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GENERATION OF BIOLOGICAL PATTERNS AND FORM: SOME PHYSICAL, MATHEMATICAL, AND LOGICAL ASPECTS

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SUMMARY

While many different mechanisms contribute to the generation of spatial order in the development of an organism, the formation of morphogenetic fields within initially near- uniform cells and tissues which in turn direct cell responses giving rise to pattern and form are of major importance and an essential part of the biological generation cycle. The chemical nature of such fields is not yet known, but it is most likely that they represent spatial concentration patterns of substances (or states of substances) produced by molecular kinetics. Short-range autocatalytic activation in conjunction with longer range inhibition or depletion effects suffice to generate such patterns. These conditions are necessary for the simplest two-factor case and are likely to be fair approximations in multi-component systems in which activation and inhibition may be system parameters, subsuming the action of several agents. The conditions of autocatalysis and lateral inhibition can be shown to be closely related to the analytical results obtained by general stability analysis, although this correspondence is not trivial and not immediately obvious. Very different molecular mechanisms are consistent with the concept, but fairly simple mechanisms known in molecular biology, in suitable

combination, would be sufficient. Gradients, symmetric and periodic patterns in one or several dimensions, stable or pulsing in time can be generated in this way.

The pattern-forming systems account for striking self-regulatory features often observed in biological systems, including the reproducible formation of structures from near- uniform initial conditions, polarity, size-regulation and symmetry changes; quantitative aspects of biological systems, such as regenerating hydra and developing insects have been modelled on this basis. The theory is applicable to intercellular as well as intracellular patterns.

Generalization to more than two components, with activation and inhibition representing system features, shows that "lateral activationm-mutual activation of two locally exclusive states in adjacent regions, mediated by diffusible substrates-can be subsumed under a generaliza-tion of the condition of lateral inhibition in conjunction with autocatalysis. Chains of induction as they seem to occur in intercalary regeneration can be modelled by further generalizations along these lines.

In multicellular tissues, cells may respond to morphogenetic fields by cell determination and differentiation, changes of cell form and interaction, and the regulation of growth and proliferation. Cell determination occurs in development as a sequence of decisions between relatively few alternatives at each stage. There are indications that determination may be combinatorial, with a combination of control circuits turned "on" defining each state of determination. Morphogenetic fields are expected to contribute to control these decisions. In this way, areas may become subdivided into discrete subareas. Combinatorial regulation would imply that there could be an area code in morphogenesis (not necessarily for all tissues, cell layers, and cell types), each area being defined by a different combination of control circuits turned "on" to define the regulatory state of the cells.

Real form of an organ or organism can often be traced back to the curvature of cell sheets resulting from evagination or invagination of initially nearly flat sheets in the course of development, the position of evagination being determined by morphogenetic fields. Experimental evidence indicates that often bending moments rather than tangential forces govern the process and that evagination is self-regulating, reversible and sometimes re- peatable. This in turn suggests that the process is not time-controlled but rather describable as approach to a stable steady state maintained some time (though not indefinitely) in the course of development and depending on the local value of the morphogenetic field. The form assumed upon evagination may then be describable as minimizing a formal generalized potential. lntracellular mechanisms, e.g. involving intracellular fibres, and contact-mediated cell interaction with other cells, and with medium may contribute to potential in a complex manner. It can be shown that linear relations between potential and (idealized) surface areas of a cell are inconsistent with a freely evaginating stable cell sheet, but simple non-linear features (e.g. due to capping of surface components) are consistent with stability. Local activation of

cell sheets leads to bending moments, curvature and the generation of form if the sheets are anisotropic in the inside-outside dimension (as many cell sheets obviously are). Shell theory as developed by architects and engineers is useful for calculating form of cell sheets. Elongated structures can be modelled on this basis. It is not claimed that the concept of minimal potential is always applicable-there will be cases requiring explicit treatment of friction, shear and pressures arising from growth; but in the cases where the self-regulatory properties mentioned are experimentally observed it is likely that the concept of minimal potentials is adequate.

The essential requirement for non-linear effects of cell form and contact areas for under- standing stability, curvature and structure of cell sheets suggests that these parameters may also contribute in a subtle non-linear manner to the regulation of cell proliferation and tissue growth. This notion might have consequences for the understanding of malignant growth as well.

The theoretical treatment of morphogenetic fields discussed in this paper indicates that relatively simple molecular kinetics (consistent with conventional molecular biology) suffice to explain basic empirical features of biological pattern formation and regulation. Pattern formation based on autocatalysis and lateral inhibition is formally analogous in some respects to the generation of structures in inorganic physics and economics. However, biological pattern formation has the distinct feature of leading to highly complex re-producible structures, under the control of genes, by the invariance of the pattern formed with respect to many details and fluctuations of initial conditions, and by the defined localization and orientation of patterns, subpatterns, sub-subpatterns etc. in the course of development.

Clearly the concepts used in the theories proposed and discussed have to be substantiated and confirmed by biochemical evidence; however, if the mechanism is within the scope of general kinetics on which the theory is based, even a complete list of bio- chemical structures involved would not in itself lead to an explanation of biological patterns. Rather, the mathematics linking molecular kinetics with developmental regulation con-stitutes, by itself, a necessary aspect for the understanding of spatial order in biological development.

CONTENTS

I. GENERATION AND REGULATION OF BIOLOGICAL PATTERNS: BASIC TYPES

One of the most impressive features of higher organisms is their highly specific form, reproducible through virtually unlimited numbers of generations. In each generation, starting from an egg cell, a complex multicellular animal is produced under the instruction of the genes. Many different mechanisms are involved in this process, including proliferation, determination, differentiation, interaction, movement and death of cells with defined order in space and time.

Though patterns are essentially determined by genes, it is obvious that nucleotide sequences cannot affect directly the pattern of the organism to be formed. Rather, this determination is mediated by general metabolism; how can biochemistry impose spatial order on developing organisms? It is true that spatial order does not start from scratch upon fertilization. Oocytes may be internally structured to a considerable extent, but the specificity of internal structure is lower by several orders of magnitude as compared with that of a complete animal, and oocytes themselves develop from almost unstructured ooblasts. Though some spatial cues may be derived from preexisting structures (such as cues to orientation), the spatial order in an organism is essentially generated de novo by internal mechanisms in the course of embryogenesis. How can this occur under the direction of the genes on the basis of molecular and cellular biology?

We may distinguish a few basic types of mechanisms which can generate order in space: Self-assembly, order in time, and internal generation of structure within initially near-uniform areas. If different components, such as molecules or cells, are produced at random positions, they may nevertheless move and interact, forming various contacts until they assume a well defined, energetically favoured configuration. An example is crystallization. Subcellular structures such as ribosomes, chromatin, membranes and fibres are probably formed by self-assembly. At the cellular level, different cell types mixed at random can often form defined configurations by sorting out, leading for instance to a structure with one cell type in the center, the other at the periphery, or to multiple-layered structures. Probably such processes also occur in the course of embryogenesis, but the major events in forming multicellular organisms are not based on self-assembly of preexisting randomly distributed cell types. It appears that self-assembly of cells reflects more the property of stabilizing certain (e.g. layered) structures and of expressing specific chemical markers on the surface of different cell types, rather than an important embryological mechanism itself.

Order in time is essential to understand development in general. Many different cell types are produced by subsequent steps of determination of precursor cells; each step can be considered as a decision between alternative pathways in cell lineage. Development of spatial order depends on a defined sequence of the formation of structures, substructures, sub-substructures etc. Moreover, mechanisms are conceivable that convert order in time directly into order in space: if a structure grows out with growth proceeding predominantly near a marginal (say distal) zone, different parts are laid down at different times, the most distal part being made last. If the growth zone changes in time (for instance, as a function of the number of cell divisions) different chemical regulatory substances may be active at different stages of outgrowth leading to different properties in proximo-distal order (Summerbell *et al.*, 1973). There is not yet a specific proof for such mechanisms, but they are highly likely to be involved somehow in embryology.

On the other hand, most primary structures of the early embryo as well as early rudiments of substructures are neither made by self-assembly nor by sequential marginal growth; rather, small initially near-uniform areas of embryonic tissue generate strikingly different parts by internal mechanisms, be it with or without growth. This process appears to be an important part of the generation cycle and is noteworthy for its striking self-regulatory properties. The conventional explanation which is most likely to be correct is that in a first stage a morphogenetic field is formed, made up of a spatial distribution of some physical properties. In the simplest case it may be a gradient of a morphogenetic substance (Child, 1929) giving rise to a one-to-one correlation between substance concentration and position to specify "positional information" (Wolpert, 1971) in the tissue. Symmetric, periodic and other distributions can also be envisaged, forming an abstract, invisible "prepattern" of the structure to be formed. (We will use the notion of morphogenetic field and prepattern synonymously for any morphogen distribution affecting patterns, because a distinction is somewhat ambiguous and intuitive, the term "prepattern" suggesting a relatively high degree of isomorphy with the real patterns eventually formed.) Cells are assumed to respond to local values of the morphogenetic fields by differentiation, proliferation, movement, interaction, death etc. giving rise to visible structure and form.

Candidates for morphogenetic substances have been found (e.g. Tiedemann, 1968),

and it has been established that very low levels of organic substances occurring naturally in animals suffice to influence morphogenesis (Schaller, 1973; Berking, 1977). However, it is still difficult to prove beyond doubt that a given substance is involved in the establishment of the primary morphogenetic fields and not, for instance, in mediating and regulating their effect on cells, because there is not yet a direct assay for such primary morphogens. Therefore, at present, the chemical substrate of morphogenetic fields is still unknown.

On the other hand, there is much and most impressive empirical evidence on biological properties, especially the self-regulatory capacities of morphogenetic fields. Structure forms from near-uniform initial conditions and must be insensitive to details and fluctuations of such conditions. A striking feature is size-regulation: A part often forms a whole at reduced size, with correct proportions of the sub-structures; for instance, certain early embryos cut in halves produce two complete animals. An important regulatory feature is response to cues to orientation, called polarity. Many structures and substructures (e.g. the legs) are asymmetric, and develop with predictable orientation within the animal. Therefore, they cannot arise by random symmetry breaking, but must be oriented by some cue from the preexisting structures.

A further regulatory feature is induction: Certain transplants can induce a secondary structure; in the gastrula, a secondary embryo can be induced (Spemann and Mangold, 1924). The host tissue surrounding the transplant forms, or at least participates in forming, the secondary structure. Aside from the activating effects of induction there is evidence for inhibitory effects in pattern formation: Existing structures are often surrounded by inhibitory fields preventing a similar structure from being formed in their immediate environment. On this basis regular spacings of structures, such as leaf rudiments in plants, can be explained.

One of the biological systems that shows all these basic self-regulatory properties in a relatively simple fashion is Hydra (Fig. 1), which was one of the earliest experimental systems in the history of developmental biology (second only to the chick embryo), and has long been known for its striking regenerative capacities (Trembley, 1744). Any tissue cut out from the body column regenerates a new animal with head and foot and does so without excessive growth and even in the absence of growth (Clarkson, 1969), essentially remodelling existing tissue of the body column into head and foot. Pieces of future head areas of early regenerates can induce secondary heads if transplanted into the body column of another host, showing that morphogenetic fields activating future head areas are actually involved in determining head structures, and develop newly and rapidly in the process of regeneration (Webster and Wolpert, 1966; MacWilliams, 1981). Depending on the section cut, the same area of the body column can produce a head or a foot upon regeneration of the section. Therefore, the decision where to form a head does not depend on a local cell property or the occurrance of a cut, but results from cell communication across the regenerating section which leads to the formation of the morphogenetic field that activates the future head. *Hydra* tissue shows proximo-distal polarity: In sections of the gastric column the head develops in most cases in the part of the regenerate which has been closest to the original head. Experiments in which animals have been reconstructed and regenerated from aggregates of previously isolated cells have shown that this tissue polarity is due to a graded scalar property, probably the concentration of cell constituents, cell types, or both (Gierer *et al.*, 1972). Polarity determines the orientation of the morphogenetic field, but the polarity-defining gradient cannot be the morphogenetic field itself, which is newly formed after the onset of regeneration. Size regulation occurs, small tissues giving rise to small heads, big pieces of tissue to big heads (Bode and Bode, 1980). Induction is observed as the capacity of certain transplants to induce secondary heads, and inhibition by the effect of preexisting heads inhibiting certain transplants from inducing second heads in their immediate environment (Webster and Wolpert, 1966; MacWilliams, 1981).

Hydra is an example of another effect of pattern regulation, a striking all-or-none change affecting symmetry. Certain chemicals can upset the asymmetry of the regenerate altogether, leading for instance to a coelenterate with feet at both ends of a regenerate and a head in the middle (Hicklin *et al.*, 1969).

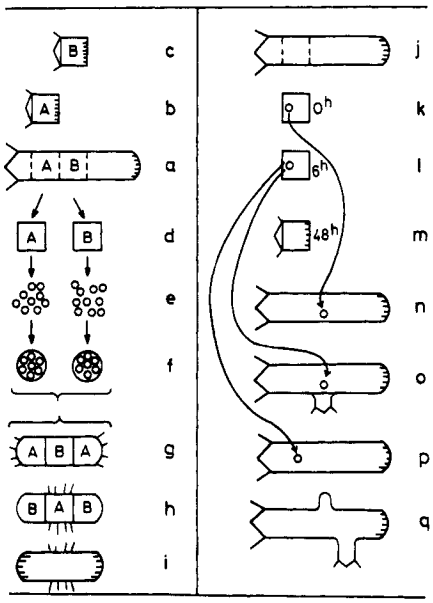

FIG. 1. Pattern regulation exemplified by hydra (regulatory properties are summarized in a schematized and simplified representation). a–c. Any section of the hydra column regenerates an animal; regeneration is polar, the head pointing toward the original head. d–h. Polarity is due to a graded property, probably a substance gradient, and not to cell orientation: animals regenerate from aggregates of previously disaggregated cells; if cells are derived from parts of the gastric column more close (A), or more distant (B) from the head (d, e), and aggregates (f) are grafted in serial order A-B-A (g) or B-A-B (h), heads regenerate predominantly in the A region. i. Certain chemical treatments can induce regeneration of symmetric animals with feet at the ends and a head in the middle. j–p. Morphogenetic fields determine future head areas. They are formed rapidly upon regeneration: a transplant including the future head area of a regenerate (l) can induce head formation after 6 hours of regeneration at a position of the host animal (o) where a 0-hour transplant is ineffective (k, n). Transplants are ineffective in positions too close to the preexisting head (p), demonstrating an inhibitory field surrounding the latter. The range of mutual inhibition of centers of activation determines bud spacing at alternate sites of the column (q).

The self-regulatory properties of developing systems have been a challenge for attempts to account for biological structures on the basis of physics. They do in fact demonstrate that the pattern to be formed is rather unrelated to preexisting spatial order, however hidden. Though its orientation can be determined by preexisting spatial cues determining polarity, the pattern itself must be made anew, involving spatial communication across the entire area in which the pattern is produced.

II. PATTERN FORMATION BY AUTOCATALYSIS AND LATERAL INHIBITION

1. *Spatial Concentration Patterns and General Chemical Kinetics*

How can we account for these internally generated patterns on the basis of physics? Since we do not yet know the biochemical or physical nature of the fields, we have to introduce an assumption as to the general class of physics the mechanism belongs to. If we assumed that the basic phenomenon were magnetism, we would then try to understand it in terms of Maxwell's equations. The realistic assumption seems to be that morphogenetic fields have the same basis as other biological phenomena which have physical explanations thus far; namely that they are primarily due to the interaction and movement of molecular compounds in plasma, on subcellular structures, on membranes or in extracellular space. The laws of physics applicable to this domain are such that the concentration of any compound $c_i(x, t)$ changes in time as function f_i of the concentration of the various compounds, to account for interaction, whereas movement due to diffusion, convection,

conduction and other processes can be described by redistribution operators $\mathscr{D}_i(c_i)$ (in the simplest case by diffusion terms $D_i \cdot \partial^2 c_i / \partial x^2$

$$\frac{\partial c_i}{\partial t} = f_i(c_1 \ldots c_N) + \mathscr{D}_i(c_i), \qquad i = 1 \ldots N \tag{1}$$

Such equations are not the most general description of anything molecular, and should not be postulated in a dogmatic way. There may be directed, vectorial processes (e.g. co-operative orientation of cells or fibres or directed pumping mechanisms) and processes with an involved interaction of space as well as time parameters (e.g. involving autocorrelation, or a rapid change of distribution parameters—such as diffusion constants—in time) which may not be easily reducible to sets of equations of type (1). Moreover, to account for the self-regulatory properties of fields, emphasis is placed on near-steady state solutions of a system of type (1), and this emphasis may not be adequate in all cases; but this being said, eqn. (1) represents a vast domain of general kinetics, and it is highly likely that it covers elementary phenomena of the generation of morphogenetic fields based on molecular biology.

Equations of type (1) are rather non-committing with respect to details of molecular mechanisms. They represent an attempt to "demystify" morphogenetic fields proposing that they are due to conventional molecular biology and nothing else; and yet, they impose stringent constraints on the construction of theories and models. Many explanations that sound convincing if expressed in words, prove awkward or even inconsistent if expressed in terms of eqn. (1). One may go as far as ask whether spatial pattern can be generated on this basis at all. That this is the case was shown by Rashevsky (1940) and Turing (1952): If there are at least two components interacting auto- and crosscatalytically and if there is re-distribution by diffusion, then, under certain conditions, spatial concentration patterns can be generated. The mathematical features of such systems, especially with respect to stability analysis, have been elucidated by Gmitro and Scriven (1966), Glansdorff and Prigogine (1971), Prigogine and Nicolis (1971), Segel and Jackson (1972) and others.

2. *Pattern Formation by Autocatalysis and Lateral Inhibition: Outline of Theory*

To explain biological phenomena, general kinetics of the type eqn. (1) are too complex and varied. Aiming at a more specific theory, we have asked the following question: Can we define a set of basic conditions required to generate patterns on the basis of molecular kinetics eqn. (1), which are interpretable by features of biological molecules or systems of such molecules; and which at the same time permit us to derive and model for the self-regulatory features of morphogenetic fields described above which are the testing ground for any physical explanation? In analysing this question, we have found the condition of "lateral inhibition" in conjunction with autocatalytic activation (Gierer and Meinhardt, 1972, 1974): a requirement is a self-enhancing feature, coupled to the generation of inhibitory effects which extend into a wider area as compared to activation. Then local activation is self-enhancing, so that some slight local initial advantage can develop into a striking activation, but the production and spreading of inhibitory effects prevents an overall autocatalytic explosion, causing activation in part of the area to proceed only at the expense of deactivation elsewhere until a stable pattern is formed. The forms of patterns are essentially determined by the ranges of activation and inhibition, the range being the mean distance between production and decay or removal of respective compounds. These conditions are necessary for the simplest two-factor case; as will be discussed further below, they may be generalized to more complex systems.

For two factors, a, and, b, equations of type (1) read

$$\frac{\partial a}{\partial t} = f(a, b) + \mathscr{D}_a(a) \tag{2a}$$

$$\frac{\partial b}{\partial t} = g(a, b) + \mathscr{D}_b(b) \tag{2b}$$

$\mathscr{D}_a$, $\mathscr{D}_b$ are redistribution operators, giving rise, in the simplest case, to diffusion terms $D_a \cdot (\partial^2 a)/\partial x^2$, $D_b \cdot (\partial^2 b)/\partial x^2$. Patterns are to be generated from near-uniform initial conditions by autocatalytic mechanisms, but the form of the pattern should be relatively insensitive to details of initial conditions, activation is to be localized, and an overall autocatalytic enhancement is to be prevented. This requires that there is a uniform solution for $f = 0$ and $g = 0$ (Eqn. 2) at some values a_0 and b_0. The solution is to be stable for uniform distributions (and thus for the spatial average $\bar{a}$ and $\bar{b}$ in case of small deviations from the uniform distribution) and labile for local deviations. In formal terms, this leads to the following conditions for pattern formation:

(A) One of the components (say a) has to be activating in the sense of self-enhancement.

$$\left(\frac{\partial f}{\partial a}\right)_{a_0,b_0} > 0 \tag{3}$$

(B) The other component (b) must be cross-inhibiting to prevent an overall autocatalytic explosion and to render the average values $\bar{a}$, $\bar{b}$, near the uniform distribution stable despite the autocatalytic term. Inhibition can be substituted by depletion of a compound required for activation.

(C) The inhibitory effect must be sufficiently strong to ensure stability of the uniform solution and thus of average values of activation in case of small deviations from this solution.

This stability can easily be assessed in various ways. We may assume that two requirements for pattern formation discussed below are met: fast and wide redistribution of b. For the asymptotic case of extremely fast and wide redistribution, the solution of $g = 0$ implies, according to eqn. (2b), that b is a function of some spatial average of a.

$$b = b(\bar{a}) \tag{4}$$

After insertion of eqn. (4) in eqn. (2a) the condition for stability of the uniform solution $a = a_0$, $b = b_0$ reads

$$\left(\frac{\mathrm{d}f(\bar{a}, b(\bar{a}))}{\mathrm{d}\bar{a}}\right)_{a=a_0} = \left(\frac{\partial f}{\partial a}\right)_{a_0,b_0} + \left(\frac{\partial f}{\partial b}\right)_{a_0,b_0} \cdot \left(\frac{\partial b}{\partial \bar{a}}\right)_{a_0} < 0 \tag{5}$$

(D) The inhibitory reaction must be relatively fast as compared to the activating one.

It is evident that, if the inhibitory reaction were extremely slow, it would not be able to prevent an autocatalytic explosion. At intermediate levels inhibition may follow activation with a lag phase leading to patterns pulsing in time.

Further conditions refer to ranges, defined as mean distances between production and decay of compounds; for a diffusion constant D and decay rate β, range is of the order of $\sqrt{(D/\beta)}$. It is not surprising that such space parameters essentially determine the spatial characteristics of the pattern to be generated. In terms of ranges, the conditions read:

(E) Range of activation must be below a limiting value of the order of total field size.

(F) Range of inhibition must be sufficiently large in comparison to range of activation.

If range of activation were larger than total field size, any activating effect would be redistributed within the field, preventing the formation of a pattern. If the range of inhibition were too small as compared with the range of activation, the inhibitory effect would not be redistributed sufficiently to permit the local activation to proceed under conditions where average activation is stable. This aspect, which has been called "lateral inhibition" in analogy with the use of this term in neurophysiology and visual perception is basic to an understanding of such self-generating patterns.

3. *Generating Principle for Models of Pattern Formation: Power Laws*

For the generation of equations and models of the type eqn. (2), and their assessment with respect to pattern formation it is useful to give them more detailed and specific forms. In particular, we may distinguish production terms P and removal terms Q:

$$\frac{\partial a}{\partial t} = P_a(a, b) - Q_a(a, b) + \mathscr{D}_a(a) \tag{6a}$$

$$\frac{\partial b}{\partial t} = P_b(a, b) - Q_b(a, b) + \mathscr{D}_b(b) \tag{6b}$$

If production and removal follow power laws,

$$P_a \sim a^{i_1} b^{i_2}; \quad Q_a \sim a^{i_3} b^{i_4}; \quad P_b \sim a^{i_5} b^{i_6}; \quad Q_b \sim a^{i_7} b^{i_8} \tag{7}$$

then with

$$n = (i_4 - i_2) \cdot (i_5 - i_7)/(i_8 - i_6); \quad m = i_1 - i_3 \tag{8a}$$

conditions for pattern formation equivalent to condition *A* and *C* are, near the uniform solution $P_a = Q_a$; $P_b = Q_b$,

$$n > m > 0 \tag{8b}$$

$m > 0$ is the condition for destabilization eqn. (3), and n > m the condition for stability of the uniform solution eqn. (5). Cross- stabilization corresponding to condition B by inhibition or depletion requires $i_8 - i_6 > 0$. Inhibition implies $i_2 < i_4$, $i_5 > i_7$; depletion corresponds to $i_2 > i_4$, $i_5 < i_7$.

Models combining short-range activation, long-range inhibition, and auto- and cross-catalysis can be generated and assessed for stability on the basis of conditions A–F, especially with the help of the simple form of eqn. (8). It allows the assessment of orders of reactions (representing, e.g. types of allosterism for enzymes or receptors) leading to a non-trivial classification of models that do or do not lead to patterns. If removal kinetics of activator is linear, autocatalysis must be "overlinear", e.g. of second order. Two examples meeting conditions eqn. (8) are a model with activator $a(x, t)$ and inhibitor $h(x,t)$

$$\frac{\partial a}{\partial t} = \mu\left(\frac{a^2}{h} - a\right) + D_a \cdot \frac{\partial^2 a}{\partial x^2} \tag{9a}$$

$$\frac{\partial h}{\partial t} = \nu(a^2 - h) + D_h \cdot \frac{\partial^2 h}{\partial x^2} \tag{9b}$$

and a depletion model where autocatalysis is counteracted by depletion of a substrate $s(x, t)$ required for activation

$$\frac{\partial a}{\partial t} = \mu(a^2 s - a) + D_a \frac{\partial^2 a}{\partial x^2} \tag{10a}$$

$$\frac{\partial s}{\partial t} = \nu(1 - a^2 s) + D_s \frac{\partial^2 s}{\partial x^2} \tag{10b}$$

In eqn. (9) and (10) scales for concentrations have been arbitrarily chosen so that the uniform solution for eqn. (9) is $a = h = 1$, and for eqn. (10) $a = s = 1$. Pattern formation requires that inhibition or depletion is a relatively fast process ($\nu > \mu$), and is redistributed widely ($D_h \gg D_a$ in eqn. (9); $D_s \gg D_a$ in eqn. (10)).

Further, conversion models have been proposed which introduce a simplification of the two-factor mechanism of pattern formation, assuming that they are not produced independently, but one is the degradation product of the other (Meinhardt and Gierer, 1974). In depletion models, the depleted substrate may be the precursor of the activator; in inhibition models, the inhibitor may be the product of activator degradation. We have shown that

such models lead to pattern formation by autocatalysis and lateral inhibition. An example is described by the equations

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

$$\frac{\partial h}{\partial t} = \mu a - \nu h + D_h \frac{\partial^2 h}{\partial x^2} \qquad (\nu > \mu)$$

The inhibitor h produced by activator degradation is assumed to have a larger diffusion rate as compared with activator, for example because of a reduction in size of the molecule or because of removal of charged groups that may interfere with passage from cell to cell.

4. *Models Based on Asymptotic Validity of the Power Laws*

In assessing mechanisms and models it is particularly useful to apply the principle of asymptotic validity of the power laws outlined in the preceding section. Any equations with any number of terms lead to patterns for finite ranges in parameter space if they can be approximated by equations with power terms as described above. Additional terms should be small if they are additive, and vary only slightly in space if they are multiplicative. Thus an extension of eqn. (9) reads:

$$\frac{\partial a}{\partial t} = \rho_0(x) + \frac{c\rho(x)a^2}{h} - \mu a + D_a \frac{\partial^2 a}{\partial x^2} \tag{11a}$$

$$\frac{\partial h}{\partial t} = c'\rho'(x) \cdot a^2 - \nu h + D_h \frac{\partial^2 h}{\partial x^2} \tag{11b}$$

and further extensions lead to

$$\frac{\partial a}{\partial t} = \rho_0(x) + c\rho(x) \cdot \frac{a^2}{1 + \kappa a^2} \cdot \frac{1}{\gamma + h} - \mu a + D_a \frac{\partial^2 a}{\partial x^2} \tag{12a}$$

$$\frac{\partial h}{\partial t} = \rho_0'(x) + c' \cdot \rho'(x) \cdot a^2 - \nu h + D_h \frac{\partial^2 h}{\partial x^2} \tag{12b}$$

By comparison with eqn. (9) it is evident that patterns will be formed if ρ_0, ρ_0', κ and γ are small, $\rho(x)$ and $\rho'(x)$ do not vary strongly in space. Equations (11, 12) have the following molecular implications: activation is bimolecular, inhibition corresponds to Michaelis-Menten kinetics. Often, the approximation $1/h$ (Eqn. 11a) instead of $1/(\gamma + h)$ (Eqn. 12a) is adequate since the range of $h \to 0$ is never approached because of eqn. (11b). All extensions incorporated in eq. (11, 12) relate to biological phenomena, in particular to induction, polarity and size regulation. Inclusion of terms ρ_0' and/or γ (Eqn. 12) above a certain threshold leads to a simple model for induction: pattern formation does not occur spontaneously but proceeds under the influence of a stimulus of sufficient strength, say a local pulse of a. ρ_0, ρ_0', ρ, ρ' describe small variations, e.g. random fluctuations, or shallow gradients of sources of activation and inhibition, such as concentrations of enzymes producing or receptors releasing a or h, respectively. Such gradients, however shallow, can define and explain polarity in the context of the theory by specifying the orientation of the emerging pattern.

The basic term $\rho_0(x)$ for activator production can give rise to fast initiation and regular spacing of activation.

The function of a saturating term for a described by the factor $1/(1 + \kappa a^2)$ is also of general interest: Such saturation leads to size regulation. The area of activation can adapt in proportion to total size of the field. For small or zero κ, maximal a is limited indirectly by the diffusion term D_a and the range $r_a = \sqrt{(D_a/\mu)}$ determines the size of the activated area. If the area increases, the maximal value of a in the field increases as long as total field is within the range of inhibition, $r_h = \sqrt{(D_h/\nu)}$. If, on the other hand, κ is larger, it leads to a limitation of a to a saturating value. If total area is increased, but is still not much in excess

of the range of inhibitor, inhibition can spread into a wider area, permitting the activated area to extend into a wider area, too. The mechanism is such that a reaches near maximal values in an area nearly proportional to total size as long as total size is within the range of inhibition.

Equations (11, 12) based on the asymptotic equation (9) have proved to be particularly suitable to model for biological systems, especially with regard to regulating properties, such as tissue polarity, size regulation, induction and regular spacing of structures (Gierer and Meinhardt, 1972; Meinhardt and Gierer, 1974).

5. *Models Based on General Conditions of Destabilization*

Asymptotic validity of the power laws eqn. (8) does not always lead to a straightforward assessment of equations for pattern formation. An example for the application of the more general laws eqn. (3, 5) is given by the following problem. In the example eqn. (9, 11, 12), inhibitor is produced by a second-order reaction with respect to activation. Can one obtain patterns based on eqn. (9a) or (12a), in a simple manner with monomolecular catalysis of inhibitor production by activator, so that inhibitor distribution differs from activator distribution only by a more extensive spreading effect in space? Then

$$\frac{\partial h}{\partial t} = \nu(a - h) + D_h \frac{\partial^2 h}{\partial x^2} \tag{13b}$$

The power rules eqn. (8) show that eqn. (9a) is almost sufficient to ensure stability of the uniform solution ($m = n$ instead of $m > n$). Eqn. (9a) extended by a saturating term κ as in eqn. (12a) reads

$$\frac{\partial a}{\partial t} = \mu \frac{a^2(1 + \kappa)}{1 + \kappa a^2} \cdot \frac{1}{h} - \mu a + D_a \frac{\partial^2 a}{\partial x^2} \tag{13a}$$

(with the uniform solution $a_0 = 1$, $h_0 = 1$). Eqn. (13a) is easily shown, in conjunction with (13b), to meet the inequalities (eqn. 3, 5) for $0 < \kappa < 1$. Eqn. (13a, b) represents a pattern forming mechanism with bimolecular autocatalytic activation and first-order cross-inhibition in conjunction with a linear relation between activation and inhibitor production. A computer demonstration of the formation of such patterns will be given below (Fig. 3).

6. *Pattern Regulation*

The main body of experimental evidence to test and support a theory of biological pattern formation is the set of self-regulatory properties described in Section II.1 related not only to the form of patterns generated, but to the response of the pattern-forming system to distortions and interferences, such as excisions and transplantations. The theory based on autocatalysis and lateral inhibition can account for these properties. This has been demonstrated by computer calculations (see Gierer and Meinhardt, 1972, 1974). Some computer simulations are given in Figs. 2–4.

(a) Self-generation and polarity of patterns. The model accounts for pattern formation starting from near-uniform distributions. Initiation requires either random fluctuations or some initial asymmetric distributions (such as a shallow gradient or a small local stimulus). The simplest form of the pattern to be generated is a gradient. The form of the gradient is near-independent of the mode of initiation (Fig. 2a–c).

While the orientation of an asymmetric pattern may be due to symmetry breaking caused by random fluctuations, many biological structures formed in the course of embryogenesis show a predictable orientation with respect to the tissues generating them. There must be a preexisting cue to orientation called polarity. In the context of the theory any initial asymmetry however slight can orient a pattern. This asymmetry is introduced as a slightly asymmetric distribution, for instance a shallow gradient of some sources ρ_0, ρ_0', ρ, ρ' in eqn. (11, 12)) of activators and inhibitors. Polarity-defining source distributions may occur in the tissue forming the pattern or underlying the pattern-forming tissue; slightly asymmetric boundary conditions by effects extending from tissues bordering the pattern-forming area

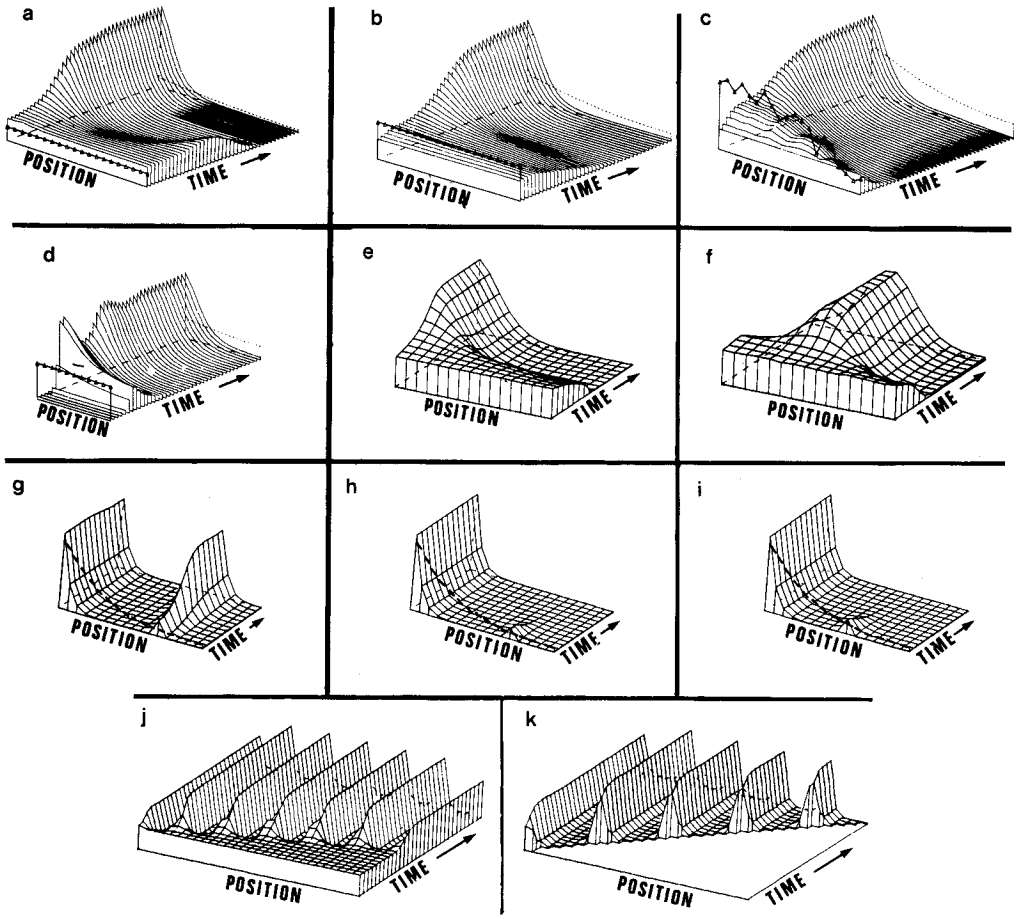

FIG. 2. Pattern regulation based on autocatalysis and lateral inhibition (computer simulations for patterns in one dimension according to eq. (11); (——) activator distribution; dimension left to right describes position within the tissue; development of the pattern in the course of time is plotted front to rear in each subpicture.) a. A monotonic gradient is generated starting from near-uniform initial distributions; a slight fluctuation (left) suffices for initiation. Activation (left) proceeds at the expense of deactivation (right) leading to a stable gradient. Its form is nearly independent of initial conditions: A shallow gradient (—▲—▲—) of sources (ρ and ρ', eq. 11), (b), or a more bizarre asymmetric source distribution (c), gives rise to essentially the same pattern as in (a). d. A section cut from (b) regenerates a gradient, the polarity being retained. If the field is sufficiently large compared to the range of activator, form and symmetry of the pattern depend on the mode of initiation: a gradient is formed if initiation is due to a shallow initial activator gradient, even in the presence of slight random fluctuations (e), but increased fluctuations can lead to a symmetric pattern (f). g–i. Model for induction (--- inhibitor distribution extending from an activated area (left) at the onset of induction): if, at some distance from the activated area a small stimulus of activation is applied, this can lead to a secondary centre of activation (g); a still smaller stimulus, at the same site, cannot overcome the inhibitory effect extending from the activated ("head") area (h), nor can the same stimulus as in (g) lead to induction at positions closer to the head (i). j. In fields large as compared to activator range, a stimulus in one part (left margin) leads to the subsequent formation of peaks and thus to a periodic pattern; such periodic peaks can also be formed in a marginally growing area (k).

could also determine polarity. Polarity-defining gradients do not sensitively affect the pattern of activator to be formed and some simple models (such as eqn. (11) with ρ' proportional to ρ) are nearly neutral even with respect to absolute values of source distributions (Fig. 2b, c). In an excised section a gradient is regenerated, the polarity being retained (Fig. 2d). All demonstrations in Fig. 2 are based on eqn. (11), but other pattern-forming equations generated on the basis of the criteria given in sections II.3–5 show similar properties. An example for pattern formation with linear cross-activation of inhibitor production (Eqn. 13) is given in Fig. 3.

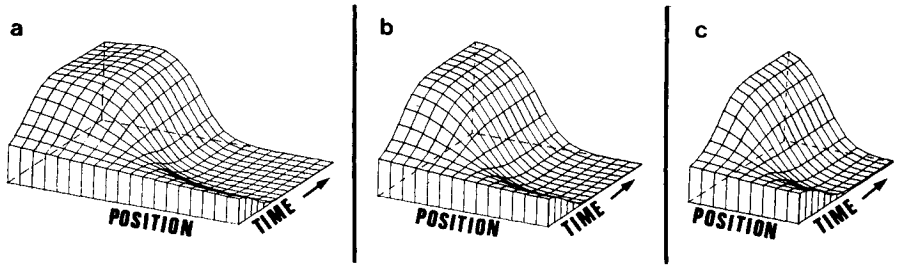

FIG. 3. Pattern formation based on a model with inhibition produced proportional to, but with wider redistribution than activation (Eq. 13), calculated for three different field sizes a–c.

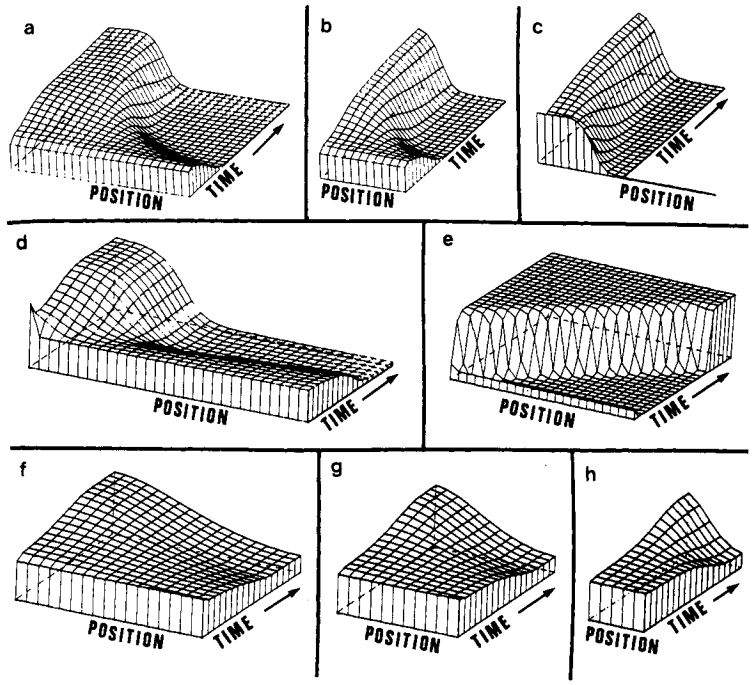

FIG. 4. Size regulation. (a, b) Activator saturation (Eq. 12) leads to size regulation, with activated area nearly proportional to total size. c. Sections cut from (a) after the pattern is established regenerate a size-regulating pattern (as in (b)), even if they contain the area (Fig. a, left) which was fully activated already. In (a), where initiation is due to a shallow gradient, the transition towards near maximal activation extends into the field in wave-like fashion. The effect is more striking if initiation of a pattern with activator saturation is due to local induction at the (left) margin, as shown in (d). Lateral inhibition causes the wave of transition to high activation to stop when the activated area has reached a certain part of the total field, whereas a wave not coupled to lateral inhibition (e) eventually covers the entire field. f–h. Proportion regulation by gradient formation coupled with a decrease in cell communication (e.g. diffusion). Diffusion rates are assumed to decrease exponentially in time until activation somewhere in the field exceeds a threshold; then a signal, perhaps a wave of type (e) prevents further changes in diffusion rates. The model leads to good proportion regulation throughout the field. Since the metric of the tissue is adapted in this way, secondary patterns formed subsequently by a second pattern-forming system, for instance periodic patterns of type Fig. 2j, would also be proportion regulating.

(b) Symmetry changes. Experimental interference can change the symmetry of patterns to be formed; thus Hydra with feet at the ends and a head in the middle, or symmetric double-abdomen configurations derived from insect embryos as well as hands with thumbs on both sides have been described. According to the theory, minor changes in parameters can lead to the formation of a symmetric pattern instead of a gradient if the field is sufficiently large. This can be the result of boundary effects (such as leakage of inhibitor across the boundary), or an increase of random fluctuations superimposed on a shallow polarity-defining gradient as simulated in Fig. 2e, f.

(c) Induction and inhibition. Pattern formation from near-uniform distributions may require an external inducing signal exceeding a certain threshold. Equations of type (12) with sufficiently large values of ρ_0' or γ can account for this property. Further, the induction of a secondary centre is possible by a stimulus of sufficient strength at distances sufficiently out of reach of inhibition from the primary centre. A model for such a distance-dependent induction is simulated on the basis of eqn. (11) in Fig. 2g–i.

(d) Multiple peaks. The formation of multiple peaks is closely related to the induction of secondary centres: they can arise if the total area is sufficiently large in relation to ranges of activators and inhibitors. Upon initiation by random fluctuations peaks form at variable distances but with second-order statistics avoiding distances below a certain threshold. If initiation occurs only at one point, such as at a margin, or if the field grows marginally, peaks develop in sequence in a recursive manner leading to regular spacing and thus to a periodic pattern (Fig. 2j, k). (A closely related mechanism for the generation of periodic structures was proposed independently by Wilcox, Mitcheson and Smith, 1973.)

(e) Size regulation. An important aspect of pattern regulation is size regulation. For some organisms, part of an early embryo can form a complete embryo of reduced size; a small section of the gastric column of Hydra will regenerate a small hydra with a correspondingly small head etc. The simplest models based on the theory, such as eqn. (9, 11) do not automatically lead to size regulation. However, if the saturating term for activation κ in eqn. (12) is introduced, the activated area adapts in proportion to total size as long as the latter is within the range of inhibitor (Fig. 4a–c). This size regulation can explain the regulation of an activated area. Another related model for proportion regulation is a four-component system with mutually exclusive lateral activation discussed below in the section (IV.2) on multi-component systems.

As seen in Fig. 4a, initiation by a shallow gradient leads to a time course of pattern formation in which the area of near-maximal activation extends into the field from the (left) margin until a stable state is reached; the model may thus be described as a wave of transition toward a state of high activation extending into the field. It is essential for pattern formation, however, that the wave front stops moving when the wave covers part of the total field. The wave-like effect is more pronounced if initiation occurs by induction at a margin rather than by a shallow gradient (Fig. 4d). The self-limiting effect which causes the wave-front to stop is due to the effect of lateral inhibition. If activation is not coupled to an inhibitory effect of wide range a wave would not be self-limiting and would eventually cover the entire field. This is demonstrated in Fig. 4e with uncoupling modelled by setting $h = h_0 = \text{const}$ in eq. (12a).

The models based on activator saturation lead to proportion regulation of the activated area, but the subarea of intermediate levels of activation does not regulate in proportion to total size. If the gradient specifies positional information at such intermediate levels, proportion regulation would not be exact. There is experimental evidence in favour of such coarse size regulation in cellular slime molds (MacWilliams and Bonner, 1979).

Whether exact regulation of intermediate levels also exists in biology, is not yet known, but the theory can be adapted to account for it. In embryogenesis, intercellular communication is found to be reduced at the time when patterns are laid down, probably by gradual closure or removal of intercellular junctions (Loewenstein, 1968; Hayes, 1976). This would correspond to a gradual reduction of diffusion rates D_a, D_h in equations of type (11) given, for example, by

$$D_a = D_a^0 \, e^{-\sigma t}; \qquad D_h = D_h^0 \, e^{-\sigma t}$$

The reduction of cell communication is expected to lead from a state where no pattern is formed to a state where a gradient is formed. (Upon further reduction of communication, the gradient would become steeper.) When anywhere in the field the activator level exceeds a certain threshold in the course of a gradient formation, it is assumed to trigger a signal extending into the entire field terminating further reduction of cell communication ($\sigma = 0$), stabilizing the gradient formed. The process is size-regulating, leading to a nearly proportional gradient in smaller fields at a stage of more reduced communication (Fig. 4f–h).

This model requires the addition of a control mechanism to the simple version of eqn. type (11), namely the mechanism for stopping the closure of junctions. The corresponding signal, however, can be relatively simple (Fig. 4e), with a local stimulus initiating a wave of transition across the entire field.

This model has an interesting property that most other models of size regulation do not show. It causes proportion regulation by changing the metric of the system: Intercellular communication adapts, so that a small piece behaves as if it were large in any aspect of pattern formation. Therefore, if, after size regulation has occurred, a secondary pattern-forming system is initiated which generates a periodic pattern as in Fig. 2j, the periodic pattern is also size-regulating. The small area would accommodate the same number of peaks as the large one with reduced distances.

A biological example of a periodic pattern with size regulation is somitogenesis: Xenopus embryos of reduced size can form the normal number of somites with reduced spacing (Cooke, 1975). Possibly, regulation of the metric as proposed above is involved in this case. A different though related model with size regulatory properties is sequential induction of a defined number of elements as described in a later section (IV.3, 4).

Another type of model for size regulation is based on a source of a substance at one margin of a field and a sink at the other, generating a linear gradient in between (Crick, 1970). Such mechanisms require primary patterns determining sources and sinks in the first place. They do not by themselves lead to proportion-regulating gradients; this requires additional assumptions. Size regulation would occur if sinks were not confined to a margin but were also distributed within the tissue (Meinhardt, 1978), or if the highest value of the gradient, which occurs in the source region, were homeostatically regulated to a level independent of total size.

The calculations on various aspects of pattern regulation, such as polarity, induction and size regulation, demonstrate that the theory of pattern formation by autocatalysis and lateral inhibition accounts for the set of striking self-regulatory properties often observed in developing systems. Since no explanations on an essentially different physical basis are known, the correspondence provides considerable experimental support for the theory. In more detail, regulatory properties of specific biological systems have been modelled for, such as pattern regulation in Hydra, early insect embryogenesis and pattern formation in single cells (Gierer and Meinhardt, 1972; Meinhardt and Gierer, 1974; Meinhardt, 1977) and spatial patterns in dividing bacterial cells (Meinhardt, 1978). Further, it has been shown that pattern regulation in the slug of cellular slime molds (giving rise to stalk and spore cells) has properties in accordance with the theory (MacWilliams and Bonner, 1979).

7. *Form of Patterns*

If a pattern is initiated in an area just exceeding the minimal size, the form of the pattern is always a gradient. This has been demonstrated by computer simulations (Meinhardt and Gierer, 1974) and can also be shown by an heuristic argument: Assume that the form of the pattern were not a gradient; then it would have one or several maxima (and/or minima) within the field for activator and, therefore, there should also be a maximum for inhibition at, or close to such points. Each internal maximum would be a point of zero net flow of material; nothing would be altered with respect to the pattern if one inserted an impermeable boundary there. Thus the area could be subdivided into subareas, each capable of forming a monotonic gradient. This, however, would contradict the assumption made that total area is close to the minimal area capable of supporting a pattern. It follows that patterns formed in fields of a size just beyond the minimum for pattern formation are always gradients. For the same reason a growing field always produces a gradient when its size exceeds the threshold for pattern formation. Decrease of diffusion rates in a field of constant size, for instance by gradual closure of intercellular junctions, has the same effect as an increase of field size by growth. A similar effect would result from increasing degradation rates of activators (and inhibitors). The gradient is thus the simplest and most straightforward pattern expected on the basis of the theory. In fields large compared with activator range

gradients can be formed only if initiation is sufficiently asymmetric, range of inhibition is large and basic production ρ_0 (eqn. 11, 12) is low.

Otherwise, symmetric or multiple-peak patterns are generated. Width of activated areas is essentially determined by the range of activator and the spacing of peaks by the range of inhibitor (see Section III.3).

The theory can be generalized to more than one dimension in a straightforward manner by introducing diffusion terms for several dimensions. Isotropic as well as anisotropic diffusion can be envisaged. While the most general case is the three-dimensional one, many biological patterns arise in two-dimensional systems such as cell sheets. Figure 5 demonstrates different types of patterns in two dimensions by computer simulation, all on the basis of the same equation (11). In (a) a gradient is formed in one dimension within a two-dimensional field rendering positional information (Wolpert, 1971), that is a one-to-one correlation between value and position in this dimension. Evidently a second pattern-forming system can specify positional information in a second dimension. In a wider field an inducing stimulus can give rise to a peak of activity (b) and in still larger fields or in fields with low ranges of activation and inhibition, multiple-peak patterns can arise with

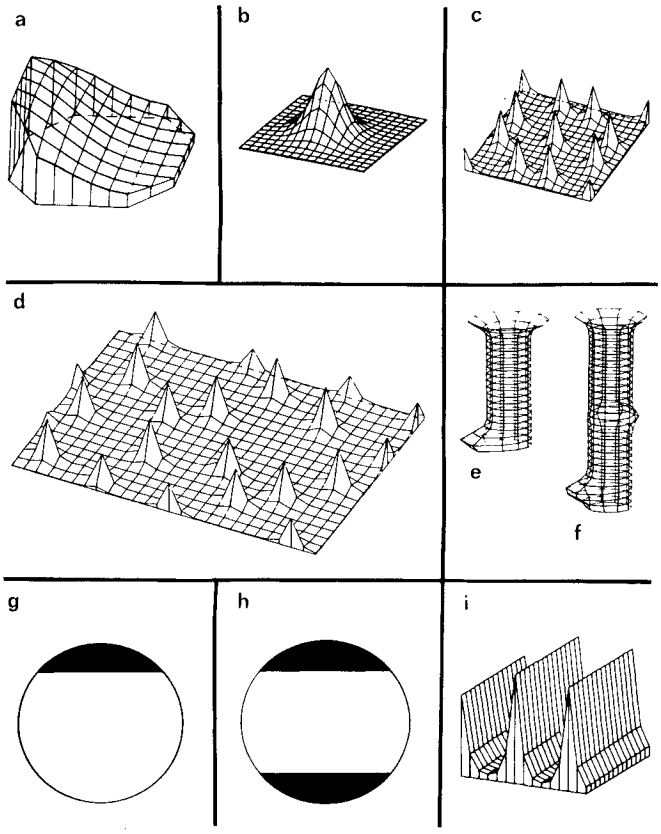

FIG. 5. Pattern formation in two dimensions, e.g. within cell sheets. All computer simulations are based on eq. (11), diffusion terms being extended for two dimensions. Final distributions are plotted a. monotonic gradient; b. single peak of activity; c. multiple-peak pattern initiated at one point leading to near-regular distances; d. multiple-peak pattern initiated by random fluctuations leading to random positions of peaks, but with a texture avoiding small distances between peaks due to the inhibitory fields; e, f. on a uniformly growing cylinder peaks of activity can be produced on alternating sites; g. on a sphere, a peak of activity can be produced; the model may apply to closed shell sheets, but also to intracellular and intra-membrane patterns generating polar cells. Their orientation can be determined by some shallow external gradient initiating the intracellular pattern; h. Opposite peaks of activity on a sphere modelling for a bipolar cell; i. If diffusion rates are anisotropic, e.g. if inhibitor diffusion is small in one of the two dimensions of a cell sheet and large in the other, a pattern may form only in the latter dimension; on this basis, periodic patterns in one dimension within the two-dimensional field can be formed.

regular distances (c). (d) shows a pattern of peaks of activity initiated by random fluctuation. The pattern exhibits some irregularity, but small as well as large minimal distances are avoided because of the effect of inhibitory fields around peaks of activation. For a growing cylinder regularly spaced peaks appear at alternating sites (e, f) as it is observed for instance for bud spacing in Hydra or leaf spacing in many plants. On a sphere, mono- or bipolar activation, as simulated in Fig. 5 (g, h) can be obtained. This may model for determination of areas within cell sheets as in the gastrula or for determination of subareas

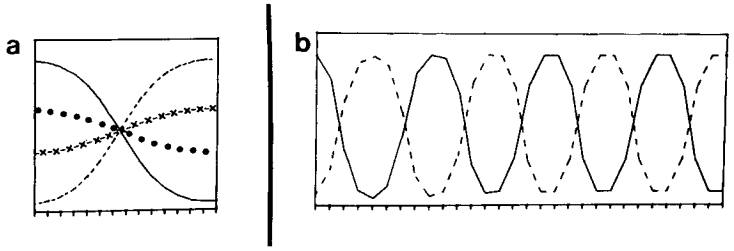

FIG. 6. Pattern formation by lateral activation of mutually exclusive states (Eq. 37, final distribution plotted; dimension left to right: position in an array of cells.). a. Subdivision of the area into two subareas with high activator of a_1 (——) and a_2 (– – –) respectively, mediated by lateral activation b_1 (. . . .) and b_2 (x-x-x). b. Larger fields and/or reduced diffusion ranges lead to alternating areas of activation of a_1 (——) and a_2 (– – –).

within a single cell or the membrane of a cell leading to intracellular patterns, such as mono- or bipolar cells. Periodic patterns in one dimension in a two-dimensional field require that either the field is narrow in one of the dimensions, or that the tissue is anisotropic (as many tissues are): If, for instance, spreading terms D_a or D_h (Eqn. 11) are different in the two dimensions, the condition for lateral inhibition—that range of activator is small as compared to range of inhibitor—may hold in one dimension only. Then, a periodic pattern in one dimension (i) is formed (Gierer, 1977a). However, as will be discussed below, we have found that systems with more than two components can generate striped patterns in isotropic tissues (Meinhardt and Gierer, 1980).

Combination and modulation of gradients and periodic patterns can evidently lead to more complex fields. Moreover, the response of the cells to the fields may be highly specific; very complex real patterns may arise on the basis of elementary field-forming mechanisms and their combinations.

8. *Molecular Interpretations*

The formalism of the theory is consistent with many different molecular interpretations and only biochemistry can decide between them. Thus production of components may be due to synthesis or release; removal to degradation or leakage; spreading to diffusion, convection, mechanisms involving transport along intracellular or intercellular fibres, and/or transducing effects across membranes. It is, therefore, not adequate to limit considerations to molecular diffusion. What matters is that activation and inhibition effects spread from the place of origin in an attenuated manner. Effects may involve nuclei, plasma, membranes or intercellular space. The autocatalysis may be directly due to a feedback of components on allosteric enzymes or receptors involved in the generation of activators and inhibitors or to much more complex reaction schemes, including cooperative effects in membranes.

It is perhaps surprising that our knowledge of the molecular basis of pattern formation is still so limited. In the thirties, when the biochemistry of induction was widely studied, it turned out that very many different effects are capable of inducing secondary centres, such as secondary amphibian embryos. Some researchers concluded that the effect is unspecific in the sense that many different biochemical effects contribute to it. It is unlikely, however, that natural induction and natural pattern formation are indeed unspecific. It appears much more plausible that there are very few specific factors which are involved in the natural formation of a morphogenetic field and its induction, but induction can be mimicked in the

laboratory by many different stimuli. What are then the natural factors forming morphogenetic fields? There are clear cases of morphogenetic effects resulting from components naturally occurring in the tissue interfering with pattern formation at extremely low concentrations, at the level of hormone action (Schaller, 1973; Berking, 1977), though it is not yet known whether these compounds are involved in forming primary morphogenetic fields or in other functions such as mediating cell differentiation. An assessment is difficult, because the inhibitory and especially the activating effects involved in the generation of morphogenetic fields might require the integrity of the cytoarchitecture (for instance of intracellular fibers and their anchorage in membranes). The homeostatic interactions in pattern formation introduce further complications into the assessment of the action of exogenous chemicals on cells and tissues. Such experimental difficulties have thus far prevented an effective assay for morphogens involved in the formation of primary morphogenetic fields. Irrespective of these difficulties, however, the experimental findings suggest that specific organic substances at low concentrations are involved in pattern regulation.

The models and equations describing pattern formation and the regulatory properties require only well-known features of molecular biology. In particular, allosteric enzymes or receptors or cooperative effects in membranes could easily provide the autocatalytic features. On the other hand it is emphasized that a large variety of different mechanisms are consistent with the reaction kinetics of pattern formation proposed, which are not limited to molecular interaction in solution and spreading by molecular diffusion. Further, it is possible that more than two components are involved in the reaction and that activation and inhibition are system parameters which may approximately subsume the combined action of several components each. This aspect will be discussed more explicitly in the context of multicomponent pattern forming systems.

9. *Combination of Patterns and Metric in Two Dimensions*

One expects that the same biochemical pattern-forming system is used at different stages and locations in the development of a complex organism and a few such systems would be sufficient for development. There must be more than one—at least two for two-dimensional pattern formation in cell sheets. Even for a single dimension there is evidence that more than one system may be involved: In Hydra, head and foot formation appear to be under the control of different pattern-forming systems (MacWilliams *et al.*, 1970). Two systems may interact: for instance, two systems may have a tendency to determine different structures (such as head and foot) at two terminal portions of an area; a system with limited range of inhibitor, regulating minimal distances between centres of activation, may be combined with a system with large range of inhibition and size regulating features; or one gradient may render the tissue anisotropic so that a second gradient forms at nearly right angles to the first, leading to a Cartesian coordinate system for positional information in a two-dimensional field. If a gradient in one dimension affects, in addition, diffusion or decay rates of the morphogens that specify positional information in the other dimension, a metric deviating from Cartesian coordinates can result. Another conceivable type of combination of patterns is a primary gradient that activates pattern formation in the second dimension only in part of the field, such as the edge of highest value of the primary gradient. Further, a gradient may modulate distance and height of peaks of activation in a periodic pattern. Generally, a single pattern-forming system with an activator and an inhibitor is not expected to provide for all aspects of complex biological systems. Rather, it may be considered as a subroutine for modelling development. As long as interactions between elementary pattern-forming systems are slight, they can be easily incorporated into the formalism of weakly connected systems with an activator an inhibitor each. (Strong interactions would require treating the problem as a multicomponent system from the outset. Some such multicomponent models will be discussed below.)

After formation of a pattern, different parts may develop subareas separated by boundaries of cell communication. Within subareas, subpatterns can be generated followed by formation of sub-patterns until the complete structure is laid down. Possibly the same pattern-generating physico-chemical system is used repeatedly in such sequences.

10. *Effects of Electrical Fields*

The equations for pattern formation on the basis of lateral inhibition given above do not include terms for the migration of components in electrical fields. Electrical fields arising in the course of development have been reported and discussed (e.g. Jaffe, 1968). Mechanisms of pattern formation involving electrical effects are conceivable which cannot be subsumed under the general reaction kinetics (Eqn. 1) without extensions, modifications, and specifications with respect to the distribution operators. An example would be pumping mechanisms for ions directed "uphill" to generate concentration gradients and electrical fields. Such mechanisms could generate asymmetric structures such as the polar fucus eggs studied by Jaffe (1968). On the other hand, it appears unlikely that the self-regulatory properties characterizing many biological systems (as discussed in the previous sections) could be explained by ion pumping as primary cause of morphogenetic fields.

To deal with electrical terms *within* general reaction kinetics of type eqn. (1, 2), one may include terms for mobility in electric fields in distribution operators $\mathscr{D}_i$ (Eqn. 1) to be coupled with equations for the electric fields as function of the various ions in the cell or tissue. While the general problem of including all conceivable types of electric field effects is difficult, their biological relevance is doubtful except for a few simple cases.

(a) Electrical fields existing in the tissue or arising from its environment, however slight, may define the polarity of the pattern, because any systematic deviation of the concentration of substances interfering with pattern formation, e.g. near a margin of the field, can determine the orientation of an asymmetric pattern. Since form and strength of the electric field would not matter much, a detailed incorporation of such electrical fields into the formalism of pattern formation would not be essential to understand the formation of the morphogenetic field.

(b) When a morphogenetic field is formed, it is expected to affect cell determination and other cell properties, and the resulting spatial order may then lead to an electric field which may feed back on the pattern-forming system. One would expect this process to be delayed with respect to the initial stages of formation of the morphogenetic field, but exact modelling would require the inclusion of the electrical field as function of the distribution of differentiated states in space. Within the formalism, this would require the inclusion of equations for the change of ρ_0, ρ, ρ' (eqn. 11) in time, of an electrical field as function of a, h, ρ_0, ρ, ρ', and of terms for movement of activator and inhibitor in the electrical field.

(c) Of more general interest is the question whether electrical terms can strongly influence the generation of morphogenetic fields in the first place. In other words, if we start with no electrical fields and if an electrical field is generated only in the course of pattern generation, feeding back on the latter, does the feedback contribute to a major extent to destabilization of uniform distributions or its prevention and to determining the general type of pattern to be formed? We will show that for mechanisms describable by general kinetics such as eqn. (2) the answer to this question is most likely "no", because effects of the morphogens are buffered by the main ionic constituents of the medium. This will be explained by a simple consideration on the orders of magnitudes involved. Let us consider some (intermediary or final) stage of pattern formation. If there are concentration differences between different parts of the field, there is an electrical potential between these parts which is given by the theory of diffusion potentials, according to Henderson:

$$\varepsilon_D = \chi \cdot \frac{RT}{F} \cdot \ln \frac{\sum u_j \cdot c_{j1}}{\sum u_j \cdot c_{j2}} \quad \text{with} \quad \chi = \frac{\sum \frac{u_j}{z_j} \cdot (c_{j2} - c_{j1})}{\sum u_j (c_{j2} - c_{j1})} \tag{14}$$

(R Gas constant, T absolute temperature, F Faraday's constant, u_j electrical mobilities, z_j charge, c_{j1} and c_{j2} concentrations of all ions, whether morphogens or not in the two areas).

$$\sum u_j \cdot c_{j1}, \qquad \sum u_j \cdot c_{j2}$$

are the electrical conductivities in the two areas of the field. The χ term is complicated only because of the charges z_j, and is of the order of, or lower than, $1/\bar{z}$ where $\bar{z}$ is some average over the charges of the compounds.

The order of magnitude of the ratio of conductivities in different parts of the area is essentially determined by the ratio of ion concentrations. We expect that the concentration of morphogens c_m which vary in space is only a very minor fraction of the concentration of uniformly distributed ions, c_0. The order of magnitude of relative variations of conductivity in space resulting from morphogenetic fields is thus estimated by

$$\frac{\sum u_j c_{j1}}{\sum u_j c_{j2}} \approx 1 + \frac{c_m}{c_0} \tag{15}$$

The order of magnitude for ε_D is given by

$$\varepsilon_D \approx \frac{RT}{\bar{z}F} \cdot \ln \frac{\sum u_j c_{j1}}{\sum u_j c_{j2}} \approx \frac{RT}{\bar{z}F} \cdot \frac{c_m}{c_0} \tag{16}$$

This potential difference by itself would lead to an equilibrium state of unequal concentrations of compounds c_1', c_2' in which electrical mobility is counteracted by diffusion; except for extreme assumptions on the charge distribution, the concentration ratio of the compounds would be of the order of

$$\ln \frac{c_1'}{c_2'} \approx \frac{zF}{RT} \cdot \varepsilon_D \approx \frac{z}{\bar{z}} \frac{c_m}{c_0} \ll 1 \tag{17}$$

Electrical fields due to diffusion potentials arising during formation of concentration patterns of morphogens are much smaller than those required to generate significant concentration ratios. The electrical effects are "buffered" and diluted by the ions which are not morphogens. It thus appears likely that for mechanisms describable by general kinetics of type eqn. (1, 2) electrical effect can be neglected in modelling for primary generation of morphogenetic fields starting from near-uniform distributions (except perhaps for the determination of polarity mentioned above).

11. *Cellular Response to Morphogenetic fields*

The effects of morphogenetic fields on cells may include determination, differentiation, changes of form, proliferation, movement, death and other properties. Cell responses can be graded or all-or-none. The terms activator and inhibitor refer to the function of these substances in the generation of morphogenetic fields; in their effect on cells, activators could be inhibiting and inhibitors activating. A morphogenetic gradient can give rise to a stable gradient in the density of subcellular components or cell types in the tissue. This would occur if a relatively stable substance is produced in proportion to the value of the morphogenetic field, or if the field determines the per cell probability for precursor cells to differentiate in a certain direction (e.g. into nerve cells). The stable graded distribution thus produced may in turn determine the source densities of enzymes and other sources ρ_0, ρ, ρ' (Eqn. 11) involved in the production of morphogens, and thus feed back on the pattern-forming system.

Such source gradients may then determine the polarity for pattern formation upon regeneration of subsections.

The source density changes are expected to be a relatively slow process compared to the primary formation of the morphogenetic field and can therefore be incorporated into the equations of pattern formation (e.g. eqn. 11) by adding slow reactions of the type:

$$\frac{\partial \rho}{\partial t} = \chi(a, h) \tag{18a}$$

$$\frac{\partial \rho'}{\partial t} = \chi'(a, h) \tag{18b}$$

For some equations, such as eqn. (11) with $\rho \sim \rho'$ this feedback does not significantly affect the morphogenetic field at all. Growth can also be included in the formalism. An application to Hydra has shown (Meinhardt and Gierer, 1974) that such models can account

for the indefinite maintenance of a spatial pattern in a continuously growing and budding animal. Not all types of feedback of sources on morphogens lead to stable patterns, but indefinite stability is not generally required for developing systems. If the feedback is slow, it may not significantly affect the field during the limited period in which it exerts its action in actual biological development of an organism.

Determination and differentiation are essentially all-or-none events. Discontinuous patterns result if cells respond to a threshold of morphogen concentrations, or to a set of multiple thresholds for different steps of determination. A continuous (for instance graded) distribution of morphogens will then lead to a subdivision of the tissue into subareas with distinct boundaries. In the pure "positional information" model (Wolpert, 1971), a morphogenetic gradient determines the development of each part of a tissue with local values of the graded distribution leading to defined cell responses. In this way, in principle, any complex subdivision of an area in response to a simple gradient is logically possible. However, there is the alternative possibility that the primary morphogenetic gradient determines only one or both terminal structures within a field and that other areas are then determined by secondary mechanisms in a recursive way, for instance by a wave of induction. Biological evidence on pattern regulation suggests that both the pure positional information case and recursive pattern formation are realized in developmental biology, the former, for instance, by pattern formation in Hydra, the latter in intercalary regeneration of segments of insect appendages (Bohn, 1970, 1971; Bryant, 1975). The recursive mechanisms require a multicomponent approach and are discussed further below.

12. *Intracellular Patterns*

It is emphasized that there is no principle difference between intercellular and intracellular patterns. The same type of kinetics giving rise to intercellular patterns can lead to patterns in the plasma or on the membrane within a single cell, although the molecular components involved are expected to be different. Cells may develop into internally patterned mono- or bipolar cells, orient in response to a shallow gradient, develop processes, or migrate chemotactically. The elementary process is postulated to be the activation of an area of the plasma or the membrane by autocatalysis and lateral inhibition, its position in the cell or on its membrane being defined either by random fluctuations or by any slight polarity-defining cues from the environment, such as a shallow external gradient.

13. *Steady States, Pulsing States and Order in Time*

In the context of the theory, it is the steady state distribution of morphogens which is assumed to determine the spatial order within the cell or tissue. Rapidly changing transient states occurring in the course of the formation of a stable morphogen distribution would not show the self-regulatory properties typically found in biological development and modelled for by the theory described.

A variant of models leading to a steady state of morphogen distributions is to assume that the inhibitory reaction is relatively slow, so that the spatial pattern produced is not stable, but pulses regularly in time. Such pulsing spatial patterns can be as good in defining positional information as stable patterns, because the time average of the morphogen concentration may be capable of determining positional information and thus the spatial organization of development (Meinhardt and Gierer, 1974). This example shows that autocatalysis and lateral inhibition subsumes models in which activation and/or inhibition are time averages of oscillating systems. Other mechanisms of this type may also be considered: It is conceivable that spreading of the inhibitory effect may be due to oscillations in time and space, generated as a function of activation and spreading much faster than diffusible molecules. (However, conditions for such mechanisms, their molecular feasibility and their regulatory properties have not been studied thus far.) On the other hand, not all conceivable mechanisms involving oscillation (such as the phase shift model of Goodwin and Cohen, 1969) are formally related to kinetics involving lateral inhibition.

The morphogen distribution assumed in the steady state (or the time average in the case of oscillation) need not be stable forever, because its effect is mostly restricted to a defined stage of development. Thereafter, further events of differentiation, growth etc. upset the conditions for pattern formation of one stage and initiate the formation of secondary patterns and structures in subsections of the first (such as the formation of appendages of insects after the primary formation of the segments) until the complete organism has developed. Large-scale development is therefore mostly irreversible.

III. ANALYTICAL ASPECTS OF PATTERN FORMATION

1. *Heuristic and Analytical Approaches to Pattern Generation*

In outlining the theory of pattern formation based on lateral inhibition it was shown that destabilization of a near-uniform distribution to generate a pattern requires a set of properties including short-range autocatalytic activation and longer range inhibitory effects. It was evident from the considerations given that destabilization must occur if all conditions are met to the extreme and cannot occur if any one of these conditions is extremely violated. On the other hand, intermediate cases and a general quantitative assessment of parameter space with respect to destabilization and pattern formation require an analytical treatment.

With respect to the form of patterns it is evident that ranges of activators essentially determine areas of coherent activation and ranges of inhibitors the spacing or exclusion of multiple peaks of activation. Again an analytical treatment of the form of patterns would be desirable. Some aspects of stability of patterns are also of biological interest.

At present knowledge of analytical aspects of destabilization is at an advanced stage, whereas stringent analytical treatment of the form and stability of patterns is still difficult. In the following section it will be shown that the results of general destabilization theory are closely related to the set of conditions heuristically introduced for pattern formation on the basis of lateral inhibition, although this correspondence is not immediately obvious. In relation to analytical problems of the form of patterns, biological considerations will be discussed which bear on the relevance (or irrelevance) of some specific mathematical approaches.

2. *Relation of Pattern Formation by Autocatalysis and Lateral Inhibition to General Stability Theory*

Stability characteristics of equations type (1, 2) have been analysed on the basis of Ljapunov's stability theory, by Glansdorff and Prigogine (1971), and Babloyantz and Hiernaux (1975). Our equation (11) was studied in detail by Granero *et al.* (1977) confirming the conditions (A–F) described in section II.3.

Stability of uniform solutions of the two-factor equation (2) can be assessed as follows: if $a'(x, t)$ and $b'(x, t)$ are small deviations from the uniform solutions $a = a_0$, $b = b_0$ of the equations $f = 0$ and $g = 0$, linear approximations of eqn. (2) with diffusion read

$$\frac{\partial a'}{\partial t} = c_{11}a' + c_{12}b' + D_a \cdot \frac{\partial^2 a'}{\partial x^2} \tag{19a}$$

$$\frac{\partial b'}{\partial t} = c_{21}a' + c_{22}b' + D_b \cdot \frac{\partial^2 b'}{\partial x^2} \tag{19b}$$

The spatially uniform, time dependent solutions a', b' of (19a, b) with $D_a = D_b = 0$, are linear combinations of terms

$$\text{const. } e^{\lambda_i t} \qquad i = 1, 2$$

By insertion into eqn. (19a, b) the two values λ_1, λ_2 are obtained; they are the two roots of the determinant

$$\begin{vmatrix} c_{11} - \lambda & c_{12} \\ c_{21} & c_{22} - \lambda \end{vmatrix} = 0 \tag{19c}$$

If one or two of the values λ_1, λ_2 are positive (or have a positive real part), there are distortions deviating from equilibrium that increase further with time, and the system is thus unstable. If both values λ_1, λ_2 are negative (or have negative real parts), any small distortion decreases, in the long run, exponentially with time and the solution is stable. This is the case if the sum of the roots is negative, and their product positive:

$$\lambda_1 + \lambda_2 = c_{11} + c_{22} < 0 \tag{20a}$$

$$\lambda_1 \cdot \lambda_2 = c_{11}c_{22} - c_{12}c_{21} > 0 \tag{20b}$$

Redistribution by diffusion terms ($D_a > 0$, $D_b > 0$) can be assessed by a quantitative Fourier analysis (Glansdorff and Prigogine, 1971; Prigogine and Nicolis, 1971; Babloyantz and Hiernaux, 1975). Deviations from the uniform solutions can be considered as composed of Fourier components. For closed boundaries and an area of size L Fourier components for a' and b' take the form

$$\text{const.} \cos\left(\frac{\pi n}{L} x\right), \qquad n = 1, 2 \ldots$$

(terms with $n = 1$ render a gradient). The corresponding contributions of the Fourier components to the diffusion terms of eqn. (19a, b), are of the simple form

$$D_a \cdot \frac{\partial^2 a'}{\partial x^2} = -D_a \cdot \left(\frac{\pi n}{L}\right)^2 \cdot a'; \qquad D_b \cdot \frac{\partial^2 b'}{\partial x^2} = -D_b \cdot \left(\frac{\pi n}{L}\right)^2 \cdot b' \tag{20c}$$

$n = 1, 2 \ldots$

This implies that positive values are to be subtracted from the diagonal terms c_{11}, c_{22} of the determinant eqn. (19c). Destabilization of the uniform distribution and generation of a spatial pattern occurs if one of the roots λ_1, λ_2 becomes positive for at least one of the Fourier components contributing to the diffusion terms. This is the case if, for some n,

$$\left[c_{11} - n^2\left(\frac{\pi}{L}\right)^2 D_a\right]\left[c_{22} - n^2\left(\frac{\pi}{L}\right)^2 D_b\right] < c_{12}c_{21} \tag{20d}$$

Introduced into specific equations of type (11, 12), the relations eqn. (20) can lead to quite involved expressions, and the correlation with the conditions of pattern formation by lateral inhibition is not immediately obvious. However, one can show that the mathematical content of both sets of conditions is very similar. This correspondence is not too surprising, since assessment of destabilization (eqn. 3, 5) is almost equivalent to a matrix treatment, eqn. (20b) being closely related to eqn. (5).

To show this correspondence, we rewrite the equations (2) in terms of the concepts on which the conditions A–F (see Section II.2; summarized in Table 1 below) for pattern formation by autocatalysis and lateral inhibition are based, namely, rates, ranges and orders of reactions. Rate is defined as proportional to the reciprocal mean lifetime of a molecule between production and decay, with the dimension sec^{-1}. Range is defined as an average

TABLE 1. CONDITIONS FOR PATTERN FORMATION BASED ON AUTOCATALYSIS AND LATERAL INHIBITION (SEE SECTION II.2) (FOR TWO-COMPONENT SYSTEMS)

A.	One 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 sufficiently strong to ensure stability of the uniform solution.
D.	The inhibitory effect must be relatively fast compared to the activating effect.
E.	Range of activation must be below a limit of the order of total field size.
F.	The range of inhibition must be sufficiently large in relation to the range of activation.

over the mean distances between production and decay, with the dimension cm. Orders of reactions are defined in terms of logarithmic derivatives of production and decay rates such that in simple cases they are given by power terms, irrespective of absolute concentrations as in the condition eqn. (8). Near the uniform solution a_0, b_0, mean production and decay rates per molecule are given as

$$\mu = \frac{P_a}{a_0} = \frac{Q_a}{a_0}; \qquad \nu = \frac{P_b}{b_0} = \frac{Q_b}{b_0} \tag{21}$$

where P_a, Q_a, P_b, Q_b are production and decay rates as defined in eqn. (6).

Range, that is mean distance between production and decay or removal of a molecule, is given, for the case of diffusion, by the rules of physical chemistry relating range r to diffusion constant D and mean decay rate β:

$$r = \text{const.}\sqrt{\frac{D}{\beta}}$$

We may thus define ranges of activation and inhibition as

$$r_a = \sqrt{\frac{D_a}{\mu}}; \qquad r_b = \sqrt{\frac{D_b}{\nu}} \tag{22}$$

Further, the orders of reactions may be defined as

$$\begin{aligned} k_{aa} &= \frac{\partial \ln P_a}{\partial \ln a} - \frac{\partial \ln Q_a}{\partial \ln a}; \qquad k_{ab} = \frac{\partial \ln P_a}{\partial \ln b} - \frac{\partial \ln Q_a}{\partial \ln b} \\ k_{ba} &= \frac{\partial \ln P_b}{\partial \ln a} - \frac{\partial \ln Q_b}{\partial \ln a}; \qquad k_{bb} = \frac{\partial \ln P_b}{\partial \ln b} - \frac{\partial \ln Q_b}{\partial \ln b} \end{aligned} \tag{23}$$

In terms of rates, orders and ranges we may rewrite eqn. (19):

$$\frac{\partial a'}{\partial t} = \mu\left(k_{aa}a' + \frac{a_0}{b_0}k_{ab}b' + r_a^2 \cdot \frac{\partial^2 a'}{\partial x^2}\right) \tag{24a}$$

$$\frac{\partial b'}{\partial t} = \nu\left(\frac{b_0}{a_0}k_{ba}a' + k_{bb}b' + r_b^2 \cdot \frac{\partial^2 b'}{\partial x^2}\right) \tag{24b}$$

and eqn. (20a, b) can be written as

$$\mu \cdot k_{aa} + \nu \cdot k_{bb} < 0 \tag{25a}$$

$$k_{aa} \cdot k_{bb} > k_{ab} \cdot k_{ba} \tag{25b}$$

According to eqn. (20d) rewritten in terms of eqn. (21–24) destabilization occurs upon redistribution if, for at least one choice of n, the term

$$T(n) = \left[k_{aa} - n^2 \cdot \left(\frac{\pi r_a}{L}\right)^2\right]\left[k_{bb} - n^2 \cdot \left(\frac{\pi r_b}{L}\right)^2\right] < k_{ab}k_{ba} \tag{25c}$$

Often orders of reactions are of the order of 1, but small values may also apply in some biological cases. Eqn. (25) permits a direct assessment of conditions for pattern formation in relation to rate, ranges and orders. In particular, combinations of signs of reaction orders (k_{aa}, k_{bb}, k_{ab}, k_{ba}) can be tested as to whether they are consistent with the conditions (25). (25a) requires that k_{aa}, or k_{bb}, or both are negative; we therefore choose k_{bb} to be negative in any case. (25b, c) can hold only if

$$T(n) = \left[k_{aa} - n^2 \cdot \left(\frac{\pi r_a}{L}\right)^2\right]\left[k_{bb} - n^2 \cdot \left(\frac{\pi r_b}{L}\right)^2\right] < k_{aa}k_{bb} \tag{25d}$$

k_{bb} being negative, this relation requires that k_{aa} is positive. Positive k_{aa} corresponds to the

condition A for autocatalysis, because it is equivalent to the condition that $\partial f/\partial a$ (eqn. 3) is positive (eqn. 19a, 24a)

$$\left(\frac{\partial f}{\partial a}\right)_{a_0,b_0} = c_{11} = \mu \cdot k_{aa} > 0 \tag{26a}$$

Positive k_{aa} and negative k_{bb} implies that $k_{aa} \cdot k_{bb}$ is negative. This is consistent with condition (25b) only if the product $k_{ab}\, k_{ba}$ is negative, too. Negative $k_{ab} \cdot k_{ba}$ can result in two ways, either from inhibition or depletion mechanism: Inhibition is defined by $k_{ab} < 0$ and $k_{ba} > 0$, whereas depletion is defined by $k_{ab} > 0$; $k_{ba} < 0$. These statements correspond to condition *B*.

Equation (5) can be rewritten in terms of reaction orders and is then identical with eqn. (25b). This corresponds to the stability condition for the uniform solution C. The conditions of stability theory corresponding to conditions A–C are thus expressible in terms of the orders of the reactions only, without reference to absolute rates or other features.

With positive k_{aa} and negative k_{bb}, (25a) corresponds to

$$\frac{\nu}{\mu} > \frac{k_{aa}}{(-k_{bb})} \tag{26b}$$

This implies that the ratio of the rate constants for the inhibiting to the activating reaction must be above a certain threshold (which will be of the order of 1 if the parameters describing orders of reactions k_{aa} and $(-k_{bb})$ are of the order of 1) corresponding to condition *D*.

Conditions *E* and *F* can be expressed in terms of orders and ranges. According to eqn. (25c), destabilization requires that $T(n)$ must be negative for at least one choice of n, that is at least for $n = 1$. This sets an upper limit for range r_a in terms of field size L, corresponding to condition *E*

$$\frac{r_a}{L} < \frac{\sqrt{k_{aa}}}{\pi} \quad \text{implying} \quad L > \frac{\pi r_a}{\sqrt{k_{aa}}} \tag{27}$$

If (27) holds, condition (25c) can always be met if the range of inhibition r_b is sufficiently large, leading to destabilization and pattern formation (condition *F*). If field size L is considerably above the minimal size required for pattern formation, condition *F* can be expressed in terms of the ratio of ranges of inhibition and activation. It is useful for the derivation to define positive values

$$p_a = k_{aa}; \qquad p_b = -k_{bb}; \qquad p_{ab} = -k_{ab}k_{ba}$$

then, according to (25b, c)

$$p_a \cdot p_b < p_{ab} \tag{28a}$$

$$-T(n) = \left[p_a - n^2 \cdot \left(\frac{\pi r_a}{L}\right)^2\right]\left[p_b + n^2 \cdot \left(\frac{\pi r_b}{L}\right)^2\right] > p_{ab} \tag{28b}$$

A necessary condition for the distribution terms to cause the change from (28a) to (28b) is evidently, independent of n, that the ratio R defined as following is >1:

$$R = \frac{r_b^2}{r_a^2} \cdot \frac{p_a}{p_b} > 1 \quad \text{implying} \quad \frac{r_b}{r_a} > \sqrt{\frac{p_b}{p_a}} \tag{29}$$

The ratio of range of inhibition to range of activation must exceed a threshold (which is above 1 if parameters p_a, p_b characterizing the reaction orders are around 1).

Combining inequalities (29) and (26b) leads to

$$D_b = \nu \cdot r_b^2 > D_a = \mu \cdot r_a^2$$

Diffusion rates of inhibition must exceed that of activation. This relation was derived directly and independently by Segel and Jackson (1972). Conditions (29, 26b) for ranges and rates are more stringent than conditions for diffusion constants only.

For fields of sizes just above those required for pattern formation according to eqn. (27), strong redistribution of inhibition may be required to generate patterns. In larger fields

($L \gg r_a$) it is only the ratio of ranges r_b/r_a and not the individual ranges that is decisive for pattern generation. The wave length of the Fourier component which is most effective for destabilization corresponds to an integer close to the value n_m for which the term $-T(n)$ is maximal. n_m is easily calculated to be

$$n_m = \frac{L}{\pi}\sqrt{\frac{1}{2}\left(\frac{p_a}{r_a^2} - \frac{p_b}{r_b^2}\right)}$$

Unless we are interested in exact numerical evaluations, we may disregard the difference between n_m and the closest integer. Insertion into eq. (28b), using the term R eqn. (29) essentially determined by the ratio of ranges of inhibition and activation ("lateral inhibition"), leads to

$$\frac{p_a \cdot p_b}{4} \cdot \left(2 + R + \frac{1}{R}\right) > p_{ab} \tag{30}$$

which is equivalent to the relation

$$\frac{(R-1)^2}{R} > 4\left(\frac{p_{ab}}{p_a \cdot p_b} - 1\right) \tag{31}$$

Therefore, if the other conditions A–E are met, destabilization occurs if R exceeds a certain threshold; because of eqn. (28a) the threshold is >1 in any case. Equation (29, 31) imply that destabilization depends on the ratio of range of inhibition to the range of activation. Pattern formation occurs if this ratio exceeds a threshold which is above 1 on simple assumption on reaction orders (though it may be lower in extreme cases in which according to the criterion (28a) the uniform solution is almost unstable, that is, if $p_{ab}/(p_a p_b) - 1 \ll 1$). This is essentially the condition of lateral inhibition F.

A numerical example for condition (31) is the simplest asymptotic approximation, eqn. (9), of eqn. (11) that was used for the computer demonstrations of pattern formation in the previous sections. In this case, $p_a = p_b = 1$, $p_{ab} = 2$, and one calculates as lower limit of ratio R consistent with eqn. (30) $R = 5.83$. This value has been derived previously by Granero *et al.* (1977) in their analysis of this particular model. Since for eqn. (9) $p_a = p_b = 1$, R represents the lower limit of r_h^2/r_a^2. Range of inhibition must exceed range of activation by at least 2.4. For areas L for which n_m is not an integer, the lower limit of R is somewhat higher.

It follows from the discussion of this section that conditions A–F introducing autocatalysis and lateral inhibition directly (see Table 1) have a very similar mathematical content to eqn. (20) based on general stability analysis. This correlation, though neither trivial nor obvious can be derived by rewriting the basic dynamic equations (19) in terms of orders, ranges and rates of reactions (Eqn. 24), combined with a casuistics of signs of the various parameters. Therefore, we may consider conditions A–F for autocatalysis and lateral inhibition as general requirements for the formation of patterns on the basis of eqn. (2) for the simplest case when two factors are involved; no qualitatively different mechanism, say short-range inhibition and long-range activation, could lead to patterns starting from near-uniform distribution.

3. *Analytical Aspects of the Form of Patterns*

(a) *Gradients*

The simplest form of a concentration pattern to arise on the basis of the equations for pattern formation is a gradient. In a certain size range beyond the minimum size required for patterns to be formed at all, as defined by the range of activator (eqn. 27), and for closed boundaries, the pattern is always a gradient. This has been demonstrated by computer simulations and by the heuristic line of thought given in section II.7. A stringent analytical proof has been given by Babloyantz and Hiernaux, (1975). Further it has been proven analytically that such gradients formed on the basis of equations type (11) can reach an absolutely stable steady state (Mimura and Nishiura, 1979).

(b) *Patterns with multiple peaks: Regularities of spacing and texture*

In fields large enough to produce multiple-peak patterns, analytical solutions present a difficult problem. Many different solutions of equations of type (2) are possible, depending on initial and boundary conditions. Two cases already discussed in a previous section on the form of patterns are of biological interest: Random initiation leads to irregular spacings, but peaks of activity are distributed according to second-order statistics, avoiding small distances. Each pattern is different from the other, depending on the details of initiation, but the general texture of the pattern based on second-order statistics is preserved. This is actually the case in patterns like those of the stomata in plant leaves. Any leaf on a tree is different with respect to details in the distribution of stomata but may follow the same rule of general distance distribution. Analytically, only the second-order statistics and not the details of a particular pattern are then of biological interest.

Regular spacings are obtained if peaks of activity are generated in sequence, for instance by initiation at a margin, or marginal growth; the distance between peaks of activity is defined essentially by the range of the inhibiting effects extending from the peak that has been formed last.

An analytical treatment of the generation of multiple-peak patterns has been given by Haken and Olbrich (1978).

(c) *Biological evidence for relative simplicity of morphogenetic fields*

One may further inquire whether a complex pattern may arise within a large field as result of complex and highly specific initial and boundary conditions. Could, for instance, the shape of bones of a limb be determined by a bone-shaped prepattern resulting from very subtle initial and boundary conditions, thus selecting with high fidelity, one solution among the large variety of possible ones? Though mathematically challenging, this problem does not appear to be significant for biological patterns: There is abundant evidence that prepatterns and morphogenetic fields are simple, whereas the response of cells to such patterns is complex (and not the other way round).

The experimental support for this notion is mainly derived from genetic mosaics (Stern, 1968): In certain cases, mainly in insects, cell mosaics from mutants with different morphological properties can be constructed. The cells of different origins can be marked by colour or other features. One can then decide experimentally whether, in mosaic embyos, cells of both strains respond alike at the same position, or whether cells of each strain develop as they would in a given position in embryos made up exclusively of their own cell type. The former results would indicate that the mutation affects the morphogenetic field, the latter that it affects the response of the cells to the local value of the morphogenetic field which itself is not affected by the mutation. In the vast majority of cases studied, the mutants affect cell responses, and only very few of them change the morphogenetic fields. This indicates that fields are simple and responses of cells to values of the fields are highly complex. Otherwise there should be many mutants affecting boundary conditions which would, in turn lead to dramatic changes of the morphogenetic field. Simplicity suggests that fields may be gradients, symmetric distributions, periodic distributions, and combinations thereof (such as periods modulated by gradients), but not highly complex distributions of morphogens. Therefore, the analytical problem of selection of specific complex patterns by subtle boundary conditions does not appear to be of much biological significance. On the other hand, models for cell responses based on reasonable kinetic assumptions have been proposed showing that cell responses to simple morphogenetic fields, such as two orthogonal gradients, can lead to a complex structure, like the bone pattern in limbs (MacWilliams and Papageorgiou, 1978).

(d) *Stability of multi-peak patterns*

The conditions for stability of solutions for multi-peak patterns are not yet analytically resolved, and are expected to depend on models, parameter choices and modes of initiation. One may ask, for instance, whether the peaks obtained by random initiation gradually

shift, leading to a more regular spacing. Computer simulations on models of type eqn. (11) show that such shifts, if they occur, are very slow compared with the time required to establish the pattern in the first place. Since the time of cell response is usually restricted to some stage of development, such slow changes need not be biologically important.

Generally, though analytical criteria for stability of solutions would be of interest, absolute stability is no biologically acceptable criterion for the quality of mathematical models; such criteria would make sense only if they would permit assessments of relative stability—the maintenance of a state for a period exceeding the time required for the formation of a pattern by some factor, say, 10.

IV. GENERALIZATION OF THE THEORY OF PATTERN FORMATION FOR MORE THAN TWO COMPONENTS

1. *A Simple Generalization of Lateral Inhibition based on distinction of Short-Range Activation and Long-Range Inhibition*

We may ask whether the principle of lateral inhibition can be generalized to systems with more than two components. This can easily be done for systems of pairs of components, each meeting the conditions for pattern formation, be it in one or several dimensions, if these pairs interact with each other only weakly, or not at all; further, slow changes of various parameters ρ, ρ' (Eqn. 18) (describing for instance effects of cell differentiation, enzyme synthesis, etc.) in time can be introduced in a straightforward manner. On the other hand, the case of many strongly interacting parameters is more intricate, especially with respect to the definition of activating and inhibiting components. Nevertheless, a generalization of the principle of lateral inhibition to multicomponent systems is possible. It is not expected, however, that the extension discussed below is the most general one.

We assume an interacting system of parameters $p_1(x, t) \ldots p_N(x, t)$

$$\frac{\partial p_i}{\partial t} = f_i(p_1 \ldots p_N) + D_i \cdot \frac{\partial^2 p_i}{\partial x^2} \qquad i = 1 \ldots N \tag{32}$$

redistribution operators being exemplified by diffusion terms $D_i \cdot (\partial^2 p_i)/\partial x^2$. If there is a uniform solution, and p_i' are deviations from the uniform solution, the linear approximation for the kinetics in the vicinity of the uniform solution is of the form:

$$\frac{\partial p_i'}{\partial t} = \sum_{k=1}^{N} c_{ik} p_k' + D_i \frac{\partial^2 p_i'}{\partial x^2} \tag{33}$$

We further assume that the uniform solution $p_i' = 0$ for $f_i = 0$ is stable without redistribution ($D_i = 0$) and ask for conditions under which the inclusion of redistribution terms leads to destabilization and thus to patterns. In particular, we would like to demonstrate that localized self-enhancement in conjunction with widely distributed inhibitory effects lead to spatial patterns, thus generalizing the principles of lateral inhibition. For this purpose it is assumed that the system of N components can be divided into two subsets: One subset (a) with small redistribution of components, if any, and one (b) with wide redistribution and fast reaction rates. These properties are conserved upon linear transformations within the subsets so that we can always choose a linear transformation of parameters in such a way that there are no internal (non-diagonal) interaction terms within the subsets. After such transformations, equations for the subsets (a) and (b) take the form

$$\frac{\partial p_i'}{\partial t} = c_{ii} p_i' + \sum_{k=j+1}^{N} c_{ik} p_k' + D_i \frac{\partial^2 p_i'}{\partial x^2} \qquad i = 1 \ldots j \tag{34a}$$

$$\frac{\partial p_i}{\partial t} = c_{ii} p_i' + \sum_{k=1}^{j} c_{ik} p_k' + D_i \frac{\partial^2 p_i'}{\partial x^2} \qquad i = j+1 \ldots N \tag{34b}$$

and the corresponding matrix of the interaction terms c_{ik} is of the form (exemplified for $j=4$, $N=7$).

$$\begin{pmatrix} c_{11} & 0 & 0 & 0 & c_{15} & c_{16} & c_{17} \\ 0 & c_{22} & 0 & 0 & c_{25} & c_{26} & c_{27} \\ 0 & 0 & c_{33} & 0 & c_{35} & c_{36} & c_{37} \\ 0 & 0 & 0 & c_{44} & c_{45} & c_{46} & c_{47} \\ c_{51} & c_{52} & c_{53} & c_{54} & c_{55} & 0 & 0 \\ c_{61} & c_{62} & c_{63} & c_{64} & 0 & c_{66} & 0 \\ c_{71} & c_{72} & c_{73} & c_{74} & 0 & 0 & c_{77} \end{pmatrix}$$

The subset of parameters (a) with small redistribution is assumed to contain at least one autocatalytic term among the c_{ii} which is positive (or has a positive real part) and thus embodies activating properties. This can occur even if all diagonal terms of the non-transformed set are negative; for example, inhibition of inhibition is equivalent to activation, the latter showing up after diagonalization. To meet the condition that the uniform solution is stable, the other subset (b) must then act, via the non-diagonal terms, in a cross-inhibiting manner on the subset (a).

We may now extend the two-factor theory of section II.2 to the two subsets (a) and (b). If the components of the cross-inhibitory subset (34b) reach equilibrium $f_i = 0$ fast and their redistribution by diffusion or other mechanisms occurs widely, leading to a near-uniform distribution in space, we can express any $p_i'(i=j+1, N)$ of the cross-inhibiting subset approximately as linear combinations of spatial averages of the components of the activating subset $\overline{p_k'}(k=1, j)$.

$$p_i' = -\frac{1}{c_{ii}} \cdot \sum_{k=1}^{j} c_{ik} \overline{p_k'}$$

Upon insertion into eqn. (34a) one obtains for the activating subset, $i = 1 \ldots j$.

$$\frac{\partial p_i'}{\partial t} = c_{ii} p_i' - \sum_{k=j+1}^{N} \sum_{l=1}^{j} c_{ik} \frac{c_{kl}}{c_{kk}} \cdot \overline{p_l'}$$

Local distortions in a wide field will affect these space averages $\overline{p_l'}$ only to a minor extent. Therefore,

$$\frac{\partial}{\partial p_i'} \left(\frac{\partial p_i'}{\partial t} \right) \approx c_{ii}$$

Because at least one of the c_{ii} $(i = 1 \ldots j)$ was assumed to be positive, local distortions are self-enhancing, leading to destabilization of near-uniform distributions and thus to spatial patterns.

It follows that, if within a multi-component system, subsets with small and large redistribution in space can be distinguished which are characterized by self-enhancing and cross-inhibitory properties as described above and if the system is stable in the absence of redistribution effects, sufficient redistribution of the terms of the inhibitory subset leads to destabilization and thus to spatial patterns as long as the redistribution of the self-enhancing subset is sufficiently small. This is a generalization of the case of two factors, one activating and one inhibiting, to two subsets with several components each. It is probably not the most general version of the principle of lateral inhibition in systems with more than two components which would require much more involved mathematical analysis, but it suggests that the notion of pattern formation by autocatalysis and lateral inhibition is not only applicable to the interaction of two components; it is also a fair approximation for many more complex cases. The main problem in multi-component systems is to define activation and inhibition without ambiguity. This is not possible for all types of general kinetics (eqn. 1). The analysis given above requires a distinction of components with wide and little

redistribution from the outset. Assessment of pattern formation and assignment of activation and inhibition to subsets depends on this distinction and will lead to different results, if, within the same reaction scheme, different subsets of components are widely redistributed.

The notions of activation and inhibition have proved to be most useful and adequate in analysing biological pattern regulation in phenomenological terms. The distinction of activating and inhibiting features in its mathematical formulation is therefore biologically meaningful. On the other hand, one has to be open-minded in considering activation and inhibition as systems parameters, subsuming the combined action of several components, not necessarily concentrations of individual substances. This is evident in cases with activation resulting from inhibition of inhibition. A less obvious example of a simple model where this notion is applicable, is given in the following section.

2. *Example for a Multi-Component System: Lateral Activation and Striped Patterns*

It is doubtful whether general multicomponent dynamics is of much use to understand biological development considering the indefinite variety of models and conceivable mathematical solutions. Systems of several differential equations of type (1) give rise to ordered solutions only under restrictive conditions (see Smale, 1967). However, if the components interact with each other in a systematic way, ordered patterns with specific properties corresponding to biological features may be obtained. This will be exemplified by a symmetric four-component system and by chained reactions to model for chains of induction.

It is conceivable that two neighbouring states require mutual support by diffusing substances, that is, mutual lateral activation. Then there arises an area where one type of activation, say a_1, is high, excluding locally an alternative state a_2 but leading to the production of a product b_1 more diffusible than a_1 which supports activation of the neighbouring area with high a_2, and vice versa:

$$\frac{\partial a_1}{\partial t} = f(a_1, a_2, b_1, b_2) + D_a \frac{\partial^2 a_1}{\partial x^2} \tag{36a}$$

$$\frac{\partial a_2}{\partial t} = f(a_2, a_1, b_2, b_1) + D_a \frac{\partial^2 a_2}{\partial x^2} \tag{36b}$$

$$\frac{\partial b_1}{\partial t} = \nu(a_1 - b_1) + D_b \frac{\partial^2 b_1}{\partial x^2} \tag{36c}$$

$$\frac{\partial b_2}{\partial t} = \nu(a_2 - b_2) + D_b \frac{\partial^2 b_2}{\partial x^2} \tag{36d}$$

with b_1 crossactivating a_2 and b_2 crossactivating a_1. For certain functions f patterns are generated (Meinhardt and Gierer, 1980). An example of a pattern-forming function f of this general type is the following:

$$\frac{\partial a_1}{\partial t} = b_2 \cdot \frac{a_1^2(1+\varepsilon)}{1+\varepsilon a_1^2} \cdot \frac{1}{\left(\frac{a_1+a_2}{2}\right)^2} - a_1 + D_a \frac{\partial^2 a_1}{\partial x^2} \tag{37a}$$

$$\frac{\partial a_2}{\partial t} = b_1 \cdot \frac{a_2^2(1+\varepsilon)}{1+\varepsilon a_2^2} \cdot \frac{1}{\left(\frac{a_1+a_2}{2}\right)^2} - a_2 + D_a \frac{\partial^2 a_2}{\partial x^2} \tag{37b}$$

with the uniform solution $a_1 = a_2 = b_1 = b_2 = 1$. The b_1, b_2 terms are cross-activating, whereas the $(a_1 + a_2)^2$ terms take care of local mutual exclusion. Diffusion constants D_a, D_b must be such that spreading of b terms is of a wider range than spreading of a terms.

In the simplest case, an area is divided into two parts, one with high a_1 and another with high a_2 (Fig. 6a). In wider areas, alternating states of a_1 and a_2 are formed in space (Fig. 6b, see page 15). In two dimensions, striped patterns can be generated.

In biology, such systems may be involved in the subdivision of an area into segments and compartments, for instance of insect segments into anterior and posterior parts, or of imaginal discs into quadrant compartments (Garcia-Bellido, 1975). Pattern-forming systems with these properties can be treated by the multi-component generalization of destabilization analysis given above (Section IV.1) and it can be demonstrated that the lateral activation mediated by b_1 and b_2 is formally isomorphous to lateral inhibition. To show this, consider the function f, eqn. (36a, b), which is of the type

$$f(x, y, \bar{x}, \bar{y})$$

In the example eqn. (37a, b) f is given by

$$f = \frac{\bar{y}x^2(1+\varepsilon)}{1+\varepsilon x^2} \cdot \frac{1}{\left(\frac{x+y}{2}\right)^2} - x \tag{37c}$$

f depends on the localized autocatalytic feedback of the substrate x produced and on the locally exclusive cross-effect of the alternative substrate y, as well as on products catalysed by these substances and spreading more widely in space, namely the cross-activating term $\bar{y}$, and, possibly, a self-inhibiting term $\bar{x}$. We assume that there is a uniform solution $a_1 = a_2 = \text{const}$, $b_1 = b_2 = \text{const}$ and analyse the solution for stability to local distortions. For small deviations a_1', a_2', b_1', b_2' from the uniform solution, a linear approximation of eqn. (36) reads, with lower indices describing partial derivatives of f near the uniform solution,

$$\frac{\partial a_1'}{\partial t} = f_x a_1' + f_y a_2' + f_{\bar{x}} b_1' + f_{\bar{y}} b_2' + D_a \frac{\partial^2 a_1'}{dx^2} \tag{38a}$$

$$\frac{\partial a_2'}{\partial t} = f_y a_1' + f_x a_2' + f_{\bar{y}} b_1' + f_{\bar{x}} b_2' + D_a \frac{\partial^2 a_2'}{\partial x^2} \tag{38b}$$

$$\frac{\partial b_1'}{\partial t} = v a_1' - v b_1' + D_b \frac{\partial^2 b_1'}{\partial x^2} \tag{38c}$$

$$\frac{\partial b_2'}{\partial t} = v a_2' - v b_2' + D_b \frac{\partial^2 b_2'}{\partial x^2} \tag{38d}$$

A parameter transformation can then be introduced, with the sum and difference between a_1 and a_2, b_1 and b_2 as new parameters:

$$\begin{aligned} A &= a_1 - a_2; \qquad A' = a_1 + a_2 \\ B &= b_1 - b_2; \qquad B' = b_1 + b_2 \end{aligned} \tag{39}$$

From eqn. (38) the following equations are easily derived for the linear approximation

$$\frac{\partial A}{\partial t} = (f_x - f_y)A + (f_{\bar{x}} - f_{\bar{y}})B + D_a \frac{\partial^2 A}{\partial x^2} \tag{40a}$$

$$\frac{\partial B}{\partial t} = v(A - B) + D_b \frac{\partial^2 B}{\partial x^2} \tag{40b}$$

$$\frac{\partial A'}{\partial t} = (f_x + f_y)A' + (f_{\bar{x}} + f_{\bar{y}})B' + D_a \frac{\partial^2 A'}{\partial x^2} \tag{40c}$$

$$\frac{\partial B'}{\partial t} = v(A' - B') + D_b \frac{\partial^2 B'}{\partial x^2} \tag{40d}$$

If D_a is small and D_b large, eqn. (40) can be assessed according to the criteria explained in the

preceding section. Equation (40) has the form of eqn. (34), with (40a, c) as the activating, and (40b, d) as the cross-inhibiting subset. Analysis of eqn. (40) is particularly simple because in this case there are two completely uncoupled parameter systems (A, B, eqn. (40a, b) and A', B', eqn. (40c, d)) assessable for stability separately on the basis of conditions outlined in section III.2. Conditions for the uniform solution ($D_a = D_b = 0$) to be stable (Eqn. 20a, b) are

$$f_y - f_x + f_{\bar{y}} - f_{\bar{x}} > 0 \tag{41a}$$

$$f_x - f_y < v \tag{41b}$$

$$f_x + f_y + f_{\bar{x}} + f_{\bar{y}} < 0 \tag{41c}$$

$$f_x + f_y < v \tag{41d}$$

and the condition for destabilization of A or A', say A (upon wider redistribution of B), is (26a)

$$f_x - f_y > 0 \tag{41e}$$

The condition for pattern formation (41b, d), fast reaction rate v, can easily be met. The equations (41a, c) now permit an assessment of systems for pattern formation. It is easy to demonstrate that the example given above eqn. (37) leads to patterns for small values of ε ($0 < \varepsilon < 1$): In this case

$$f_x = -\frac{2\varepsilon}{1+\varepsilon}; \qquad f_y = -1; \qquad f_{\bar{x}} = 0; \qquad f_{\bar{y}} = 1$$

meeting the conditions eqn. (41a, c).

Several general aspects are illustrated by this example: Multi-component systems may be reducible by parameter transformations in such a way that pattern formation is essentially determined by two systems parameters, one activating and one inhibiting. The biological implication is that activation and inhibition as defined phenomenologically in biological systems need not be represented by the concentration of single chemical components, but rather by features of multi-component systems.

In the example given, activation "is" the difference between two activators; the inhibitory effect is due to (mutually exclusive) activation mediated by diffusible cross-activators b_2, b_1. It is formally represented in the linear approximation (40a, b), by the difference $B = b_1 - b_2$ of the two cross-activators. By comparison with eqn. (24a, b) it is seen that B plays the role of an inhibitor: The interaction parameter k_{ab} (eqn. (24a)) is proportional to, and of the same sign as $f_{\bar{x}} - f_{\bar{y}}$ in eqn. (40a); it must be negative because of eqn. (41a) in conjunction with eqn. (41e), whereas $k_{ba} = 1$ is positive.

It follows that mutual exclusive lateral activation is isomorphous with lateral inhibition rather than representing a different class of pattern-forming mechanisms. This notion is expected to be extensible to multi-component systems with chains of induction to be discussed below. In two-dimensional isotropic fields, the mutual support of neighbouring states can lead to stable striped patterns (Meinhardt and Gierer, 1980), whereas stripes are found to be unstable, in isotropic fields, for most two-component systems. Stripes occur frequently in developmental biology, as exemplified by insect segmentation.

3. *Chains of Induction and Intercalary Regeneration*

There is a class of phenomena of pattern formation and regeneration which has properties differing from those of simple morphogenetic fields based on lateral inhibition, and for which essentially different explanations have been discussed. An example is a pattern in which one or two terminal structures in an array are produced first and the internal parts are subsequently determined in a recursive manner by a wave of inductions with an n^{th} section determining the $n + 1^{th}$ section in the neighbourhood until the sequence of structures making up the pattern is complete. Regeneration of planaria (Chandebois, 1975) and regeneration within segments of certain insect appendages (Bohn, 1970, 1971) seem to be of this type. Within such segments, excised internal sections are regenerated, but

transplants producing segments with excess parts lead to further regeneration, resulting in a mirror-symmetric duplication of excess parts. Duplication is also found for certain small sections cut from imaginal discs.

These phenomena can be formally described as regeneration leading to a distinct sequential order. We enumerate the natural array of elements $1 \ldots N$, a designation that may but need not correspond to a substance gradient. Upon intercalary regeneration of parts removed, missing sequences are inserted until all (or nearly all) discontinuities in the natural sequence $1 \ldots N$ are eliminated. For instance, upon removal of an internal array 3-4-5-6 from a sequence 1-2-3-4-5-6-7-8, regeneration of 1-2-7-8 leads to the sequence 1-2-3-4-5-6-7-8 (underlined elements newly regenerated). Transplantation of excess sequences, exemplified by the array 1-2-3-4-5-6-7-3-4-5-6-7, lead to further interpolating regeneration giving rise to 1-2-3-4-5-6-7-6-5-4-3-4-5-6-7. Simple morphogenetic gradients formed on the basis of lateral inhibition do not easily explain the phenomenon of intercalary regeneration and do not account for duplication in a satisfactory way. The observations rather resemble a wave of induction: it appears that whenever an element n has neighbours different from natural neighbours $n-1$ or $n+1$, this discontinuity gives rise to regeneration; the process is recursive, leading to the state $n-1$ (or $n+1$) in the neighbourhood of n. Upon formation of $n-1$, the element $n-2$ can then be formed etc. until the complete sequence is re-established.

Such chains of induction, while easily described in words, are quite difficult to formalize in terms of general kinetics. Subtle conditions are to be met so that one of the elements does not eventually occupy the entire area, and two elements (say n, and $n-1$) do not alternate in space instead of restoring a complete sequence $1 \ldots N$. Models appear to require spreading (for example, diffusible) effects defining the range of induction of areas of type $n-1$ (or $n+1$) by areas of type n, or spatial self-limitation of element n, or both.

The spreading effect is accounted for by products $b_i(x, t)$ which are catalysed by, but more diffusible than, activators a_i. $\partial a_i/\partial t$ has to be a suitable function f of terms describing self-activation (a_i), mutual exclusion of alternative states (a_k, $k \neq i$)) at the same location by a suitable function r, lateral cross-activation by b_{i+1} and/or b_{i-1}, and, possibly, lateral self-inhibition by b_i. Not all variables are required for chains of induction in any particular case. We have demonstrated the pattern-forming capacity of various types of functions f. The choice of f is dependent on the special biological properties which are to be modelled for: for instance, whether regeneration proceeds upwards ($n \to n+1$), downwards ($n \to n-1$) or in both directions, and whether the regenerate is derived from cells further downwards or further upwards from the junction, or both, whether the system is size-regulating, producing a complete array of small sections in a small field, or non-regulating, leading to incomplete regeneration if total area is small (Meinhardt and Gierer, 1980).

An example of a model giving rise to waves of induction is the following:

$$\frac{\partial a_i}{\partial t} = \frac{c_i}{r}(a_i + \delta^- b_{i-1} + \delta^+ b_{i+1})^2 - \mu a_i + D_a \frac{\partial^2 a_i}{\partial x^2}; \qquad (c_i > c_{i+1}) \tag{42a}$$

$$\frac{\partial b_i}{\partial t} = \nu(a_i - b_i) + D_b \frac{\partial^2 b_i}{\partial x^2} \tag{42b}$$

$$\frac{\mathrm{d}r}{\mathrm{d}r} = \sum c_i \cdot (a_i + \delta^- b_{i-1} + \delta^+ b_{i+1})^2 - \gamma r \tag{43}$$

Fig. 7 demonstrates intercalary regeneration and duplication on the basis of eqn. (42, 43).

Such formalisms are generalizations of the four-component model of the previous section. Two systems with lateral activation (a_1, b_1) and (a_2, b_2) are substituted by N such systems generating an array of N states. f must contain terms that lead to chaining from n to $n+1$ or $n-1$, and terms that ensure that each section is limited in size. This limitation can be due to direct lateral inhibition of each state by a self-inhibitory terms b_n, or the indirect lateral inhibition mediated by lateral crossactivation of finite range b_{n-1}, b_{n+1} or both. Such chains of induction are formally related to the notion of "hypercycles" introduced by

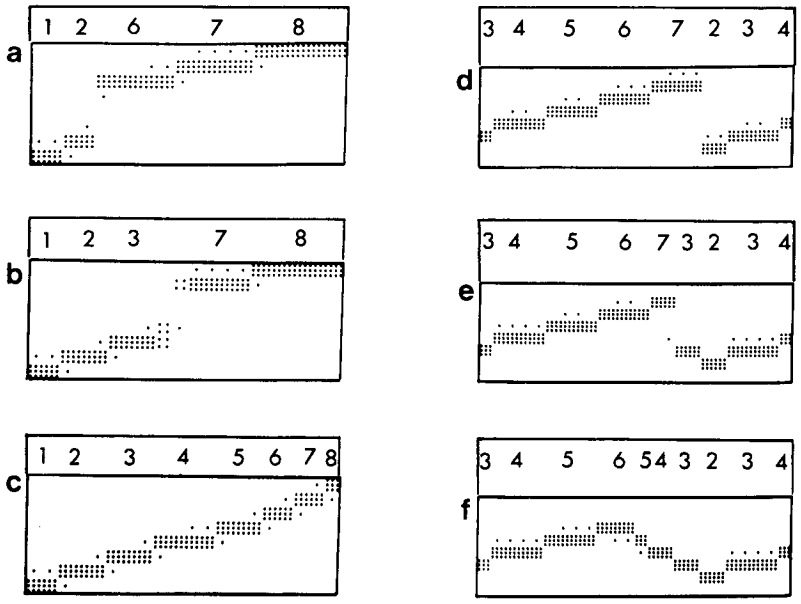

FIG. 7. Model for intercalary regeneration based on waves of induction eqn. (42). In each subpicture, dimension left to right represents position in the tissue, the dimension bottom to top the number of the state (a_n) activated. The dotted field ⁞⁞⁞⁞⁞⁞⁞ describes the activated state for each position in the tissue, as calculated by computer simulation. The resulting spatial sequence of states is given by the sequence of numbers on top of each subpicture. A sequence of activated states 1 . . . 8 is formed by sequential induction (not shown). After removing an internal part (3–4–5) (a), this can be restored by sequential induction (b. intermediate, c. final state). Transplants introducing excess sequences (d) lead to further regeneration until the discontinuity is smoothed out. (e. intermediate state, f. final state) (Adapted from Meinhardt and Gierer, 1980).

Eigen and Schuster (1978) for mutual stabilization of the production of macro-molecules involved in evolution. Proliferation and growth can be introduced into such models, but they are no logical prerequisite for intercalary regeneration. Preexisting areas of tissue may be respecified as well.

Alternative (though somewhat analogous) possibilities for models of a chain of states would be to postulate additive instead of exclusive states or—less likely—a sequence of states of a single multi-stable system involving very high orders of reactions.

In molecular terms, chaining systems suggest a system of different but related control circuits. An example involving control at the gene level would be a set of neighbouring genes on the chromosome with chemically related products and with some facilitation of the activation of a given gene if a neighbouring one is activated; another possibility would be auto- and cross-catalysis of a set of related RNA or protein molecules with cross-activation by molecules of related sequences. In each case special assumptions have to be made on the orders of the auto- and cross-catalytic reactions involved and on the range of molecules due to diffusion or other modes of spreading.

4. *Discontinuity Sensing*

A relatively simple model for recursive pattern formation presumes that the natural sequence of states in space corresponds to a monotonic gradient of some substrate ρ (x). Catalytic mechanisms can be envisaged that give rise to a local signal wherever there is a discontinuity in the distribution of ρ exceeding a certain threshold (Gierer, 1977a). If one component, say an activator a has a longer range than another component, say an inhibitor h, both produced in proportion to ρ, then the ratio a/h is below average at the high and above average at the low side of any discontinuity of ρ, whereas elsewhere the value of a/h is constant, irrespective of the value of the gradient. The discontinuity signal (which may be static or pulsing in time) can be envisaged as stimulating a gradual increase of ρ at the low side, or a decrease of ρ at the high side or both until the discontinuity falls below a

threshold of signalling, or disappears altogether. This process may, but need not, be accompanied by growth. It can lead to terminal as well as intercalary regeneration.

The model does not in itself lead to stable levels of ρ transferred to progeny cells upon cell proliferation and further growth, whereas chains of induction, as discussed in the previous section, lead to discrete stable states within subsections which could easily be preserved upon proliferation. We have shown that gradient discontinuity sensing can be linked to chaining mechanisms in such a way that the discontinuity signal drives the sequence of inductions until the signal falls below a threshold, because the complete sequence has been formed. The model combining discontinuity sensing and chains of induction has good size-regulatory capacities (Meinhardt and Gierer, 1980), and allows for mechanisms transferring the determined state to progeny upon cell proliferation.

V. READOUT AND INTERPRETATION OF MORPHOGENETIC FIELDS

1. *Types of Cell Responses*

Morphogenetic fields in multicellular tissues are expected to affect different processes, such as determination, differentiation, changes of form and interaction, orientation, proliferation and death of cells. In the simplest "positional information" model (Wolpert, 1971) cell response occurs with respect to the local value of the morphogenetic field and can be conceptually separated from the formation of this field. The morphogenetically active component may be the activator or inhibitor distribution, the latter extending farther into the tissue from centers of activation than the former. The terms activator and inhibitor refer to the function of components in the formation of the morphogenetic field. With respect to its effects on cells, activators can be inhibitory and inhibitors activating. The formation of secondary diffusible morphogens catalysed by the primary activators and inhibitors can also be envisaged. In the multi-component system discussed above, cell responses can occur to any of the components (a_n, b_n).

2. *Determination and Differentiation*

In the course of development of an organism, multipotent cells undergo a sequence of determining steps, each deciding between few (possibly, but not necessarily two) alternative pathways to generate eventually a diversity of differentiated cells. Alternative pathways for a cell can be determined by intracellular mechanisms, random fluctuations, or by intercellular morphogenetic fields in the tissue; in the latter case the morphogenetic field can lead to a subdivision of an area with different states of determined or differentiated cells in different regions. The simplest conceivable mechanism for cell determination would be to switch a bistable system from an "off" to an "on" state if the local morphogen concentration is above a certain threshold (see Meinhardt, 1976; Lewis, Slack and Wolpert, 1977; Gierer, 1977a). The state remains switched "on" if the morphogen is then removed. Multiple steps with different thresholds can be formalized along similar lines.

There are theoretical reasons suggesting that cell determination may be combinatorial, each state of a cell being defined by a combination of control circuits at the gene level turned either "on" or "off". A limited set of circuits would then suffice to specify a large number of states of cells. This suggestion is supported by analysis of the rules for transdetermination (Kauffman, 1973), compartmentalization (Garcia-Bellido, 1975), and sequential branched determination defining cell lineage as well as the expression of states of differentiation on the cell surface (Gierer, 1973). The chaining rules required to obtain sequential branched determination are somewhat analogous to those proposed for the chain of induction in the preceding section.

According to combinatorial models, cell determination in response to a morphogenetic field leads to the subdivision of an area into different sections; thereafter, new morphogenetic fields, perhaps of the same biochemical type as the preceding ones, are formed within the sections, leading to further subdivisions into subsections. This would lead to the specification of an "area code" in morphogenesis subdividing not only cell types but also

areas in a combinatorial fashion: there would be a limited set of control circuits and the combination of the circuits turned "on" is specific for a given subarea.

Some developmental processes can be described as a wave of differentiation extending into a tissue (see Zeeman, 1974). In the context of the theory of morphogenetic fields and cell response by differentiation, such waves can be modelled for on the basis of the size-regulating versions of pattern-forming systems as described in Fig. 4a, d. If the field is initiated by a gradient (Fig. 4a) or induced marginally (Fig. 4d), near-maximal activation extends into the field in wave-like fashion. This may be looked upon as a transition of cells from a state of low to a state of high activation, spreading in space from cell to cell until lateral inhibition stops further spreading. If irreversible cell determination and differentiation occurs above a threshold near maximal activation, differentiation also extends into the field as a wave. A simplified version of this concept is that activation *is* determination or differentiation, implying that the wave front of activation progresses irreversibly, by transition of cells from low to high activation, induced by the activated state of neighbouring cells, until lateral inhibition prevents further spreading. Such models are distinctively different from concepts in which a wave of transition towards differentiation extends into the tissue to eventually cover the entire area ("domino theory") or to be stopped by an external or internal time-controlled event. The essential point is that the spatial extent of the wave is self-limiting by lateral inhibition originating in the activated area but spreading into the nonactivated domains, so that the wave of transition stops at a defined extension of activation by effects arising internally within the activated area itself.

3. *Generation of Tissue Curvature and Real Form*

(a) *Self-regulating properties in the generation of form*

Morphogenesis proper is the generation of real form of cells, tissues, organs and organisms in the course of development. This is a complex process in which various combinations of different mechanisms are involved, and one does not expect explanations which are universally applicable. Nevertheless there is a relatively simple prototype in multicellular organisms: form often originates as a pattern of curvature of cell sheets and its generation can be traced back to processes of evagination or invagination at defined positions within originally nearly flat cell sheets in the course of development; this applies to gastrulation, neurulation, the development of structures in the central nervous system, the formation of rudiments of appendages in imaginal discs of insects etc. For a physical understanding of this process the following features are particularly relevant that have been experimentally found in various simple model systems: (i) Excised pieces of tissue often generate a structure equal or similar to the one that can be produced without excision; thus a piece of hydra tissue cut from the budding region can produce a bud. This suggests that bending moments rather than tangential forces dominate the generation of form. (ii) The inner and outer part of (single or multiple) cell layers are often different; in many cases the inside-outside anisotropy is obvious in the microscope. This anisotropy implies that a morphogenetic field activating a subarea in the tissue can generate a bending moment different from that in the environment, leading to curvature. (iii) Theoretically, evagination of a tissue could proceed in different ways: Either cells retain their nearest neighbours, but undergo gross changes in the shape of the cross-section tangential to the cell sheet—say from round to elongated; or cells retain this shape, but rearrange to a considerable extent. Studies on cell forms support the latter mechanism (Fristrom, 1976; Graf and Gierer, 1980). It requires that friction and shear are sufficiently low not to inhibit evagination. (The shape of the cross-section perpendicular to the surface changes upon evagination in any model, because curvature requires different inner and outer surface areas.) (iv) Certain inhibitors reversibly delay the process of evagination and others can even lead to reversion and repetition of evagination (Spooner and Wessels, 1970; Spooner, 1975). The generation of form is thus not due to a time-controlled all-or-none irreversible event (such as a contraction and fixation of inner or outer cell surface areas); it rather appears that a morphogenetic field locally changes the conditions for a stable steady state which is then approached by the generation of curvature. On

this basis, distortions and delays can be corrected by self-regulation. The capacity for self-regulation is maintained for some time during development, but usually ceases when substructures are formed, so that development at large is an irreversible process.

The self-regulatory properties suggest that the curvature and form assumed by the tissue corresponds to a state of a minimum of generalized potential. In the simplest case, potential would be additive with respect to the cells. Potentials have been introduced into theories and models of cell arrangements and of tissue form by D'Arcy Thompson (1952), Gustafson and Wolpert (1963) and Steinberg (1963) with reference to the interaction between cell surfaces. Generalized potentials as introduced by Ljapunov are a generalization of the concept of minimal free energies to steady states and dissipative structures which require a flux of energy. Such generalized potentials are expected to be a function not only of the interaction of cells with other cells, with external media and with extracellular substrates, but also of internal parameters determining cell form, e.g. in relation to anchorage, formation, elongation and degradation of intracellular fibres. In the steady state, such fibres may continuously be formed and broken down. Contraction may but need not be involved; if it is, it is not considered to be a single irreversible event, but one contribution among others to the stable steady state.

(b) *Stability of cell sheets and induction of bending moments by morphogenetic fields*

The theory summarized below (Gierer 1977b) is based on generalized potentials, stability criteria for cell sheets and the generation of bending moments to produce curvature at positions activated by morphogenetic fields. For tissue evagination to occur in a reproducible fashion, cell sheets must be free to undergo the movements of evagination and must nevertheless be stable. A prototype is the free cell sheet facing different inside and outside media, but cases in which cell sheets rest on a flexible inner cell mass instead of inner media are formally similar. The problem of stability of such free cell sheets is an intricate one. If the shape and contact areas of the cell (idealized as a cell model with flat surfaces) are expressed in terms of external, internal and intercellular surfaces, f_a, f_b and f_c (with two values determining the third for a given cell volume), it is found that no linear relation between potential and surfaces

$$P = c_a f_a + c_b f_b + c_c f_c \tag{44}$$

can lead to a stable cell sheet. Sheets would be unstable either to decay into outside or inside medium or to clumping. In molecular terms, this implies that no stable isotropic distribution of substances on the cell surfaces, however complex in composition, can possibly lead to a free stable cell sheet.

On the other hand, very simple non-linear relations, such as

$$P = c_a f_a + \frac{c_a' \kappa_a f_a}{1 + \kappa_a f_a} + c_b f_b + \frac{c_b' \kappa_b f_b}{1 + \kappa_b f_b} + c_c f_c \tag{45}$$

suffice to ensure stability not only with respect to clumping and decay, but also with regard to a set of further distortions of the sheet (Fig. 8). The non-linear features required may result from a variety of molecular mechanisms, including capping of components on the membrane, limited supply of molecules involved in cellular interaction, and effects of anchorage and formation of intracellular fibres.

A morphogenetic field which locally changes parameters such as c_a', κ_a leads to local changes of bending moments, and thus to curvature and form; the state of minimal potential is a state of evagination. Bending moments and resistance to bending are derivatives of potential with respect to curvature of the cell sheet. The state of minimal potential of the sheet is then given by the laws of mechanics.

However, the parameters introduced into the calculations of the form of the sheet corresponding to a state of minimal potential, need not determine the mechanical properties of the tissues. These are influenced by stabilizing structures that may be produced secondarily. For instance, the mechanical stability of Hydra tissue is due partially to extracellular mesoglea at the interface between ectoderm and entoderm, and mainly to tight

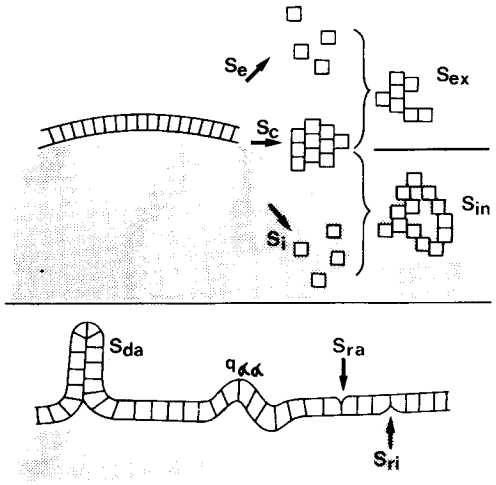

FIG. 8. Stability conditions for cell sheets. A freely moving cell sheet should be stable against clumping (S_c), decay into inside and outside medium (S_i, S_e), spongy states in inside or outside medium (S_{in}, S_{ex}), backfolding (S_{da}), undulation ($q_{\alpha\alpha}$) and intercellular cleavage from outside or inside (S_{ra}, S_{ri}). The set of conditions is inconsistent with linear relations between generalized potential and cell contact areas with other cells, inside and outside medium; but simple non-linear relations of type eqn. (45) can lead to stability according to all criteria listed in the figure.

junctions between ectodermal cells. Relaxation of such stabilizing structures may occur only slowly, and may itself depend on morphogenetic signals and fields.

(c) *Application of shell theory to tissue form*

To calculate real form, shell theory developed by architects and engineers is particularly suitable. It is based on the assumption that the thickness of the (cell-) layer is considerably smaller than the radius of curvature. For the biological application, the equations for bending moments are most relevant. Shell theory is easy to apply for curvature in one dimension, and for rotationally symmetric structures. The latter include free closed shells in three dimensions. Effects of local generation of bending moments in response to morphogenetic fields can be simulated by computer.

A few model cases with rotational symmetry are demonstrated in Fig. 9. Figure 9a shows that activating a single area can lead to a complex structure because of the interaction of curvatures in the two dimensions tangential to the sheet. Figure 9b shows a simulation of evagination starting from a spherical shell. It demonstrates the generation of form based entirely on internal mechanisms within the cell sheet, without reference to boundary conditions. The prepattern can be formed internally, the position of the activated area being determined either by some unspecific polarity-defining gradient or even by random symmetry breaking leading to activation as modelled in Fig. 5g; the cell response then causes a defined structure to be formed by evagination (b), or invagination (c), depending on the effect of the morphogenetic field on potential P (eqn. 45).

Closed configurations (Fig. 9b, c) are models not only for morphogenesis in multicellular organisms but also for the generation of form of single cells. In this case, the layer generating cruvature is to be interpreted as a cell membrane or boundary layer rather than a cell sheet. If the membrane shows an inside-outside anisotropy, because molecules inserted on the inside and outside surface are different, a focus of intracellular or intramembrane activation may affect the distribution of such molecules causing bending moments which lead to evagination or invagination, possibly followed by detachment. It is not claimed, however, that all processes affecting cell form can be modelled in this way.

(d) *Elongated structures*

Most structures generated in the course of embryogenesis are elongated rather than spherical. Elongated structures can be produced in various ways: If a cap area is activated

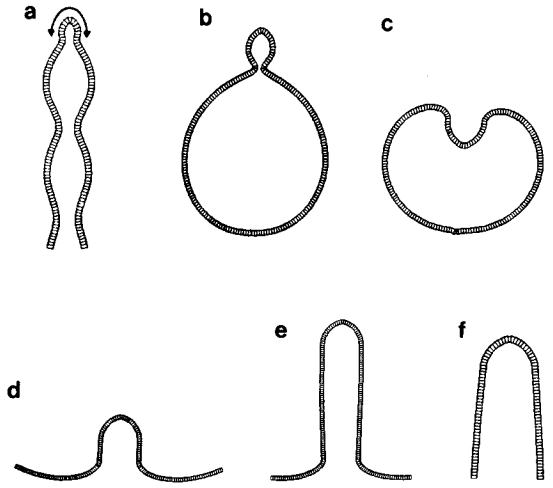

FIG. 9. Rotationally symmetric structures formed by local activation of an area (centre-top of the structures drawn) generating excess bending moments. Pictures represent sections through (three-dimensional) cell sheets. Axis of rotation is vertical. Calculations are made on the basis of shell theory. a. A single circular area of activation (top) can lead to a complex structure due to interaction of curvature in two dimensions. b. Evagination, c. invagination from a closed sphere, resulting from activation of a small area on the top. Such models can be applied to cell sheets to model tissue form as well as to membranes and boundaries of single cells to model cell form. If there are two degrees of activation, a strong one in a small circular area (top) and a weaker one extending into the surrounding area, an elongated structure such as a protruding bud can be produced (d, e). Elongated structures can also be formed if an activated area (top) develops a strong local bending moment, and, in addition, extends a gradient into the surrounding tissue giving rise to anisotropy, with bending moments preferentially in the circular dimension; a simple model case is calculated in Fig. f.

strongly and the environment of this cap area more weakly, an elongated form results (Fig. 9d, e). Another possibility is that a cap area is primarily activated and extends a gradient into the environment; there the gradient causes the tissue to become anisotropic, with bending moments predominantly in the circular dimension perpendicular to the gradient, leading to elongated structures (Fig. 9f). Coordination effects (e.g. a preference of cells to form close-to-hexagonal arrangements) can also contribute to elongation.

Further, elongated structures can be formed if resistance to bending (flexibility) rather than bending moments themselves is affected by morphogenetic fields. For example, if activation of a (cap) area reduces resistance to bending there, or increases this parameter in the region surrounding the primarily activated area, elongated structures can be produced. This type of mechanism is capable of good proportion regulation: if activated area is proportional to total area, length can be nearly proportional to width. Many other mechanisms producing elongated structures, such as those described in Fig. 9, do not by themselves lead to proportion regulation of real form.

An example of the generation of form with proportion regulation is the regeneration of Hydra from excised pieces of the body column, leading to nearly constant proportions of head to body tissue (Bode, and Bode, 1980) as well as of length to width (Sugiyama, personal communication). Possibly, this proportion regulation results from an effect of the morphogenetic field on resistance to bending during the generation of form, for example by increasing flexibility in the area activated to produce a new head.

(e) *Multicellular layers*

Some biological structures are determined by cell sheets consisting of several layers. The theory based on generalized potentials has been extended explicitly to two-layered structures (for which hydra tissue consisting of entoderm and ectoderm cell layers is an example). For sheets consisting of a small, but undefined number of cell layers, it is conceivable that the contribution of inner and outer surface areas to generalized potentials (e.g. by providing access to molecules of the medium) may have a finite range of several cell layers. Formally,

one may then consider a multicellular unit extending across the sheet as one cellular element and apply the concepts of the theory to this unit: Bending moments generated by the morphogenetic field lead to an increase of one (say, external) surface and a decrease of the other (say, internal) surface, and thus to curvature and form.

(f) *Kinks*

Tissues often show kinks in the contour of the surface; for instance, early insect embryos and rudiments of appendages form kinks at segment borders. Generally it appears that such kinks are easily formed at border lines separating differently determined sections of a tissue.

Such borders have properties different from that of the internal part of the different sections. Local morphogenetic signals could arise at such borders by discontinuity sensing based on autocatalytic mechanisms (Gierer, 1977a; Meinhardt and Gierer, 1980; see section IV.4) or by the cooperation of substances produced in different sections (Meinhardt, 1980). Such local signals could strongly affect parameters relevant for potentials and the generation of bending moments and in this way lead to a kink in the border region.

Within the framework of the theory based on generalized potentials, an attractive possibility would be that differently determined parts of the tissue do not recognize each other as "self"; cells with an intercellular contact area at the boundary, f_c', may respond in some respects to the foreign cell as if it were a medium. Such behaviour could easily result in kinks. If for instance, in eqn. (45), f_a in the non-linear term is substituted by $(f_a + f_c')$ and the interaction term c_c' with respect to f_c' is assumed to be slightly different from c_c, the resulting potential of cells at the boundary is given by

$$P = c_a f_a + \frac{c_a' \kappa_a (f_a + f_c')}{1 + \kappa_a (f_a + f_c')} + c_b f_b + \frac{c_b' \kappa_b f_b}{1 + \kappa_b f_b} + c_c f_c + c_c' f_c' \tag{46}$$

Computer calculations based on an idealized geometrical model for a kink show that three interesting types of behaviour can be obtained for different parameter ranges: The most stable configuration can either be a kink with an angle between 0° and 180°, or a kink of 180° where the sheet folds back on itself; further, there are parameters leading to a state of near-zero resistance to curvature resembling an ideal joint.

(g) *Waves*

As discussed in section V.2. on differentiation, cells in an activated area may undergo a transition toward a differentiated state, and this transition may spread, from a centre of initiation, into the surrounding tissue in a wave-like fashion until lateral inhibition prevents further spreading. The same mechanism may apply to the spreading of a wave of transition of cells with respect to form, orientation, interaction, bending moments and/or resistance to bending, and may lead to progressive recruitment of cells participating in form-generating processes like budding, invagination, evagination or folding. Again, as in the case of waves of cell differentiation, wave mechanisms with self-limitation by lateral inhibition are different from waves eventually covering the entire tissue, or terminated by external or time-controlled events.

Budding Hydra may provide an example of such self-regulation of a limited area participating in tissue evagination. Budding is initiated by activation of a near-circular area of the body column in which cells are recruited for the future bud. The cells assume a relatively columnar shape before they participate in evagination and forming the bud. Recruitment of cells extends into the tissue in a radial fashion (Otto and Campbell, 1976; Graf and Gierer, 1980), but the extent of recruitment is self-limiting, possibly by effects of lateral inhibition arising in the activated area.

The theory of tissue evagination based on generalized potentials, stability criteria for cell sheets and the generation of form resulting from bending moments induced by morphogenetic fields is not proposed as generally applicable in biology. On the contrary, one expects that there are other mechanisms primarily determined by tangential forces, pressure, friction and shear as well as mutual steric hindrance of structures, which cannot be subsumed under the theory. However, in cases that show the self-regulatory properties

mentioned, it appears likely that the theory is a fair approximation independent of details of the molecular mechanisms affecting cell surfaces and cyto-architecture.

In the application of shell theory to engineering, emphasis is mostly placed on tangential forces; an example is the design of thin concrete roofs capable of supporting enormous weights while avoiding strong bending moments which would cause the roof to collapse. In the biological applications proposed, tangential forces are considered to be small, the emphasis being on bending moments. Logically these can be produced only because cell sheets have a feature that most technical materials do not show: a striking inside-outside anisotropy. It is this property which is the prerequisite for the generation of excess bending moments in response to localized scalar signals, such as high morphogen concentrations.

4. *Growth*

Whereas growth *per se* is capable of generating order in space if different parts of a structure grow out in sequence, embryonic structures are often preformed in small rudiments which grow out to larger sizes only in later stages. In these cases, growth patterns affect proportions of parts laid down previously, transforming rather than generating form. Morphogenetic fields are expected not only to cause the subdivision of the rudiment into different parts but also to affect the growth pattern programming cells as to the occurrence (and perhaps number) of proliferations.

The decision for a cell within the tissue to proliferate is probably influenced by a combination of parameters. The theory of pattern and form discussed above does not explicitly refer to growth, but draws attention to the possibility that the parameters which are essential in determining patterns and form are also involved in the control of proliferation. Extracellular and intracellular humoral factors, including those active in morphogenesis, feedback mechanisms controlling and stabilizing the density of cells of certain types in the tissue, chemical signals defining the occurrence and distance of structures within this tissue, as well as contact areas of cells with other cells, with media and with extracellular material may be expected to contribute to control growth.

In this context, two general experimental findings are of interest: (a) Cells isolated from tissues (other than bone marrow) are difficult to grow in suspension. In many cases cell cultures are possible only after rare changes, such as somatic mutations; but then, the cells produced often differ from the original tissue cells. It appears that tissue cells are difficult to grow in suspension not because the ideal media are not yet known, but because the cells require contact with other cells or extracellular material. In simple cases, as for fibroblasts, contact with a surface of a dish is sufficient for growth, but often a more complex environment appears to be necessary resembling that within tissues more closely. (b) Cancerogenesis often appears as a multiple-step process, passing through latent and pre-cancerogenic states. This suggests that the release of cells from growth control may be a multiple-step process. Growth of a tissue cell may depend on the combination of several conditions to be met. Release from all these conditions leads to uncontrolled growth.

Several lines of experimental evidence suggest effects of external factors, cell contacts, and/or cell form on proliferation. Growth can be stimulated by soluble growth factors, and inhibited by cell contact resulting from crowding of cells on the dish. However, the latter inhibition can be overcome by larger amounts of growth factors (Holley, 1975). Negative effects of cell contact could but need not be the result of reduced access of the cells to soluble factors in the medium. Attachment to surfaces might be an independent parameter affecting growth control (Dulbecco and Elkington, 1973).

The relation between cell form and growth is further clarified by results of Folkman and Moscona (1978). By contact with different artificial surfaces to which fibroblast cells adhere, cell form can be varied between flat and near-spherical. Proliferation of cells is found to be mainly determined by cell form rather than by intercellular contact. It remains undecided whether growth regulation is primarily due to the effect of the contact area between cell and surface, or to cell shape and internal cytoarchitecture or to a combination of these features. The results do not preclude that in other systems cell form *and* contact with other cells codetermine the control of proliferation. In particular, effects of cell

interaction on growth control are likely to occur in tissues made up of cells of different types.

In analogy to the conditions controlling stability and form of cell layers we expect that the control of proliferation of tissue cells also requires a non-linear algorithm. This may involve the concentration of external humoral factors, different cell surface areas, and possibly other form parameters related to the internal cytoarchitecture. Such non-linearity implies that the combined effect of factors involved in growth control cannot be inferred from studying the effect of the individual factors by themselves.

VI. SOME GENERAL AND PHILOSOPHICAL ASPECTS OF BIOLOGICAL PATTERN FORMATION

1. *Physicalism and Biological Form*

One of the aims of modern biology is to understand basic biological phenomena in terms of physical laws. This does not imply reductionism; the features distinguishing living from non-living entities are not to be denied but to be explained. Along these lines much progress has been made in recent decades in the field of molecular genetics with results on the structure, replication, mutation and primary function of genes that apply to all living organisms. At present, much attention is directed towards the specific features characterizing higher organisms. Their most interesting and striking aspects are behaviour and form. Behaviour is determined by the function of nervous systems which, in turn, is expected to be understandable in terms of physical laws. This expectation is partially based on the theorem of Pitts and McCulloch stating that any formalizable capacity can be modelled on the basis of digital computers. Though nerve nets are not made up of digital elements, the capacity of the nerve cell is certainly not below that of a digital unit, but may embody the capacity of a set of digital units. Therefore, one expects physicalism to hold for neurobiology in that any formalizable function can be explained as a property of neural networks. An open question is whether all properties of brains (including those relating to consciousness) are fully formalizable.

With respect to form, the generation of patterns starting from near-uniform states and their striking self-regulatory features (such as the formation of two small, but complete organisms from two halves of an early embryo) have long been considered a challenge for physical explanations in biology.

The theory of pattern formation described which is capable of modelling these regulatory properties, is based on very conventional physical laws—general chemical kinetics requiring only features which are common in molecular biology, though in some special combinations. Even if it were necessary to amend the theory considerably in the course of further experimental developments, it is evident already at the present stage that biological patterns and their properties of self-regulation are within the scope of conventional present-day physics: physicalism and biological facts are consistent.

However, one would not do justice, historically, to the vitalist branch of thinking in describing it as "unscientific" and obscure. The basic laws of physics have been discovered and experimentally confirmed first in the non-living domain, and the extension of physical laws beyond a given domain is always an empirical question which cannot be decided by thinking alone. For instance, classical mechanics had to be revised and extended to quantum mechanics to be applicable to atomic dimensions and properties; on the other hand, no further revision was necessary to understand molecules in general and chemical bonds in particular. It is experimental evidence, especially in the field of molecular biology that has shown the physical laws are fully applicable and sufficient for biology. Further, at the stage the vitalists put forward their arguments, physicalism was often associated with reductionism, postulating that the basic laws of physics and chemistry would, in themselves, provide explanations for biology. Our present notion is somewhat more liberal: though the basic laws of physics hold in the entire biological domain, one has to take into account that a system of components has properties that the components themselves do not have. Most of the interesting biological properties are such systems features as are revealed only by specific studies of biological systems. It was the merit of the vitalist school of thought to

emphasize the holistic view, though this was mainly done on the basis of intuition. Afterwards, with the advent of systems theory, the holistic approach became fully integrated into science, thus complementing and justifying rather than invalidating the physicalist position that the basic laws of physics apply fully to the biological domain.

2. *Common and Distinct Properties in the Formation of Biological and Non-Biological structures*

The generation of structures by autocatalytic mechanisms in conjunction with depletion or inhibition effects is not restricted to developmental biology; it is evidently involved in many other processes, such as the formation of stars and galaxies, waves and clouds, crystals and dunes in the domain of physics, and it can be recognized in social effects as in the generation of socio-economic inequalities, urban congestion, or traffic jams. The reason why such effects have been inferred for developmental biology only relatively late is that most chemical reactions in solution lead to homogeneous distributions of compounds in liquids. Only since Turing (1952) discovered that spatial concentration patterns can be generated in solution on the basis of catalytic reaction kinetics, were conditions for such pattern formation studied in more detail and related to biological properties. Our principle of autocatalysis in conjunction with lateral inhibition which emerged from biological studies provides a link to autocatalytic structure formation in the inorganic as well as social domains. Other conceptual approaches to structure generation have been developed which also elucidate general features common to biological and non-biological structures in space and time. Theories emphasizing energy dissipation (Glansdorff and Prigogine, 1971), bifurcation, catastrophes (Thom, 1972), and the understanding of synergetic processes in terms of general "order parameters" (Haken, 1977) are particularly relevant.

However, studies of properties of biological pattern formation which are common to the generation of physical structures, by themselves, do not adequately deal with the specificity of biological phenomena. In biology complex structures are generated in a reproducible fashion under the instruction of genes. A structure is produced in a sequence of patterns, subpatterns, sub-subpatterns etc. and each subpattern arises in a reproducible location and orientation with respect to the pattern of which it is a part. In this process, the reproducible orientation of subpatterns with respect to the initial structure, that is the polarity effect, is of particular importance. If we look at an elephant, we realize that no interesting structural feature is due to true symmetry breaking. The tusk always turns up, the toes point forward etc. For asymmetric patterns formed on the basis of autocatalysis and lateral inhibition, this polarity effect is expected: the orientation, though not the shape, is determined by preexisting spatial order.

In the formation of many inorganic structures true symmetry breaking is essential. Random fluctuations determine position and often other properties of the structure to be formed, say a crystal, a cloud or a dune. In biology, formal general results on bifurcation and symmetry breaking are also useful mathematical tools. Nevertheless, if we ask for essential *distinguishing* properties of biological versus inorganic structure formation, it is specific for biology that most interesting features do *not* arise on the basis of true, that is, random symmetry breaking.

3. *Pattern Formation and Pattern Recognition*

The concept of lateral inhibition was originally introduced in the fields of neurophysiology, visual perception and pattern recognition (Kuffler, 1952; Kirschfeld and Reichardt, 1964; Wilson and Cowan, 1973). Short-range activation in conjunction with long-range inhibition leads to contrast sharpening, such as edge enhancement, emphasizing the contour rather than the area of objects. Neural correlates, such as activating and inhibiting synapses, have been found. The importance of short-range activation and long-range inhibition in pattern formation suggests a formal relation between the generation and recognition of patterns, despite the fact that the biochemical mechanisms of (mostly non-neural) cells in one case and synaptic electrical interaction of neurons in the other are very different. The formal relation suggests that often patterns which are easy to produce are easy to recognize;

and that the corresponding hidden regularities are immediately experienced as "aesthetic", although their formal analysis need not be easy. Combinatorial interaction of simple patterns can be obvious, as in the case of a periodic pattern modulated in intensity or spacing or both. A less obvious example is given by certain textures: Julesz (1962) has studied various arrangements of objects with respect to figure-ground discrimination, testing which arrangements are immediately (that is in fractions of a second without conscious thought) perceived as a figure if placed on a ground with a different arrangement. One of his results is that textures with different second-order statistics are directly perceived. For instance if a field of dots in positions, which are random except for a tendency to avoid small distances, is placed on a background of completely randomly distributed dots of the same density, there is immediate figure-ground discrimination (Fig. 10). The "granularity"

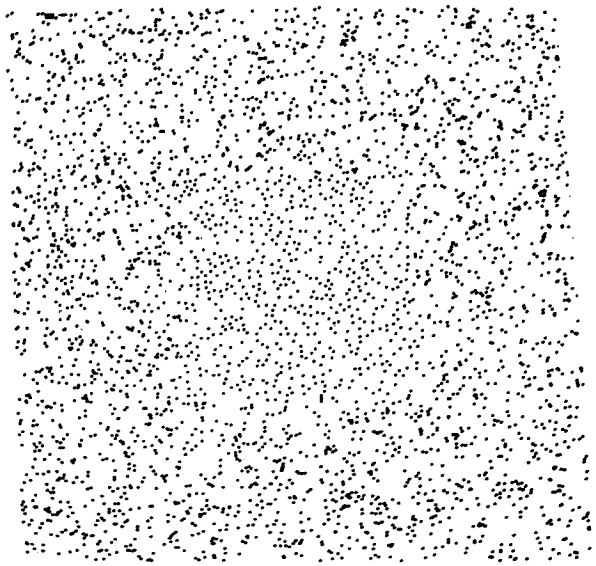

FIG. 10. The central part of the figure is distinguished by immediate perception from the surrounding area, although dots are distributed randomly and at the same density throughout the field. The centre is distinguished, however, by the avoidance of small distances. According to Julesz (1962), this texture leads to immediate figure-ground discrimination. The same feature—inhibitory fields surrounding peaks of activation—characterizes the formation of patterns by autocatalysis and lateral inhibition upon random initiation in a two-dimensional field, as demonstrated in Fig. 5d.

avoiding small distances, however, is just the type of spacing of peaks obtained by pattern formation on the basis of lateral inhibition upon random initiation within a field (Fig. 5d). It follows that this texture which can be formed in a most simple way on the basis of lateral inhibition is also perceived easily within seconds and without conscious thinking although a formal analysis of the properties characterizing the texture requires considerable time and scientific training.

With respect to real form of organisms and organs given by variable curvature of contours it is conceivable that, again, hidden regularities characterizing the formation of pattern and form (such as relation between curvatures in two dimensions as in Fig. 9a) may play a major role in the perception of the form, and in aesthetic experience as well.

One expects that further insights into mechanisms of pattern formation and recognition would lead to more subtle formal relations which are anthropologically interesting. Overt as well as hidden regularities produced by biological pattern formation can be immediately experienced by our brain and often appeal to our aesthetic sense. The same is true if such hidden regularities are found in works of art, or in natural or artificial landscapes. It is not claimed, however, that the relation between pattern formation and recognition can by itself explain aesthetic visual experience: this has many aspects, including the interrelation of symbols, experiences and emotions; the aesthetics of hidden regularities is just one of them.

4. *Logic of the Generation Cycle*

In the eighteenth century a popular theory of biological development proposed by Bonnet and Haller was to assume that the organism to be formed is contained in a miniature, but fully specified form within the egg cell, in analogy to the Russian "puppets in the puppet" (for an excellent presentation and discussion of the early history of embryology, see Jacob, 1970). Since the egg cell is much smaller than the organism from which it is derived, to provide organisms for many generations ahead would imply that their structure is contained in unbelievably small volumes. This point has already been made in the eighteenth century by Buffon. While the argument was always plausible, in modern physics it can be based on a law of nature: according to quantum mechanics structures involving chemical compounds cannot be stable in dimensions less than an atom's volume, that is 10^{-24} cm^3. Since an egg cell has a volume several orders of magnitude below that of the organism in which it originates, organisms cannot contain structures providing for progeny many generations ahead.

It follows that structure is newly generated in each generation. However, as discussed in the context of polarity, biological pattern formation is not essentially based on random symmetry breaking in initially uniform distributions. Rather, to generate a reproducible complex organism, the orientation of structures is to be reliably derived from preexisting spatial cues. This is a necessary requirement for the reproducible formation of substructures of the embryo. It would not be logically necessary for the egg cell itself, except for the handedness (causing the heart to be on the left) which is probably determined by asymmetric molecules and molecular compounds, though in an indirect and still non-understood manner. However, experimental evidence shows that even the egg cell itself can develop (as in the case of many insect eggs) in a predictable orientation with respect to the organism from which it derives: asymmetric oriented flies can produce asymmetric oriented eggs and they in turn can develop into asymmetric flies.

In verbal discussions explanations of orientation by orientation may sound like a circular argument. However, the formal treatment shows that this is not the case. While the puppet in-the-puppet theory of biological pattern formation is inconsistent with physical laws, no such laws are violated if the orientation of structures is reliably and indefinitely determined by preexisting structures. In the framework of the theory of pattern formation by autocatalysis and lateral inhibition, the orienting feature is incorporated as polarity-defining gradient which can be an unspecific gradual cue orienting a pattern; on the other hand, the form of the pattern is not affected: this means that most of the information contained in the polarity-defining distribution is not embodied in the pattern newly formed, but is lost in the generation cycle. In terms of the theory of pattern formation described, this loss of information is due to the non-linear features of the pattern-generating equations. The partial destruction of information in the course of pattern formation is as important in understanding the logic of the generation cycle as is the conservation of cues to orientation. The logic of the generation cycle implies that structures are determined by genes in the long run. This in turn requires that minor deviations of structures other than genes should not matter: for instance, a deviation in the plasma of an egg cell at present is usually not expressed in organisms a few generations from now.

5. *Mathematics and Matter as Explanatory Basis of Morphogenesis*

The discussion of biological pattern formation in this article presumes that the combined consideration of material and mathematical aspects is required for a satisfactory explanation. This is in contrast to those reductionalist and materialist viewpoints which consider material aspects, especially chemical structures as facts, whereas mathematics provides hypotheses which have only transient value, if any in the search for the structure of relevant molecules. The idea that only molecules (or more generally, structures) are essential cannot apply to development, because properties characterizing development are systems features. The structure of an organism is related to the structure of molecules involved in its generation only in a most indirect manner. Even if we were to have a complete list of all molecules

involved in pattern formation with any structural detail, we would not be able to derive the patterns they generate without general physical kinetics and without making use of mathematical facts as well.

The other extreme view held by some mathematicians is that molecules are of no interest at all; the formal principles involved in a systems approach provide the explanation. Conceptually, such principles include catastrophes, energy dissipation, bifurcation, network interactions, etc. This view, however, is not fully satisfactory either. The concepts mentioned, though valuable explanatory principles, do not explain specifically biological phenomena, but relate to structure formation in general. But even if the formalisms could deal adequately with features specific to biology, experimental confirmation by biochemical evidence, in addition to phenomenological evidence on pattern formation and regulation, would be much more satisfactory than support by phenomenological facts alone. Moreover, the knowledge of molecular structures involved in pattern generation would be a prerequisite for studying certain questions of general interest, such as the degree of complexity of these molecular systems, their relation to molecules with other functions, and the evolution of pattern-forming systems. Therefore, only a combination of knowledge of mathematics and matter is expected to lead to a satisfactory understanding of biological pattern formation. It is psychologically understandable that most biochemists and molecular biologists prefer the materialist and most mathematicians the formal aspect of the problem. Philosophically, it appears that the formal, mathematical aspect is more fundamental than the structural one for understanding, but is insufficient for experimental confirmation. However, it is worth noticing that the relative explanatory value of mathematics versus matter is the subject of an age-old controversy traceable to Pythagoras and Plato in favour of mathematics, and Demokrit (as well as Marx) in favour of the materialist notion, and perhaps not objectively resolvable.

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