrophil adhesion, paradoxically inhibits the same cell response at low doses when used in primed cells.

A phenomenon similar to desensitization is *tolerance*, which can be defined as the acquisition of nonreactivity of the immune system to given antigens. Tolerance is a fundamental mechanism whereby the immune system learns to distinguish the substances of the body itself from extraneous substances, though in certain conditions there may be tolerance of nonself substances (acquired tolerance). The state of tolerance may involve either B lymphocytes (antibody response) or T lymphocytes (cell-mediated response). It is possible to induce tolerance of foreign agents by exposing the body to higher doses than those which are normally immunogenic (we are dealing therefore with a lymphocyte desensitization or inactivation phenomenon) or to very low subimmunogenic doses of antigen (in this case the tolerance is due apparently to the intervention of T suppressor lymphocytes). It has been claimed that the tolerance induced by low doses bears witness to the great ability of the body to react to subliminal stimuli and may be one of the mechanisms of action of many preparations with a weak primary action [Speciani, 1991]. Other authors have proposed that the immune modulation implemented with microdoses of antigens (in some experimental models immune responses have been observed to fractions of a microgram of protein) constitutes a field in which a great deal of evidence furnished by “orthodox” science exists to support the claims of homeopathic medicine [Grange and Denman, 1993].

The examples given here above refer mainly to the field of immunity and the biology of leukocytes, but, with the necessary modifications, the same principles could also apply to the biology of platelets, liver cells, muscle, and even nerve cells.

Another example comes from the vascular pathophysiology sector. It was discovered fairly recently that the walls of blood vessels produce nitric oxide, which is essentially an "endogenous nitrovasodilator." Practically speaking, this molecule is nothing other than the active component of drugs long known as vasodilators, namely amyl nitrite, trinitroglycerine, and the other nitrovasodilators [Collier and Vallance, 1991]. In this connection, it is worthwhile stressing the fact that this discovery shares certain features with the discovery of endogenous opioids (enkephalins and endorphins) and with the discovery of endogenous digitalis-like factors: the body knows how to produce many of the drugs it needs! There are clear indications that in various cardiovascular diseases there is a reduced production or increased catabolism of nitric oxide, which may thus predispose the vessels to a more marked and longer-lasting spasm and to consequent ischemia. The other side of the coin is that there may be excess production of nitric oxide in shock and hypotension.

In equilibria of this type, we would stress once again that disease changes sensitivity: when the arteries suffer endothelial damage, they produce less nitric oxide, but at the same time the underlying vascular wall becomes hypersensitive to nitrovasodilators [Moncada *et al.*, 1992]. The "sick" wall is thus sensitive to the drug of the healthy wall, which would account, at least partially, for the efficacy of nitroglycerine in angina.

The existence of inverse effects on changing the dose has long been known in pharmacology, where terms such as "*hormesis*" and the "*Arndt-Schulz law*" have been used to identify positive, stimulatory effects of low doses of inhibitors or toxins, or low doses of radiation. These stimulatory effects have been detected with regard to cell vitality and growth, muscular contractility, breathing, nervous transmission, and other functions. [Schulz, 1888; Towsend and Luckey, 1960; Stebbing, 1982; Calabrese *et al.*, 1987; Furst, 1987; Sagan, 1989; Wolff, 1989; Linde, 1991; Oberbaum and Cambar, 1994].

The fact that such phenomena exist is therefore indisputable, but to date not enough attention has been paid to them, since the tendency has been to view them either as pharmacological curiosities or as simply marginal phenomena compared to the main action of the toxin or inhibitor. Within the framework of a theory which seeks to provide a rational explanation of the therapeutic effect of low doses of substances which in themselves are often poisonous, as indeed is the case in homeopathy, our traditional knowledge of hormesis takes on new and major significance [Linde, 1991; Bellavite *et al.*, 1993c]. The connection between homeopathy and hormesis has been thoroughly discussed in a recent review [Oberbaum and Cambar, 1994]. The authors suggest that the common basis might be the process of information in biological systems. Every toxic compound induces a series of characteristic biochemical modifications in the target. These modifications have a toxic, damaging, and even lethal effect on the system when the concentration of the agent is high. On the other hand, when the dose of the toxin is decreased, the same modifications have an "informative" effect which enables the biological system to adapt by counteracting a particular agent, using specific defense mechanisms for that agent. The adaptability of the living system indicates that it is able to recognize the aggression, and receive it as information regarding the properties of the toxin. This information helps the organism to react and reinforces its defenses.

Others have suggested that one of the most important cellular mechanisms of the hormetic effect is the production of "heat-shock proteins" or "stress proteins," a class of proteins that are coordinately synthesized after exposure to heat, radiation, heavy metals, and oxidizing agents [Smith-Sonneborn, 1993]. In fact, the same agents that have been identified as hormetic also induce the stress response, and the stress response preferentially includes the synthesis of products that repair both protein and DNA, which may stimulate growth and longevity.

In the following section we shall attempt to illustrate in greater detail some of the biological elements upon which these apparently paradoxical effects are based.

5.6.3. Receptors and transduction systems

One specific field in which the complexity of living systems can be studied and documented in laboratory systems is that of cell biology. Among the various aspects which could be adduced by way of examples, particularly significant would appear to be the studies on receptors and communication systems. These cell

functions constitute a *homeostasis of signals*, made up of competitive actions, nonlinear responses, feedback loops, and spatiotemporal oscillations.

In the previous sections of this chapter and in the chapter on research in homeopathy (Chapter 4), on repeated occasions experiments were cited illustrating the following phenomena:

a) The same agent can induce opposite effects if used at different doses.

b) Two different agents can produce the same effect on the target system.

c) The same agent can determine or induce different responses in a healthy organism compared to a sick one.

d) The same agent can prove stimulatory, inhibitory or have no effect according to the mode of administration.

e) The effects of the same agent vary according to the conditions of the target system, which in turn are caused by previous or concomitant contact with other agents.

f) The effect of a certain treatment can vary according to chronobiological factors.

These are all characteristics peculiar to living systems, and are in many respects paradoxical; their general basis lies within the framework of the complexity of receptor dynamics. The topic is of cardinal importance for homeopathy because the flow of biological information depends not only on the nature of the signal (be it physical or chemical), but also on the behavior of the receptors. It is therefore advisable to briefly summarize here the recent advances made by research in this field.

Receptors are present both inside the cell and on the outer membrane (see also, for example, Figure 6).

The *intracellular receptors* are those which receive the signal in the form of molecules which cross the plasma membrane by virtue of their hydrophobicity (e.g. steroid or thyroid hormones). Once these receptors have bound the signal, they undergo activation, or a conformational change which makes them suitable for binding to specific DNA sequences or to other intermediate protein structures. In any event, eventually there will be activation of a series of genes and the triggering of a series of specific cell responses for that molecular signal.

The *membrane receptors*, on the other hand, are designed to receive molecules which do not enter the cell because of their particular size or electrical characteristics (the membrane is impermeable to small electrically charged molecules). For membrane receptors to be able to function they need a transducer, i.e. they are coupled to another transmission system which transduces the signal from the membrane to the system that has to be activated within the cell. The transducers, also called second messengers, are very important because they can intervene by modifying the signal either quantitatively (amplification, suppression) or qualitatively (altering its "meaning" in the sense of being able to re-route it towards functions other than its normal ones).

There are many types of receptors and transducers, some of which are illustrated in Figure 13. It can be seen that a number of receptors are directly coupled to an ion channel, i.e. when they bind the signal molecule they open up a passageway for the ions, which thus cross the membrane in large amounts according to their electrochemical gradient. It is worth noting that among these receptors there are some which are not activated by the signal molecule, but by variations in the electrical potential of the membrane. The signal, in this case, consists of electrons or electromagnetic fields. This can easily be explained, if we consider that almost all proteins have at least some electrically charged portion.

Other types of receptors are coupled to protein kinases, i.e. to proteins which operate a phosphorylation of other proteins, with a consumption of energy, but also with very significant consequences for the cell biology. Phosphorylation, in fact, often constitutes a dramatic change in the physicochemical properties of the protein, with a consequent change of function (activation or deactivation of enzymes or of the receptors themselves).

Figure 13. Three main functioning modes of membrane receptors. **1** = receptors directly coupled to an ion channel, which is opened (or closed) whenever interaction with a signal molecule occurs or there is a change in membrane potential; **2** = receptors directly coupled to an enzyme with protein-kinase catalytic activity (there are also those with protein-phosphatase activity), which is activated by the signal; **3** = receptors which, once they have bound the signal molecule, in turn transmit the signal - via GTP-binding proteins - to other systems such as the enzymes adenylate cyclase or phospholipase; these latter enzymes, in turn, produce other intracellular biochemical signals.

The activation of protein kinases can come about as an event directly related to the receptor (see Figure 13, **2**) or with a series of intermediate steps associated with the coming into operation of other enzymes such as adenylate cyclase, phospholipase, and various ion pumps. (Figure 13, **3**). In the signalling process which forms the second messengers, G-proteins (short for GTP-binding proteins) appear to be of great importance; these proteins constitute a kind of shuttle running from the activated receptor to the other enzyme, which in turn has to be recruited into the transmission system. G-proteins are particularly important in determining the outcome of a certain signal reaching the cell. Various receptor systems contain stimulatory and inhibitory G-proteins. Depending upon which of these come into action, a given signal may have opposite effects. Typical, in this connection, is the adenylate cyclase system which is coupled via G-proteins to alpha$_2$- and beta-adrenergic receptors [see, for example, Alberts *et al.*, 1989].

For reasons of simplicity, Figure 13 does not contemplate another important receptor activation mechanism, consisting in the *aggregation* of various receptors on the membrane as a result of signals constituted by macromolecules with multiple coupling sites. This aggregation can occur, as we shall see in Chapter 7, as a result of electromagnetic currents - even very weak ones - crossing the cell.

The systems for receiving and processing extracellular signals are very sophisticated and flexible, as well as being closely linked together at various levels by feedback mechanisms. The signal transducers enable the action of any single receptor to be amplified enormously, though inhibitory feedback effects are also possible. Figure 14 shows an example of how complicated the network of events resulting from signal molecule-receptor binding is. We see that, when a signals reaches the receptor, enzymes of the protein-kinase type are directly or indirectly activated, which, via the phosphorylation of proteins, trigger a series of functions. At the same time, however, the protein-kinase activity does not disdain an attack even on the very receptor from which the signal departed: the receptor is phosphorylated in a precise amino acid site, with consequent disactivation of its function. A subsequent dose of stimulant will no longer find the receptor ready to function as the "virgin" receptor did, but will find it unable to bind the stimulant itself or unable to promote phosphorylation.

Figure 14. Example of how a receptor can be inactivated by a negative feedback mechanism triggered by its own activation.

This latter example explains only one of the countless possible molecular modifications associated with positive or negative variations in receptor sensitivity. It is well known that excessive receptor occupancy expresses itself in many cases as an effective *disappearance* of the receptors, which are *internalized* or "*sequestered*" in cell sites inaccessible to the signal molecules (so-called receptor *down-regulation* mechanism).

Another important way in which the cells and systems regulate receptor sensitivity is the so-called "*shedding*" of receptors (loss as a result of detachment and dissemination). In this case what happens is that, after cell activation, the receptors are lost because they detach themselves from the membrane, passing into the physiological fluids (blood, lymph). Consequently, in addition to cell desensitization, there is also the possibility that *soluble receptors* will bind with signal molecules outside the cell, blocking transmission of the signal. In practice, the receptor transforms itself into its opposite, namely a specific inhibitor.

To conclude this section, it may be useful to present a model of receptor dynamics which illustrates the possible oscillatory variability of cell sensitivity (Figure 15). The resting cell (*A*) has multiple receptors on the membrane, represented here for reasons of simplicity as receptors for two different types of stimulants, depicted as "triangular" and "circular" molecules. When the cell encounters a small amount of the triangular stimulant, it undergoes an initial stimulus (*B*), which, however, does not evoke any response other than an increase in receptors, both *homologous and heterologous*, i.e. for different types of molecules (*B'*). Whenever the cell encounters a greater stimulus, it presents a substantial activation of the cell response, with various specific effects (*C*). Whenever the stimulus is of considerable intensity and long-lasting, the receptors for the triangular molecule are rapidly inactivated, with the result that a state of desensitization or adaptation (*D*) comes about. Finally, if a new stimulus should arrive, produced by other molecules (depicted here as circular), the cell will be very ready to respond, since only the heterologous priming (*E*) will have persisted in it.

Figure 15. Receptor dynamics and consequent variations (potentiation or desensitization) in cell activity. For an explanation, see text.

After a varying period of inactivity, it is possible (though not the rule) that the situation of the cell will revert to its initial state, with reappearance of the initial receptors. Another possibility is that the cell will remain "impressed" by its experience forever (memory), with modifications of gene expression, receptor expression and enzyme activities. In practice, we will have a cell which is different from the one we started with.

To these considerations should be added a related subject: that of the synergisms and antagonisms existing between substances which simultaneously occupy different cell receptors, these being aspects of major importance in physiological and pharmacological regulation. This topic, however, is perhaps among the clearest and most extensively documented areas in modern biomedical culture, and therefore there is no point in dwelling on it here any further.

The foregoing comments constitute a representative cross-section of researchers' "daily bread" in the advanced biomedical field. What has all this got to do with homeopathy? We have to proceed step by step in our analysis and thus the answer will be clearer later on (see Chapter 6). For the time being, it is important for us to have fixed the following concept firmly in the forefront of our minds: *receptor dynamics vary in physiology and pathology and are so complex that a given substance can behave as an activator or an inhibitor on the same cell or on the same organism, according to the doses of the substance itself in relation to the receptor sensitivity at a given time.*

In other words, this concept can be translated as follows: from the biological point of view, *there are no substances which are in themselves* "*good*" *(medicines) or* "*bad*" *(poisons, toxins)*. Every substance is "*two-faced*" because its possible biological effect depends both on its molecular structure and dose and on the system with which it comes into contact. The quality of "goodness" or "badness," in the sense of its positive or negative effect on the homeostatic system as a whole, is "attributed" to the substance by the system with which it interacts, by its particular state of sensitivity and responsiveness. Several objections to this conclusion can be raised, based on glaring exceptions (it is difficult, for instance, to see a positive role for HIV virus at any dose or in any system), but the thesis remains essentially valid as a general concept (as far as viruses are concerned, however, it is well known that they are not always pathogenetic, infecting only cells which are susceptible to them and which present suitable receptors).

5.7. Chaos and fractals

5.7.1. The "discovery" of chaos

In fairly recent times, theories explaining the dynamics of complex systems have come up with a new protagonist: chaos.

The co-existence of casual phenomena alongside strictly deterministic and predictable phenomena has always been recognized, but until only fairly recently the attention of scientists was focused only on the latter. The laws of classical physics, in fact, are deterministic: if we know the state of a system at a certain moment and the laws governing its modification, we are in a position to predict its future behavior.

On the basis of the classic laws of gravity, for example, we can predict eclipses, or the trajectory of a satellite, or the motion of a pendulum. On the basis of Mendel's laws of genetics, we can predict the characters of a plant deriving from the union of two plant gametes of known character. Other natural phenomena, however, are not so readily predictable: the motion of the atmosphere, the population density of animals in a certain environment, the form of fingerprints, and the turbulence of a liquid running through a pipe, elude our predictive ability. According to the classical view, one possible solution saving determinism was to ascribe failure to a dearth of information, so that all that was needed was to collect and process a sufficient amount of information on the system studied in order to get progressively closer to an exact description. As the mathematician Pierre-Simon Laplace pointed out, the laws of nature imply a rigid determinism and total predictability, though the incompleteness and imperfections of our observations make

it necessary to resort to the theory of probability. With the aid of this theory, the exploration of the physical world should have no problem developing in a progressive, linear fashion.

This "optimistic" view of the possibilities of traditional science has been modified in the course of the twentieth century, for two basic reasons: the first is the development of quantum mechanics and, in particular, the discovery of Heisenberg's principle of indeterminacy, whereby there is an insurmountable limit to the precision with which the position and velocity of elementary particles can be determined.

The second reason, more closely related to the question of chaos, is the discovery that systems consisting of three or more bodies may present chance behavior. This element of chance does not disappear with the collection of further information about the system itself. The example cited in this connection is that of a system of hard spheres (billiard balls) which represents, in a simplified fashion, the interaction between atoms or molecules in a gas. If one billiard ball is fired against other balls, it is impossible to predict the path it will take after only a few ricochets. The nonpredictability is maintained even if we assume that the surfaces of the balls are perfectly smooth and the trajectories perfectly straight.

The reason for the unpredictability in such a simple system lies in its extreme dependence on the initial conditions. The phenomenon is known, in fact, as *sensitivity to initial conditions*. A minimal variation in the direction of incidence of the first impact is amplified on the curved surface of the ball, so that the trajectory after the first impact will be appreciably different from the expected one, and likewise after subsequent ricochets. After several impacts, the trajectories will be unpredictable. There is no instrument capable of firing the ball without the slightest imperfection (it would be a perfect instrument, but absolute perfection doses not exist in the physical world; there will always be some minor perturbation or fluctuation in the state of a physical system, though, macroscopically, this imperfection may not be apparent). For this reason, the apparently "simple" system described by the billiard-ball model has an intrinsic tendency towards chaotic behavior. The chaos does not derive from a mistake in firing the ball, or from ignorance of the laws governing trajectories and rebounds, but is a characteristic property of the system. David Ruelle, the "father" of the strange attractors and one of the world's leading experts on chaos, defines the latter as "*a temporal evolution with appreciable dependence on initial conditions*" [Ruelle, 1992, p. 77].

Another way by which the behavior of chaotic systems is exemplified is the so-called "*butterfly effect*," the principle embodied in which is conveyed by the dictum that the flapping of a butterfly's wings in Brazil may trigger off, or stop in its tracks, a tornado in Texas [Lorenz, 1979; see also Nicolis and Prigogine, 1991, p. 144 and Shinbrot *et al.*, 1993].

In this section we intend to give a brief overview of the problems raised by the "discovery" of chaos, these being topics to which we shall return later on account of their implications in the interpretative models of homeopathy presented here.

No description of complex systems in which several components interact (figuring among these without the slightest doubt are living systems) can afford to underrate chaotic phenomena, regarding them merely as factors disrupting what in other respects is a perfect theory, but any such description must find the instruments and the ways to integrate these phenomena in a theory previously regarded as adequate.

Even mathematics, once regarded as the exact science *par excellence*, has discovered chaos: there are various functions with a single variable, which, by means of an iteration process (insert the result of the first evaluation in place of the variable in the next and so on), can yield highly variable, nonperiodic results. An example is provided by the mathematical function:

$$\mathbf{A_{n+1} = A_n + A_n\, k\, (A_{max} - A_n)}$$

where $\mathbf{A_{n+1}}$ is the value of the next cycle **n+1** which can be calculated on the basis of the value resulting from the previous cycle ($\mathbf{A_n}$) added to the growth due to the cycle itself, which is equal to $\mathbf{A_n}$ multiplied by a coefficient of growth **k** and by a factor given by the difference between the maximum allowed ($\mathbf{A_{max}}$) and $\mathbf{A_n}$. An equation similar to this was described in 1845 by the mathematician B.F. Verhulst to analyze population trends [cited in the studies by Garner and Hock, 1991 and by Cramer, 1993]. We use it here to describe the time course of the value "**A**", which could be any real or imaginary parameter. What matters here is not to assign any precise value to "**A**", but to calculate its variations in successive iterative cycles, fixing an initial value and a maximum allowed value.

The additive growth (or decline) of each cycle depends on the result of the preceding cycle and is limited by the fact that there is a maximum achievable value: in fact, with the increase in the A_n trend the number to be subtracted from the maximum achievable value increases and the multiplication factor (A_{max}-A_n) decreases. Consequently, it is only logical that A_{n+1} tends to increase at the beginning, but then the increase progressively declines until a plateau is reached where no further increase takes place. The formula, then, describes a fairly simple mathematical feedback. In actual fact, however, it "conceals" a very substantial measure of complexity: Verhulst noted that the results the iterative calculation allows are very different depending upon what value is assumed for the coefficient k and depending upon the initial value you start out with.

A number of such calculations are illustrated in Figure 16 in an example which assumes initial $A_n = 1$ and $A_{max} = 3$. Assuming the value of coefficient k to be fairly low compared to A_{max} (e.g. 0.1), and proceeding with the iterative calculations, the following solutions are obtained: at the beginning, as long as A_n is much lower than the maximum, the growth is effectively linear. When, however, the value starts to rise and to approach the maximum (A_n approaches A_{max}), the difference A_{max}-A_n tends towards zero and growth comes to a halt. Plotting the number of iterations (successive cycles) on the x axis against the value of A on the y axis, we have a growth curve which reaches the maximum asymptotically (Figure 16 A). So far there is nothing odd about the curve. If, however, we insert a coefficient of growth greater than a certain value (0.6 approx.) in the equation, we have a qualitatively different curve: at the beginning the A_{max} value is exceeded, and then subsequent iterations lead to values oscillating slightly above and below A_{max}, after which the curve stabilizes at the maximum value without any further changes (Figure 16 B). At even higher coefficients of growth, a phenomenon occurs which is called "*bifurcation*": the function oscillates between the two fixed values which are repeated alternately in successive cycles (Figure 16 C). This oscillating function is fairly stable and is maintained even if the (initial) A_n value changes appreciably (e.g. a 20% change from 1.0 to 1.2) (Figure 16 D). On exceeding another critical k value (0.88 approx. in the example given), we have another bifurcation because the order is lost completely and an infinite number of possible solutions present themselves: practically all configurations are admitted and the oscillations of the variable A appear irregular or *chaotic* (Figure 16 E). We have entered a sphere of mathematics where chaos manifests itself in the form of totally nonperiodic results.

Figure 16. Verhulst iterations. The successive panels show variations in the parameter "A" in successive iterations of the equation $A_{n+1} = A_n + A_n\, k\, (A_{max} - A_n)$. A_{max} in all cases is assumed equal to 3.0. The (initial) A_n and k values in the various panels are as follows: panel A: $A_n = 1.0$, $k = 0.1$; panel B: $A_n = 1.0$, $k = 0.6$; panel C: $A_n = 1.0$, $k = 0.8$: panel D: $A_n = 1.2$, $k = 0.8$; panel E: $A_n = 1.0$, $k = 0.88$; panel F: $A_n = 1.001$, $k = 0.88$. For further explanations, see text.

It is important to note that, in these conditions, in which the time trend of the A values is chaotic, the mathematical feedback is extremely sensitive to the initial conditions: on making very minor changes in (initial) A_n, for example from 1 to 1.001 (i.e. a 0.1% change) we obtain a curve which shows no difference over the first 10-15 cycles, but then diverges dramatically, with the result that after the 20th iteration the trend is completely different in the two tracings, which no longer present even a single point in common (Figure 16 F). Despite this, some basic similarities between curves 16 E and 16 F must be stressed: both are chaotic and, even more importantly, the values remain confined between two extremes, i.e. a maximum upper and a minimum lower value. Though plotting different trajectories, these point trajectories "belong" to the same area. This area may be regarded as the *attraction basin* of the function described (with the parameters k and A_{max} fixed).

On further increasing the value of k, the oscillations are increasingly high and irregular so that eventually the value of A_n may become 0 or even negative. At this point, the evolution of the function undergoes a dramatic change because the negative value of A_n produces a more negative A_{n+1} value and so on, to infinity (not shown in Figure 16). Also in this case, it can be calculated that minimal changes in initial A_n determine whether the function diverges to infinity or remains "trapped" in the attractor.

The contribution made by the physico-mathematical approach to the problem of complexity is much greater than might be imagined: while it is true to say that a living system with its thousands of subcomponents will never resemble a chemical system with two or only a very few components and can

never be described by a mathematical formula, on the other hand it is also true that the study of the complexity of "simple" systems may enable us to discover "basic rules" of behavior which are repeated in substantially identical forms in systems with a different evolutionary status. In other words, the complexity of biological systems (and their pathological aspects) may perhaps be tackled and better understood by reference to a framework common to all complex systems, i.e. to a model developed in physicochemical reference systems.

Basically, the characteristic of the equation capable of generating chaos is that it carries within it the presence of a feedback, so that the result of the calculation is used as factor for the reiteration of the calculation itself. This fact is very interesting from the biological viewpoint, in the sense that the Verhulst formula essentially describes the dynamics of a mechanism operating in living beings: as we have seen, living systems, in fact, are regulated by reaction and counterreaction cycles which constitute the so-called *homeostasis*. These "cycles" are nothing more or less than the repetition of the same operation (by analogy with mathematical iteration) in which the result of the previous cycle serves as the basis of the next one. For example, at the end of the systole-diastole cycle the heart reverts to the end-diastolic condition; at the end of a mitotic cycle the condition of the two daughter cells becomes in turn the starting condition for a new mitosis; thus, every rhythmic modification of the organism hinges upon the previous state and occurs according to fixed rules (in the analogy we have adopted, the rule is the mathematical formula). The physiological variables controlled by the homeostatic systems oscillate continually between a maximum and minimum allowed value (cf. the A_{max} values considered above), but this variability may be more or less regular or rhythmic, depending upon the initial conditions (cf. the A_n value) and upon multiple conditioning factors performing the function of coefficient "k" in the formula examined above. Now it clearly emerges that all systems endowed with these characteristics are subject to chaos and thus that this type of chaos must be regarded as a physiological phenomenon, at least within certain limits.

The chaotic behavior of billiard balls and similar systems is due to the extreme dependency on initial conditions owing to the existence of a principle which amplifies the error (the curved surface of the billiard balls). The chaotic behavior of mathematical systems is related to considering equations as nonlinear, i.e. terms appear which are not simply proportional to the variable. For an analysis of feedback in mathematical terms and its possible applications in biology, the reader is referred to the work of Nicolis and Prigogine [Nicolis and Prigogine, 1991].

The idea that deterministic systems are not always sustained by equations with regular solutions has taken a long time to come home to people [Croquette, 1991; Ruelle, 1992]. Obviously, the discovery of chaos in physical systems and in simple mathematical equations has aroused the interest of scientists, spurred on in the hope that these might afford a model for understanding the functioning of more complex systems as well. No-one would dream of denying the existence of unpredictable phenomena in systems such as meteorology, economics, the behavior of the earth's crust (earthquakes), the beating of the heart (arrhythmias), or ecology (cf. the forecasts of terrestrial temperatures or the hole in the ozone layer). This unpredictability might, however, be due only to the lack of sufficient information about the details of such systems, or to inadequacy of the experimental conditions, but if chaotic behavior is also an intrinsic characteristic of physical and mathematical systems, it can be postulated that this chaos is due to a basic principle of the physical world which cannot be ignored.

Recently, researchers have provided an elegant demonstration of the fact that an experimental chaotic system (represented, in this case, by the oscillations of a flexible rod coupled to a magnet which generates impulses at desired frequencies) can be regulated by minimal perturbations of the system control parameters [Shinbrot *et al.*, 1993]. By slightly modifying the frequency of the impulses emitted by the magnet, the chaotic behavior of the vibrations can be made to appear and disappear. Though the mechanism utilized is fairly simple, it presents complex chaotic dynamics, thus prompting the authors to suggest that these experiments may furnish indications of a general nature about chaotic systems. Amongst other things, it is claimed that the control of chaos (in this case the appearance of periodic rhythmic oscillations) can be obtained by means of the repetition of minor corrective adjustments at intervals.

5.7.2. Attractors and fractals

Physicists and mathematicians lost no time in checking to see whether or not "laws of chaos" existed, or, in other words, whether what might have seemed to be the triumph of randomness in the deterministic domain

was not actually an expression of some kind of order or regularity. Effectively speaking, this has led to the discovery and study of the so-called *strange attractors*, which, on the mathematical plane, are geometrical figures which describe the long-term behavior of a number of dynamic systems. To put it another way, an attractor is something to which the behavior of a system is attracted or whose stabilizing effect it undergoes. It therefore possesses an important property - stability. *In a system subjected to perturbations, movement tends to be towards the attractor.*

The attractor may be a single point, as for example in the trajectory of a pendulum when it reaches the stationary state (for a mathematical example of a single-point attractor, see Figure 16 A and B), or a finite number of points reflecting a periodic-type behavior (mathematical example, Figure 16 C and D), or an infinite system of points generating a figure in the form of an orbit which never repeats itself identically, as may happen in chaotic systems ("strange attractors") (mathematical example, Figure 16 E and F). It is difficult to imagine a *strange attractor* in the field of geometry because its characteristics imply that an orbit of this type must have an infinite length contained in a finite surface (the attraction area). These are therefore objects of "nonentire dimension" or *fractals* (see below) [Ruelle, 1992].

The concept of the attractor has also been analyzed in relation to pharmacology, and in particular to pharmacodynamics [van Rossum and de Bie, 1991]. In classic pharmacokinetic theory, the situation is simple because the attractor is a single point, and adequate information can be obtained by measuring a single variable such as the concentration of a drug or one of its metabolites in the blood. In the field of pharmacodynamics, which also examines the effects of drugs, the situation is more complex and the attractor can be of the chaotic or strange type. The effect induced by a drug is not a single entity, or a single modified mechanism, but a series of simultaneous changes in several variables, each of which interrelates with the others in a nonlinear manner. This implies that a dose of drug may induce unpredictable changes in a complex system, such as, for instance, the cardiovascular system. The same dose of drug can produce different effects on different occasions owing to the great sensitivity to initial conditions. It is true that the variability can be overcome by referring to the statistical mean of many observations, but the above-mentioned authors claim that, if the system is chaotic (which is something different from randomness and biological variability), the use of means is not appropriate.

Chaotic systems also present elements of regularity. For example, the equations described previously, though yielding unpredictable results at given coefficient values, do not furnish infinite solutions; the amplitude of the oscillations remains within a certain range. Moreover, in several chaotic functions, on continuing the iterations and further increasing the coefficient value, after the periods of chaos periods of order may reappear, followed by new zones of chaos and then order. There is thus a "recurrent regularity" [Hofstadter, 1991] in successive generations of transitions from chaos to order, with the reappearance of single solutions or regular oscillations which undergo cascade duplication on increasing the coefficient value. This recurrent regularity creates figures with regularity and irregularity "bands" which are repeated and resemble one another, with a fractal type pattern.

The study of the behavior of mathematical and physical systems with transitions from order to chaos and vice versa has witnessed a major upsurge with the analysis of the dynamics involved in the formation of fractal objects. Fractal geometry, in fact, is the most suitable for describing chaos and complexity [Jurgens *et al.*, 1990]

The term "*fractal*" was coined in 1975 by B.B. Mandelbrot and gained extensive notoriety in scientific circles in the early '80s [Mandelbrot, 1982]. What is meant by this term are those mathematical or geometrical entities which are endowed with a fractional dimension (from the Latin *fractus*, meaning "broken"). Many fractal figures have a repetitive configuration on changing scale, a sort of self-similarity between details and the general pattern.

Fractal shapes can be generated by the computer using algorithms (lists of instructions which specify the operations to be performed to solve a given problem) starting from mathematical functions which are suitably iterated. By means of these operations, two- or three-dimensional figures with the following characteristics appear:

a) An enormous variety of details of different shapes.

b) The presence of subtle ramifications that can be pursued in the finest detail.

c) Self-similarity, whereby, on magnifying part of the structure, details can be detected which repeat themselves on different scales of magnification.

"Eidiomatic" experiments, performed on the computer, show that figures with a fractal dimension are endowed both with a fantastic variety of shapes and with self-similarity [Dewdney, 1991]. In very complex

structures such as the sets of Julia and Mandelbrot, extremely varied and fanciful details can be observed (circles, spirals, helixes, stars, various ramifications), within which other, different details can be discerned on magnifying the image (Figure 17). Within some of these particular images one finds, surprisingly, "mini-sets" very similar to the macroscopic ones from which they originated. In the close-up detail we rediscover an image which appeared to have been lost in the variety of details and ramifications. By increasing the number of iterations, a better definition of the fractal image is obtained (figure 17, C--> E).

Similar figures, showing increasing definition with the increase in iterations, have been reported also by others [Garner and Hock 1991]. Interestingly, these authors outlined an analogy between this type of fractal behavior and the homeopathic concept of dilution/dynamization, in which process the information is claimed to spread and increase its "potency" in serial dilutions (see also Chapter 7, Section 4.2).

Figure 17. Mandelbrot set. The image is produced on a personal computer by iteration of the formula: $Z_{n+1} = Z_n^2 + C$ in the complex field, using *Fractint* software. Every point on the graph represents a complex number of the form: *x coordinate + i * y coordinate.* The x coordinate is an ordinary real number, and the y coordinate an imaginary number, i.e. a real number times *i*, where *i* is the square root of -1. C is set as a constant for each point, while Z varies with the iteration. Every black point is the set of all points C for which the value of Z is less than 2 after the indicated number of iterations; the white points represent points for which Z exceeds 2 after the indicated number of iterations. Figure 17 **A** is the entire Mandelbrot set, the result of 18 iterations per point. In 17 **B** we see a 20 times magnification of the detail indicated in the box shown in 17 A, and in 17 **C** a further 10 times enlargement of the boxed detail in Figure 17B. In 17 **D** the same detail as in Figure 17C is shown with a further 5 iterations per point. In 17**E** with a further 30 iterations per point; and in 17 **F** with a further 100 iterations per point. From 17C to 17F an increase in the definition of the specific characteristics and details of the image is evident. In 17F, the "fragment" of the set shows a pattern similar to the "whole" (17A).

Mandelbrot and, more recently, other investigators have observed that many apparently disorderly natural objects possess this fractal property. This is having a very considerable impact in the scientific world. Despite the fact that these forms were discovered by a mathematician and are still studied mainly by mathematicians and computer scientists, fractals are useful instruments for describing a whole variety of physical phenomena and natural forms [Sommaruga, 1992].

One example of a fractal form is a tree, whose trunk is divided into branches; the branches themselves then divide into smaller branches, twigs, and so on until you get to the leaves, which in turn present veins with multiple subdivisions.

Figure 18. A flower (Celosia) with a fractal structure. The same pattern as in the entire flower (**A**) can be seen in a close-up of a part and in all its further subdivisions (**B**).

Other examples of natural fractals are clearly illustrated by flowers (Figure 18) and snowflakes, as well as by noncrystalline molecular aggregates, viscous ramifications in unmiscible fluids, corals, electrical discharges such as lightning, the ramifications of the airways and blood vessels [Sander, 1987], the dendrites of the neurons, the Purkinje system conducting electrical signals in the heart, and the folds of the intestinal mucosa [Goldberger *et al.*, 1990]. It has also been demonstrated that, in many different physical situations, particles floating on the surface of an irregularly moving fluid display a fractal arrangement [Sommerer and Ott, 1993].

The formation and growth of such structures is well described by the laws and formulas of fractal geometry, so that today, with the indispensable aid of the computer, many objects which, on account of their complexity, formerly eluded any kind of formal or quantitative analysis can be simulated graphically. We can calculate the fractal dimension of real objects such as coastlines, mountains, clouds, etc. The human arteries have a fractal dimension of 2.7 [Jurgens *et al.*, 1990].

In the formation (= taking shape) of objects with fractal dimensions we witness a particular interaction between stochastic (random) events and events determined by the state of the physical system which is

growing. This type of growth, also called *aggregation by diffusion*, very probably played a fundamental role in the birth of life on earth and continues to do so in the processes of physical and biological growth [Sander, 1986].

Aggregation by diffusion depends on the random motion of dispersed particles and on the "attraction" or "conditioning" produced by the first microaggregate, or by the temperature conditions, or by the motion of the fluid, or by other physical factors. Aggregation by diffusion produces fractal structures, fascinating geometrical shapes in which determinism and randomness, order and diversification coexist. Fractal geometry thus refers to some form of *conditioned randomness*, so much so, indeed, that some people talk about a determinism of chaos (deterministic chaos).

We are thus investigating a field which is one of the frontiers of science: identifying the "laws of disorder," or certain fundamental rules of behavior of complex systems, which reveal the way in which the chaotic system can organize itself in a broad-ranging order, at the same time maintaining a certain degree of randomness. This randomness, discovered recently even within the atom [Gutzwiller, 1992], remains as an uneliminable factor in ontogenetic and philogenetic evolution, a factor which, coupled to information capable of generating order, constitutes a means of continuously generating novel forms and novel diversities. Fractal geometry tends to reveal that "hidden order" which is not always apparent in natural objects. This order is believed to be represented precisely by what has been termed *scale invariance symmetry* [Sander, 1986].

5.7.3. Boolean networks and self-organization

A peculiar property of complex systems is the ability to evolve in the course of time. This is observed both in the biological development of any organism (ontogenesis) and in the development of living species in general throughout history (evolution). In the classic Darwinian theory of evolution, the emergence of increasingly complex species is the fruit of *random variability* and *selection*, which operate to the advantage of those species which, by virtue of characteristics acquired by chance mutations, better succeed in adapting to increasingly difficult environmental conditions and in surviving the competitive struggle for vital space and food. This well-known concept of natural selection and the survival of the fittest in evolution has also been applied on a molecular and cellular scale as well as in embryology. The classic view of the origin of order and diversification of biological species - based on natural selection - has recently been contested on the basis of mathematical studies and computer models showing that, alongside natural selection, other mechanisms are involved, which have been grouped together under the term *self-organization* [Kauffman, 1991; Kauffman, 1993].

As a result of the laws of chaos, nonlinear dynamic systems can easily present transition from order to disorder and vice versa, following even only minimal perturbations in control parameters or in the energy flow across such systems (cf. butterfly effect, dissipative systems, see also Chapter 5, sections 8.1 and 8.2). Nevertheless, in these cases, we are invariably in the presence of changes somehow induced from the outside. There may, however, also be a phenomenon whereby the complex, disorderly system spontaneously "crystallizes" in an orderly state. From disorder to order thanks to *an intrinsic original property* of the system itself and with no input of outside energy; quite rightly, this phenomenon has been termed *anti-chaos*.

The mathematical models of self-organization were initially developed with the aim of explaining how the cell genome is organized. The genome can be viewed as a complex computer in which there is a data memory (information stored in the DNA for approximately 100,000 different proteins), but also the parallel processing of some of this information (a few hundred or a few thousand data units simultaneously). What is more, many of these protein data units influence the genome itself in its activity, in multiple control sites. In this way, many genes are "coupled" with the functioning of others, influencing one another reciprocally, and constituting a *network*. The coordinated and sequential behavior of this network is the basic factor responsible for the functioning and differentiation of the cell, with the result that a liver cell is different from a heart muscle cell and performs different functions, despite containing the same genetic information, being composed of the same elementary materials (amino acids, sugars, lipids, carbohydrates), and obeying the same "general functioning rules" (biochemical reactions).

We cannot go into any detail here about the mathematical parallel processing systems constructed to explain self-organization; such details can be found in the literature cited. We will confine our attention here to the essential notions. To describe the behavior of a great many elements coupled together (network), each

of which may be in an active or an inactive state, we resort to the use of models based on stochastic Boolean networks (after the logician George Boole). In such a network, formed by a number of elements N, the behavior of each element (active or inactive) is determined by the input variables which connect ("AND" variable) or disjoin ("OR" variable) it from the behavior of the other elements. Each element can have a number of inputs according to choice. If the number of inputs is K, the possible combinations of the two variables (AND + OR) will be 2^K. Networks of this type are called NK networks because they contain a number of elements N, each of which has K inputs, and *stochastic* because the choices *AND* and *OR* and the number of inputs of each individual element are selected at random at the start. Since the elements are connected, by activating (*AND*) or disactivating (*OR*) one element in the network, we can observe the evolution of the states of the network in subsequent cycles. For the sake of simplicity, each passage from one state to another is implemented by synchronous modification of all the elements involved. The system passes from one state to another in a deterministic manner and then, in view of the fact that the possible combinations are infinite, however complex the network, it will always end up sooner or later by finding itself in a state previously formed, thus resuming the cycle of transformations. The cycles of states which Boolean networks pass through in the course of time are called *dynamic attractors*, and each network, if left to its own devices, will sooner or later finish up in one of these attractors and will stay there.

Experiments can be performed on these mathematical systems, introducing *perturbations*, some of which modify the network only locally and for a short time, until it resumes its original cycle, returning to the same attractor. Other perturbations, on the other hand, are not absorbed; these destabilize the network and force it towards another attraction basin, whence it can no longer return to the previous one. Experiments can also be performed by modifying the *structure* of the network itself, altering the number of elements N, or the number of inputs K for each element. It can be observed that the attractors are modified accordingly. When the number N is equal to K (N = K), we have the maximum disorder, and the system is totally chaotic and unpredictable. Chaotic behavior patterns can also be observed when K < N, provided that K is equal to or greater than 3. If K = 2, the properties of stochastic Boolean networks are such that a tendency towards spontaneous collective order is easily manifested. In practice, groups of elements are formed which are interconnected so that not every perturbation causes them to shift towards the attractor. It is as if there were a kind of homeostatic behavior in the system, and this is one of the main reasons why such networks tend to simulate a number of properties of biological systems.

The basic reason for the birth of spontaneous collective order in stochastic Boolean networks lies in the fact that a "frozen core" forms randomly in these networks, that is to say a set of elements which are blocked, by the crossfire of opposing control elements, in a certain active or inactive state. This core creates uniform, interlaced "walls," which are propagated to a broader zone, "infiltrating" themselves into the system. As a consequence, one orderly zone isolates itself from other elements which remain variable and disorderly. Since, in these networks where K = 2, there is not much communication (in other words, the system is not very *connected*), the modifications described remain confined to islands, which do not communicate with one another, but are homogeneous aggregates, distinct from the rest. By suitably modifying the values of parameters N and K, moments of transition can be found between order and disorder, changes in size of the "frozen islands" can be observed, and so on. With an increase in the number of inputs K (and thus of connections), the well organized and rigid frozen components "melt" and the network gains in complexity and dynamism.

These mathematical models, endowed with great flexibility, are used to simulate the natural selection and the self-organization capabilities of complex systems. Networks can be designed, for example, which simulate the human genome, assuming the number N equal to 100,000 (i.e. the number of genes) and the coefficient K equal to 10 (i.e. the number of inputs, or controls, which every gene is subject to). Or we can simulate cell populations, on the basis of an empirical knowledge of the number of cells and the signals (e.g. hormones) which they exchange. In such networks, which "hover" between order and disorder, alterations to an element (appropriately called "mutations") can be introduced (for example, by changing it from active to inactive), and one can observe what happens in the evolution of the system. In some cases, the Boolean networks adapt to the mutation with minimal adjustments, as in a homeostatic system; in other cases, the mutations cause cascades of impressive alterations, substantially modifying the structure of the network and the shapes and sizes of the islands.

Using similar methods, it has been possible to calculate approximately how long the life cycle of a cell should be for it to pass through all the forms of expression of its genes (i.e., in mathematical terms, to pass through the entire attractor and return to the starting point. The results obtained (from 370 to 3700 minutes)

closely match the data provided by experimental observations of the mitotic cycle. It has also proved possible to calculate, on the basis of the possible attractors in a genome, how many cell types are feasible (a cell type, in fact, distinguishes itself from the others precisely because it activates some genes and represses others). These estimates, too, have been found to correspond to experimental findings in organisms of increasing complexity, from bacteria to the human being. Lastly, the models also allow us to predict the stability of the cell types and the effect of genetic mutations, which is of considerable importance in the study of tumors.

In conclusion, then, thanks to this interesting type of mathematical approach, it has proved possible to gain deeper insights into complex systems and the relationships between stochastic phenomena and the birth of order. Above all, this kind of approach has been able to demonstrate a phenomenon which has been of undoubted importance in evolutionary theory, namely that microscopic order may originate *at random* from the chaos of multiple interconnected elements; this initial core may give rise *spontaneously* to macroscopic order in the form of islands of order or cycles of modifications which are repeated according to cycles described by the attractors. This theory, therefore, amplifies (rather than replacing) that of natural selection and that of dissipative structures (see Chapter 5, Section 8.1.) to explain biological order.

5.7.4. Chaotic systems in medicine

The models of chaos and interconnected networks can be applied to physiology and pathology. The pathogenesis of many diseases, at least in the initial phases, are characterized by communications defects arising in the complex networks of integrated systems, such as those considered in other sections of this book (control of cell proliferation, immune system, equilibrium between pro- and antiinflammatory factors, etc.), for which models can be created like the Boolean networks. In a network in which many homeostatic systems (molecular, cellular, systemic) are interconnected, the information of the entire system "passes through" cycles (attractors) which have variable, fluctuating spatiotemporal forms, but which can always be traced back, in states of normality, to a harmonic pattern where the whole is viewed in its entirety, aimed at the survival of the organism with the least possible consumption of energy. If one or more elements in these networks lose their information connections, i.e. something snaps in the homeostatic system itself, or the flow of information is cut off between different systems, a pathological process occurs precisely because chaos is generated, or rather the chaotic system goes over to another attractor, as was seen to be possible in the Boolean models. According to these models, the new attractor, regarded as "pathological" in the case in question, may be preserved even if the initial perturbation (loss of connection) is only temporary (in pathology, one could speak of a *tendency towards chronicity*).

What is certain is that chaotic dynamic patterns are normally present in the homeostasis of networks with multiple crossed components such as cytokines, neuropeptides, the endocrine system, idiotype-anti-idiotype networks, and immune HLA-receptor equilibrium.

The rate of enzymatic activities oscillate when two enzymes compete for the same substrates, and small changes in the concentrations can lead to changes in the frequencies and amplitudes of oscillations, causing them to become chaotic if previously harmonic or to become harmonic if previously chaotic [Cramer, 1993].

Analysis of temporal variations in hormone levels in healthy subjects has revealed chaotic situations in this sector, too [Nugent *et al.*, 1994].

We have already mentioned that chaos is not in itself negative, since it is an element of flexibility and a generator of diversity. Oscillations of the control parameters of the various physiological systems are the norm in biology and in medicine. If, however, the coordination is lost, i.e. the *connectivity* of the system as a whole and in relation to the rest of the body, certain subcomponents may oscillate in an excessive, unpredictable and pointless manner, thus generating localized disorders which may, however, be amplified (the amplification of fluctuations is a typical behavior of chaotic systems). Oscillation thus becomes disorder and takes on the aspect of disease, in that it causes the emergence of substantial symptoms and damage. It is as if chaos were amplified and formed "nuclei" of pathological interrelations between cells or systems, also involving the connective system; these nuclei then in some way isolate themselves from the global control system and prove self-maintaining.

Mental disease, too, often originates from, and is then consolidated by, the loss of ability to communicate with one's fellow human beings.

There are many ways in which an integrated system loses complexity and connectivity, some of which are listed here by way of examples (basically, all pathology could be viewed in this light):

a) Reduction of the number of cell elements involved (cf., for example, processes of senile atrophy or atrophy due to cellular anoxia).

b) Loss of number or sensitivity of receptors when they are occupied for too long or too intensely (cf. what was said in the section on receptors), or when they are directly attacked by the disease (e.g. myasthenia gravis), or when they are genetically defective (e.g. familial hypercholesterolemia).

c) Lack of production of the signal (e.g. anatomical defect or endocrine glandular disease) or its interception en route (interruption of nerves, presence of autoantibodies to the signal protein).

d) Defect of intracellular signal transduction mechanisms (from the receptor to the intracellular space): we need only mention, for example, the action of bacterial toxins which put G-proteins out of action, or the adaptation of the G-proteins themselves in cardiac failure, or the action of many pharmacologically active substances such as calcium antagonists or agents which elevate cyclic AMP. Many oncogenes act precisely on these delicate proliferation control pathways.

e) Distortion or suppression of homeostatic responses by ingestion of excess alcohol, or by overmedication with enzyme inhibitors, antibiotics, anaesthetics, narcotics, and similar drugs.

Other applications of the theory of chaos have been described in cardiology. For example, it has been reported [Goldberger *et al.*, 1990] that the heart rate of a healthy individual varies over time with an intrinsically chaotic periodicity and not, as was believed in the past, according to a normal sinus rhythm influenced only by the homeostatic systems. On observing these variations according to different time scales (minutes, ten minute periods, and hours), we see similar fluctuations reminding us of a fractal behavior in the temporal rather than in the spatial domain. Obviously, these are not arrhythmias, but oscillations in normal rhythm. The electroencephalogram also shows similar chaotic patterns as normal aspects of its functioning [Freeman, 1991].

The above-mentioned authors claim that the physiological systems with intrinsically chaotic dynamics have functional advantages, in that they are more flexible and adaptable to variations in conditions and demands on the part of an environment in a state of continual change. In other words, it might be said that *a chaotic system can be more easily modulated than a system presenting a substantial measure of order*. By way of confirmation of this theory, we would mention a paradoxical finding: many electrocardiograms in patients with severe heart disease reveal disappearance of the chaotic fluctuations, as if disease were a state of stability [Goldberger *et al.*, 1990]. The nervous system, too, may show a loss of variability and the onset of pathological periodicity in disorders such as epilepsy, Parkinson's disease, and manic-depression syndrome.

Walter J. Freeman, professor of neurobiology at the University of California, Berkeley, reports: “Our studies have led us as well to the discovery in the brain of chaos, complex behavior that seems random but actually has some hidden order. The chaos is evident in the tendency of vast collections of neurons to shift abruptly and simultaneously from one complex activity pattern to another in response to the smallest of inputs. This changeability is a prime characteristic of many chaotic systems. It is not harmful in the brain. In fact, we propose it is the very property that makes perception possible. We also speculate that chaos underlies the ability of the brain to respond flexibly to the outside world and to generate novel activity patterns, including those that are experienced as fresh ideas” [Freeman, 1991. p. 34].

In the immune system, too, chaos may play a very important role, especially because this system continually needs to generate new forms of receptors to cope with all the possible antigens that the outside world and the inside of the body may present. *Fantasy*, then, is a fundamental property of the immune system, without which the body would lack the necessary adaptabilty to a world in a constant state of change and the ability to defend itself against potential aggressors. Chaos and fractals are essential in the dynamics of idiotypical networks, as modern immunology is increasingly demonstrating.

In the hematopoietic system there are cells, called stem cells, which can give rise to all the possible cell lines of the blood (erythrocytes, granulocytes, lymphocytes, megakaryocytes, monocytes-macrophages). What makes the cell “decide” the evolutionary path it is going to take? There are two interpretations: according to the deterministic conception, the cells obey external signals such as hormones and growth factors; according to the stochastic conception, the choice is made at random. Probably, both theories contain elements of truth: it would appear, in fact, that the stem cell differentiates itself at random, but that, after this choice, it expresses receptors for growth factors, with the result that it is the latter which make the cell

proliferate (otherwise it would remain differentiated but useless, in that it would yield no progeny) [Golde, 1991].

It is very likely that in the near future studies on fractals and on chaos will be applied to physiology and pathology to an increasing extent. In fact, if chaotic dynamics is a *normal* aspect of physiological processes, investigating this may furnish more complete predictive information for characterizing dysfunctions due to old age, pharmacological or toxic substances, and other pathogenetic processes.

Recently, an example of how to control chaos in a living system has been provided by elegant experiments performed on an *in vitro* neural network prepared from a hippocampal slice of rat brain [Schiff *et al.*, 1994]. In this brain area, focal neuronal activity is represented by discharge burst with typical chaotic behavior (unstable periodicity), that can be monitored by computerized recording under suitable experimental conditions. Intermittent electric pulses delivered at appropriate time intervals ("periodic pacing") to the bath where the slice is kept can increase and regularize the periodicity of such neuronal population bursting. Moreover, periodic behavior in certain preparations can be *anti*controlled in order to induce chaos. The authors claim that this model could be applied to the control of *in vivo* epileptic spike foci, which share similar characteristics of unstable periodicity.

The existence of chaos and particularly of the possibility of at least studying some of its rules carries much more far-reaching implications than one might think. In this connection, it is worth quoting the conclusive part of a study on chaos by a group of American researchers: "Chaos brings a new challenge to the reductionist view that a system can be understood by breaking it down and studying each piece. This view has been prevalent in science in part because there are so many systems for which the behavior of the whole is indeed the sum of its parts. Chaos demonstrates, however, that a system can have complicated behavior that emerges as a consequence of simple, nonlinear interaction of only a few components.

The problem is becoming acute in a wide range of scientific disciplines, from describing microscopic physics to modeling macroscopic behavior of biological organisms. The ability to obtain detailed knowledge of a system's structure has undergone a tremendous advance in recent years, but the ability to integrate this knowledge has been stymied by the lack of a proper conceptual framework within which to describe a qualitative behavior. For example, even with a complete map of the nervous system of a simple organism, such as the nematode studied by Sidney Brenner of the University of Cambridge, the organism's behavior cannot be deduced. Similarly, the hope that physics could be complete with an understanding of fundamental physical forces and constituents is unfounded. The interaction of components on one scale can lead to complex global behavior on a larger scale that in general cannot be deduced from knowledge of the individual components.

Chaos is often seen in terms of the limitations it implies, such as lack of predictability. Nature may, however, employ chaos constructively. Through amplification of small fluctuations it can provide natural systems with access to novelty. A prey escaping a predator's attack could use chaotic flight control as an element of surprise to evade capture. Biological evolution demands genetic variability; chaos provides a means of structuring random changes, thereby providing the possibility of putting variability under evolutionary control.

Even the process of intellectual progress relies on the injection of new ideas and on new ways of connecting old ideas. Innate creativity may have an underlying chaotic process that selectively amplifies small fluctuations and molds them into macroscopic coherent mental states that are experienced as thoughts. In some cases the thoughts may be decisions, or what are perceived to be the exercise of will. In this light, chaos provides a mechanism that allows for free will within a world governed by deterministic laws" [Crutchfield *et al.*, 1986, pp. 48-49].

5.8. General discussion on complexity

We have sought here to expound, in broad outline at least, the main concepts embodied in the complexity paradigm because they constitute the basis for a proper understanding of homeopathy. The relationship between the discussion on chaos and fractals, on the one hand, and homeopathy, on the other, is neither immediate nor simple. We will return to this subject and discuss it in some detail in Chapters 6 and 7 after presenting the general frame of reference for the hypothesis as to the mode (or modes) of action of homeopathic remedies. Without this frame of reference, any kind of speculation at this point would seem vague and largely unfounded.

There can be no doubt that living phenomena belong to the category of complex phenomena. Even a single bacterium contains such a large amount of constituent elements interacting with one another in a coordinated manner that there can be no objection to defining it as a *complex system*. On the contrary, the phenomena involved in the physics of nonliving systems (such as gravity and the motion of the pendulum), the various pure chemical substances (in the form of solid, liquid or gaseous matter), and the geometrical forms are usually regarded as fundamentally simple systems, which are at any rate amenable to exact description and whose behavior can be predicted. In recent decades, however, new physical research instruments and new theories applied to classical mechanics show that the dividing line between "simple" and "complex" is much narrower than was once supposed. Even simple systems can present complex behavior in certain conditions. For this reason there has been a whole crop of studies by physicists and mathematicians providing us today with a fairly objective view of complexity and its main properties, which is also undoubtedly of importance in biology and medicine.

Introducing the concepts of complexity and chaos into the fields of biology and medicine is tantamount to introducing a new way of thinking because it is not merely a matter of understanding that things are not as simple as one might have hoped (i.e. that they are highly complicated), but a matter of acquiring a number of categories of thought whereby it is possible to "find one's way" around the world of complexity with a certain measure of familiarity. To put it another way, understanding the peculiar properties of complex systems may help us to avoid being overwhelmed by the infinite variety and the degree of complication of the individual mechanisms involved. This, it goes without saying, is not important only for the homeopathic approach, but it allows us to view that approach within the framework of a paradigm which is now gaining ground and establishing itself in many fields of science.

Nicolis and Prigogine declare: "Our physical universe is no longer symbolized by the regular, periodic motion of the planets which underlies classical mechanics. It is rather a universe of instability and fluctuations, which underlie the incredible wealth of forms and structures we see in the world around us. We therefore need new concepts and new instruments to describe a nature in which evolution and pluralism have become the basic watchwords" [Nicolis and Prigogine, 1991, p. XI].

5.8.1. Birth of a complex behavior

In an attempt to illustrate the concept of complexity from a purely physical standpoint, we have to refer to the properties of those dynamic systems which are in a state of continual change or exchange with other systems. One form of complexity, or rather of complex behavior, may emerge from a dynamic system in equilibrium, when it is subjected to a *flow* of energy (termed by some authors a *constraint*) which reaches a critical threshold (also called *symmetry break*).

For example (Figure 19), let us consider an open system which presents two states A and A' which are theoretically in a state of equilibrium and reversible (A and A' may be state of health and state of disease, or, more simply, a physiological or cellular parameter with values oscillating from a minimum A to a maximum A'. The system is open in the sense that it receives an input (x) of matter, energy, and information from the environment (other systems) and produces an output (x') of matter, energy, and information to the environment. Prigogine defines this system as a "*dissipative*" system, whereas Reckeweg speaks of a "*flow system*."

Figure 19. Schematic representation of an open system.

The status of A and A' in any given time period will be conditioned by the *constraint* consisting in the change in x and x' in that period. Thus a system of this kind will hardly be stable, but will undergo continual changes.

Unlike the system considered, isolated systems, in which no exchange with the environment is allowed, tend in an irreversible manner towards a final state of equilibrium, in which there is no longer any diversity, asymmetry, or change. In this case, *equilibrium* does not coincide with *order*, but with disorder of all the elements of the system.

This behavior of matter is expressed by the famous second law of thermodynamics:

$$dS/dt \geq 0$$

whereby the change in entropy *dS* over the time period *dt* is equal to or greater than zero, which is as if to say there is an inevitable tendency towards disorder. In technical terms, *entropy* is a measure of the degree to which the energy in a closed thermodynamic system or process has ceased to be available for some type of work or modification.

On the other hand, open systems are subject to continual modifications; here the entropy is kept under control. In a certain sense, open systems, and among these particularly living systems, evade the second law of thermodynamics, precisely because of the dissipation of entropy in the environment. Internally, these systems undergo the increase in entropy, in the sense that their internal entropy d_iS is ≥ 0, but they also depend on an exchange of entropy with the outside environment. If we call this exchange d_eS, we obtain a reformulation of the law whereby:

$$dS/dt = d_iS/dt + d_eS/dt$$

Whereas physical law imposes $d_iS \geq 0$, there is no law imposing the sign of d_eS, in that the flow of entropy of a system can be positive or negative according to the system considered. It is thus possible that, in some particular system, d_eS may become negative enough to exceed the value of d_iS, with the result that the system presents:

$$dS/dt < 0$$

which is a situation where the disorder decreases and the degree of organization increases. Basically, this would be the fundamental mechanism whereby disorder is diminished in the course of evolution. From the environment the system takes or receives energy, matter, and information; the open system supplies the environment with energy, matter, and information in another form. Within the system, entropy decreases, whereas the total entropy of the universe continues to increase, thus saving the general validity of the second law.

The real situation of physical and biological systems, however, cannot be encompassed within schematic frameworks of this kind, since it would appear that, even within the system itself, large-scale spatial interactions of the various elements may somehow "spontaneously" elude disorder and generate orderly forms and complex behavior patterns [Nicolis and Prigogine, 1991]. More thorough analysis of these issues goes beyond the scope of this book, the intention here being merely to provide an overview of certain as yet unsolved problems in the field of complexity.

An effective example of what is meant by complexity in a physical system might be the field of thermal convection phenomena. If we take a receptacle, fill it with water, and then leave it in isolation ("closed" system), after a short time the turbulence of the liquid which occurred during filling will subside and disappear, the air bubbles will rise to the surface and we will have a homogenous liquid in which all the parts are identical, homogeneous, and at the same temperature. The water molecules move in a state of maximum disorder (Figure 20 A).

Figure 20. Formation of Bénard convection cells. The vectorial movements of water molecules are depicted schematically in a receptacle enclosed between upper and lower partitions, sufficiently far apart for the side walls to have no effect. For the sake of simplicity, only the section of the receptacle is represented. A: situation of equilibrium; B: new equilibrium, caused by flow of thermal energy; C: disruption of the equilibrium, with onset of complex behavior (Bénard convection cells).

The tendency of the system towards equilibrium is fairly strong: if a perturbation is applied, for example by stirring (kinetic energy) or dipping a finger into the water (thermal energy), after a short time the water will settle down again and disperse the thermal energy: not a trace of the perturbation will remain. If, however, we subject this system to a *flow* of energy (constraint), for example by applying stable heat which comes from below the receptacle and is dispersed above it (assuming that the side walls have no effect), two different situations can be observed: up to a certain point the system transfers energy from the warmer water (below) to the colder water (above) according to "simple" thermal convection, i.e. a new equilibrium will be created, with molecules moving in a disorderly fashion, faster in the lower layers and more slowly in the upper layers (Figure 20 B). In this new equilibrium, too, however, the molecules move in disorderly fashion, at higher or lower speed according to the temperature. At a certain point, called the critical point, massive, collective movements of the molecules begin to occur in the liquid. The continuity of the thermal gradient from top to bottom is interrupted and rising (warmer water) and descending (colder water) currents are generated, forming the so-called Bénard convection cells (Figure 20 C).

At this point, the water is no longer in the state of disorder which predominated previously; a certain type of order appears in the system, as if the molecules had received their marching orders and had started to move in rank and file, obedient to some form of *coordination* from on high. It is as if each molecule of the fluid "knew" its own position and the movement of the neighboring molecules and took due account of this in its contribution to the overall pattern. The result of these broad-ranging interactions is that the motion is not caused only by kinetic energy and by random impacts between molecules, but also by the collectivity. To this we should add the fact that the new rules not only influence the molecules *within* a convection cell, but also the behavior of adjacent cells: in fact, two adjacent cells present motion in opposite rotation directions.

If we continue to supply energy to this ideal receptacle, above a certain critical value the order is disrupted and so-called turbulence phenomena appear (collective, but chaotic movements) whereupon the motion reverts to being almost completely disorderly. Obviously, the maximum molecular disorder is reached in the gaseous state.

The example we have given, despite its schematic simplification, embraces the main elements of complexity: the birth of the *organization* or *structure* of a system, despite the existence of strong tendencies towards the growth of entropy. This order in the system considered consists in the appearance of *broad-ranging* interactions between many water molecules (it has been estimated that a Bénard convection cell contains roughly 10^{20}). This has been achieved by subjecting the open system to a flow of energy.

How complex and still not entirely understood the behavior of water is can also be deduced from another phenomenon, namely its crystallization. In the phase transition from liquid to ice during the drop in temperature, volume expansion takes place (unlike what happens in the vast majority of other liquids), but, more importantly, in suitable conditions, the occurrence of orderly figures such as snowflake crystals is possible. In the snowflake, the crystal lattice produced by the intermolecular hydrogen bonds has little to do with the development of the snowflake branches in such a great variety of forms, in that the sizes of the latter are of an incomparably greater order of magnitude than the molecular diameters. What happens, then, is a complex behavior of the water vapor in the phase in which it is subjected to nonequilibrium during the drop in temperature. This complex behavior is capable of generating orderly, organized forms on a much greater scale than the molecular crystal lattice.

Many systems have been described as being capable of generating order in complex systems subjected to given constraints: the order may be spatial (waves, spatial structures such as rings or spirals) or temporal (oscillations). There are even chemical reactions which are capable of self-organization in nonequilibrium states in the course of time. The prototype of these reactions is the one described by Belusov-Zabotinskij: in this reaction, the reagents (we will ignore the details) generate a colored product which, however, is not always constant, nor always on the increase, but continues to disappear in the course of time, thus giving rise to talk of a *chemical clock*.

If orderly systems are formed in space (e.g. circles, target-type structures, spirals) this means that each element is affected in its position and speed by the others in the system: it takes its "orders" or information from the adjoining element and behaves accordingly. This results in phenomena of cooperation and coherence, so that the elements arrange themselves in orderly structures. If orderly structures are formed in time (e.g. chemical oscillators, biorhythms, pulsations) this means that the state of the system in any given instant "depends" on the previous one and "conditions" the next one in the series. In complex systems, this is not equivalent to a certain type of inertia of motion according to the classic laws of physics because these

orderly structures in time may present, and indeed usually do present, a nonlinear trend. There is transmission of information processed in a complex manner in time, and thus a form of memory.

In brief, it has been claimed that it is the very appearance of *spatiotemporal structures*, due to *broad-ranging* interactions among the elements of a system, that constitutes the most typical feature of complexity [Nicolis and Prigogine, 1991]. It is in this that complex behavior consists in the true sense of the term, and not merely, as one might be tempted to believe, in the continual increase in the factors involved. In other words, complexity contains not only a quantitative factor related to the number of elements involved, but also a qualitative factor associated with the appearance of "structure," or "form."

In cells, *structures* have absolutely indispensable functions: we need only mention the cell membranes, which divide the cells into compartments and separate the cell itself from the surrounding environment. Via the membranes a marked *dysequilibrium* of substances and electrical charges is maintained, which is necessary for a whole series of functions, such as the production of energy (mitochondria), the production and transmission of signals (neurons), and the activation of movement (muscles).

Biologists are accustomed to regarding these types of order as the rule in cell structures (membranes, filamentous proteins, circadian rhythms, cell cycle, and many others), but recently chemists and physicists, too, have devoted their attention to phenomena of this type which occur in nonliving nature. This is important because if the ability to generate order is also found outside the biological setting, this means that we are in the presence of a type of behavior basic to nature itself and thus in a certain sense ancestral or atavistic.

Order, information, and complexity are aspects of one and the same problem, which would appear to be of increasing importance in fields ranging from biology to physics, to the study of evolution and of social and economic systems. The contribution deriving from a physico-mathematical approach to the problem of complexity is much greater than might at first sight be imagined: while it is true that a living system with its thousands of different subcomponents will never resemble a chemical system with two or only a few components and can never be described by a mathematical formula, on the other hand it is equally true that the study of the complexity of "simple" systems may enable us to discover a number of "basic rules" of behavior which are repeated in substantially identical form in systems at different levels of evolutionary development.

In other words, the complexity of biological systems (and their pathological aspects) may be tackled and better understood by reference to a pattern common to all complex systems, i.e. to a model developed in physicochemical reference systems. Knowing the behavior of a system on a small scale may help us to identify some of the properties of a vaster and more complex system. Such a procedure exploits the knowledge accumulated in the study of fractal geometries, which shows us that there is kind of *self-similarity* in many aspects of nature, whereby the fundamental rules (even as expressed in mathematical formulae) remain similar on changing the scale of operations. In the detail, we rediscover the image of the vaster context in which that detail occupies a place (see Chapter 5, Section 7).

5.8.2. Résumé of the properties of complex systems

Without wishing, obviously, to dismiss here in few words such a broad-ranging and difficult topic, we can summarize the particular characteristics, properties, or modes of functioning of complex systems as emerging today in many disciplines:

a) *A complex system presents properties which amount to more than simply the sum of its component parts*. From the interrelations between subsystems new functions are generated, such as, for example the electrical membrane potentials in the field of physiology, or the control of blood pressure at vascular system level, or thought and emotions at central nervous system level. The new functions cannot be deduced from an analysis of the subcomponents, though they are conditioned and determined by them.

b) *In a complex system not all behavior patterns are theoretically predictable and experimentally reproducible*. For example, in the Bénard convection cell system described above, we can predict and reproduce the phenomenon of the appearance of convection motions at temperature values beyond a certain threshold, but we cannot predict what will be the direction (e.g. clockwise or counterclockwise rotation) of the motion of the water of a cell in a given space in the receptacle. This latter parameter is selected by the system at random, probably in response to imperceptible fluctuations of the random motions of the molecules at the critical moment when the orderly flow tends to begin. Thus, the behavior of a complex

system is the fruit of cooperation between determinism and chance. The immune system, too, is characterized by this property: the lymphocytes continually generate new receptors with random sequences, and then the information which enters in the form of an antigen selects the corresponding clones and induces them to proliferate, thanks to the intervention of many other components of the system itself (receptors, immunological memory, other lymphocytes, cytokines).

c) *A system which obeys the laws of complexity does not always behave in a linear manner, i.e. the effects are not always proportional to the doses of a given factor which modifies the equilibrium*. Theoretically, the consequences of this factor may be extremely variable. For example, it may happen that a perturbation is "absorbed" without leaving a trace, i.e. it causes the modification of a parameter, immediately followed by a return to the initial state (negative feedback).

It is also possible that the reaction of the system may be such that there are oscillations of the parameters, even in the opposite direction to that of the initial perturbation (what in pharmacology may be defined as a rebound effect). On the other hand, another possibility is that minimal changes in initial conditions or minimal perturbations may activate autocatalytic cycles ("positive" feedback), or interlinked amplification cascade systems, with major consequences (see the butterfly effect).

The field of meteorology, in fact, is one of those in which the problem of complexity and chaos has been grasped and tackled in a highly systematic way owing to the notorious difficulty encountered in forecasting the weather. Amplification systems, however, are present at all levels in the equipment of a complex system receiving external stimuli. "*Pronounced dependency on initial conditions*" is a fundamental property of complex systems, accounting for the occurrence of chaotic dynamic patterns, unpredictability and nonlinearity of responses [Ruelle, 1992].

d) *Complex systems exhibit another singular property - bistability*. Given the same external and internal conditions (temperature, chemical concentrations, mathematical parameters), the system may assume different states. The choice between one state and another possible state often depends on the *past history* of the system itself. For instance, when we consider a liquid "perfect," the melting point and freezing point coincide, but when we consider aggregates of molecules which are midway between liquid and solid (microaggregates), the two points may differ very considerably: on heating a solid composed of microaggregates, it will melt at a certain temperature; on cooling the solution, the microaggregates remain in the liquid phase up to lower temperatures than those they had when they were in the solid phase prior to melting. At one and the same temperature, the molecules may be in the solid or liquid phase according to their previous state: if they come from a liquid phase they tend to remain liquid; if they come from a solid phase, they tend to remain solid.

These models are regarded as being very useful for more thorough study of phase transition processes, which, as is well known, are also of decisive importance in living systems (cf. the continual polymerization and depolymerization phenomena of cellular macromolecules).

e) *A complex system is regulated by communication modes suited to the degree of complexity*. For example, communication between two molecules (fairly simple system) consists in electrostatic attraction or repulsion; communication between several groups of molecules (complex system) consists in ondulatory dynamics and spatiotemporal variations (oscillations of particular signal molecules); communication between organs and systems is entrusted to further complex systems which use both chemical (hormones) and physical (action potentials) means of communication. Lastly, communication between different individuals is entrusted to other means such as words, writing, looks, and broadcasting by cable or over the air. This means that if we want to "enter the communications network" (with a view to understanding and eventually influencing it) we have to use the same method or methods of communication as the system we are interested in. This fact has been clearly appreciated by psychiatrists who, though armed with a vast array of highly effective molecules such as neurotransmitters and transmission inhibitors, know perfectly well that this molecular approach may condition, but not solve a psychiatric problem, the root of which lies in an affective, relational or behavioral disorder.

The comparison, however, can be extended to other fields of medicine because the more we study them, the more these other systems, too, prove to be composed of interactive networks between cells and multicellular centers which, in qualitative terms, resemble the neural networks. If a system is regulated by modes of communication consisting in synergisms between several molecules acting at low doses, to enter this network in an effective way theoretically you would need to use the same method: low-dose modulators exploiting synergisms and antagonisms. If, on the other hand, we use modulators of only one molecular type and at high doses, we obtain effects, admittedly, and even effects in the desired direction, but not in complete

harmony with the system itself, the result of which therefore will be a high incidence of unwanted side effects.

f) *Systems far from equilibrium are susceptible not only to dramatic changes but also to having to conserve a spatiotemporal memory of such changes.* Memory in this context means the possibility of an *irreversible change*. Unlike what happens in a system in a state of reversible equilibrium on changing the external or internal parameters (see Figure 19), in a complex system a situation can be reached in which there is a *symmetry breaking*, or irreversible change. Minor localized attempts to deviate from the equilibrium are not necessarily made to fail by the instantaneous development of some form of counteraction, but may be accepted or even amplified by the system, thus becoming sources of innovation and diversification. In mathematical terms, this phenomenon is also called bifurcation. While it is true that random fluctuations and perturbations can usually be damped, beyond certain values, or in the presence of appropriate environmental conditions, these effects are not annulled, but with the system acting as an amplifier, a reaction is triggered which removes the system from the reference state. We thus have the setting up of a new state of the system, which can no longer be restored to the previous state. This phenomenon has proved very important, amongst other things, in the evolution of cells: the spatial symmetry breaking occurring, for example, when the membranes started to separate what was inside the cell from what lay outside the cell marked an irreversible leap forward in the organization of living systems, since it was precisely through these partitions that a gain in information and complexity was achieved, favoring the existence of a new state compared to the previous one.

Complexity, then, is associated with memory, or rather with the storage of information: a previous event may influence other subsequent events. Information exhibits a special propensity for being transmitted, and thus is particularly capable of generating consequential effects on the receiving system, but also of being stored, and of persisting as a memory of subsequent events. The evolution both of the macrocosm and of each individual (embryogenesis, followed by development and growth) is a progressive increase in the storage and handling of information.

g) *In the behavior of complex systems the quality of the information is far more important than the quantity, or the energy consumed to provide it.* The biological system, in particular, has developed and has integrated within itself the systems of production and use of energy, utilizing various well coordinated metabolic pathways and the rapid availability of phosphorylated intermediates. The functional reserve of these systems is considerable (except in highly pathological cases of cell damage or anoxia), so that normally the functional oscillations and the behavior patterns of cellular or organic biological systems do not depend on addition or subtraction of energy, but on control mechanisms at the information level. Information of such a nature as to be received and processed efficiently by receptors and signal transduction systems is capable of activating energy metabolism and of evoking functional and mechanical response cascades which may be incomparably greater than the initial stimulus in terms of amount of energy and information produced.

The more complex and “flexible” a system is, the scantier may be the amounts of energy capable of altering its behavior. We need only mention, for instance, the brain, which is in all probability the most complex system existing in nature. It can be “mobilized” - and, as a result, so can the entire body - by nonmolecular stimulations, which in a certain sense may be devoid of energy or matter. Biochemical, metabolic, electrical, receptor, and even anatomical systems (in the sense of the physical structure of the neurons) can be activated and modulated not only by chemical or pharmacological substances and electrical stimulations, but also, in an optimally efficient and specific manner, by sounds, visual images, words, ideas, and thoughts. What matters, in this case, is not the quantity or intensity of the stimulus, but its “meaning” in terms of information.

h) *When it comes to describing and understanding complex systems it may be very useful and perhaps indispensable to use archetypes and analogies*. This approach has been strongly emphasized by Nicolis and Prigogine [Nicolis and Prigogine, 1991]. What is meant by analogy is that similarity between two distinct systems which may serve to understand one of them better on the basis of knowledge already gained about the other. Analogy can therefore be used to construct more advanced models compared to those in current use and to make forecasts about unknown systems starting from known systems (usually physicochemical or mathematical) which act as *archetypes*, i.e. as reference systems.

The above-mentioned authors state the case: “The physicochemical systems which give rise to transition phenomena, to broad-ranging order, or to far-from-equilibrium symmetry breaking may serve as archetypes for understanding other types of systems which exhibit a complex behavior, for which the laws of evolution of the variables involved are not known at any comparable level of detail. (...) The analysis proceeds in two

steps. Firstly, certain analogies are traced between the observations and the behavior of physicochemical "reference" systems. This defines the type of model which is likely to be the most appropriate representation of the system concerned. Then, an attempt is made to go beyond the stage of plain analogy, to pinpoint, *within the framework of the model adopted*, the specificity of each problem and to incorporate it in the description. Lastly, the predictions of the analysis are compared against experience with past behavior and, assuming an agreement is reached on quality, these predictions are used to foresee future tendencies. If all goes well, the natural outcome of the process should be an ability to come up with concrete, practical suggestions as to how to *dominate complexity*" (authors' italics) [Nicolis and Prigogine, 1991, p. 251].

As we have seen earlier (Chapter 5, Section 7), fractal geometry appears to be the most suitable for describing the forms and properties of many natural systems. The existence of self-similarity on changing the scale, a typical property of fractals, in a certain sense justifies the analogy procedure: an image or behavior pattern is observed in the part which resembles the whole to which that selfsame part "belongs." The part then is significant, and contains information and properties which allow analogies to made with the entire system and with other parts of that system. In a nutshell, the analogy highlights that "hidden order" which exists in complex systems, consisting in scale invariance and thus in the presence of elements of similarity between apparently very different systems (see also Chapter 5, Section 7).

The following chapters will serve to illustrate how the complexity paradigm helps us to get the basic features and principles of homeopathic medicine into proper perspective.