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Biological Pattern Formation and Physico-Chemical Laws

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Abstract:

Physical principles underlying biological pattern formation are discussed. In particular, the combination of local self-enhancement and long-range ("lateral") inhibition (Gierer and Meinhardt, 1972) accounts for de-novo pattern formation, and for striking features of developmental regulation such as induction, spacing and proportion regulation of centers of activation in tissues and cells. Part I explains physical principles of spatial organisation in biological development. Part II demonstrates in mathematical terms that and how short-range activation and long-range inhibition are conditions for the generation of spatial concentration patterns. The conditions can be expressed in terms of ranges, rates and orders of reactions. These conditions, in turn, can also be derived by analysis of dynamic instabilities by means of Fourier waves, showing the neither obvious nor trivial relation between the latter approach and the theory based primarily on autocatalysis and lateral inhibition.

Biological Pattern Formation and Physico-Chemical Laws.

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PART I

Physical Principles of Spatial Organization.

1. – Morphogenesis and molecules.

Nature produces many types of spatial structures which evolve *de novo* from nearly uniform initial conditions: stars and galaxies, waves and clouds, crystals, mountains, valleys; but the most specific, most impressive generation of spatial order is the development of the animal from the egg cell. This process shares some features of « self-generation » with the inorganic domain, but it is quite unique in that structural characteristics are reproduced in minute detail under the control of the genes. It is for this reason that elephants have innumerable spatial properties in common, whereas clouds share only few. How can genes determine spatial organization? Genes are themselves spatial structures, namely sequences of nucleotide pairs. Yet these structures bear no resemblance to the form of the animal, say a mouse, generated in the course of embryogenesis. Primarily, genes specify nucleic acids and proteins including enzymes, receptors and membrane components. They are involved in the complex network of biochemical reactions, which, in turn, lead to spatial order. In this process, the relation of genes to their immediate products is much better understood than the mechanisms by which a set of molecules produced under the control of genes eventually generates spatial patterns. If we concentrate on the latter aspect, this is because it is the more challenging one, not because we underrate the role of genetic control of pattern formation. The question is: How can molecular interactions and movements lead to spatial order in cells and tissues?

Not all scientists will agree that this is an adequate way of stating the main problem. One may object that, in development, one observes phenomena, not molecules. Physics encompasses many processes like electromagnetic waves which are nonmolecular. Is it conceivable that pattern formation is due to some strange physical effect, or even to processes beyond known physical

laws? Such possibilities cannot be strictly refuted by logical argument. However, very few would question that unsolved biological problems, *e.g.* photosynthesis, muscle contraction, or chromosome segregation, have molecular solutions. Why then maintain doubts with respect to pattern formation? Everything we know about cells and tissues indicates that their basic properties can be explained on a molecular basis. We expect this to apply also to their spatial organization.

2. – Basic physical types of the generation of spatial order.

Several physical mechanisms are conceivable for the generation of spatial order in cells and tissues. One of them is self-assembly: constituents such as molecules, molecular compounds or cells may be produced at random positions, move at random, collide, associate and dissociate until an energetically favourable, stable spatial arrangement is reached. In the inorganic domain this occurs during the formation of crystals from molecules in a liquid. In biology, self-assembly is involved in the formation of many intracellular structures, such as ribosomes, chromatin and membranes. Even whole cells of different types can sort out, forming distinctly different layers by self-assembly starting from random positions. However, the main spatial features, say of a mouse, are not generated in this way. Rather, cells in different positions of the embryo develop in different directions from the outset.

A second basic mechanism is a conversion of order in time into order in space. This occurs if, in a marginal zone of a tissue, cells of different types are produced in a certain time sequence. Embryological evidence suggests that time order is involved in pattern formation, but it often modifies rather than generates structures. The initial process in the production of a rudiment of an organ or its substructures appears to be the generation of strikingly different parts *within* originally near-uniform cells and tissues. For example, the early embryo becomes subdivided into ectoderm, mesoderm and endoderm. A particularly suitable system for studying such internal self-organization is the regeneration of the fresh-water polyp hydra. Sections of its body column regenerate a new animal with head, gastric column and foot. This process does not require cell proliferation or growth; originally near-homogeneous tissue is re-specified, differentiating into distinctly different parts.

This « self-organization » can be described as a *de novo* generation of unequal distributions of molecular components within a given spatial domain of a tissue. Many embryological structures can be traced back to invisible primary patterns. For instance, if a piece of hydra tissue is allowed to regenerate, the future head area newly acquires head-activating properties only a few hours after the onset of regeneration, and much before a new head is actually formed. This can be shown by the capacity of the future head area to induce a new head upon transplantation into the body column of another hydra. This

evidence demonstrates that the primary event is the formation of an (invisible) spatial distribution of a physical property which then activates the formation of visible structures. There is no doubt about the existence of such primary patterns which are often called « pre-patterns » or « morphogenetic fields » specifying « positional information » [1]. Though their chemical nature is not yet known, one expects that such fields are spatially distributed concentrations of morphogenetic substances. The simplest conceivable field would be a monotonic concentration gradient, eliciting concentration-dependent and thus position-dependent responses of the cells. Obviously, the notion of morphogenetic fields does not, in itself, solve the problem of pattern formation; rather, this is shifted to another level: How might one explain by physico-chemical schemes the generation of reproducible spatial distributions of morphogens from near-uniform conditions?

Several aspects of this question will be discussed: Do chemical reactions and molecular movements in cell plasma, membranes and intercellular space suffice for the generation of spatial concentration patterns? If yes, can the mechanism be described in terms of simple physical and mathematical properties of molecular systems? How do they relate to the facts of molecular biology? Are they suitable to explain experimentally observed features of embryological development and its regulation?

The simplest type of molecular movement is passive random movement which can be described by diffusion terms. In most cases diffusion tends to smooth out and destroy patterns rather than generate them. Nevertheless, reaction-diffusion systems are also capable of *generating* patterns *de novo* under certain conditions. This was first shown by RASHEVSKY [2], using a crude two-component model in a paper rarely quoted today. The more general continuous theory was introduced by TURING [3], and the mathematical analysis, which includes nonlinear features, has been developed by several groups, particularly by PRIGOGINE and NICOLIS [4] and their co-workers.

We can now ask: What are the conditions of pattern formation in systems of interacting molecules? One requirement emerged gradually and has a long record in the literature: the generation of structures has self-enhancing features. This principle is implicit in embryological thoughts of Spemann, Kühn and Waddington. It can also be found in the generation of structures in inorganic and social domains. Crystals and clouds develop by self-enhancement from minute « nuclei ». Economic inequalities may also be autocatalytic, as explained by MARX for the accumulation of capital, or by MYRDAL in his analysis of the causes of underdevelopment. On the other hand, autocatalysis by itself will not lead to structure but to an overall explosion. The formation of a stable pattern requires that autocatalysis is coupled to another effect, that of « lateral inhibition » [5]; its features can be analysed and explained by introducing the notion of « range ».

We define « range » as the mean distance r between the production and the

decay of a molecule. If a molecule's diffusion rate is D (dimension cm²/s), and if its decay rate is $\varkappa$ (dimension 1/s), then, according to the rules of physical chemistry, its mean range r is of the order of $\sqrt{D/\varkappa}$ (dimension cm). This relation converts reaction constants such as D and $\varkappa$ into spatial parameters with the dimension of distance, width, or length. In some types of mathematical analysis such parameters are introduced as wavelengths, but their immediate molecular meaning is the mean distance between the position where a molecule is found during its lifetime and the position where it was produced. This notion of range is very useful for specifying conditions of pattern formation. Spatial pattern can be generated by two suitably coupled reactions: one autocatalytic, the other cross-inhibitory. Direct inhibition can be substituted by depletion of a substance required for and consumed by activation. The inhibitory reaction must be faster than the activating one. The two most specific and least trivial conditions for pattern formation depend upon ranges: The range of activation must be sufficiently small as compared to the total field size; and the range of inhibition must be sufficiently large as compared to the range of activation. If these conditions are met, small random or systematic advantages within an otherwise uniform distribution are self-enhancing. Autocatalytic activation at one location causes deactivation nearby because the inhibitory effect which originates in the activated area extends into a wider range. Pattern formation proceeds until a stable distribution is reached. In the simplest case this is a graded distribution, but symmetric and periodic patterns can be formed by the same general type of mechanism with different values for ranges and rates. Range of activation specifies the size of the activated area, and range of inhibition codetermines whether secondary areas of activation are formed, and, if yes, how they are spaced. Mechanisms of this type may generate patterns not only in multicellular tissues, but also within individual cells.

The general conditions for pattern formation mentioned are consistent with many different molecular mechanisms, and quite simple schemes combining known features of molecular biology would suffice. We have shown [6] that the conditions listed above are mathematically necessary for the simplest two-factor systems. The same general considerations also apply to multicomponent systems if they can be divided into subsets of molecules with short and long ranges. Then, activation and inhibition are system properties of the respective short-range and long-range subsets, and not of individual substances. These mathematical aspects are the subject of part II of this lecture.

3. – Pattern formation, pattern recognition and the principle of « lateral inhibition ».

The principle of long-range inhibition (coupled to short-range autocatalytic activation) was suggested to us by certain analogies between pattern formation

and pattern recognition. In the latter domain, KUFFLER [7] and others demonstrated that lateral inhibition is crucially involved in edge enhancement and other phenomena of visual perception. In the visual system of the brain activating connections of short range and inhibitory ones of longer range were detected between nerve cells. While the physical mechanisms of transmission of electrical signals across neural synapses bear no relation to reaction-diffusion mechanisms, the formal relations between lateral inhibition in pattern recognition by neural networks and long-range inhibition in biochemical reaction networks are close. In both cases, local activation is enhanced, whereas activation in a wider environment is repressed. The formal relationship may even be of interest for a theory of aesthetic experience. Patterns which are produced by simple molecular mechanisms may also be recognized by simple processing of information in the brain. For instance, hidden regularities in seemingly complex patterns, such as second-order statistics (« granularity ») in textures, are easily produced by autocatalysis and lateral inhibition. Such textures are known to be detected by « immediate perception » [8] in fractions of a second and without conscious thought.

4. – Developmental regulation.

In simple biological systems, such as cellular slime molds or hydra, there is good experimental evidence for the involvement of morphogenetic substances in pattern formation. However, the assays available do not yet allow us to construct the complete network of reactions which generates spatial patterns; therefore, the molecular mechanisms are basically still unknown. Nevertheless, there is a well-defined and impressive set of experimental facts, some of them of a quantitative nature, to test theories and models: the unique features of developmental regulation [9-12]. These include polarity (the capacity of slight asymmetric cues to reliably orient developing structures), induction (the capacity of weak localized external or internal signals to initiate the all-or-none formation of a defined, spatially confined structure, such as a second head of a hydra), inhibition (such as the prevention of the formation of a secondary structure in close neighbourhood to a primary one, a principle which also explains the regular spacing in periodic arrangements including the pattern of leaf rudiments in plants), symmetry changes (such as the formation, under certain circumstances, of a hydra with a head in the middle and feet at both ends), and, last but not least, proportion regulation (such as the capacity of part of a sea urchin embryo to form a whole animal at reduced size, or of a small piece of hydra tissue to regenerate a whole animal with a correspondingly small head). It is this set of properties, in particular proportion regulation, upon which vitalist thinkers based their claims that « normal » physics is insufficient for an understanding of biological development. Modern systems

theory shows, however, that these self-regulating « holistic » properties are within the scope of conventional physical laws and processes, emerging as system properties of suitable reactions. Our analysis of pattern formation by short-range activation and long-range inhibition (ref. [5]; for review see ref. [6, 11]) demonstrated that all regulatory features can be explained in a rather straightforward manner, independent of the details of the molecular mechanisms involved. The simplest form of a concentration pattern, formed spontaneously if a tissue grows beyond the lower limit specified by the range of activation, is a monotonic gradient which is capable of specifying « positional information » [1] across the field. Reproducible orientation, that is polarity of such fields, is due to the fact that any asymmetric initial activation, however slight, can reliably orient an asymmetric pattern such as a graded distribution. Induction occurs if a small local stimulus initiates the formation of a centre of activation. A second centre may be induced out of the range of inhibition of a primary one. The range of inhibition is capable of determining regular spacing in periodic structures. Within fields considerably larger than the range of activation, graded as well as symmetric distributions can be generated, depending on boundary conditions.

Proportion regulation occurs if activation is saturating and range of inhibition exceeds the total size of the field; under these conditions, activation invades the field until a certain level of inhibition is reached which stops further invasion. This occurs at a fixed proportion of the activated area in relation to total field size, thus leading to proportion regulation. While this simple mechanism leads to proportion regulation with respect to two subareas only, other models are capable of proportion regulation for all parts of a field specified by a graded distribution of morphogens [6]: their steepness adapts to total field size. This occurs if gradient formation is initiated by gradually closing intercellular junctions between cells of the tissue and thus reducing diffusion constants of activators and inhibitors. At a critical stage when range of activator is sufficiently small to destabilize the initially near-uniform distribution, a gradient is formed. If this gradient elicits a signal across the field stopping further closure of junctions, the gradient becomes stabilized shortly after its formation. Such mechanisms would lead to strict proportion regulation independent of field size. They correspond to an adaptation of the « metric » of the tissue, fixing ranges of activation and inhibition in relation to total area of the field: in a small field, the mean distance between production and decay of the molecules is correspondingly small because a smaller part of the junctions between cells is open. Since such mechanisms affect the ranges of molecules in general, secondary patterns formed at later stage are also regulated in proportion to the entire area; in particular, spacing of periodic structures could adapt to total field size, a feature which is difficult to account for by other models. Whether such proportion regulation is realized in biological development—for instance, in the regulation of the spacing of somites, with

small sizes of somites in vertebrate embryos of reduced size—is an open question.

In any case, pattern formation requires certain nonlinear features of auto- and cross-catalysis. These features lead to stable spatial patterns. Their orientation may be determined by pre-existing asymmetric spatial cues, but their forms are essentially determined by the physico-chemical characteristics of the pattern-forming system, namely the ranges of activation and inhibition. In this way, a basic logical requirement for biological pattern formation is met: The pattern formed in each generation is not hidden in initial spatial distributions within the early oocyte or embryo, but generated *de novo* in each generation.

5. – Pattern formation within single cells.

Pattern formation by autocatalysis and lateral inhibition appears to occur in the course of development within multicellular tissues (mostly as two-dimensional fields in cell sheets) but also within single cells. There is no difficulty in applying the theory to membranes and plasmas of individual cells [5, 13]. For instance, allosteric enzymes, if activated by their products, could give rise to the autocatalytic feature required for pattern formation, and inhibition could be due to depletion of the precursor of the enzymatic product. Range of activator—the mean distance between production and decay—would have to be small compared to the size of the cell. Then, a focus of activity can be produced which may be oriented by slight asymmetric cues from the environment and which may lead to asymmetric development of the cell, to systematic orientation, directed movement, oriented cell division and other polar features. On the other hand, it is to be emphasized that polar development of single cells can have other causes as well. In particular, cells differ from tissues in that they are produced as potentially asymmetric structures from the outset: the plane of cell division distinguishes part of each daughter cell; this would suffice for explaining a polar development of the cell. Other mechanisms for polar development of single cells may involve directed pumping mechanisms across membranes, whereas such mechanisms seem to be less likely for pattern formation in animal tissues.

6. – Cell response to primary patterns.

Morphogenetic fields are mediators of pattern formation, but real spatial structures result only from the response of cells to such fields. Local concentrations of morphogenetic substances are expected to give rise to local responses of cells, such as proliferation, differentiation, shape changes, orienta-

tion, movements and death. Two types of responses are of particular interest: the cell differentiation and the change of cell shapes. These, in turn, determine structures and forms of tissues, organs and organisms.

Cell differentiation is the transition of the cell from one stable state to another. It is a feature of many interacting systems that a transition between stable states may occur in response to a transient stimulus above a certain threshold, the light switch being a simple example. Correspondingly if cells in a tissue respond to the concentration of a morphogenetic substance above a certain threshold by differentiation in a certain direction, then a *graded* distribution of a morphogen can give rise to a *subdivision* of a tissue into two distinct regions with sharp boundaries. Multiple thresholds would lead to more refined subdivisions. Once a primary division is formed, new morphogenetic effects may arise from boundaries between subareas, or within individual subareas, leading to more refined structures. Aside from threshold effects, graded distributions of morphogens may also affect the probabilities of cellular differentiations, leading to cell distributions which are smoothly graded within the tissue.

In the generation of biological form, many different mechanisms are involved in a complex fashion. Nevertheless, in many cases, defined biological structures can be traced back to an elementary process, namely the evagination or invagination of an initially almost flat cell sheet at a well-defined position. Two aspects of this process may be distinguished: activation of a defined subarea of a cell sheet, and the generation of curvature leading to invagination of the activated area. Spatially confined activation can be due to a morphogenetic field generated by internal processes within a cell sheet, leading to a localized concentration peak. In the course of embryogenesis, activation may also be induced by contact of a given area of one tissue with another.

How can local activation affect cell and tissue shapes? The generation of tissue form often shows self-regulatory features (such as reversibility of certain types of inhibition) suggesting that cell form corresponds to a stable steady state of processes within the cell and on its boundaries. These include insertion, removal, production and decay of membrane components, as well as the formation and disassembly of intercellular fibres, intracellular fibres and of the structures mediating the anchorage of fibres within membranes. Contraction and expansion of fibres are also expected to occur. Probably all such mechanisms interact with each other, and codetermine cell form in tissues. Models relying exclusively on fibres (by postulating that cells change shape only because fibres near a surface contract) or on adhesive membrane-membrane associations are probably oversimplified and insufficient. Form is a system property involving, in most cases, the dynamics of fibres as well as membranes. Nevertheless, a simple physical principle can be stated for tissue evagination in the generation of biological structures: If the sheet shows an inside-outside asymmetry, a local activation of a subarea of the cell sheet will, independent

of the details of the mechanisms involved, give rise to an asymmetric response with different effects on the inside and outside boundary of the cell sheet. This, in turn, leads to local bending moments, curvature and thus to new structures. Shell theory, as developed by engineers and architects, is suitable for model calculations on the generation of form in sheeted structures [14]. The logical requirement for reproducible evagination (in contrast to random symmetry breaking with equal chances for evagination and invagination) is inside-outside asymmetry. This is a biological characteristic of epithelial cell sheets. It is often directly visible in the microscope, whereas most technical materials do not show such an anisotropy.

7. – Development of the nervous system.

The most interesting organ is obviously the brain; therefore, the most intriguing aspect of development is how a neural network is produced during the course of embryogenesis. The mechanisms involved are understood only to a very limited extent, but all the evidence suggests that basic principles for the development of nonneural tissues also hold for neuroembryology. Originally nearly uniform tissues, often organized in cell sheets, become subdivided into different areas and subareas. It is highly probable that each subarea acquires different chemical characteristics, either qualitatively or quantitatively. In other words, one expects that there are cell and surface markers characteristic not only for cell types but also for cell position within the nervous system. The main problem of neuroembryology is connectivity—the generation of a specific, highly complex pattern of connections between cells which are not close neighbours. Though part of the connections in higher animals are formed postnatally and influenced by learning, many features of connectivity are genetically determined, and are produced in the course of embryogenesis. How are axons guided toward their targets? It is likely that the markers characteristic for different parts of the developing nervous system contribute to the selection of specific pathways by growing axonal processes. Pathway and target search probably involve fibre-fibre, fibre-pathway and fibre-target interactions, but it may also be codetermined by the time order of outgrowth of fibres.

In higher organisms, there are many more nerve cells than genes. Therefore, the individual neural connection cannot be due to a specific genetic instruction; instead, genes crudely specify similar repeating substructures; further there must be genetically encoded rules for the spatial ordering of large numbers of fibres. A simple example for a topological relation between the order of fibres in the area of origin and the order of their terminals in the respective target tissue is the generation of a projection from one area of the nervous system onto another (as in the connections of retinal cells of the eye with the first relay station in

the brain such as the tectum in birds, amphibia and fish) [15, 16]. Many different fibres have correspondingly different targets; nevertheless, a few spatial determinants could suffice, because in a projection the spatial orders in target and source area are closely interrelated. In terms of information theory, all that is necessary are a few appropriate signals converting the order « please project from here to there » into chemistry. The mechanism of such conversions is one of the challenging problems of neuroembryology. One possibility is that reliable projections are generated by suitable interactions between axonal components graded with respect to position of origin and spatially graded components in target tissue [17].

8. – Morphogenetic fields and the logic of the generation cycle: a summary.

Morphogenesis of an animal is the result of many genetically determined mechanisms and our present knowledge is still very limited. A crucial aspect for an understanding of the logic and physics of the generation cycle is the subdivision of cells and tissues into different parts. The primary process, the generation of morphogenetic fields within initially near-uniform cells and tissue, is proposed to result from short-range autocatalytic activation coupled to long-range (lateral) inhibition. This notion provides a link between biological development and its regulation, on the one hand, and the properties of systems of interacting molecules, on the other.

I would like to summarize the relation of the theory described to empirical evidence by replying to two typical questions.

i) The theory is about morphogenetic fields, such as gradients, but has anybody ever observed such a gradient? The answer is « no » for biochemical identification of the components involved in pattern generation (though this may change soon with the advance of developmental genetics), but clearly « yes » for indirect detection by transplantation experiments.

ii) The molecular basis of pattern formation is still unknown and the theory can describe, by proper combination of elementary mechanisms, any pattern; how can it then be supported or refuted by experimental facts at all? The answer is that developmental regulation, the striking effects produced upon artificial (mostly surgical) interference with development, is a testing ground for theoretical concepts. We have shown that the specific features of developmental regulation are rather straightforward consequences of mechanisms of pattern formation based on autocatalysis and lateral inhibition. This is not the ultimate proof, which could emerge only in conjunction with further molecular studies. However, the fact that simple reaction-diffusion mechanisms explain basic properties of developmental regulation is unlikely to be incidental. Of course, there could be more than a few substances involved,

and there may be surprises with respect to mechanisms; they could involve the transduction as well as the transport of cell-to-cell signals across membranes, directed transport of molecules along fibres and across junctions, pulsing instead of stable patterns of morphogens, and many other features. In particular, movement and signalling need not always be constrained to random redistributions describable by diffusion terms. Independent of details and of the complexity of mechanisms, we expect the general principle of short-range autocatalysis and long-range inhibition to be a suitable framework for explaining a wide range of phenomena of spatial self-organization.

PART II

Short-Range Activation and Long-Range Inhibition as Conditions for Pattern Formation: Analytical Derivation and Specification.

9. – Mathematical analysis of conditions for pattern formation.

After discussing features of biological pattern formation and their explanation on the basis of autocatalysis and lateral inhibition in semiformal terms, we will now consider the generality and stringency of the principles involved by analytical reasoning.

The capability of coupled reactions for the generation of spatial patterns was first shown by TURING [3]. He mainly analysed linear kinetics, but he already realized the importance of a nonlinear approach as well. Nonlinear equations such as the « Brusselator » were proposed and investigated [4, 18]. In our studies on mathematical models for biological development we found that pattern formation by coupled reactions, as a rule, requires a long-range inhibiting effect, as antagonist of short-range autocatalysis; these effects, in turn, explain rather directly the impressive « holistic » aspects of developmental biology including regeneration, proportion regulation, induction, regularities of spacing and symmetry changes [5], exemplified by regeneration of hydra and developmental regulation in other biological systems. We set out with an analysis of spatial destabilization for equations with power terms for reaction orders of activation, inhibition and depletion; in this way, simple relations *between* power terms can be derived as conditions for pattern formation, as will be shown below. These relations were used for the generation of a variety of models adapted to specific biological problems and exhibiting specific physico-chemical properties. *Any* such model, however, is capable of explaining the main features of developmental regulation mentioned above; only biochemistry could prove a particular model. For one of our activator-inhibitor models the mathematics of gradient formation was studied by

BABLOYANTZ and HIERNAUX [19]. A detailed analysis of the parameter range leading to pattern formation was conducted by GRANERO, PORATI and ZANACCA [20], and the distribution resulting from the nonlinear interactions was derived in terms of analytical mathematics by MIMURA and NISHIURA [21]. The development of multipeak patterns was investigated by HAKEN and OLBRICH [22].

10. – Requirements for pattern formation in terms of rates and orders of reactions, and ranges of diffusion.

Beyond the analysis of particular models it is possible to demonstrate that the conditions of autocatalysis and lateral inhibition are *general* mathematical requirements for pattern formation by two coupled reactions, and this approach has been further extended to systems with more than two reactions [6]. It turned out that short-range activation and long-range inhibition or depletion are conditions for pattern formation starting from spatially near-equal distributions as revealed by the mathematical theory of instabilities. In the following we will briefly demonstrate this point.

Two coupled equations with production terms P and removal terms Q for the components of concentration $a(x, t)$, $b(x, t)$ and with redistribution operators (*e.g.*, diffusion terms) $\mathscr{D}_a$ and $\mathscr{D}_b$ read as follows:

$$\frac{\partial a}{\partial t} = P_a(a, b) - Q_a(a, b) + \mathscr{D}_a(a)\,, \tag{1a}$$

$$\frac{\partial b}{\partial t} = P_b(a, b) - Q_b(a, b) + \mathscr{D}_b(b)\,. \tag{1b}$$

We assume that there is a spatially uniform solution $a = a_0$, $b = b_0$ for a stable state characterized by

$$P_a(a_0, b_0) = Q_a(a_0, b_0)\,, \tag{1c}$$

$$P_b(a_0, b_0) = Q_b(a_0, b_0)\,. \tag{1d}$$

For small space-dependent deviations $a' = a - a_0$, $b' = b - b_0$ from the uniform solution, the linear approximation of eq. (1) can be derived. For a domain of length L with closed boundaries, Fourier components of the distribution of a', b' are of the form

$$a'_n(x, t) = a'^0_n(t) \cdot \cos \frac{\pi n x}{L}\,, \qquad n = 0, 1, 2, \ldots\,, \tag{2a}$$

$$b'_n(x, t) = b'^0_n(t) \cdot \cos \frac{\pi n x}{L}\,, \qquad n = 0, 1, 2, \ldots\,. \tag{2b}$$

In the simplest case redistribution terms of eq. (1) can be described by diffusion terms; for the n-th Fourier component, they read

$$\mathscr{D}_a(a) = D_a \frac{\partial^2 a'_n}{\partial x^2} = -D_a \left(\frac{\pi n}{L}\right)^2 a'_n , \tag{3a}$$

$$\mathscr{D}_b(b) = D_b \frac{\partial^2 b'_n}{\partial x^2} = -D_b \left(\frac{\pi n}{L}\right)^2 b'_n . \tag{3b}$$

We now convert all kinetic terms of (1) into terms of the lateral-inhibition theory: Rates μ, ν are reverse mean lifetimes τ_a, τ_b of molecules, ranges r_a, r_b are defined as mean distances between production and decay as functions of diffusion and decay rate, and orders of reactions are given as pure numbers which indicate, for instance, whether and to which extent reactions are self-enhancing.

Rates in terms of the reciprocal mean lifetimes of molecules are given as

$$\mu = \frac{Q_a(a_0, b_0)}{a_0} = \frac{P_a(a_0, b_0)}{a_0} , \tag{4a}$$

$$\nu = \frac{Q_b(a_0, b_0)}{b_0} = \frac{P_b(a_0, b_0)}{b_0} . \tag{4b}$$

Ranges are determined by the rules of physical chemistry (well known, for instance, in the context of Brownian movement) as proportional to, and approximately equal to, the square root of diffusion constant times the time allowed for diffusion (that is, in our case, the reverse decay rate). We thus define ranges r_a, r_b as

$$r_a = \sqrt{\frac{D_a}{\mu}} , \tag{5a}$$

$$r_b = \sqrt{\frac{D_b}{\nu}} . \tag{5b}$$

Reaction orders are defined as pure numbers:

$$\begin{cases} k_{aa} = \dfrac{\partial \ln P_a}{\partial \ln a} - \dfrac{\partial \ln Q_a}{\partial \ln a}\Big|_{a_0,b_0}, & k_{ab} = \dfrac{\partial \ln P_a}{\partial \ln b} - \dfrac{\partial \ln Q_a}{\partial \ln b}\Big|_{a_0,b_0}, \\ k_{ba} = \dfrac{\partial \ln P_b}{\partial \ln a} - \dfrac{\partial \ln Q_b}{\partial \ln a}\Big|_{a_0,b_0}, & k_{bb} = \dfrac{\partial \ln P_b}{\partial \ln b} - \dfrac{\partial \ln Q_b}{\partial \ln b}\Big|_{a_0,b_0}. \end{cases} \tag{6}$$

For instance, if production P_a of component a is proportional to a^2 (as in the examples eq. (21)-(24) below), and removal Q_a proportional to a^1, k_{aa} is the difference of the powers of a in P_a and Q_a, $2 - 1 = 1$.

We begin by assessing the stability of the spatially uniform solution for a and b; its stability is required because the formation of a reproducible pattern will normally depend on a reproducible near-uniform initial situation. It implies that the uniform distribution is protected against an overall explosion, and has also implications for nonuniform distributions: in that case, the spatial average of a and b is stable in the linear approximation even if local values are not.

Formally, stability of the uniform solution is assessed by setting redistribution terms $\mathscr{D}_a(a)$, $\mathscr{D}_b(b)$ in (1) to 0. By making use of (4)-(6), we may now rewrite (1) in terms of deviations a', b' from a_0, b_0.

The linear approximation reads

$$\frac{\partial a'}{\partial t} = P_a(a,b) - Q_a(a,b) = \left(\frac{\partial P_a}{\partial a} - \frac{\partial Q_a}{\partial a}\right) a' + \left(\frac{\partial P_a}{\partial b} - \frac{\partial Q_a}{\partial b}\right) b' =$$

$$= \mu \left\{ \left(\frac{a_0}{P_a}\frac{\partial P_a}{\partial a} - \frac{a_0}{Q_a}\frac{\partial Q_a}{\partial a}\right) a' + \frac{a_0}{b_0}\left(\frac{b_0}{P_a}\frac{\partial P_a}{\partial b} - \frac{b_0}{Q_a}\frac{\partial Q_a}{\partial b}\right) b' \right\} =$$

$$= \mu \left\{ \left(\frac{\partial \ln P_a}{\partial \ln a} - \frac{\partial \ln Q_a}{\partial \ln a}\right) a' + \frac{a_0}{b_0}\left(\frac{\partial \ln P_a}{\partial \ln b} - \frac{\partial \ln Q_a}{\partial \ln b}\right) b' \right\}.$$

Therefore, with definitions (6) of reaction orders,

$$\frac{\partial a'}{\partial t} = \mu \left(k_{aa} a' + \frac{a_0}{b_0} k_{ab} b' \right). \tag{7a}$$

The corresponding equation for $\partial b'/\partial t$ reads

$$\frac{\partial b'}{\partial t} = \nu \left(\frac{b_0}{a_0} k_{ba} a' + k_{bb} b' \right). \tag{7b}$$

Spatially uniform solutions $a'(t)$, $b'(t)$ are linear combinations of $\exp[\lambda_1 t]$ and $\exp[\lambda_2 t]$, λ_1 and λ_2 being eigenvalues given by the following equation:

$$\begin{vmatrix} \mu k_{aa} - \lambda & \mu \dfrac{a_0}{b_0} k_{ab} \\ \nu \dfrac{b_0}{a_0} k_{ba} & \nu k_{bb} - \lambda \end{vmatrix} = 0 . \tag{8}$$

The two-factor system (1) leads to a stable uniform distribution $a' = b' = 0$ (that is $a = a_0$, $b = b_0$) if both eigenvalues λ_1, λ_2 (8) are negative (or have a negative real part). This is the case if

$$\lambda_1 + \lambda_2 = \mu k_{aa} + \nu k_{bb} < 0 \tag{9a}$$

and

$$\lambda_1 \lambda_2 = \mu\nu(k_{aa} k_{bb} - k_{ab} k_{ba}) > 0 \,. \tag{9b}$$

We may now determine the conditions under which the system is capable of developing spatial patterns starting from near-uniform initial distributions, that is from the uniform solution a_0, b_0 with very slight superimposed variations. Pattern formation occurs if, in case of a slight spatial variation, at least one of the Fourier terms is self-enhancing. Because terms $\partial^2 a/\partial x^2$ and $\partial^2 b/\partial x^2$ are no longer 0, the dynamics of pattern formation is governed by the redistribution terms of eq. (1). The corresponding diffusion constants are expressed according to (5), in terms of ranges $r_a^2 = D_a/\mu$, $r_b^2 = D_b/\nu$, introduced into (3). Rewriting eq. (1) in terms of ranges, rates and orders leads, in the linear approximation, to eq. (7) extended by diffusion terms (3) expressed by ranges r_a, r_b:

$$\frac{\partial a'}{\partial t} = \mu\left(\left(k_{aa} - r_a^2\left(\frac{\pi n}{L}\right)^2\right)a' + \frac{a_0}{b_0} k_{ab} b'\right), \tag{10a}$$

$$\frac{\partial b'}{\partial t} = \nu\left(\frac{b_0}{a_0} k_{ba} a' + \left(k_{bb} - r_b^2\left(\frac{\pi n}{L}\right)^2\right)b'\right). \tag{10b}$$

The corresponding determinant for the assessment of instabilities is

$$\begin{vmatrix} \mu\left(k_{aa} - \left(\frac{\pi n}{L}\right)^2 r_a^2\right) - \lambda & \mu \frac{a_0}{b_0} k_{ab} \\ \nu \frac{b_0}{a_0} k_{ba} & \nu\left(k_{bb} - \left(\frac{\pi n}{L}\right)^2 r_b^2\right) - \lambda \end{vmatrix} = 0 \,. \tag{11}$$

Spatial destabilization and thus pattern formation occurs if there is at least one n for which, because of the diffusion terms described by r_a, r_b, one of the eigenvalues λ_1, λ_2 becomes positive. This is the case if the product $\lambda_1 \lambda_2$ changes sign as compared to the situation (9b) without redistribution:

$$\lambda_1 \lambda_2 = \mu\nu\left\{\left(k_{aa} - r_a^2\left(\frac{\pi n}{L}\right)^2\right)\left(k_{bb} - r_b^2\left(\frac{\pi n}{L}\right)^2\right) - k_{ab} k_{ba}\right\} < 0 \,. \tag{12}$$

If (12) holds, the n-th Fourier wave is self-enhancing. The condition of sign change allows us to combine inequalities (9b) and (12), leading us to a relatively simple necessary condition for pattern formation

$$\left(k_{aa} - r_a^2\left(\frac{\pi n}{L}\right)^2\right)\left(k_{bb} - r_b^2\left(\frac{\pi n}{L}\right)^2\right) < k_{ab} k_{ba} < k_{aa} k_{bb}$$

and thus

$$k_{aa} r_b^2 > -k_{bb} r_a^2 + r_a^2 r_b^2 \left(\frac{\pi n}{L}\right)^2 . \tag{13}$$

11. – Correspondence of inequalities derived from the theory of dynamic instabilities to autocatalysis and lateral inhibition as conditions for pattern formation.

We may now restate the set of conditions for pattern formation by autocatalysis and lateral inhibition, and demonstrate point by point that they are equivalent to the conditions for pattern formation as given by the theory of dynamic instabilities in reaction-diffusion systems.

The lateral-inhibition theory of pattern formation is based on the following conditions *A*)-*E*):

A) One out of the two components a, b (say a) must be self-enhancing.

B) The other component (b) must be cross-inhibiting; inhibition can be substituted by depletion of a substrate required for and consumed by activation.

C) The inhibitory effect must be relatively fast compared to the activating effect.

D) Range of activation must be below a limit of the order of total field size.

E) The range of inhibition must be sufficiently large in relation to the range of activation.

The basis of the derivation are inequalities (9*a*), (9*b*), (12) and (13), which can be rewritten as

$$\mu k_{aa} + \nu k_{bb} < 0 \,, \tag{9a}$$

$$k_{ab} k_{ba} < k_{aa} k_{bb} \,, \tag{9b}$$

$$\left(k_{aa} - r_a^2 \left(\frac{\pi n}{L}\right)^2\right)\left(k_{bb} - r_b^2 \left(\frac{\pi n}{L}\right)^2\right) < k_{ab} k_{ba} \,, \tag{12}$$

$$k_{aa} r_b^2 > - k_{bb} r_a^2 + r_a^2 r_b^2 \left(\frac{\pi n}{L}\right)^2 . \tag{13}$$

The combined inequalities (9*a*), (9*b*), (12) are sufficient for pattern formation. Equation (13) derived from (9*b*) and (12) is necessary but not sufficient. According to (9*a*) at least one of the k_{aa}, k_{bb}—say k_{bb}—must be negative:

$$k_{bb} < 0 \,. \tag{14a}$$

Then both terms on the right-hand side of eq. (13) are positive. Therefore,

$$k_{aa} > 0 \tag{14b}$$

must be positive—implying that one of the reactions has to be autocalatytic. This corresponds to condition *A*).

It follows from (14) that $k_{aa}k_{bb}$ is negative and, therefore, according to eq. (9*b*),

$$k_{ab}k_{ba} < 0 \tag{15}$$

must also be negative; either k_{ab} is negative and k_{ba} positive—this means that activation leads to the production of an inhibitor which inhibits activation; or k_{ab} is positive and k_{ba} negative in which case substance b is required but also depleted by activation. There is no third possibility in the two-factor case. This corresponds to condition *B*).

From (9*a*) and (14*a*), it follows that

$$\frac{\nu}{\mu} > -\frac{k_{aa}}{k_{bb}}. \tag{16}$$

Since k_{aa} is positive and k_{bb} negative, and reaction rates μ and ν are always positive, the ratio ν/μ must be above a positive threshold (which will be of the order of 1 if the parameters describing orders of reaction k_{aa} and $-k_{bb}$ are of the order of 1). This corresponds to condition *C*).

Inequality (12) implies that it is not sufficient for pattern formation that k_{aa} is positive. The entire bracket

$$k_{aa} - \left(\frac{\pi n}{L}\right)^2 r_a^2$$

must be positive for at least one choice of n, that is $n = 1$, and thus

$$\frac{r_a}{L} < \frac{\sqrt{k_{aa}}}{\pi}. \tag{17}$$

It follows that there is an upper limit for the range of activation, in relation to total field size L, corresponding to condition *D*).

The condition of lateral inhibition follows from eq. (12): Pattern formation occurs if the range of inhibition exceeds a threshold. Taking into consideration the signs of the terms in (12) (first bracket positive, k_{bb} negative, the product $k_{ab}k_{ba}$ also negative), inequality (12) is assured if r_b is sufficiently large, corresponding to condition *E*).

A necessary, but not sufficient requirement is eq. (13) which says that

$$r_b^2 > r_a^2 \frac{-k_{bb}}{k_{aa}} + \frac{r_a^2 r_b^2}{k_{aa}} \left(\frac{\pi n}{L}\right)^2 > r_a^2 \frac{-k_{bb}}{k_{aa}}. \tag{18a}$$

It follows that, in any case, the ratio r_b/r_a has to exceed the threshold

$$\frac{r_b}{r_a} > \sqrt{\frac{-k_{bb}}{k_{aa}}}. \tag{18b}$$

More detailed assessments of these conditions have been described elsewhere [6]. It can be shown that, for field sizes which are large compared to the ranges of activation and inhibition, pattern formation depends on the *ratio* of the ranges of inhibition and activation r_b/r_a, and occurs if this ratio exceeds a threshold.

Except for rather special parameter ranges, range of inhibition must exceed range of activation in absolute terms if patterns are to be generated. However, strictly speaking, a smaller range of inhibition would also lead to patterns in cases with strong activation and weak inhibition, cases in which the uniform state is close to instability with respect to an overall explosion of the system. Such parameter ranges are probably more of a mathematical than of a biological interest, but even then the general conditions (17), (18) hold that there is a *lower* limit for the range of inhibition and an *upper* limit for the range of activation. In intuitive terms, this can be explicated by the fact that the range of inhibition *adds* to the spatial extension of the activator distribution; the inhibitor covers an area approximately given by the *sum* of the ranges of activation and inhibition.

We conclude that conditions *A*)-*E*) are necessary for the « self-generation » of a spatial pattern by a two-component reaction-diffusion system. The list of conditions for pattern formation as given by the lateral-inhibition theory is thus equivalent to conditions of destabilization derived by analysis of instabilities in terms of linear approximations for two coupled reactions. This correspondence, however, is neither trivial nor obvious because it depends on the systematic analysis of signs within a set of inequalities.

12. – Pattern formation by more than two components: generalization of autocatalysis and lateral inhibition as system properties.

Is it possible to generalize the conditions derived for the two-factor case to systems with more than two coupled reactions? One might think of classifying the reactions into those with and without autocatalytic terms. This direct method, however, has proven to be inadequate. For instance, activation can be produced by inhibition of inhibition; therefore, a pattern-forming system can be constructed with three components, two of which activating indirectly, in combination, by inhibition of inhibition, while the third gives rise to an inhibitory effect proper; no reaction with direct positive feedback need be involved. On the other hand, an analysis of multicomponent systems is pos-

sible if we resort to the main feature of the lateral-inhibition theory, the ranges of molecules (as given by their diffusion and decay rates), from the outset: By *first* classifying the components into short-range and long-range molecules and *then* determining whether the short-range subset would be unstable if left to itself. This analysis can be carried out by assessing the eigenvalues of the corresponding matrix of the short-range subset. The subsystem is unstable and thus self-activating, if and only if at least one of the eigenvalues is positive. This is one requirement for pattern formation. Further the system as a whole has to be stable in the absence of redistribution just as in the two-factor case (eq. (9)). All eigenvalues have to be negative. This implies that the long-range components exert a *stabilizing inhibitory* effect on the activating subset of reactions which would explode, if left to itself. If these conditions are met, then redistribution by diffusion leads to destabilization and generation of the spatial patterns, if and only if the ranges of molecules of the activating subset are sufficiently small and if the ranges of molecules of the inhibiting subset are sufficiently large. This consideration, demonstrated in detail elsewhere [6], shows that the concepts of autocatalysis and lateral inhibition are widely applicable; on the other hand, we are warned that activation and inhibition need not be features of individual types of molecules. They can be system properties of several reactions; pattern formation may occur even in systems in which no single reaction has a directly self-enhancing property.

13. – A simple « recipe » for the generation of models of pattern formation: power laws.

For the purpose of constructing and assessing models for pattern formation it has proved very useful to employ power laws in the kinetic equations [5]; as further simplifications, we may introduce the assumption, reasonably sustained by enzymology, that removal reactions Q_a, Q_b (eq. (1)) are linear relations with respect to a, b, and that the powers are integers specifying orders of reaction. This leads to equations of the type

$$\frac{\partial a}{\partial t} = c\,\frac{a^k}{b^l} - \mu a + D_a \frac{\partial^2 a}{\partial x^2}\,, \tag{19a}$$

$$\frac{\partial b}{\partial t} = c'\,\frac{a^m}{b^n} - \nu b + D_b \frac{\partial^2 b}{\partial x^2}\,. \tag{19b}$$

We may now state the conditions for relations between the powers k, l, m, n which lead to patterns for suitably chosen parameter ranges of the other constants involved, such as D_a, D_b, and c, c'. The condition for autocatalysis (14) is simply

$$k_{aa} = k - 1 > 0\,, \quad \text{and thus} \quad k \geqslant 2\,. \tag{20a}$$

In the simplest case, $k = 2$, condition (9*b*) reads

$$ml > n + 1 \,. \tag{20b}$$

A few particularly simple models will now be listed.

i) Activator (a)—inhibitor (h) model; no self-inhibition of inhibitor production ($n = 0$):

$$\frac{\partial a}{\partial t} = c\,\frac{a^2}{h} - \mu a + D_a \frac{\partial^2 a}{\partial x^2}\,, \tag{21a}$$

$$\frac{\partial h}{\partial t} = c'\,a^2 - \nu h + D_h \frac{\partial^2 h}{\partial x^2}\,. \tag{21b}$$

This is one of the simplest models conceivable; it is the one on which many of our biological simulations were based and which has been analysed and applied to a considerable extent in the literature.

ii) Model with inhibitor production in proportion to activation ($m = 1$, $n = 0$):

$$\frac{\partial a}{\partial t} = \frac{ca^2}{h^2} - \mu a + D_a \frac{\partial^2 a}{\partial x^2}\,, \tag{22a}$$

$$\frac{\partial h}{\partial t} = c'\,a - \nu h + D_h \frac{\partial^2 h}{\partial x^2}\,. \tag{22b}$$

iii) Model with similar production terms for activator and inhibitor ($k = m$, $l = n$); this could be interpreted, for instance, as kinetics of release of both activators and inhibitors from the same type of vesicles:

$$\frac{\partial a}{\partial t} = c\left(\frac{a}{h}\right)^2 - \mu a + D_a \frac{\partial^2 a}{\partial x^2}\,, \tag{23a}$$

$$\frac{\partial h}{\partial t} = c'\left(\frac{a}{h}\right)^2 - \nu h + D_h \frac{\partial^2 h}{\partial x^2}\,. \tag{23b}$$

Depletion models ($k_{ab} > 0$, $k_{ba} < 0$) implying that inhibition is brought about indirectly by depletion of a substance (concentration $s(x, t)$) which is required for and consumed by activation can be analysed along similar lines. An example is given by

$$\frac{\partial a}{\partial t} = ca^2 s - \mu a + D_a \frac{\partial^2 a}{\partial x^2}\,, \tag{24a}$$

$$\frac{\partial s}{\partial t} = c' - \nu' a^2 s + D_s \frac{\partial^2 s}{\partial x^2}\,. \tag{24b}$$

Further, one may inquire whether the two-factor system can be conceptually simplified by assuming that the inhibitor is a product of activator degradation, or that the depleted substrate is a precursor of the activator. The answer is positive. For instance, in the model equation (22) we may set $c' = \mu$ in eq. (22*b*) and then interpret the model as describing a system in which the product of activator degradation is the inhibitor. Pattern formation requires that activator degradation strongly increases the diffusion range of the molecule, for instance by reducing its size or by the removal of charges such that it is able to pass easily through junctions from cell to cell.

The basic models that we have listed meet the conditions for pattern formation easily and not just by a narrow margin. This is because we assumed integer values $k = 2$ for the autocatalytic effect, leading to k_{aa} (eq. (20*a*)) $= 1$ and not, say, 0.05, just above the critical threshold 0. This makes sense in biological terms because mechanisms should be robust against minor fluctuations and distortions. It is also useful for the construction of more involved models, because, then, the equations can be used as asymptotic criteria for complex systems which incorporate detailed and realistic features. For instance, mathematicians will be quick in pointing out that they do not like models of the type (21)-(23) because terms $1/h$ go to infinity at low concentrations of h, and physical chemists will insist that enzyme kinetics leads to inhibition terms of the Michaelis-Menten type $1/(1 + \text{const} \cdot h)$, and not $1/h$. However, substitution of terms $1/h$ by $\text{const} \cdot 1/(1 + K_m h)$ does not affect the pattern-forming capacity of the system if K_m is sufficiently large. Another feature that can easily be introduced is a constitutive production of activator, or inhibitor, or both. Adding such terms is again consistent with pattern formation if the terms are sufficiently small. Therefore, the simple equations with power terms are very useful in assessing even complex reaction systems, incorporating many features, as to the capacity for pattern formation.

14. – Biological pattern formation, synergetics and the theory of dynamic instabilities.

Since this lecture is part of a course on synergetics and dynamic instabilities it appears appropriate to ask for the relevance of these concepts for understanding biological development. The answer is that biological pattern formation is closely related to synergetics in that self-generating mechanisms with autocatalytic characteristics are involved, whereas the relation to dynamic instabilities is less direct: true random symmetry breaking is not a dominating feature in the development of an animal. The most conspicuous spatial structures, say of a rhino, arise reproducibly at defined stages in defined places with defined orientation, being generated under the control of the genes. Nevertheless, the theory of dynamic instabilities is of interest for modelling develop-

ment, because spatial order often depends on the amplification of slight pre-existing cues causing induction or orientation of the structure. For instance, the reproducible orientation of a substructure may be directed by a very shallow initial gradient; in logical terms, this is in contrast to symmetry breaking because the orientation is strictly included in the initial conditions; mathematically, however, the process is very closely related to true symmetry breaking because in the latter case a random fluctuation produces a very slight asymmetry in the first place, which in turn leads to a polar pattern of *unpredictable* orientation. For this reason, the mathematics of dynamic instabilities is useful for models of primary biological pattern formation.

Generally speaking, the explanation of properties of biological systems including development of spatial organization of an organism eventually requires the *combination* of molecular biology and mathematics in order to reveal how systems of interacting and moving molecules generate a given spatial order. Up to now the relation between mathematicians and theoreticians, on the one hand, and experimentalists, on the other, has not been as fruitful in developmental biology as it was in physics and physical chemistry. This has, in part, psychological reasons: many biologists still believe that every issue will eventually be settled by molecular biology alone, whereas actually even the complete list of all molecules involved in pattern formation would not elucidate the actual pattern produced by them unless we analyse the corresponding system in mathematical terms. On the other hand, theoreticians often refrain from adapting theoretical concepts to biological facts. The main issue nowadays is not how structures can be formed altogether but how the specific and impressive features of biological regulation are to be accounted for. This requires stating main biological features at the outset and then proceeding to the specific search for adequate mathematical theories and models. Further, it is necessary to avoid distortions of biological facts for the sake of analytical mathematics, and to refrain from raising artificial problems which are irrelevant for biology. Two examples of the latter type will be mentioned: Primary patterns such as morphogenetic gradients have to be relatively stable to exert their organizing effect in the course of biological development. However, life is finite, and, therefore, absolute stability of solutions of equations for pattern formation is not a biologically acceptable criterion for the quality of mathematical models. Another example is sensitivity of patterns to boundary conditions. Pattern formation by reaction-diffusion mechanisms strongly depends on boundary conditions in cases in which the ranges of activation and inhibition are small compared to the size of the field at the stage of pattern initiation. In such cases, multiple-peak patterns are formed; small distances between peaks are avoided, but otherwise the distribution of peaks is highly sensitive in detail to initial and boundary conditions including slight random fluctuations. However, this feature of reaction-diffusion systems should not be taken as a challenge to its applicability

in biology by questioning whether the reproducible structure of organisms can be determined in this way: There is no biological evidence that reproducibility, multiple-peak pattern and random initiation occur together.

The complexity of biological structures appears to result from the complexity of cell responses to simple fields such as gradients, and from the combination of patterns and subpatterns. Gradients themselves, as primary specifiers of « positional information », are rather robust with respect to minor variations in initial and boundary conditions which may affect polarity, but generally they do not change the form of the gradient; polarity reversal is indeed observed in some biological systems, such as regeneration of coelenterates under certain artificial conditions. In exceptional cases, symmetric distributions are formed instead of polar structures, for instance a hydra with feet at both ends and a head in the middle; this feature can also be explained on the basis of pattern formation by autocatalysis and lateral inhibition: if total field size is sufficiently large compared with the range of activation, symmetric instead of polar fields can be produced. In cases in which *periodic* patterns are *reproducibly* formed, as, for instance, in the formation of leaf rudiments in plants, peaks of activation are *not* randomly initiated but produced sequentially in a recursive manner in the course of growth, the position of the youngest rudiment being defined by the range of inhibition extending from the preceding one. Certain multipeak patterns such as that of stomata in plant leaves appear to be randomly initiated; but in this case the pattern is not reproducible: whereas the density of stomata and their tendency to avoid small distances (« second-order distribution ») is determined, each leaf on a tree is different in detail with respect to the distribution of the stomata. In other words, the theory of pattern formation by autocatalysis and lateral inhibition is in remarkable agreement with the biological results on robust as well as sensitive features of patterns formed in actual morphogenesis.

Generally, one expects that the understanding of spatial organization in biological development is to be based on a combination of embryological, biochemical, physical and mathematical studies. As far as the contribution of mathematical physics is concerned, synergetics may contribute in an important way, and the theory of dynamic instabilities in a helpful manner towards a better understanding of biological development.

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