AN EIGENSTATE APPROACH TO CELLULAR DIFFERENTIATION

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
Philip S. Ulinski
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ABSTRACT

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The problem of the Thesis is to study the relationship between the morphological type of a given cell and its biochemical system. This problem is shown to be a particular example of a more general set of problems typical of biology. The literature leading up to B. C. Goodwin's theory of cellular temporal organization is reviewed and criticized. This theory attempts to transfer physical concepts to the study of biological systems. It is argued that this transference is not warranted because physics and biology characteristically use different modes of analysis.

It is assumed that a given cell can be in one of a finite number of discrete states and there is an isomorphism between morphological states and cellular biochemical states. These assumptions imply that the probability density function for the proteins and the messenger ribonucleic acids of a cell satisfies a linear partial differential equation. This equation is solved for a simple example. It is shown that in addition to states resulting from the activitation and inactivation of genes, cellular states may result from the total organization of the cell.

The assumptions are related to experimental data by discussing the ontogeny of the cerebral cortex and the embryology of the neural crest. The possibility of cellular organization states is related to current concepts of gene action. Suggestions are made for making the theory more directly testable.

AN EIGENSTATE APPROACH TO CELLULAR DIFFERENTIATION

By

Philip S. Ulinski

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CHAPTER I. INTRODUCTION: THE PROBLEM OF CONCRESCENCES

"All science is philosophical, and the only philosophies capable of validation are those of scientists."

— G. G. Simpson

A. The Problem of Concrescence

1. The origin of the problem -- A liver cell, a cat, a tulip, a virus, and a mushroom are all examples of integrated biological systems. The purpose of this Thesis is to find some way of handling, both conceptually and experimentally, a problem which arises in studying integrated biological systems. This problem will be called the problem of concrescence and it becomes important in the following way. The initial step in most scientifically conducted inquiries is to conceptually divide the system of interest into its constituent units. The proper method of inquiry, Descartes instructs us, is to "reduce involved and obscure propositions step by step to simpler ones, and then attempt to ascend by the same steps from the intuition of all those that are entirely simple to the cognition of all the others". Biologists have, to date, been predominantly engaged in applying the first part of the algorithm to living systems, delineating and studying the "simplest units" of animals and plants. Exactly what these units are depends on which aspect of living systems interests a given biologist: the geneticist has designated,

at various times, the gene, the "cistron", the "recon", etc. as the simplest unit of the genome; the neurologist divides his system into neurons, fiber tracts, and nuclei; the anatomist takes cells to be the elemental units of all organisms; the biochemist gives primacy to proteins, sugars, coenzymes, etc.; the population biologist considers systems of gene pools and of animal and plant populations.

The second part of the algorithm, the ascension to the cognition of all the other entities, those that are not entirely simple, is potentially much more difficult; but it is this undertaking which principally interests the biologist, for no living thing is a simple unit and all living things are composed of interacting simple units. This idea of "organism" is, of course, not at all new. The philosophy of Organicism[1] developed in the first decades of this century championed exactly this idea, arguing that the total understanding of biological systems depends on studying simultaneously the action of all their components. Most groups of experimental biologists have long been aware of the problem. Very few, if any, phenotypic traits are determined by a single gene. Rather, phenotypes are usually determined by a series of multiple alleles subject to the influence of various modifier and suppressor genes. To some extent, every trait is determined by the entire genome. The importance of interactions between the components of developing systems is reflected in Roux's idea of Entwicklungsmechanik and was

[1] The doctrine of the Organismic school is less well known than that of the Mechanists and the Vitalists. Bibliographies and elementary statements of the argument are to be found in the books by Bertalanffy (1933,1952). A more detailed presentation is the one by Goldstein (1963). Discussions particularly relevant to this Thesis are by Weiss (1961,1963). A historical discussion is by Needham (1931).

emphasized by the discovery of embryonic inductions[2]. The interconnexity of the nervous system forces one to assume that all but the simplest physiological and behavioral processes result from the operation of many, interacting anatomical or functional units. Most neurologists seem to appreciate the need for what is called, following C. S. Peirce, a "calculus of relations", but — indicating a sober appreciation of the difficulty of the problem — tend to place the search for such a calculus in the same category as the medieval serach for the Holy Grail. In the past ten years, biochemists have elaborated many examples of how interactions between metabolic processes result in their control. But, they still face the problem of understanding how all metabolic processes affect each other in vivo.

Although the importance of interactions is generally appreciated and a descriptive analysis of many examples has been effected, the complexity of most systems has frustrated attempts to quantitatively study integrated biological systems. The impressive exception is in the study of populations where an entire science has crystallized around the quantitative analyses of ecological and genetic populations. The problem is difficult just because it is confusing to think about more than a few things at the same time. Most people in Western societies, for example, find it almost impossible to keep their family tree straight past the second cousin level. The task of keeping track of one's kinfolk is, however, unrepresentatively simple because it is a purely relational one and a static one: the relationship Father-Son is constant once it has been ex-

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[2] A clear and recent review of the various interactions operative in embryogenesis is by Ebert (1965).

tablished and has no quantitative aspects. A developing embryo, however, is formed by virtue of a concatentation of changing interactions which have quantitative as well as relational aspects. For instance, the eye lens of an adult salamander is only distantly related, spatially and functionally, to the heart of the animal. At one point in development, however, the heart anlage comes into contact with the lens anlage and, in some way, contributes to its subsequent differentiation (Jacobson, 1966). Presumably, some inductor substance passes between these two components; if this is so, the differentiation of the lens would depend on how much inductor is transferred from the heart anlage to the lens anlage. Living systems are a reticulum of constantly changing correlations between subsystems.

The point is, that the result of traditional inquiries into biological systems is an assemblage of subsystems and the problem of finding out what the relation is between the original integrated biological system and the conceptual or manufactured subsystems. We would like some way of specifying exactly what that relationship is. In particular, we would like that way of thinking to allow us to design experiments exploring the results of systematic variations of the subsystems upon the behavior of the integrated system. We will call the problem of finding such relations the <u>problem of concrescence</u>. To begin with, we introduce a vocabulary designed to discuss this kind of relationship.

2. <u>The general nature of the problem</u> -- The notion of concrescence is borrowed from the work of A. N. Whitehead (1925, 1929). For Whitehead, the universe is a process merged from complex and

interdependent processes, called actual entities, much as the sea is an amalgam of water drops. In the process of becoming an actual entity the potential unity of many entities — actual and non-actual — acquires the real unity of one actual entity. The actual entity is the real concrescence of many potentials. An electron, for example, is a process which the physicist may represent, say, as a plane wave. An atom is also a process, represented by a different, more complicated kind of wave. A hydrogen atom is an actual entity which is the real concrescence of its component electrons and protons, each of which is an actual entity in its own right. The process of becoming a hydrogen atom is a series of eliminations of alternate possibilities: protonic, neutronic, and electronic processes have the potentialities of becoming sulfur atoms, hydronium ions, carbon atoms, etc.

The initial analysis of an actual entity reveals it to be a concrescence of prehensions which have originated in its process of becoming. A prehension consists of three factors: (a) the "subject" which is prehending, namely, the actual entity in which that prehension is a concrete element, (b) the "datum" which is prehended, and (c) the "subjective form" which is how that subject prehends that datum. There are two kinds of prehensions: (a) positive prehensions are termed feelings, (b) negative prehensions are said to "eliminate from feeling". An electron and a proton and a neutron at the vertices of an infinite equilateral triangle, for example, are not a hydrogen atom because they do not prehend each other. A hydrogen atom results from a progessive concrescence of prehensions, the subject — say, a proton — incorporating various data such as an electron and a neutron into the real actual entity of the atom

and eliminating from feeling, considering as inoperative in the progressive concrescence of prehensions, data such as other hydrogen atoms and the walls of the container.

A nexus (plural nexūs) is a set of actual entities in the unity of the relatedness constituted by their prehensions of each other. A society is a nexus of actual entities which are ordered among themselves in such a way that the nexus is self-sustaining. A bottle of hydrogen gas, for instance, is a nexus but is not a society. A mouse is a nexus which is also a society. It is clear that the universe consists of a hierarchy of nexūs ordered such that a given nexus consists of actual entities, each of which is a concrescence of a subordinate nexus, and such that the specified nexus is a datum in the concrescences of a superordinate nexus. For practical purposes, it is convenient to consider only some small portion of this hierarchy.

For the metaphysician, Whitehead's thought is tremendously useful because it lays bare in full generality the structures of all possible processes. For the scientist, Whitehead's thought is a mask for ignorance because it does not specify the details of processes interesting to the scientist. Instead, the scientist must supply these details — subject to the constraint that, insofar as Whitehead is correct and the scientist does his work adequately, the scientific description of a specific process must emerge as a particularization of Whitehead's description of the general process. The scientist describes the processes of a specific nexus. In the example of the hydrogen atom, this description can be effected (in the "Heisenberg representation") by representing the electronic and the protonic processes by separate Hamiltonian

operators, H_{elec}, H_{prot}, and, rougly speaking, representing the prehensions by an interaction Hamiltonian, H_{int}. The atomic process is then represented by the Hamiltonian

$$H = H_{elec} + H_{prot} + H_{int}.$$

Whitehead's cosmology can be especially useful to the scientist when he attempts to compare the specific descriptions of two distantly related processes.

3. Specific problem of the Thesis -- In this Thesis, we will restrict our attention to the relation between the nexus of chemical molecules and the special set of societies which are integrated biological systems and, more particularly, are individual cells. The classical biologists consider a cell to be a unit of structure and describe it in terms of its shape, its size, its spatial relation to other cells, its extracellular matrix, its staining properties, its embryological potentialities and prospective fates, etc. The biochemist, typically, does not deal with the cell as a unit, but homogenizes it to produce a variety of components which react with each other by certain types of chemical processes. The cell that the classical biologist studies and the abstracted cellular elements that the biochemist studies are not the same kind of thing. The cell is a society and the chemical processes are a nexus. In Whitehead's terms, it seems clear that an intact, living, individual cell may be regarded as the concrescence of all its biochemical processes. The goal of this Thesis will be to solve the problem of this concrescence. That is, we seek to spe-

cify the relation between the biochemical state of a cell and its morphological state.

We will consider the problem solved if a rule is found which will allow us to specify what the morphological type of a given cell is if we are given the current state of all of its biochemical processes. Such a rule would allow one to predict the results of experiments in which the biochemical components of a living cell were systematically varied. This is exactly the kind of experiments performed by embryologists. A typical embryology experiment might be conducted as follows: First, specify a certain cell in embryo A and determine its fate in the resulting adult. Second, in embryo B, alter the environment of the corresponding cell and determine its fate in the resulting adult. One way to do this is to transfer the cell to a foreign environment in a third embryo, C. Often, one will discover that two homologous cells will have different morphological characteristics in adult A and in adult C. The interpretation of such an experiment is that the environment of the cell affects its biochemical processes in some way and that this disturbance shows up in a deviation from its normal morphological type. Unfortunately, it is not usually possible to predict the results of such an experiment unless it has been done before. Some general guidelines do exist for such predictions, but it is not easy to generalize them into an overall picture of developmental processes couched in terms of biochemical processes. A specification of the relationship between biochemical and morphological cellular states would provide such a generalization.

B. Introductory Comments

The discussion in this Thesis requires the following preliminary comments. First, although a specification of the relation between biochemical and morphological states of individual cells will be proffered, it is probably an entirely unsatisfactory one. What is really being attempted is a formulation of the problem and some of its possible avenues of attack which is explicit and heuristic enough to provide a foundation for subsequent work. Secondly, a fairly detailed knowledge of the biochemistry of the cells being studied will be assumed as "given". This is, of course, a poor assumption. A critique of how useful this assumption is will be found in Chapter IV. Thirdly, the purpose of the work is to develop a **general method** for solving the problem. In any particular case, the method presented would have to be fitted to the nature of the system under study. This would require a detailed knowledge of the system and would be a fairly involved mathematical problem. Thus, the solution of the problem for an actual biological system must be regarded as a project in itself. The example worked out in Chapter III is designed to illustrate how the calculations are to be done. Fourthly, the discussions of this Chapter and of Chapter II boarder the domain of speculative philosophy. However, they are deemed to be a necessary preface to the critical development of the theory of Chapter III. The primary goal of this theory is a theoretical scheme which the experimental biologist can use to design new experiments and to systematize the results of old ones. Fifthly: "The true method of discovery", Whitehead writes, "is like the flight of an aeroplane. It starts from the ground of par-

ticular observations; it makes a flight in the thin air of imaginative generalization; and it again lands for renewed observation rendered acute by rational interpretation." For the sake of efficiency, it seems best to relieve the people who elaborate theoretical generalizations from the reaping of particular observations. This freedom must not be interpreted as a license for the theoretician to permanantly reside in some domain of ethereal verities, but imposes the responsibility of assurring that the aeroplane will have somewhere to land. In an attempt to discharge this responsibility, the theory of Chapter III has been reworded in empirical terms in Chapter IV.

CHAPTER II. TACTICS AND STRATEGY IN A THEORY OF CELLULAR TEMPORAL ORGANIZATION

> "We shall probably fare better if we constantly recall that the physical object before us is an undivided system, that the divisions we make therein are more or less arbitrary importations, psychological rather than physical, and as such, are likely to introduce complications into the expression of natural laws operating upon the system as a whole."
>
> — A. J. Lotka

Our problem of studying the relationship between the morphological type of a cell and its biochemical system has remained almost entirely unscarred by theoretical assaults. But Brian Goodwin (1963,1964a,1964b) has made an impressive attack on the related problem of the relationship between the components of the biochemical systems of cells and the time behavior of cells. By way of establishing the tactics with which to approach our problem, we will use this Chapter to review Goodwin's theory, requisitioning those of its aspects which bear upon our problem and noting in what ways it can be altered to satisfy our needs. The Goodwin theory is also of interest in that it illustrates a strategy which has pervaded theoretical biology: Most students who have attempted

to formulate the relationships exhibited by integrated biological systems have almost tacitly made the initial assumption that they are subject to the same kinds of laws operative in the systems studied by the physicist, so that theoretical biology should parallel theoretical physics, the laws of the former being analogous to the laws of the latter. To allow a judgement of the utility of this strategy, we will digress slightly by discussing the theories — which are the grandparents of Goodwin's theory — of animal and plant populations elaborated by A. J. Lotka and Vito Volterra.

A. The Mechanics and Thermodynamics of Demographic Systems

1. Kinetic equations -- Attempts to describe the growth or evolution of populations of species by general mathematical laws date back to Malthus' essay, but one of the earliest workers to produce exhaustive theoretical and empirical studies of the behavior of populations was A. J. Lotka[3]. By analogy to chemical kinetics, Lotka considers the evolution of populations as transferences of masses and energies between the components of a system, each species of organism comprising a component and the number of individuals of a species constituting the "mass" of the component. Interactions between species are represented as stoichiometric relationships, and the time rate of change of each component is described by a first order differential equation so that a set of kinetic equations

$$(1) \qquad \frac{dN}{dt}_r = F_r(N_1,\ldots,N_n), \quad r = 1,2,\ldots,n,$$

[3] Lotka's work is summarized in his Elements of Mathematical Biology (1956) originally published as Elements of Physical Biology (1924).

where N_r is the mass of the rth species, describes an ecological system of n species. Of particular interest are systems in which a balance between prey and predator exists so that $dN_r/dt = 0$ for all r. This situation is defined as a steady state. Steady states are classified as stable or unstable depending on whether the N_r's remain at or oscillate about some mean values q_r, or whether they will gradually decrease to zero or increase to some upper limit. The task of the demographer is to find the form of the functions $F_r(N_1,\ldots,N_n)$ and to detail the conditions which will cause the system to be in a stable or unstable steady state. Lotka considers a variety of empirical examples of demographic systems, but for our purposes it is more instructive to consider Volterra's descussion of the demographic problem.

2. The Volterra equations -- In two papers (1931,1937), Volterra considered demographic systems of the Lotka type from a fairly mathematical viewpoint. He takes the kinetic equations (1) to be of the form

$$\frac{dN}{dt}r = \left(\epsilon_r + \frac{1}{\beta_r} \sum_{s=1}^{n} a_{sr} N_s \right) N_r \,. \tag{2}$$

The coefficients ϵ_r describe the growth (or death) of the rth species when it is in isolation; the coefficients $1/\beta_r$ — if one again thinks of a population of individuals as a mass — describe the mass of the rth component which is transformed in unit time into another component, i.e. $1/\beta_r$ individuals of the rth species are equivalent to $1/\beta_s$ individuals of the sth species; the constants a_{sr} describe

the interaction between the rth and the sth species, and must be antisymmetric, i.e. $a_{sr} = - a_{rs}$. We will call the Equations (2) Volterra equations. If we introduce the notation $N_r = dX_r/dt$, the Volterra equations can also be written as a second order system:

$$(3) \qquad \beta_r \frac{d^2X_r}{dt^2} = \left(\epsilon_r \beta_r + \sum_{s=1}^{n} \frac{dX_s}{dt} \right) \frac{dX_r}{dt} \quad .$$

The conditions that the system (2) have stable steady states are well known in the theory of differentiatl equations. The solutions to these equations will be of the form

$$N_r = G_{r1} e^{\lambda_1 t} + G_{r2} e^{\lambda_2 t} + \ldots + G_{rn} e^{\lambda_n t} + G_{2r1} e^{2\lambda_1 t} + \ldots$$

where the parameters λ_r are the roots of the characteristic equation

$$D = \begin{vmatrix} \epsilon_1 - \lambda & a_{21}/\beta_1 & \cdots & a_{n1}/\beta_1 \\ a_{12}/\beta_2 & \epsilon_2 - \lambda & \cdots & a_{n2}/\beta_2 \\ \vdots & \vdots & & \vdots \\ a_{1n}/\beta_n & a_{2n}/\beta_n & \cdots & \epsilon_n - \lambda \end{vmatrix} = 0$$

The nature of these roots determines the stability of the states. For example, if all the λ_r's are pure real and positive, the sys-

tem will persist near to the stable state values $q_1,\ldots,q_r,\ldots,q_n$; if they are pure imaginary and positive, they will oscillate about the stable state values; if they are negative, the state is unstable.

Of particular interest is a result which Volterra calls the law of the conservation of fluctuations and which guarantees that if a system has n even, has stable steady states, and if the N_r's are bounded between two positive limits, then at least several of the N_r's will exhibit undamped oscillations about their mean values. This means that in systems of sufficient complexity (as biological systems undoubtedly are) we may be justified in treating the system as ergodic and replacing time averages by averages over the ranges of the variables N_r.

3. Physical analogs -- In Whitehead's terms, the kinetic equations describe feelings in the demographic universe. On the face of it, the idea of an actual entity resulting from a concrescence of these feelings seems poorly conceived, for the masses N_r seem to be the only attributes which could be applied to such an entity, and they are the attributes of the feelings. However, both Lotka and Volterra suggest that such a concrescence is justifiable. Lotka, arguing by analogy to the kinetic equations of chemical kinetics, suggests that the demographic system considered as an entity should evolve in accordance with an analog of the second law of thermodynamics. His argument hinges on the construction of a quadratic form. If we define the vector $N = N(N_1,\ldots,N_n)$ in an n-dimensional space and designate the transpose of N by $\tilde{N}$, a quadratic form is a scalar function, Φ, defined by $\Phi = NA\tilde{N}$ where

A is an nxn constant matrix. After a suitable orthogonal transformation, it can be shown that the condition for the stability of the system is that Φ be a minimum at the stable state values q_r. This is analogous to the classical mechanics where it can be shown that the potential energy is a quadratic form. Thus, as in mechanics and thermodynamics, important ideas about demographic systems can be phrased in terms of a minimum principle, so there might

> exist functions Φ analogous to the functions known as thermodynamic potentials, in terms of which the behavior of the system can be concisely epitomized, after the manner of thermodynamics. If such a plan could be successfully carried out, the result would be a species of quasi-dynamics of evolving systems, in which certain parameters P played a role analogous to forces, without being in any sense identical with forces (or even with generalized forces); certain other conjugate parameters p would play a role analogous to displacements, and certain functions Φ would resemble in their relations to certain events in the system, the energy functions (free energy, thermodynamic potentials) of thermodynamics. 4/

Although Volterra sometimes follows Lotka in considering the kinetic equations of a demographic system as analogous to chemical kinetic equations, he usually considers them as the equations of motion of the system. In this case, the attributes of the actual entity are parameters analogous to those of classical mechanics. Thus, Volterra writes

$$L = \sum_{r=1}^{n} \beta_r \frac{dX_r}{dt} \quad \text{and} \quad M = \sum_{r=1}^{n} \beta_r \in_r X_r - C$$

where C is a constant, and shows that L + M = constant. L is called the demographic kinetic energy and M is the demographic poten-

4 A. J. Lotka (1956), p 321.

tial energy, so that we have a conservation of demographic energy. Similarly, demographic work is given by

$$\mathcal{V}_r = \epsilon_r + \frac{1}{\beta_r} \sum_{s=1}^{n} a_{sr} N_s .$$

Also, Volterra was able to derive equations (3) by considering the first variation of the function

$$\Phi = \sum_{r=1}^{n} \beta_r \frac{dX}{dt}r \ln \frac{dX}{dt}r + \tfrac{1}{2} \sum_{r=1}^{n} \sum_{s=1}^{n} a_{sr} \frac{dX}{dt}r \, X_s + P$$

where P is the demographic potential

$$P = \sum_{r=1}^{n} \beta_r \epsilon_r X_r + \tfrac{1}{2} \sum_{r=1}^{n} \sum_{s=1}^{n} c_{rs} X_r X_s ,$$

whence come the Euler-Lagrange equations

$$\frac{d}{dt} \frac{\partial \Phi}{\partial \dot{X}_r} - \frac{\partial \Phi}{\partial X_r} = 0 .$$

These reduce to the kinetic equations (3). Now, if we put $p_r = \partial/\partial X_r$, we have the Hamiltonian

$$H = \Phi - \sum_{r=1}^{n} p_r X_r$$

and the canonical equations of motion

$$\frac{dp_r}{dt} = \frac{\partial H}{\partial X_r} \quad \text{and} \quad \frac{dX_r}{dt} = - \frac{\partial H}{\partial P_r}.$$

It is clear, then, that given sufficient ingenuity, one could easily construct a "demographic mechanics" analogous to classical mechanics.

B. The Statistical Mechanics of Biological Associations

1. <u>The microcanonical ensemble</u> -- Lotka's suggestion is that there may exist for demographic systems analogs to the thermodynamic state parameters of chemical systems. Volterra suggests considering the kinetic equations of the demographic system as equations of motion. The obvious next step is to develop an analog to the program of statistical mechanics and to relate the equations of motion to the parameters of state, a task which has been accepted by E. H . Kerner (1957, 1959, 1964).

(a) To obtain a system of equations which satisfies the Liouville equation, Kerner introduces the change of variables $v_r = \ln N_r/q_r$. Since — at steady state — equations (2) imply the equations

$$\epsilon_r \beta_r + \sum_{s=1}^{n} a_{sr} q_s = 0 ,$$

the equations of motion become

$$(4) \qquad \beta_r \dot{v}_r = \sum_{s=1}^{n} a_{sr} q_s (e^{v_s} - 1) .$$

Now, as in the statistical mechanics, we think of these equations as guiding the movements of a point in an n-dimensional phase space. If we consider a Gibbsian ensemble of such phase spaces, we can define a density of points in phase space, $\rho(v_1,\ldots,v_n)$. We also define $\vec{V} = (\dot{v}_1,\ldots,\dot{v}_n)$. The conservation of density in phase space gives us

$$\frac{\partial \rho}{\partial t} + \operatorname{div} \rho \vec{V} = \sum_{r=1}^{n} \dot{v}_r \frac{\partial \rho}{\partial v_r} + \frac{\partial \dot{v}_r}{\partial v_r} = 0 .$$

From the equations of motion (4), we have $\partial \dot{v}_r / \partial v_r = 0$; but we note in passing that this is a consequence of the particular form of Equations (2), and will not hold for the general kinetic equations. In this case, the, we have the Liouville equation

$$\frac{\partial \rho}{\partial t} + \sum_{r=1}^{n} \dot{v}_r \frac{\partial \rho}{\partial v_r} = 0 .$$

As is done in classical statistical mechanics, Kerner restricts the discussion to the <u>equilibrium</u> case where $\partial \rho / \partial t = 0$.

(b) The starting point of statistical mechanics is some statement about the way energy is distributed between the possible state of the system. Thus, **Kerner** must (1) introduce an analog to total energy, and (2) make some statement about its distribution. He

chooses to discuss first the microcanonical ensemble in which the energy of the system is held constant, but he could equally well have elected to treat first the canonical ensemble in which the total energy varies but the temperature is fixed.

(1) By multiplying Equations (4) by $q_r(e^{v_r} - 1)$, setting $\gamma_r = \beta_r q_r$, and summing over all r, we obtain

$$\sum_{r=1}^{n} \gamma_r \dot{v}_r (e^{v_r} - 1) = 0 ,$$

and we have the integral of motion

$$G = \sum_{r=1}^{n} \gamma_r (e^{v_r} - v_r) = \text{constant.}$$

Since, if we set $\gamma_{sr} = \alpha_{sr} / \beta_s \beta_r = -\gamma_{rs}$, G satisfies the canonical equations

$$\dot{v}_r = \left(\sum_{s=1}^{n} \gamma_{sr} \frac{\partial}{\partial v_s} \right) G ,$$

Kerner takes G as the Hamiltonian of his system. Again, we note that the success of this choice depends on the particular form of Equations (2); G may not exist for some systems.

(2) It is customary in statistical mechanics to postulate that there is, a priori, an equal probability of finding each of the possible states of an isolated system to have a specific total energy, G. Then, it is shown that if the number of systems in the

ensemble is sufficiently large, the probability distribution approaches the frequency of finding the system in the "most probable" state of the system. This is expressed mathematically by writing the density function as a delta function

$$\rho = \rho_o (G - G_o)$$

where ρ_o is a constant. Kerner adopts this proceedure.

2. The canonical ensemble -- (a) Consider, now, the canonical ensemble formed by studying only ν of the n species in a system and regarding the remaining $n - \nu$ species as a "heat bath" with energy being transferred into and out of the system of ν species. In studying the canonical ensemble, it is customary to take

$$\rho_\nu = \exp \frac{1}{\Theta} (\psi - G_\nu)$$

to be the density function for the species; ψ is a parameter independent of the variables v_r, and the meaning of Θ will be made clear later; G_ν is the total energy of the ν species. Since we must have

$$\int \rho_\nu \, d\tau_\nu = 1$$

where $d\tau_\nu$ is the incremental volume in phase space, and the integration is over all of phase space, we have the phase integral

$$Z = \exp(-\psi/\Theta) = \int \exp(-G/\Theta) \, d\tau_\nu .$$

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This relationship is used in the evaluation of ensemble averages. Thus, the ensemble average $\overline{F}$ for some function $F = F(v_1,\ldots,v_n)$ is

$$\overline{F} = \int \rho_\nu F \, d\mathcal{T}_\nu = \int F \exp(\psi - G_\nu/\theta) \, d\mathcal{T}_\nu$$

$$= e^{\psi/\theta} \int F \exp(-G_\nu/\theta) \, d\mathcal{T}_\nu = \int F \exp(-G_\nu/\theta) \, d\mathcal{T}_\nu \Big/ e^{-\psi/\theta}$$

$$= \int F \exp(-G_\nu/\theta) \, d\mathcal{T}_\nu \Big/ \int \exp(-G_\nu/\theta) \, d\mathcal{T}_\nu .$$

Since the actual evaluation of such averages is a formidable exercise in integration, we will merely catalog the results.

(1) The ensemble average of N_r is $\overline{N}_r = q_r$.

(2) The demographic analog to kinetic energy is the function $T_r = v_r \partial G/\partial v_r$. Kerner shows that $\overline{T}_r = \theta$. Since this result holds for all r, this is the analog of the equipartition theorem for physical systems: the average value of the demographic kinetic energy for each of the ν species is the same, and one thinks of the kinetic energy being distributed equally between the different species.

(3) To see the significance of θ, consider the ensemble average of the function $\mathcal{T}_r^2(N_r/q_r - 1)^2$. It can be shown that

$$\mathcal{T}_r^2 \overline{(N_r/q_r - 1)^2} = \theta_r .$$

Thus, $\theta = (\mathcal{T}_r/q_r)\overline{(N_r - q_r)^2}$, so that, within a

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constant factor, Θ is the variance of the system about its mean value q_r. Since temperature plays the analogous role in physical systems, Θ is the demographic temperature.

(b) If Θ is the "temperature" and G is the "total energy", what, we might ask, are the analogs of the other themodynamic state parameters? These are all easily calculated from the formulae of statistical thermodynamics:

(1) The free energy is $\Psi = -\frac{1}{\alpha} \ln Z$

(2) Heat capacity is $C = \partial \bar{G}/\partial\theta$

(3) Entropy is $S = \overline{G - \Psi}/\theta$

(4) Work due to environmental influences if $F_i = -\partial G/\partial\alpha_i$ for some external variable α_i.

Under certain conditions, we can derive the demographic ideal gas law $\bar{F}\ T = \lambda\nu\theta$.

(c) However, ensemble averages of certain parameters relating to the oscillations of the variables N_r are of more value. We introduce the notation $x_r = \tau_r/\theta = \beta_r q_r/\theta$.

(1) First, we define T_-/T as the fraction of time, over a long interval, that the values of N_r are below the steady state value q_r. To calculate T_-/T, Kerner (1959) uses the function

$$h(v_r) = \begin{cases} 1, & v_r < 0 \\ \\ 0, & v_r > 0 \end{cases}$$

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$$T_-/T = e^{\Psi/\theta} \int_{-\infty}^{\infty} h(v_r)\, e^{-G/\theta}\, dv_r .$$

Evaluation of this integral gives

$$0.5 \leqslant T_-/T = I(\sqrt{x},\, x-1) \leqslant 1$$

where $I(\sqrt{x},\, x-1)$ is the incomplete gamma function. When evaluated numerically, this result indicates that for θ small, i.e. the system is near steady state, $T_-/T = T_+/T$, $(T_+/T = 1 - T_-/T)$. But for θ large, $T_-/T \geqslant T_+/T$. Thus, in populations which show large fluctuations, the number of individuals of any species will usually be below the steady state level.

(2) In a similar way, one can calculate the mean amplitude A_+ of oscillations above the steady state value as

$$A_+ = \frac{x^x e^{-x}}{(T_+/T)x!} .$$

(3) Also of interest is the mean frequency with which the population numbers take on a given value. It is possible to carry out a variety of calculations of this sort, but the simplest is the determination of the ratio of the mean frequency that the system takes on steady state values (i.e. $N_r/q_r = 1$) to the mean frequency that it takes on some other value, $\eta = N_r/q_r$. This is found to be

$$\omega_{rel}(\eta) = e^{xr}(\eta e^{-\eta})^{xr}$$

for the rth species. We note that $\omega_{rel}(1) = 1$ and $\omega_{rel}(\eta) < 1$ if $\eta \neq 1$, which means that N_r takes on its steady state value more frequently than any other value.

(d) Kerner offers two empirical tests of his theory. First, he (1957) notes that the probability of finding the population of r between v_r and $v_r + dv_r$ is given by

$$P_r\, dv_r = e^{-G_r/\theta}\, dv_r / Z_r.$$

Then, by setting $n_r = N_r/a_r$ he finds

$$P(n_r)\, dn_r = \frac{n_r^{\alpha\tau_r - 1}\, e^{-\alpha\tau_r n_r}\, dn_r}{\alpha\tau_r^{-\alpha\tau_r} \prod(\alpha\tau_r)}$$

and observes that this form of distribution has been used to describe actual populations by R. A. Fisher and his group. Second, Kerner (1959) makes use of data on the fluctuations in a population of Labrador foxes over a period of 91 years. From these data, he is able to calculate parameters such as T_-/T, A_+, and A_- for the system. He then solves his theoretical expression for each of these parameters for x and finds that within reasonable limits the empirically calculated values of x are in accord. Also, Kerner finds good agreement between theoretical and empirical $\omega_{rel}(\eta)$

values.

C. Theories of Epigenetic Systems

1. Waddington and the cybernetics of development -- The idea of using the tools developed to study demographic systems to elucidate embryological problems is due to C. H. Waddington. As an example of Waddinton's ideas, we will consider the first essay in his book The Strategy of the Genes (1957). As the starting point of this essay, Waddington takes an observation about developmental systems:

> The major empirical fact about the development of animals — a fact which has no theoretical inevitability, but which is so obstrusive that only the crudest observation is necessary to establish it — is that the end products which it brings into existence usually vary discontinuously. The tissues of an animal are in most cases quite sharply distinct from one another; skin, nerve, muscle, lung, kidney, etc., with of course many subtypes in the bodies of highly evolved and complex creatures, but with very few kinds of cells which could be considered as providing a range of intermediates connecting two of the major varieties. Similarly, each organ has its well defined and characteristic morphology. 5/

Waddington sees one of the central problems in developmental biology to be that of accounting for these macroscopic discontinuities in terms of genes and "gene products" which are present only in small concentrations in the organism and which vary continuously. This is a different statement of our general problem of the relationship between feelings and actual entity, the processes of gene action being feelings and morphological discontinuities being attributes of the actual entity which results from a concrescence

5 C. H. Waddington (1957), p 13.

of the genetic processes.

Waddington's approach to a solution is to regard the substances of the genetic-metabolic system as populations which can be described by kinetic equations similar to those of Lotka and Volterra; he notes

> If we regard the system as closed ... and if the supplies of raw materials are taken as constant, the equations which result are of the same type as those which arise in the study of the growth of two populations of animals which compete with one another for a limited food supply. 6/

The generalization to open systems was made by H. Kacser in his epilogue to Waddington's The Strategy of the Genes. Kacser notes that, in general, the kinetic equations of an open system show that the composition of the final steady state of the system is dependent upon the nature and the quantity of catlysts (i.e. enzymes) present, that these steady state values are independent of the initial concentrations of the components of the system, and that the flux of materials into the system enters as a factor into the steady state values. He then uses these properties of an open system to suggest explanations for some biological phenomena. For example, the independence of initial conditions might explain how in regulatory eggs a half of an embryo produces and apparently normal adult. Or, pleiotropic effects in which a single gene may lead to a variety of phenotypic characteristics might be explained by differences in enzyme concentration or state. In all likelihood, these explanations are too simple to be adequate, but the point of interest is that it is possible to use kinetic equations to study open systems.

6 Ibid., p 21.

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Waddington realizes that in using the kinetic approach to study developing systems, a likely method is to consider them as guiding the motion of points in a multi-dimensional phase space. To facilitate thinking about developmental problems in these terms, he introduces a surface, which he calls the epigenetic landscape, defined in this phase space. His description of an epigenetic landscape for two independent variables is as follows (Figure 1):

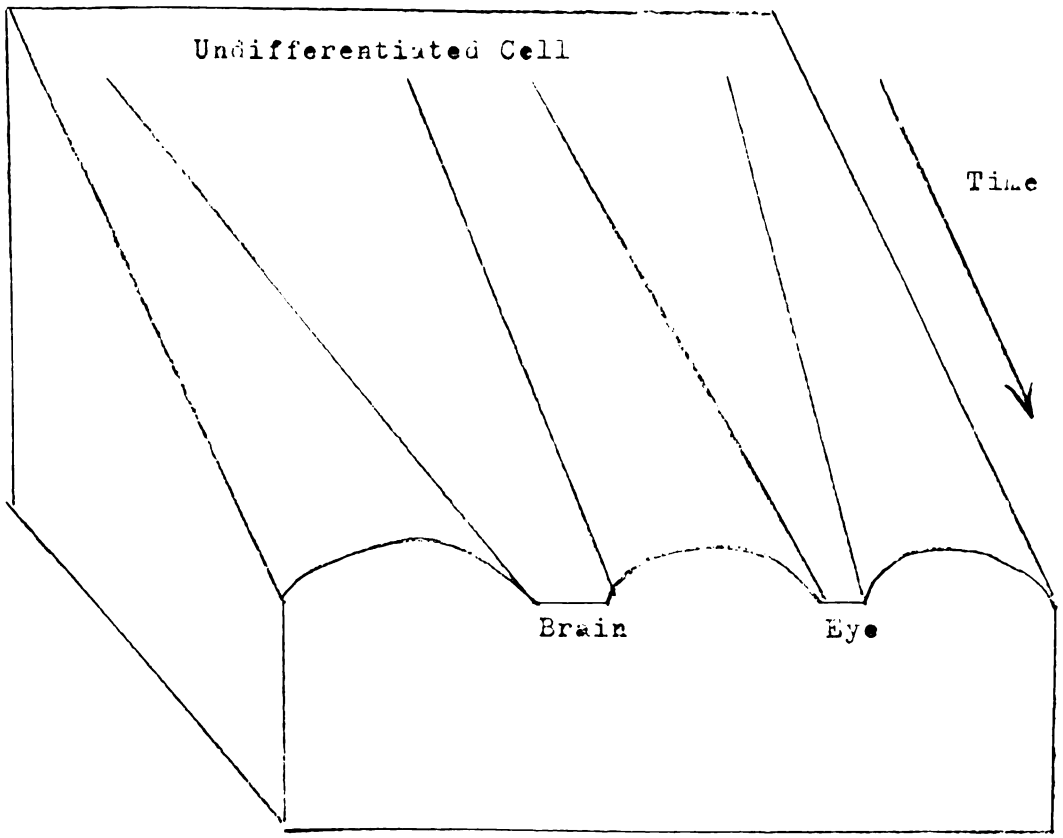
> Consider a more or less flat, or rather undulating, surface, which is tilted so that points representing later states are lower than those representing earlier ones. Then if something, such as a ball, were placed on the surface it would run down towards some final end state at the bottom edge. There are, of course, not enough dimensions available along the botton edge to specify all the components in these end states, but we can, very diagrammatically, mark along it one position to correspond, say, to the eye, and another to the brain, a third to the spinal cord, and so on for each type of tissue or organ. Similarly, along the top edge we can suppose that the points represent different cytoplasmic states in the various parts of the egg. 7/

In a rough way, one can conceive of the position of the ball representing the developmental state of, say, a cell. The depth of the trough the ball finds itself in is a measure of the competencies of the cell at that time — the deeper the trough, the more difficult it being to deter the cell from its prospective fate.

The concepts which Waddington expounds are particularly instructive because they bring to bear upon our problem of the genotype to phenotype relationship all of the ideas and methods we have discussed both in this Chapter and in Chapter I. As we have already noted, Waddinton suggests that one of the central problems in embryology is that of the concrescence of genetic processes to those of morphogenesis. He also emphasizes the importance of sta-

7 Ibid., p 29.

Figure 1. The epigenetic landscape. See text for description. Drawn after Waddington (1957).



bility and the discontinuity of the morphological aspects of organisms in this problem. He observes that genetic processes can be described by kinetic equations in the same way that Lotka and Volterra used them to describe the evolution of populations of organisms. The idea of the epigenetic landscape is similar to Lotka's potential function[8], and Waddington, apparantly independently of Kerner, used the kinetic equations to move a point in a phase space, albeit Waddington expressed himself qualitatively. A significant difference between Waddington and the demographers is that Waddington's discussion stems only from biological considerations, ignoring the use use of physical analogs.

2. A statistical mechanics of epigenetic systems -- (a) The task of following up Waddington's suggestion and attempting to establish a relationship between a set of genetic-metabolic equations and parameters which can be used to study an intact developing cell was accepted by Waddington's student, Brian Goodwin. This undertaking necessitated, as a preliminary, some attempt to resolve three problems: (1) The Lotka-Volterra-Kerner theory discusses populations of organisms, and to use it as the basis for an epigenetic theory one must first argue that the demographic and epigenetic systems have the same formal characteristics, (2) the epigenetic system is imbedded in the genetic system, in the physiological system, in the evolutionary system, etc. Before proceeding, one must decide in what way this hierarchy of nexūs is to be included or excluded from the discussion of the epigenetic system, (3) the Volterra equations describe interactions between individuals. To transfer the Volterra-Kerner theory to a consideration

[8] Cf. J. Needham (1936).

of epigenetic systems, one must first derive a set of kinetic equations which describe the genetic-metabolic system. We will examine Goodwin's answer to each of these problems.

(1) Waddington's writings tend to emphasize the similarities between embryological and evolutionary processes, and his suggestion that populations of organisms be considered as analogous to the chemical components of an organism carries this theme just one step further. Phylogeny and ontogeny are similar in that they study the rise and fall of something (populations or components) with time; they are both looking at developing systems, in the broadest sense of the term. The Lotka and Volterra theories are potentially capable of describing evolving systems of populations, but their principal application is to systems of animal and plant populations which are in equilibrium or, more precisely, in a steady state. The Kerner theory, however, does not intrinsically have the potential of describing developing systems and can deal only with steady state systems. This is, of course, because Kerner models his theory after the classical statistical mechanics. Mathematically, this condition of reversibility is introduced by setting $\partial P/\partial t = 0$ in the Liouville equation or by making the ergodic hypothesis which allows the replacement of time averages by ensemble averages. An ergodic system is not necessarily absolutely invariant with time, but may vary about some mean value; all that the hypothesis requires is the variation be periodic with at most a period of very long duration. Insofar as Kerner is interested in studying the periodic fluctuations of populations about a steady state value, the modus operandi of classical sta-

tistical mechanics is ideally suited to the problem.

Thus, since Goodwin applies the Kerner theory to the study of cellular problems, his theory must be viewed as a failure for the purposes of studying embryological systems. But, such an equilibrium theory should apply to an important class of cellular steady state activities, the most notable of which are the clock rhythyms characteristic of many biological systems. Goodwin is well aware of this limitation of his theory and applies his results primarily to problems in the study of biological clocks, realizing that applications to embryological problems would require the elaboration of a theory analogous to non-equilibrium statistical mechanics.

(2) There is nothing new in the observation that living organisms present to the biologist a hierarchy of nexūs. Comte (1858), for instance, noted this and suggested that a classification of these levels can be either "biotaxic" or "biostatic" depending on whether it is in terms of dynamic and functional or of structural characteristics. Waddington (1957) chooses a classification in terms of "time scales", the history of the ancestors of an organism constituting the evolutionary or longest time scale, the development of the individual making up the embryological or medium time scale, and the constant activities of the organism forming the shortest or physiological time scale. Goodwin also constructs a classification in terms of time scales, but uses the relaxation time of a given hierarchical level as a defining criterion. He distinguishes a <u>metabolic system</u> of cells comprising the diffusion and interaction processes and the enzymatic transformations of small molecules (i.e. not macromolecules) and having a relaxation

time of 10^{-1}-10^{-2} seconds, an epigenetic system comprising the biosynthesis, diffusion, and interaction of macromolecules and having a relaxation time of 10^{2}-10^{4} seconds, and a genetic system which has a very long relaxation time and is not relevant to his discussion.

Waddington realizes that an accurate theory of biological systems demands the simultaneous consideration of all levels of organization in a mathematical theory of living systems would induce a complexity prohibiting the comprehension of the theory by everyone save, perhaps, a Laplacian calculator ratiocinatrix, and the primary task of the theoretician becomes one of performing some einklämmerung or bracketing off of all the organizational levels except the one in which he is most interested. Goodwin meets this problem by making a distinction between parameters and variables:

> if the two systems have very different relaxation times (say one is 100 times larger than the other), then relative to the time required for significant changes to occur in the "slower" system (larger relaxation time), the variables of the "faster" one (shorter relaxation time) can be regarded as being always in a steady state. Therefore only these steady state quantities will enter into the dynamic equations describing the slower system, and a very considerable economy of motional equations can be achieved. On the other hand, the variables of the "slow" system will enter into the equations of motion of the "fast" one as parameters, not as variables. These parameters have a slow rate of change, and the faster system will gradually move in time in response to these slow changes; but for the purpose of studying the short-term dynamics of the fast system, the slowly changing quantities which define the motion of the slow system can be regarded as environmental parameters. 9/

(3) As the fundamental "unit" of the epigenetic system, Goodwin takes a control loop, pictured in Figure 2. L_j represents a certain gene locus or perhaps an operon; R is a ribosome which

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9 B. C. Goodwin (1963), p 10.

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uses the messenger RNA, r_j, of the jth species of protein, p_j; C is a cellular locus where the jth species of protein is utilized to produce the metabolite M_j from some precursors. The loop shown in Figure 2 has a feedback mechanism so that when the concentration of M_j exceeds some value S_j, the production of the enzyme p_j is halted by some mechanism of enzyme repression. Presumably the entire genetic-metabolic system of the cell could be represented by an array of such loops with varying degrees of interaction between loops (Figure 3).

For the purposes of developing an analytic theory of the epigenetic system, the array of control loops in Figure 3 must be replaced by a set of kinetic equations which will serve the same pupose fulfilled by the Volterra equations in the Kerner theory. Since these control equations are undoubtedly greatly oversimplified, we will only sketch Goodwin's derivation. The principal assumption made is that the gene-repressor interaction in enzyme repression and induction follows the same mechanism that enzyme-substrate interactions follow.

To derive the control equations for the control loop in Figure 2, Goodwin assumes, to begin with, that each protein is synthesized at a rate proportional to the concentration of its mRNA and that it is degraded at a constant rate. Thus,

$$\frac{dp_j}{dt} = \alpha_j r_j - \beta_j, \quad j = 1,2,\ldots,n \tag{5}$$

where α_j and β_j are constants and where p_j designates the concentration of the jth species of protein and r_j the concentration of

Figure 2. A single cellular control loop. L_j is a certain gene locus; R_j is a ribosome; C_j is the cellular locus where the jth protein is used; p_j is the jth species of protein and r_j is the corresponding mRNA; M_j is the metabolite produced by the protein. Drawn after Goodwin (1963).

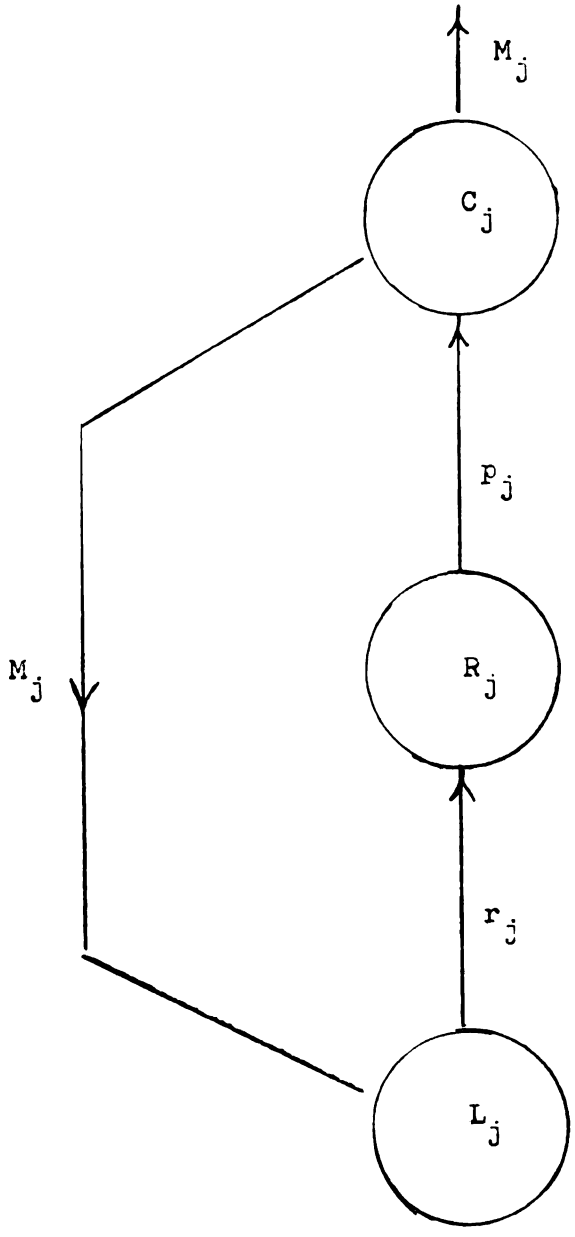
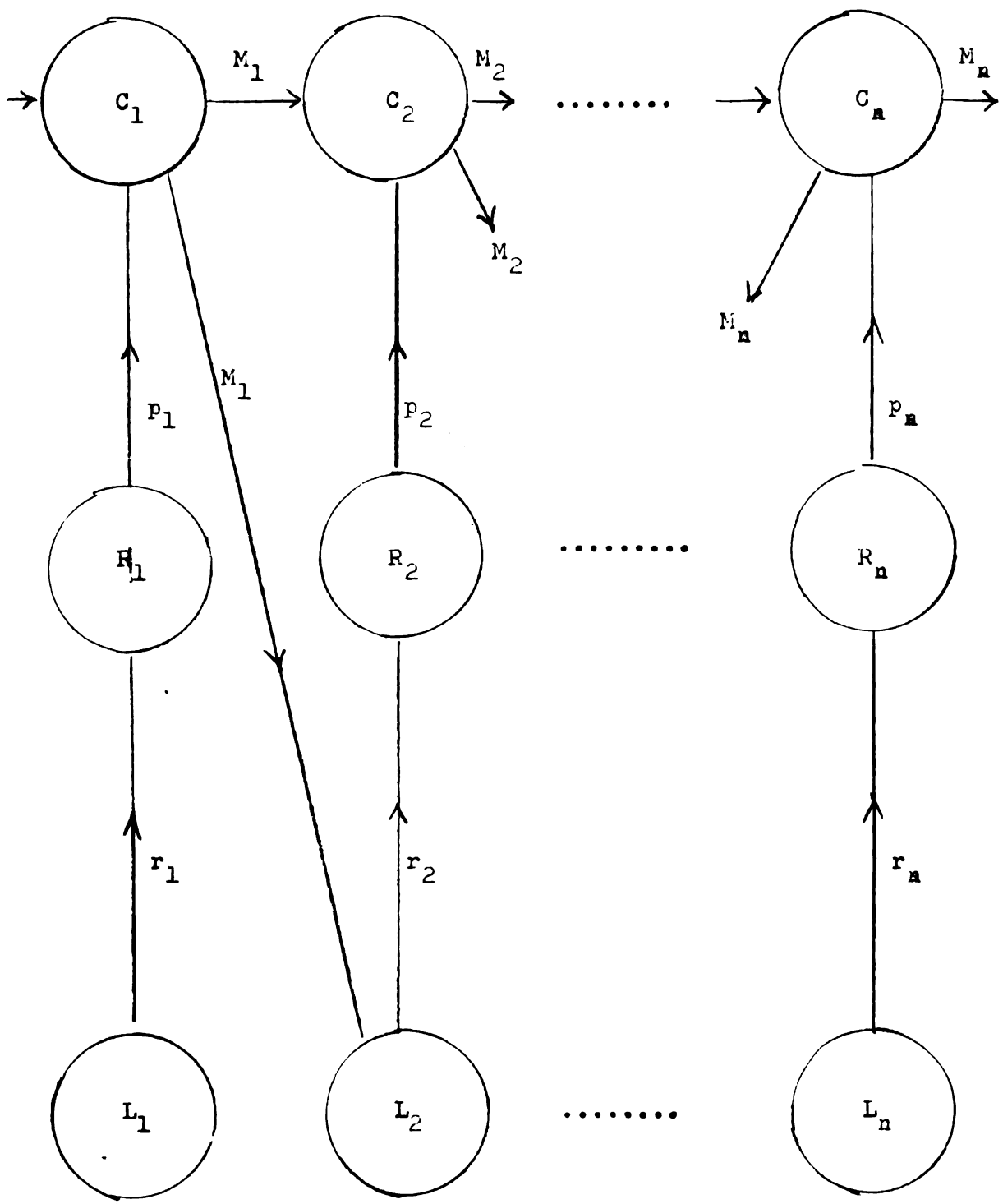


Figure 3. The biochemical system of a cell represented as an array of control loops. See Figure 2 for explanation of notation. Drawn after Goodwin (1963).



the jth species of mRNA.

To obtain a control equation for r_j, Goodwin assumes that the precursors (nucleotides) of the mRNA are present in the cell in the constant average amount $[\bar{A}_j]$, and that the precursors and the repressor (nucleohistone?) for the jth locus compete for the available gene template, T_j. Then, we have the stoichiometric equations

$$T_j + \bar{A}_j \rightleftharpoons T_j\bar{A}_j \quad \text{and} \quad R_j + T_j \rightleftharpoons T_jR_j,$$

the equilibrium constants

$$K_j = \frac{[T_jR_j]}{[T_j][R_j]} \quad \text{and} \quad L_j = \frac{[T_j\bar{A}_j]}{[T_j][\bar{A}_j]},$$

and the conservation relationship

$$[T_j]_o = T_j + T_j\bar{A}_j + T_jR_j$$

where $[T_j]_o$ is the total amount of template and R_j is the concentration of the repressor of the jth template. Thus,

$$[T_j\bar{A}_j] = \frac{L_j[\bar{A}_j][T_j]_o}{1 + L_j[\bar{A}_j] + K_j[R_j]}.$$

Now, if we assume that the concentration of repressor is proportional to the "excess" of metabolite

$$[R_j] = \sigma_j[M_j - s_j],$$

σ_j a constant, and assume that r_j is degraded at a constant rate b_j, then we have

$$\frac{dr_j}{dt} = \frac{L_j[\bar{A}_j][T_j]_o}{1 + L_j[\bar{A}_j] + K_j\sigma_j[M_j - S_j]} - b_j,$$

for the rate of synthesis of r_j may be assumed proportional to the concentration of "activated" template, $[T_jA_j]$. Setting $a_j = L_j[\bar{A}_j][T_j]_o$, $B_j = 1 + L_j[\bar{A}_j]$, and $m = K_j\sigma_j$,

(6) $$\frac{dr_j}{dt} = \frac{a_j}{B_j + m_j[M_j - S_j]} .$$

The distinction between parameter and variable discussed in Section 2.a.(2). is used to remove M_j from Equation (6). Suppose that M_j has a kinetic equation of the form

$$\frac{dM_j}{dt} = c_jp_j - s_j,$$

c_j and s_j constants. Since M_j is a parameter in the epigenetic system (i.e. it is a variable in the metabolic system), we may replace it by its steady state value, c_jp_j/s_j. Thus, Equation (6) becomes

(7) $$\frac{dr_j}{dt} = \frac{a_j}{A_j + k_jp_j} - b_j$$

where $A_j = B_j - m_j S_j$ and $k_j = m_j c_j / s_j$. Equations (5) and (7) are the control equations for the control loop shown in Figure 2. This method of removing M_j from Equation (6) is clearly just a first approximation; a more detailed study would necessitate obtaining M_j as a function of the p_j's and r_j's so that the control equations would be more complex.

(b) Starting from control equations of the type just derived, Goodwin develops a theory of the epigenetic system parallel to the Kerner theory. Because we have already discussed this development in Section B, we will only (1) discuss a detail in which the Goodwin theory differs from the Kerner theory, and (2) discuss the results and predictions of the Goodwin theory.

(1) In Section B.1.(b) we saw that a simple set of transformations serves to put the Volterra equations into a form which will always lead to a Hamiltonian function G. However, the control equations for different arrays of control loops can be expected to vary widely in form, and it may not always be possible to find a G function. In the case of Equations (5) and (7) there is no difficulty. We let $\bar{p}_j$ and $\bar{r}_j$ designate the steady state values of p_j and r_j and set $Q_j = A_j + k_j \bar{p}_j$. Then if we introduce the variables $\hat{r}_j$ and $\hat{p}_j$ defined by $\hat{r}_j = r_j - \bar{r}_j$ and $1 + \hat{p}_j = (A_j + k_j p_j)/Q_j$, Equations (5) and (7) become

$$\frac{d\hat{r}_j}{dt} = b_j\left(\frac{1}{1 + \hat{p}_j} - 1\right)$$

$$\frac{d\hat{p}_j}{dt} = \alpha_j \hat{r}_j .$$

From these equations we obtain

$$dG(\hat{p}_j,\hat{r}_j) = \alpha_j \hat{r}_j d\hat{r}_j - b_j\left(\frac{1}{1+\hat{p}_j} - 1\right)d\hat{p}_j = 0$$

and
$$G(\hat{p}_j,\hat{r}_j) = \int_{r_j}^{\infty}\int_{(A_j/Q_j-1)}^{\infty} dG(\hat{p}_j,\hat{r}_j) = \text{constant}$$

$$= \frac{\alpha_j \hat{r}_j^2}{2} + b_j\left[\hat{p}_j - \ln(1+\hat{p}_j)\right] = \text{constant}.$$

This integral is easily expressed in terms of the original variables p_j and r_j. But, in the case of the control loops shown in Figure 4, there is a serious difficulty. Goodwin shows that the control equations can be put in the form

$$\frac{d\hat{r}_1}{dt} = b_1\left(\frac{\gamma_1}{\gamma_1 + p_1} - 1\right)$$

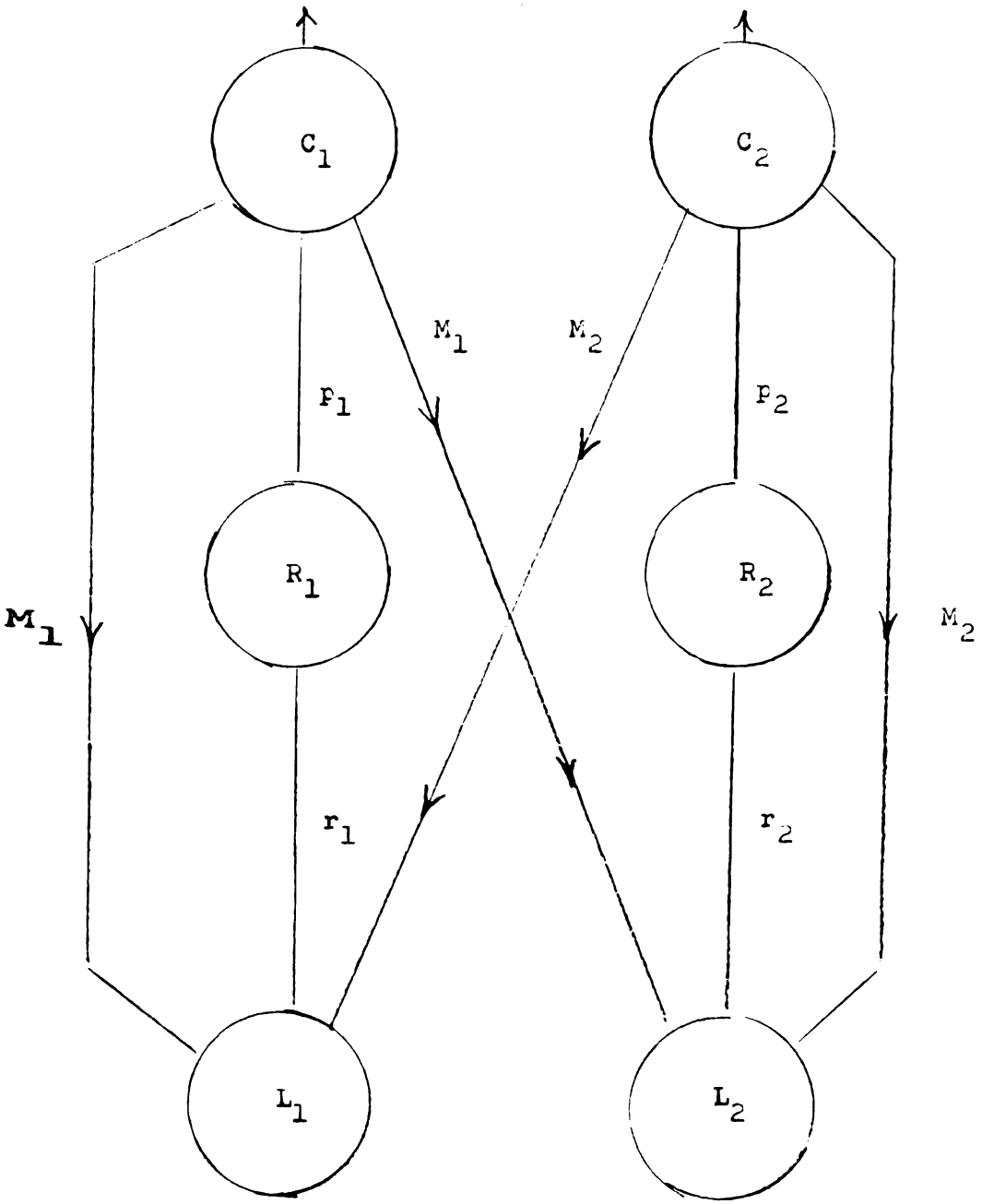
$$\frac{d\hat{r}_2}{dt} = b_2\left(\frac{\gamma_2}{\gamma_2 + p_2} - 1\right)$$

$$\frac{d\hat{p}_1}{dt} = \frac{\gamma_1}{Q_1}\left(k_{11}\alpha_1 \hat{r}_1 + k_{12}\alpha_2 \hat{r}_2\right)$$

$$\frac{d\hat{p}_2}{dt} = \frac{\gamma_2}{Q_2}\left(k_{21}\alpha_1 \hat{r}_1 + k_{22}\alpha_2 \hat{r}_2\right).$$

However, it is possible to integrate the differential $dG(\hat{p}_1,\hat{p}_2,\hat{r}_1,\hat{r}_2)$

Figure 4. Two control loops illustrating strong coupling. See Figure 2 for explanation of the notation. Drawn after Goodwin (1963).



only in the special case when $\gamma_1 k_{12} \alpha_2/\varrho_1 = \gamma_2 k_{21} \alpha_1/\varrho_2$, for then the terms

$$\frac{\gamma_1}{\varrho_1} k_{11} \alpha_2 \hat{r}_1 d\hat{r}_2 + \frac{\gamma_2}{\varrho_2} k_{21} \alpha_1 \hat{r}_2 d\hat{r}_1$$

becomes a perfect differential. In general, it will be possible to find a Hamiltonian function for any set of control equations (Kerner, 1964), but this example shows that it may not be possible to actually integrate the differential form. This is an important shortcoming, for the integrated form is needed to calculate all ensemble averages.

(2) Goodwin follows Kerner's methods and calculates the various thermodynamic functions, but replaces the adjective "demographic" by the word "talandic" meaning "oscillatory" so that his analyses are in terms of talandic energy, talandic temperature, talandic entropy, etc. As in the Kerner theory the actual use of these state functions is that the total energy is needed to calculate ensemble averages and that the talandic temperature is used to measure the variance of fluctuations about steady state values. The equilibrium assumption is reflected in the equipartition of talandic energy between the components of the system. Kerner's results on the functions A_+/A, T_+/T, ω, etc. are found also to apply in the case of epigenetic systems.

Goodwin extends his study one step further than the Kerner theory by studying the statistical properties of oscillating systems in some detail. In particular, he discusses the consequences of strong coupling between control loops; that is, situations in

which macromolecules from one control loop directly affect other control loops. This type of occurrence is illustrated by the loops in Figure 4 and is the opposite of weak coupling which occurs when control loops interact through the metabolic system. Strongly coupled control loops can exhibit the phenomenon of entrainment in which one loop may recruit other loops to its particular frequency and amplitude. As his choice of an adjective indicates, most of Goodwin's results are aimed at studies of the oscillating systems exemplified by biological clock phenomena. Thus, he suggests experiments in which the character of oscillations are changed following pulses of amino acis or RNA's. However, as Goodwin points out, these results on periodically varying systems will probably be of little use in the study of developmental problems.

D. Tactics and Strategy

The five workers we have discussed constitute something of a movement throughout which the tendency has been to develop theories and concepts which parallel those of physics. The general program of the movement has been to effect a description of a system in terms of kinetic equations and to use these equations to formulate a statistical description of the system considered macroscopically. If we use this approach on our problem of the genotype to phenotype relationship, we must decide (1) to what extent it is advantageous to continue the tactics of their statistical description, and (2) to what extent it is appropriate to follow their strategy of paralleling physics.

1. Tactics -- The Goodwin theory serves the important pur-

pose of making clear what is inadequate as a general theory of cellular development. The central result of the theory is that environmental stimuli result in changes in the talandic temperature — or variance of the epigenetic variables p_j and r_j about their steady state values — so that the epigenetic "system can exist in many different talandic energy states without any change occuring in the steady state values of the microscopic variables"[10]. But, if we think of cellular states as reflecting the enzymes present in the cell, development must consist of changes of the steady state values of the p_j's. Thus, "it is necessary to have a model which is irreversible in the sense that ... the steady state quantities p_j and q_j undergo permanent change"[11]. "The difficulty is to produce a model which switches under an environmental stimulus (a temporary parametric alteration) and then stabilizes itself by other changes of internal activities so that even when the stimulus is removed the altered state persists"[12]. Our tactics, then, will be to use the crux of the Goodwin method and represent the genetic-metabolic system of the cell by control equations and the state of the cell by points in a multidimensional phase space, but to attempt a more general theory able to describe irreversible and quantized processes.

2. Strategy -- The tendency to use physics as a model for theoretical biology is clear in Lotka's use of "stoichiometric" equations to study the "transformations of masses and energies" between demes. It is to be seen in Volterra's law of the conservation of demographic energy and his pains to show that his "equa-

10 Ibid., p 133.

11 Ibid., p 151. Goodwin uses q instead of our r.

12 Ibid., p 151. Italics in original.

tions of motion" can be derived from a variational principle. It is to be seen in Kerner's even greater pains to derive the Volterra equations from a least-action principle, in his use of the Gibbsian statistical mechanics, and in his decision to derive the various thermodynamic functions. It is important to realize that none of these workers is directly applying physics to their problems. One can no longer doubt that the laws of chemistry and physics are satisfied in living systems, so that it behooves the biologist to pay attention to entropy and force and molecule and a sizeable fraction of the physicist's armamentarium of concepts. But, each of the workers we have discussed realizes that it is inappropriate — in general — to use these concepts to discuss integrated biological systems. It is meaningless, say, to speak of the phhysical kinetic energy of a population of animals when studying population genetics. The tendency is one to proceed in analogy to physics and the outcome is an analog to temperature, an analog to entropy, and an analog to Hamilton's equations rather than a novel use of the physical quantities per se.

An example of an argument offered in support of this analogizing occurs in the first chapter of Goodwin's book. First, Goodwin points out that biologists are faced with a form of what we have called the problem of concrescence:

> From the properties of the "elementary particles" of cells, such as the cistron, the zymon, the replicon, etc., must emerge those characteristics which are the recognized attributes of living cells. 13/

This is analogous to the problem of statistical mechanics, but unlike the case in thermodynamics where a quantiative set of macro-

13 Ibid., p 1.

scopic relationships in the form of phenomenological thermodynamics exist,

> All that there is in biology is a set of concepts such as organization, adaptation, regulation, competence, homeostasis, etc., which must carry an enormous burden of more or less intuitive understanding and experience about the essential principles of biological structure and function. Although some of these concepts have been analyzed into more exact notions which could lead to quantitative definitions satisfying to some extent their intuitively-perceived content, there is certainly no set of relations which order them into phenomenological laws of cellular biology. 14/

Goodwin then asserts that phenomenological laws of cellular systems are not apt to be found:

> The singular absence of precisely-formulated laws of cellular organization suggests that there simply are no obvious general quantities for measuring cell behavior which are presented to our senses in the manner that heat, pressure, and volume are in the study of physical phenomena. 15/

Thus, Goodwin elects to introduce analogs of the known "macroscopic parameters" from thermodynamics:

> The present theory ... sets out to derive some general macroscopic or "thermodynamic" functions which arise from certain dynamic characteristics of molecular control mechanisms in living cells. The programme is, then, to use the present knowledge of the molecular organization of cells, so brilliantly exposed by molecular biologists, as the microstructure for a statistical theory from which the general behavioural consequences of this organization can be deduced in terms of functions which bear a complete formal analogy with the classical thermodynamic quantities of temperature, free energy, work etc. 16/

Again, it must be emphasized that the posited relationship between physical thermodynamics and talandic thermodynamics is one of similarity or analogy rather than one of identity.

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14 Ibid., p 2.

15 Ibid., p 3.

16 Ibid., p 3.

If we were to adopt this strategy, we would generalize the Goodwin theory to obtain the talandic analog of quantum mechanics or non-equilibrium statistical mechanics. However, we shall argue that this strategy is not valid and should be abandoned. Instead, we suggest that the problems of the mechanical-thermodynamic relationship and of the genotype-phenotype relationship are related in that they are elements of the same class of proolems — those of concrescences — and that there is no formal similarity between the systems studied in physics and those studied in holistic biology. Our reasons are that (a) the difference in the modes of analysis of the physicist and of the biologist suggest that concepts cannot easily be transferred between the disciplines, and (b) assumming that the subjects of the two sciences are similar and that similar concepts exist in the two sciences has led to no useful results.

(a) (1) To see how the analyses of the physicist and the biologist are different we must realize that all of the physicist's concepts and results are derivative from his experience as a human being in what, for lack of a better term, we shall call <u>experential space</u>, the "space in which" he perceives things and moves around in and endures in. The mathematical description of experential space is that of a Euclidean 3-space and a Newtonian inertial reference system. For some purposes, experential space is too naive of a concept and it is necessary to alter our conception of space and time to one of space-time. This change is tolerable because this new idea still has experential space as a "classical" limit and, more importantly, even in relativistic situations events are still

constrained by the most basic properties of experential space. For example, it is not possible to escape from space, the result of any movement is that the moved object is still in experential space. Also, an object can be made to move about in experential space in a continuous motion from one point to another. Even the most abstract results in physics must conform to such basic properties of experential space. Mathematically, the point is that all the things that can be done to a physical object must be expressable in terms of a group of continuous transformations of objects in experential space. There is no force of logic behind this "must", it is just the intuitively established standard of our intellectual tradition.

The mathematician remains unfettered by considerations of experential space. In fact, mathematics is difficult just because it requires one to abandon his "real" experential space for a consideration of the more abstract idea of a set of elements which may exhibit peculiar characteristics when judged by the standard of experential space. This mathematical license tends to obscure the primacy of experential space in physics, for the physicist often uses a variety of mathematical spaces as tools to study events that occur in experential space. The Lagrangian and Hamiltonian formulations of mechanics represent events that happen in experential space as points in configuration or phase space. Quantum mechanics represents "states" of objects in experential space as vectors in Hilbert space. But, the physicist never studies events "in phase space" or "in Hilbert space"; instead, he uses these spaces to emphasize the characteristics of events in experential space, and they are useful only as long as he can translate his findings to the coordinates of experen-

tial space-time. An apparent exception to this is the affine pressure-volume-temperature space of thermodynamics which imposes the odd restriction that state transformations can be made only along isobars or isotherms instead of along the shortest route from point to point.[17] Perhaps, as Bridgman (1961) points out, it is because of this degree of abstraction that it has seemed necessary to "reduce" thermodynamics to mechanics and, thus, to events in experential space. By virtue of such reductions, all of the concepts of physics become utensils for thinking about events in experential space: concepts such as "force", "energy", and "angular momentum" being clearly related to experential space, the concept of electricity and magnetism such as "charge" being less immediately stated in terms of experential space, and the parameters of thermodynamics being related to experential space only through the sophisticated transformation of statistical mechanics. In sum, physics may be defined as the study of events from the point of view of experential space.

(2) However, this is not the only point of view from which to view natural events; entirely different orientations are often useful and intelligible. As a simple example, suppose one had a pile of building materials — boards, bricks, nails, etc. These components could be used to build one of several objects; say, a house, barn, and a row boat. It would, of course, be possible to describe the construction of each item from a physicist's viewpoint as a series of transformations in experential space. Each step in construction would be described as a change in space-time, particular problems being formulated in terms of "forces" and "stresses"

17 Cf. L. Brillouin (1964).

and "strains". But, it is also possible to describe the building of any object from alternative points of view. One might define a sequence of states such as that of being unassembled materials, that of being a house, that of being a boat, etc. Now, if we wish to study the transformations between such states, this viewpoint can give unique insights. It is clear, for instance, that a direct transformation from the state of being a hous to the state of being a barn could probably be carried out by a suitable remodeling process. But, it is unlikely that a direct transformation between the state of being a house and the state of being a boat could be managed; the radical differences in the macroscopic organizations of the two objects would demand an intermediate transformation to the unassembled state. All of these transformations possible or impossible must conform to the physical laws of transformations in experential space. On the other hand, it does not seem worth the effort to try to explain the impossibility of the house-to-boat transformation in the physicist's language: it is not clear, for example, that the entropy of a boat is more or less than that of a house so that it seems to be a misplaced effort to dream up an analogy to the Second Law to describe the "evolution" of such objects. This example is important to us because much of the biologist's work is done from a point of view which emphasizes organization rather than processes viewed from the point of view of experential space. In particular, the ideas of "gene action" and "cellular state" and "induction" do not really gain much by being interpreted in terms of experential space. Biology might be characterized (but not defined) as the study of a certain class of objects from the point of view of or-

ganization. A more realistic example of this point of view is discussed in Section B of Chapter IV.

(3) This recognition of the possibility of viewing natural events from several points of view — what Whitehead calls different modes of analysis — puts us in the position to realize that an insistance on making physical analogies might tend to actually vitiate the power of the tools the Volterra-Kerner-Goodwin movement has developed. The central theme of these theories is the representation of a demographic or an epigenetic system by a point in a "phase" space. These spaces, however, are only distantly related to the physicist's phase space in which the positions of objects in experential space and their momenta are plotted, for the points in these spaces are in no way related to events represented from the point of view of experential space. Rather, they represent the results of interactions between organisms or between control loops, and are better described by a representation from the point of view of the organization of a population of organisms or of a cell. Most of the physicist's concepts are ideas about events in experential space and it is inappropriate to assume that they are meaningful when used in conjunction with the viewpoint of organization expressed by the "phase" spaces of Kerner and Goodwin. The danger in this transference of concepts between points of view is that the prevalence of "physical analogs" may obscur parameters and concepts which faithfully describe events from the organizational point of view. Since both the Kerner and the Goodwin theories have achieved some success in describing biological events it is necessary to tentatively accept their "phase" spaces as faith-

fully representing biological phenomena. But since these "phase" spaces manifest a point of view radically different from the point of view of experential space represented by phase spaces it is necessary to assume, until proven otherwise, that the systems being analyzed by the physicist and by the biologist are not formally similar so that "physical analogs" should not be expected to have anything to do with biological systems.

(b) At this point, we must raise two questions. First, has the use of physical analogs received any empirical justification? Secondly, is Goodwin justified in using physical analogs as a last resort because the complexity of biological systems has masked all phenomenological relationships? We will consider Goodwin's point first.

(1) Immediately after suggesting the use of physical analogs, Goodwin proceeds to a discussion of cybernetics and negative feedback as applied to cells. This is ironic, for "negative feedback" is the prime example of an intuitive, basically biological concept which has been rendered quantitatively precise. The notion of negative feedback is pretty much equivalent to W. B. Cannon's concept of homeostasis in that each homeostatic system is bound to exhibit negative feedback and each instance of negative feedback in an organism is apt to be associated with a homeostatic system. Furthermore, Rosenblueth, Wiener, and Bigelow (1943) have suggested that negative feedback is a precise way of describing "purposiveness", the prototype of metaphysical entities. The concept of negative feedback is also an example of an idea oriented towards the organizational viewpoint. It says something about the way in which the

organizational components (as opposed to the material components) of a system interact; although this interaction can usually be described as events or configurations in experential space, the important consideration is only that a set of components actually do interact; how the components are arranged spatially is pretty much besides the point. Because the idea of negative feedback is geared to the organizational viewpoint, it is not likely to be used and has not been used in any of the branches of physics as a fundamental concept. The utility of negative feedback in studying biological systems does not prove that a set of phenomenological relationships will soon be found for cellular systems, but since it, like the phenomenological relationships of thermodynamics, allows some definite statements about the behavior of the system of interest, the fact that theoretical biology and theoretical physics might not look alike no longer seems to be a serious shortcoming.

(2) We return now to our first question. In this regard, it must be pointed out that most of the physical analogs derived by Kerner and Goodwin are actually never used and do not contribute to the derivation of any testable results. The only physical analogs which are used are the total energy, G, and the temperature, θ. Physical total energy is defined directly in terms of the coordinates of experential space. The function G is chosen as the talandic total energy because it satisfies the Hamiltonian equations; on this basis, the probability distribution in phase space is defined. If we retain our scepticism towards the transferrence of physical concept to biological problems, this becomes a rather <u>ad hoc</u> choice of a probability distribution. Regardless, it is doubt-

ful how much physical content is actually being introduced into Goodwin's theory, for a simple calculation [18] shows that the probability that the variable r_j falls between r_j and $r_j + dr_j$ is given by just the Gaussian distribution (cf. Chapter III, Section D.1.(a)). We have already noted that the talamdic temperature is more or less simply related to the variances of the variables p_j and r_j. The other parameters (T_+/T etc.) which are actually used are measures of the various oscillatory characteristics of the system and are not physical analogs. Thus, all of the useful results of the Kerner and Goodwin theories could be obtained, devoid of physicalistic trappings, by using the usual statistical methods to study the oscillations of the variables p_j and r_j. There is no empirical justification for the transference of physical concepts to the study of biological systems.

We have argued that because the physicist and the biologist tend to look at natural events from two different viewpoints, the physicist abstracting out of nature ideas which relate directly to experential space while the biologist has found it useful to abstract out of nature the functional relationships manifest in the system he is studying, it should not generally be possible to transfer particular concepts from one science to the other. We have pointed out that, at least in one instance, a biological concept has been put into a form which allows definite statements about the behavior of intact systems. And, we have noted that the important results obtained by Kerner and Goodwin are not actually dependent on the use of physical analogs. We conclude, therefore,

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[18] See B. C. Goodwin (1963), p 63.

that the strategy of paralleling physics is an unnecessary one which is likely to obscur already opaque problems by introducing inappropriate formulae and unneeded entities. A more appropriate strategy is to begin by doubting the applicability of all physical concepts to integrated biological systems until such concepts have been shown to be actually necessary to understand living systems and to take as central to a theoretical biology those ideas and parameters which biologists have found helpful in thinking about living systems. No doubt the two modes of analysis will ultimately find some concepts mutually compatible, but by using this strategy we will feel confident that these common concepts reflect some basic similarity in the two types of systems rather than mirroring the scientist's human tendency to over generalize well understood ideas.

CHAPTER III. A THEORETICAL APPROACH TO CELLULAR STATES

"The aim is not to ape physics, but to mine the universal mathematical quarries."

-- E. H. Kerner

A. Assertions

1. The problem -- Our problem is to discover the relationship of the genetic-metabolic system of a single cell to the morphological type of the cell. We will assume that the genetic-metabolic system of the cell is adequately represented by a set of 2n kinetic equations in terms of the variables p_j and r_j. If we introduce the variable λ which measures the morphological type of the cell, the problem will be solved if we can express λ as a function of the variables p and r (we abbreviate the set of n p_j's by p and the set of n r_j's by r). In this Thesis, we will restrict the discussion to cells which persist in a cellular state and do not pass from state to state.

2. Assertions -- As the basis for a solution we will use two assertions about the nature of cellular states. These statements are assertions in the sense that experimental data can be used to support them, but will not be presented until Chapter IV. The reader who is uneasy about this is invited to read Chapter IV before proceeding in this Chapter. First, we assert that a given

cell can be in one of a finite number of discrete morphological types and that the cell is a stable system (in a sense defined below) with respect to its morphological type. Secondly, we assert that there is a one-to-one correspondence or isomorphism between the morphological type of a cell and the state of its genetic-metabolic system. In view of the first assertion, this means that a cell can only be in one of a finite number of discrete genetic-metabolic states.

B. Definitions and the Eigenvalue Equation

1. P space, biochemical states, and morphological states -- We designate the aggregate of all cellular systems as S. From now on, the genetic-metabolic system of the cell will also be called the biochemical system of the cell. The biochemical system of a cell is represented by a set of 2n kinetic equations

$$\frac{dp}{dt}j = R_j(p,r) \quad \text{and} \quad \frac{dr}{dt}j = P_j(p,r), \; j = 1,2,\ldots,n.$$

p_j is the concentration of the jth species of protein; r_j is the concentration of the corresponding mRNA. For the purposes of developing the general theory of cellular states, we will allow the functions $R_j(p,r)$ and $P_j(p,r)$ to be any well behaved functions of p and r. However, we assume that $R_j(p,r)$ and $P_j(p,r)$ do not depend explicitly on small molecules such as ions, cofactors, hormones, vitamins, phosphorylating agents, etc. In almost every interesting case it will be necessary to take account of these small molecules; this can be done without changing the general

theory by expressing some of the constants in $R_j(p,r)$ and $P_j(p,r)$ as functions of p,r, and other variables; thus, obtaining more complicated kinetic equations. A more practical description of S is suggested in Section D of Chapter IV.

We define a vector space, $\mathcal{P}$, with the basis vectors a_j and b_j, $j = 1,2,\ldots,n$, such that each axis of $\mathcal{P}$ represents the concentration of a species of protein or mRNA. A vector in $\mathcal{P}$ is given by

$$\vec{T} = \sum_{j=1}^{n} (p_j \vec{a}_j + r_j \vec{b}_j),$$

and the time rate of change of $\vec{T}$ is

$$\frac{d\vec{T}}{dt} = \vec{Q} = \sum_{j=1}^{n} (\dot{p}_j \vec{a}_j + \dot{r}_j \vec{b}_j) = \sum_{j=1}^{n} (R_j \vec{a}_j + P_j \vec{b}_j).$$

We temporaily define the <u>biochemical state</u> of S as the 2n-tuple (p,r); that is, a point in $\mathcal{P}$. This definition is implicit in Goodwin's "phase" space of epigenetic systems.

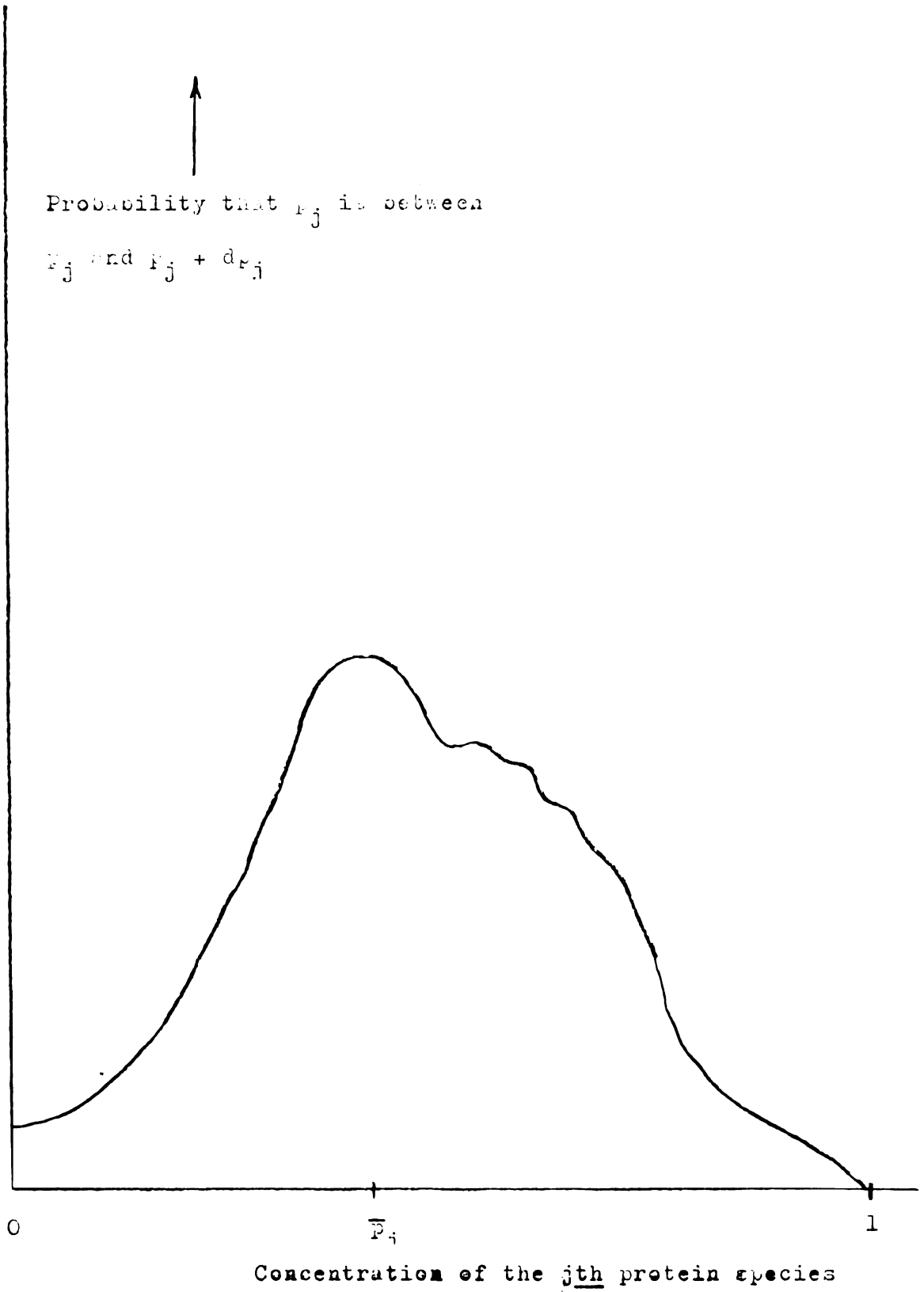
The <u>morphological state</u> of a cell is a real positive number λ_k such that each different morphological cell type corresponds to a different λ_k. (It is easy to modify the theory to accomodate the possibility of several cell types corresponding to the same λ_k). In accord with our first assertion, we stipulate that there be N morphological states and N λ_k's; that is, $k = 1,2,\ldots,N$. Our second assertion maintains that there be a correspondence between the biochemical and morphological states of a cell. To obtain

such a correspondence, we introduce a function L(p,r) defined on $\mathcal{P}$ which maps biochemical states (2n-tuples) into the set of all λ_k's; we will specify the form of L(p,r) in Section C. Our assertion requires an isomorphism between biochemical states and λ_k's, but there are an infinity of 2n-tuples in $\mathcal{P}$ and only a finite number of λ_k's. To obviate this difficulty, we will redefine our concept of biochemical state.

2. Biochemical experiments -- Experimentally, we can at least conceive of measuring the variables p and r for a given cell. Biochemical experiments consist of measuring the p and the r under normal and experimentally altered conditions. In either case, the result of any experiment is a 2n-tuple of values, (p,r), which can be represented as a point in $\mathcal{P}$. Suppose that it be possible to follow the variables p and r for a period of time in a cell of a given morphological type. The results of this experiment will be a configuration of points in $\mathcal{P}$. Suppose further that we pick one of the p or the r, say, p_j, and prepare a plot of the density of points in $\mathcal{P}$ as a function of p_j. If this plot be normalized, we can consider it as a probability density. The results of such an experiment might resemble the plot in Figure 5. To generalize this idea to the case of all 2n variables, we introduce a single valued, continuous function [19] $U_k(p,r,t)$ defined on $\mathcal{P}$; the index k means that the cell was in the kth morphological state at the time of the experiment. Denote the density of experimental points in $\mathcal{P}$ by $U_k^*(p,r,t)U_k(p,r,t)$ where $U_k^*(p,r,t)$ is the complex conjugate of $U_k(p,r,t)$. After normalization, $U_k^*(p,r,t)U_k(p,r,t)dpdr$ gives the probability that the results of a single measurement of p_j is

[19] Cf. Atkinson (1965), note 8.

Figure 5. The results of the measurement of the concentration of the jth species of protein in a biochemical experiment.



between p_j and $p_j + dp_j$ and r_j is between r_j and $r_j + dr_j$ for all j.

In general, we must expect that an actual distribution of points and not a single point will result from a series of measurements. If we were to explicitly study the influence of small molecules on the p and r, variations in the activities of small molecules would produce variations in the experimentally obtained values of p and r. But, even if we ignore these effects, the components of p and r are likely to oscillate about some mean values, so that measurements will result in values of p and r anywhere between certain limits. The necessity of recognizing such oscillations is another reason for rejecting the idea of a biochemical state being represented by a point in $\mathcal{P}$.

To meet this contingency, we now discard our first concept of biochemical state and define the biochemical state of S as a density function in $\mathcal{P}$. We can fulfill our second assertion by considering N such functions. The function $U_k(p,r,t)$ serves to define such a density function, so we define the N biochemical states of S as the N functions $U_k(p,r,t)$, $k = 1,2,\ldots,N$. Thus, we consider the function(al) L(p,r) as mapping the set of functions $U_k(p,r,t)$ into the set of the λ_k's. We will assume that all N functions $U_k(p,r,t)$ are different. The distribution of points in $\mathcal{P}$ are, in general, not disjoint.

3. Expectation values and variances -- In a biological system, p_j must have some upper bound; for example, if the average total protein concentration for a cell is p_o, it will always be the case that $p_j < p_o$. By a suitable choice of units for the p

and the r we can have $0 \leqslant p_j < 1$ and $0 \leqslant r_j < 1$ for all j. Thus, all experimental points will be within the unit sphere in $\mathcal{P}$ and for all t $U_k^*(1,1,t)U_k(1,1,t) = 0$.

Suppose that S be in its kth state; the expectation value of any function, A(p,r), defined on $\mathcal{P}$ is given by[20]

$$\overline{A_k(p,r)} = \int_0^1 \int_0^1 A(p,r)U_k^*(p,r,t)U_k(p,r,t)\, dpdr .$$

Note that $\overline{A_k(p,r)}$ does not take into account the probability that S is in the kth state. Setting $d\mathcal{T} = dpdr = dp_1 \ldots dp_n dr_1 \ldots dr_n$ and suppressing the limits of integration as understood, this can be written as

$$\overline{A_k(p,r)} = \int U_k^*(p,r,t)A(p,r)U_k(p,r,t)\, d\mathcal{T} .$$

If S has N possible morphological states, we have a similar expression for the expectation value of A(p,r) when S is in each state. Since these states are assumed disjoint, we may inquire what the expectation value is for a series of determinations of A(p,r) performed while S is in different stable states. We denote the probability that S is in the kth state when a measurement is being made by $c_k^* c_k$. Then, the expectation value of A(p,r), taking into account the probability that S is in different states, is

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20 This is the usual definition of expectation value. For example, see Frazer (1958).

$$\overline{A(p,r)} = \frac{\sum_{k=1}^{N} c_k^* c_k \, \overline{A_k(p,r)}}{\sum_{k=1}^{N} c_k^* c_k} .$$

We require, of course, that $\sum c_k^* c_k = 1$, so that

$$(1) \qquad \overline{A(p,r)} = \int \sum_{k=1}^{N} c_k^* U_k^*(p,r,t) A(p,r) c_k U_k(p,r,t) \, d\tau .$$

A more compact form of (1) can be obtained by introducing the N x N matrix

$$A(p,r)_{Ma} = A(p,r) \begin{vmatrix} 1 & 0 & \cdots & 0 & 0 \\ 0 & 1 & \cdots & 0 & 0 \\ \vdots & \vdots & & \vdots & \vdots \\ 0 & 0 & & 1 & 0 \\ 0 & 0 & & 0 & 1 \end{vmatrix}$$

the column matrix

$$U = \begin{bmatrix} c_1 U_1 \\ \vdots \\ c_k U_k \\ \vdots \\ c_N U_N \end{bmatrix}$$

and the row matrix

$$U^* = \underbrace{c_1^* U_1^* \cdots c_k^* U_k^* \cdots c_N^* U_N^*} \quad .$$

In terms of this notation, (1) becomes

$$\overline{A(p,r)} = \int U^* A(p,r)_{Ma} U \, d\tau \quad .$$

This form of the expectation value will be used throughout this work.

As a special case, note that if $A(p,r) = p_j$ or $A(p,r) = r_j$, then

$$\bar{p}_j = \int U^* p_j U \, d\tau$$

(2)

$$\bar{r}_j = \int U^* r_j U \, d\tau \quad .$$

The variance of all measurements of $A(p,r)$ in all N states is defined as

$$(3) \qquad (\Delta A)^2 = \overline{(A^2)} - (\bar{A})^2$$

$$= \int U^* A_{Ma}^2 U - \left[\int U^* A_{Ma} U \right]^2$$

where $A = A(p,r)$ for brevity. An equivalent way of writing (3) is

$$(4) \qquad (\Delta A)^2 = \int U^* [A_{Ma} - A]^2 U \, d\tau .$$

Equation (3) is easily derived from Equation (4); these definitions and properties of variance are analogous to those usually used in statistics (cf. Frazer (1958)).

4. Stability -- Let $A = A(p,r)$ be some function of p and r. S is defined to be stable with respect to A(p,r) if and only if $(\Delta A)^2 = 0$. This suggests that if a series of measurements of A are made at times $t_1, t_2, \ldots, t_h$, then S should be considered stable with respect to (wrt) A if the time average deviation of the measurements from some value A of A is zero. This definition poses two technical difficulties.

(a) The definition of expectation value introduced in B.3. is an average in ρ-space, and it is not obvious that this kind of average is equivalent to a time average resulting from an actual experiment. The connection between the two kinds of averages is achieved by interpreting, for example,

$$\frac{c_k^* c_k}{\sum c_k^* c_k}$$

as the fraction of time spent in the kth state over a sufficiently long period of time. The difficulty comes in deciding how long is "sufficiently long". Physicists meet the difficulty be resorting

to time averages extended over infinite periods of time, for which the correspondence can be more or less established (ergodic hypothesis). In our case, the most realistic approach is to leave the matter entirely up to the judgement of the experimentalist and allow him to decide how long he has to continue to make measurements to faithfully reflect the nature of the system under study.

(b) The word "stability" has a precise meaning in mathematics; but, in general, it is not clear how the definition given here is related to the mathematical definition. However, if we set $A(p,r) = p$, the condition $(\Delta A)^2 = 0$ suggests that p is arbitrarily close to $\bar{p}$ for almost all values of t. Then, the solution $\hat{p}$ of the system of equations $\dot{p} = P(p,r)$ and $\dot{r} = R(p,r)$ will be stable in the sense of Poincaré (for example, see Magiros (1966)) with $\hat{p}$ as a point of stability. Also, if A(p,r) is a quadratic form of the p and the r, then $(\hat{p},\hat{r})$ will be a stable point of the equations if A(p,r) has a minimum at $(\hat{p},\hat{r})$. Thus, it should be understood that the "stability" used in this Thesis is related to but not necessarily identical with mathematical stability.

It is very possible that S may be stable wrt A and be unstable wrt some other function $B = B(p,r)$, i.e. $(\Delta B)^2 \neq 0$. An individual cell is expected to be stable wrt morphological type but be unstable wrt some of the p and the r most of the time.

5. Embryological experiments -- From an embryological point of view, an experiment consists of "measuring" the morphological state of a cell under normal and experimentally altered conditions. Our first assertion was that cells are stable with respect to morphological type. Thus, the anticipated results of an embryologi-

cal experiment are of the form of Figure 6. In the experiment depicted, the morphological state of a cell was measured, and the parameter λ_k (the cell was of the kth type) has been equated to the expectation value of the measurement. Since L(p,r) serves to relate the variables of $\mathcal{P}$ to the values λ_k of the morphological variable λ, we choose to write $\lambda_k = \overline{L(p,r)}$. L(p,r) is a function defined on $\mathcal{P}$ so that the expectation value and the variance of L(p,r) are defined as in the previous Section. The Figure and the first assertion indicate that $\Delta L(p,r) = 0$ for the kth state (and, in fact, for all of the morphological states). Together, Figures 5 and 6 show that S is stable with respect to the function L(p,r) or the variable λ, but is not stable with respect to the variables p and r. These observations are the starting point of the theory to be developed here. In preparation for this development we will need the familiar Schwartz inequality and a certain integral.

6. Two preliminary results -- (a) In our notation, the Schwartz inequality [21] is

$$(5) \qquad \int U^* U \int (A_{Ma}U)^* (A_{Ma}U) \geq \left[\int (A_{Ma}U)^* U\right]^2$$

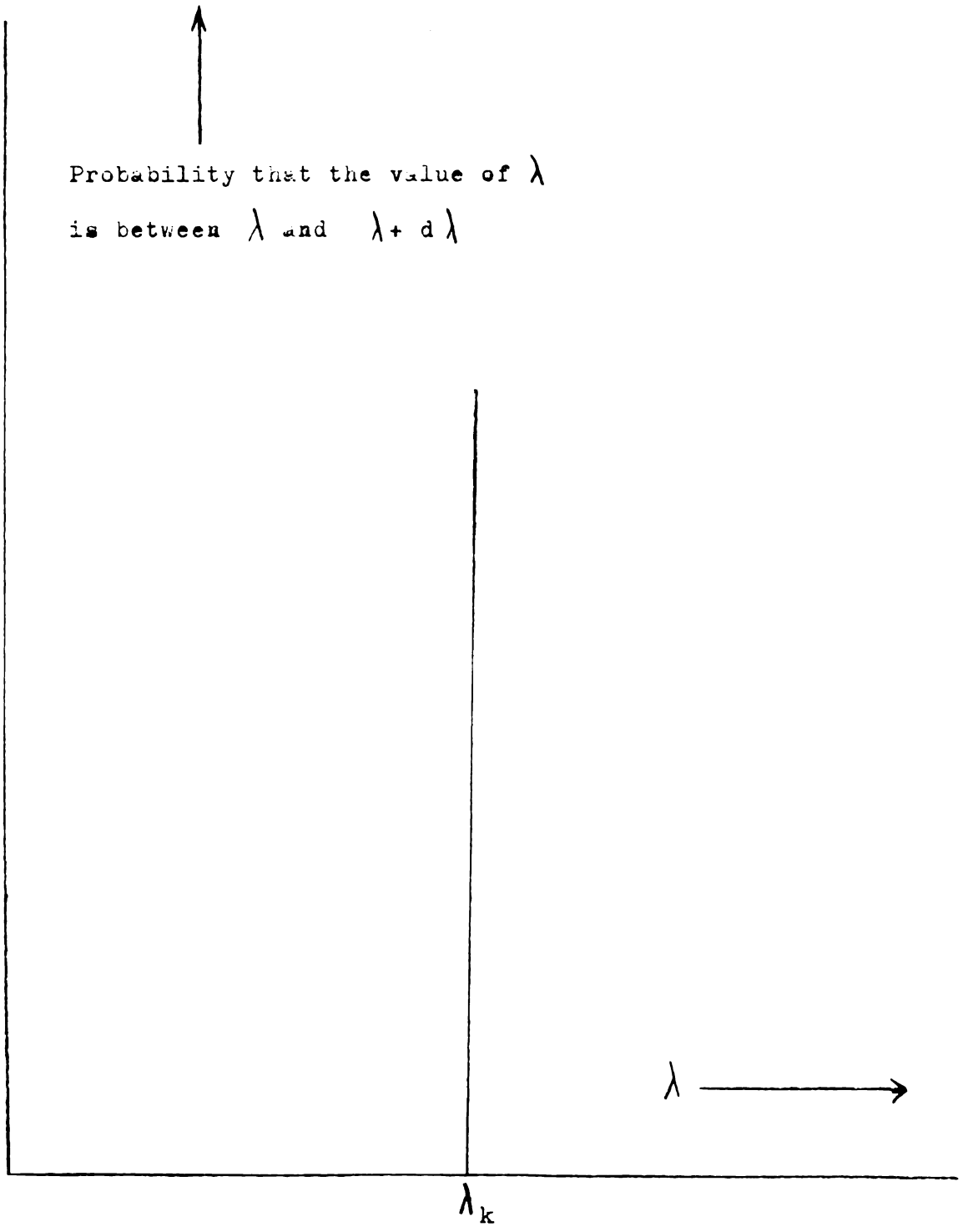
with equality holding if and only if $A_{Ma}U = CU$ for some scalar C. In particular, we may take $C = \int U^* A_{Ma} U$.

(b) We will now show that the integral

$$\int U^* A_{Ma}^2 U = \int U^* A_{Ma} A_{Ma} U$$

[21] This is a standard result in analysis; e.g., see Schmeidler (1965).

Figure 6. Results of the measurement of the cell type of a single cell in an embryological experiment. λ is the morphological variable.



can be written in the form $\int (A_{Ma}U)^* (A_{Ma}U)$. To do this, consider the column matrix

$$W = U + d(A_{Ma}U) \text{ , d is any scalar}$$

and the corresponding row matrix

$$W^* = U^* + d^*(A_{Ma}U)^* .$$

By inspecting the integrand of the integral

$$\int W^* A_{Ma} W = \int \left[U^* + d^*(A_{Ma}U)^*\right] A_{Ma} \left[U + d\,(A_{Ma}U)\right]$$

and recalling that A_{Ma} is just a compact way of writing the possible values of a biological measurement, we see that the integral itself must be a real quantity. Written explicitly, this is

$$\int W^* A_{Ma} W = \int U^* A_{Ma} U + d \int U^* A_{Ma} (A_{Ma}U) + d^* \int (A_{Ma}U)^* (A_{Ma}U)$$

$$+ dd^* \int (A_{Ma}U)^* A_{Ma} (A_{Ma}U) .$$

But, in order for this to be real, we must have

$$(\text{Im } d) \int U^* A_{Ma} A_{Ma} U + (\text{Im } d^*) \int (A_{Ma}U)^* (A_{Ma} U) = 0 .$$

Thus, we have our result

$$\int U^* A_{Ma}{}^2 U = \int (A_{Ma} U)^* (A_{Ma} U) \quad .$$

7. The eigenvalue equation -- we can now derive the fundamental equation of the present theory. Recall that $\Delta L(p,r) = 0$; that is

$$\int U_k^* L_{Ma}{}^2 U_k = \left[\int U_k^* \, L_{Ma} \, U_k \right]^2$$

for we are limiting ourselves to the measurement of a single cell in the kth state so that $c_k^* c_k = 1$ and $c_k^* c_{k'} = 0$ for all $k' \neq k$. Using the results of Section B.6.b. and the fact that $\int U_k^* U_k = 1$:

$$\int U_k^* U_k \int (L_{Ma} U_k)^* (L_{Ma} U_k) = \left[\int U_k^* \, L_{Ma} \, U_k \right]^2 .$$

However, by the Schwartz inequality

$$L_{Ma} U_k = \left[\int U_k^* \, L_{Ma} \, U_k \right] U_k .$$

But, $\lambda_k = \int U_k^* \, L_{Ma} \, U_k$ so that

$$L(p,r)_{Ma} \, U_k(p,r,t) = \lambda_k \, U_k(p,r,t) \quad .$$

This equation will be called the eigenvalue equation; and the func-

tions U_k are said to be the eigenfunctions of the matrix L_{Ma} while the λ_k's are the eigenvalues of that matrix.

8. Transition states -- The present theory has so far considered measurements only when a cell is in a stable state. To obtain a complete picture of cellular development, it would be necessary to deal with the "transition states" which occur when then cell is passing from one stable state to another by introducing a set of functions, $V_{mk}(p,r,t)$, defining the probability density functions for measurements when the cell is moving from the mth state to the kth state. We have associated two important properties with the functions U_k: (a) the definition of the expectation value of a function motivated treating the U_k's as the elements of a vector, and (b) because they are associated with stable states with respect to $L(p,r)$, they satisfy the eigenvalue equation. The functions $V_{mk} = V_{mk}(p,r,t)$ will not, in general, satisfy such an eigenvalue equation; but, they should be vectors, for if the transition process begins at t_o and ends at t_f, we must have

$$V_{mk}(p,r,t_o) = U_m(p,r,t_o)$$

and

$$V_{mk}(p,r,t_f) = U_k(p,r,t_f).$$

In general, there will be $N(N-1)$ such functions, but $V_{mk}(p,r,t)$ may be identically zero for some values of m and k.

One approach to the problem would be to consider the functions V_{mk} as vectors in an N-dimensional vector space, $\mathcal{R}_N$, which

has the functions U_k as its basis. This is the method which is adopted in quantum mechanics. It is not difficult to see that if there were a countable infinity of morphological states, then the functions U_k would form a complete set and we could express any function of p and r as a linear combination of the U_k's[22]. However, the first assertion was that there are only a finite number of morphological states, and the functions V_{mk} cannot be assumed to be vectors in $\mathcal{R}_N$ because a finite subset of a complete set is not generally complete. In quantum mechanics it is generally assumed that there are at least a countable infinity of system states so that the approach is valid. It is beyond the scope of this Thesis to consider the problem of calculating the functions V_{mk}.

9. The relation of $\mathcal{R}_N$ to $\mathcal{P}$ — linear operators on $\mathcal{P}$ --

Defined on $\mathcal{R}_N$ are a series of matrices, A_{Ma}, one corresponding to each variable of S. These matrices and the vectors of $\mathcal{R}_N$ completely determine the expectation values and variances of the variables of S in the manner detailed above. The system S, however, was originally described in terms of $\mathcal{P}$. It would be desireable to determine in terms of the coordinates of $\mathcal{P}$ what the probability distribution for each of the N stable states of S is and what the expectation values of the variables are for each state. It is not easy to do this in terms of the matrices defined on $\mathcal{R}_N$. Instead, it is shown in the Appendix that to each matrix, A_{Ma}, defined on $\mathcal{R}_N$

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22 Because U_k defines a probability density, $\int U_k^* U_k \leq 1 < \infty$. Thus, the functions U_k form a complex L^2 space. Since the L^p spaces are known to be complete by a theorem due to Riesz-Fischer, the functions U_k form a complete set (see Royden (1963)).

there is a linear differential operator, $A(p,r)_{Op}$, defined over $\mathcal{P}$. This means that to each eigenvalue equation in matrix form there corresponds a partial differential equation of the form

$$A(p,r)_{Op}\ U_k(p,r,t) = a_k\ U_k(p,r,t)\ .$$

The solutions of these equations will determine the probability distributions and allow the calculations of the expectation values of any variable of S. It remains only to choose an operator corresponding to the morphological function L(p,r).

10. Summary of argument -- The format of the argument up to this point is:

(a) Assume that the variables λ_k are finite in number and represent the expectation values of a variable with zero variance. That is, S is stable wrt that variable.

(b) Assume that there is an isomorphism between the set $\{\lambda_k\}$ and the set $\{U_k\}$.

(c) Let L(p,r) be a function which maps $\{\lambda_k\} \to \{U_k\}$. Agree to specify this mapping by requiring that $\lambda_k = \overline{L(p,r)}$.

(d) The first assumption implies that $L(p,r)_{Ma}\ U_k = \lambda_k\ U_k$.

(e) It is possible to replace this matrix equation by a differential equation $L(p,r)_{Op}\ U_k = \lambda_k\ U_k$. If the form of $L(p,r)_{Op}$ is specified, it is possible to find U_k and calculate $\bar{p}$, $\bar{r}$, and $\overline{L(p,r)}$.

C. A Choice of the Morphological Operator $L(p,r)_{Op}$

1. Definition and properties of L -- (a) We have previous-

ly postulated that the morphological cell type of S is a function, L(p,r), of the variables p and r of S. Since no experiments have ever been directed to the determination of such a function, there is no possible way of knowing a priori what form is appropriate for L(p,r). The only possible way to proceed is to guess a form for L(p,r) and to hope that suitable experiments can guide the formulation of another guess which better approximates the form of L(p,r) (if such a function is possible at all!). There are two strategies which can be used to direct the formulation of these guesses. First, one could take L(p,r) to be one of the parameters — such as entropy, temperature, information, energy, etc. — which have been useful in the study of physical systems. This is the approach which has been used by Kerner and Goodwin, and has been discussed in Chapter II. The advantages of this method are that it allows the wholesale use in biological systems of the concepts and techniques of physics and chemistry, and that it suggests a solution to the philosophical problem of the relationship between physical and biological systems by treating them as different examples of the same type of system. The risk involved in adapting this method is that biological systems may not be organized in the same way that physical systems are, so that the application of physical parameters to these systems may not lead to meaningful results. The second approach is to elect to set L(p,r) equal to some parameter which expresses some very general character of a system. Thus, in the next section we will specify that L(p,r) is to be considered as a certain function which is a measure of the time rate of change of each variable of S relative to

the movement in $\mathcal{P}$ of the probability distribution U*U. The advantage of this method is that L(p,r) will be reflecting an aspect appropriate to a biological system, for it will be a parameter applicable to any system which undergoes changes. The risk involved in this case is that this parameter may be so general in nature that it can contribute no interesting or useful information about biological systems as opposed to systems in general. The choice between these two approaches is, perhaps, really dependent on individual intuition, but the only legitimate means of arbitrating the matter is to compare the success of the experimental predictions of each approach.

(b) Since we are proceeding in an arbitrary manner at this point, there is nothing to prevent picking L(p,r) to be a differential operator defined on $\mathcal{P}$. The advantage of this choice is that, since we have assumed S to have stable states with respect to L(p,r) and have argued that there is an equivalence between the matrices defined on $\mathcal{R}_N$ and differential operators defined on $\mathcal{P}$, we can write immediately

$$L(p,r) = L(p,r)_{Op} = L(p,r)_{Ma}$$

where the equal signs mean contextual equivalence, and conclude that

$$L(p,r)\ U_k(p,r,t) = \lambda_k U_k(p,r,t) .$$

Then, the λ_k's will be the expectation values of L(p,r) in the N stable states of S; the functions $U_k(p,r,t)$ determining the probability distributions will be the solutions of a differential equation.

The total time derivative of the function U(p,r,t) is given by

$$\frac{dU}{dt} = \frac{\partial U}{\partial t} + \sum_{j=1}^{n} \frac{\partial U}{\partial p_j} \frac{dp_j}{dt} + \frac{\partial U}{\partial r_j} \frac{dr_j}{dt} .$$

Using the kinetic equations of S and multiplying by $i = \sqrt{-1}$:

$$(8) \qquad i \frac{dU}{dt} = i \frac{\partial U}{\partial t} + i \sum_{j=1}^{n} \frac{\partial U}{\partial p_j} R_j(p,r) + \frac{\partial U}{\partial r_j} P_j(p,r) .$$

We now <u>postulate</u> that the function L(p,r) is the <u>Liouville operator</u>

$$L(p,r) = L(p,r)_{Op} = i \sum_{j=1}^{n} R_j(p,r) \frac{\partial}{\partial p_j} + P_j(p,r) \frac{\partial}{\partial r_j} .$$

Then, Equation (8) can be written

$$i \frac{dU}{dt} = i \frac{\partial U}{\partial t} + L U$$

where we have set L = L(p,r) for brevity.

The Liouville operator can be written in a different form by use of standard vector notation: Recall that a position vector in $\mathcal{P}$ denoted by $\vec{T}$ has the time rate of change

$$\vec{\dot{q}} = \frac{d\vec{T}}{dt} = \sum_{j=1}^{n} R_j(p,r)\,\vec{a}_j + P_j(p,r)\,\vec{b}_j \quad .$$

U is a function defined on $\mathcal{P}$ with gradient

$$\vec{\nabla} U = \sum_{j=1}^{n} \frac{\partial U}{\partial p_j}\,\vec{a}_j + \frac{\partial U}{\partial r_j}\,\vec{b}_j \quad .$$

In terms of this notation, we have

$$L = i\,\vec{\dot{q}}\cdot(\vec{\nabla}\ )$$

so that $L\,U = i\,\vec{\dot{q}}\cdot\vec{\nabla}U$.

(c) In Section B.1., we took the function L to describe the morphological state of S. The result of an embryological experiment is an expectation value of L, and picking a form for L is the same as stipulating what is "really" being measured in an embryological experiment. In our case, if S is in its kth state, the expectation value of L is

$$\lambda_k = \int U_k^*\,L\,U_k\,d\tau = i\int U_k^*\,\vec{\dot{q}}\cdot\vec{\nabla}U_k\,d\tau \quad .$$

$\vec{\dot{q}}$ is the velocity of a point in $\mathcal{P}$; $\vec{\nabla}U$ is a vector with magnitude equal to the greatest rate of change of the function U and pointing in the direction of the greatest rate of change of the function U

and pointing in the direction of the greatest change of that function; U_k determines the probability distribution in $\mathcal{P}$. Thus, λ_k may be taken, roughly, as a measure of the motion of the vector $\vec{T}$ relative to the motion of the distribution of points in $\mathcal{P}$.

(d) Since U*U is a probability density, we require that

$$(9) \qquad 1 = \int U^*(p,r,t)U(p,r,t) = \int U^*(p,r,t')U(p,r,t')$$

for all $t' > t$. That is, we want

$$\frac{d}{dt}\int U^*U = 0.$$

Anticipating the result of Section D.1., $U^*(p,r,t)U(p,r,t) = u(p,r) \times u(p,r)$ where $u = u(p,r)$ is a time independent function of p and r, and it is sufficient to demand that

$$(10) \qquad \vec{q}\cdot\vec{\nabla}\rho + \frac{\partial \rho}{\partial t} = 0$$

where $\rho = u^*u$. The identity

$$\text{div}\,(\rho\vec{q}) = \rho(\vec{\nabla}\cdot\vec{q}) + \vec{q}\cdot\vec{\nabla}(\rho)$$

allows this condition to be written as

$$\frac{\partial \rho}{\partial t} + \text{div}\,(\rho\vec{q}) - \rho(\vec{\nabla}\cdot\vec{q}).$$

Consider now a small, arbitrary volume, $\mathcal{V}$, about the point $\vec{T}$ in $\mathcal{P}$. Designate the surface surrounding $\mathcal{V}$ by $\mathcal{S}$. dv is a differential element of $\mathcal{V}$ and $d\sigma$ is a differential element of $\mathcal{S}$. $\vec{n}$ is the unit normal to $\mathcal{S}$. The density of points in $\mathcal{P}$ at time t is given by ρ. Since $\mathcal{V}$ is small, the velocity of the points in $\mathcal{V}$ may be taken to be just $\vec{Q} = d\vec{T}/dt$. Thus, the number of points of $\mathcal{V}$ passing through $d\sigma$ in the time interval dt is given by $\rho\vec{Q}\cdot\vec{n}\, d\sigma$. The number of points passing through the entire surface in the time interval dt is then

$$\int_{\mathcal{S}} \vec{Q}\cdot\vec{n}\, d\sigma = \int_{\mathcal{V}} \operatorname{div}(\rho\vec{Q})\, dv \quad ,$$

where the divergence theorem has been used. But, the number of points passing through $\mathcal{S}$ in the time interval dt is also given by

$$-\frac{\partial}{\partial t}\int_{\mathcal{V}} \rho\, dv = \int_{\mathcal{V}} \left(-\frac{\partial \rho}{\partial t}\right) dv.$$

Thus, since $\mathcal{V}$ is an arbitrary volume,

$$-\frac{\partial \rho}{\partial t} = \operatorname{div}(\rho\vec{Q}) \quad ,$$

and the condition that the integral (9) be time invariant reduces to $\vec{\nabla}\cdot\vec{Q} = 0$.

In general, $\vec{\nabla}\cdot\vec{Q} \neq 0$. For example, if $\dot{p} = ap + br + c$, $\dot{r} = dp + er + f$, then $\vec{\nabla}\cdot\vec{Q} = a + e$. Thus, to obtain an acceptable density function we introduce the function $M = M(p,r)$, called the <u>last mul-</u>

tiplier of S, defined by the condition $\vec{\nabla}\cdot(M\vec{Q}) = 0$. Multiplying equation (10) through by M and setting $D = M\rho$, we obtain

$$M\vec{Q}\cdot\vec{\nabla}\rho + \frac{\partial D}{\partial t} = 0 .$$

Using the identity $\text{div}\,(M\rho\vec{Q}) = \rho\vec{\nabla}\cdot(M\vec{Q}) + M\vec{Q}\cdot\vec{\nabla}\rho$, gives

$$\frac{\partial D}{\partial t} + \text{div}\,(D\vec{Q}) - \rho\vec{\nabla}\cdot(M\vec{Q}) = 0 .$$

If we use D as the corrected probability density, we will have $\text{div}\,(D\vec{Q}) = -\partial D/\partial t$ and $dD/dt = 0$.

M can be determined from its defining condition

$$\vec{\nabla}\cdot M\vec{Q} = 0 = M\vec{\nabla}\cdot\vec{Q} + \vec{Q}\cdot\vec{\nabla}M .$$

This condition implies that $dt\,\vec{Q}\cdot\vec{\nabla}M = -dt\,M\vec{\nabla}\cdot\vec{Q}$. Since M does not explicitly contain t, $dM/dt = \vec{Q}\cdot\vec{\nabla}M$, and

$$\frac{dM}{M} = -dt\,\vec{\nabla}\cdot\vec{Q}, \text{ or } M = \exp\left[-\int\vec{\nabla}\cdot\vec{Q}\,dt\right] .$$

The parameter t must be removed from M by solving the kinetic equations of the system in terms of p and r. This proceedure is illustrated in the Example, below. In some instances, $t = t(p,r)$ will be a complex function; then we will use $D = (M^*M)^{1/2}u_k^*u_k$ as the corrected probability density.

(e) To be biologically meaningful, the λ_k's must be real

constants.

$$\lambda_k = i\int MU_k^*\vec{q}\cdot\vec{\nabla}U_k = i\int\left[M\vec{q}\cdot\vec{\nabla}(U_k^*U_k) - U_kM\vec{q}\cdot\vec{\nabla}U_k^*\right]$$

$$= i\int\left[\vec{\nabla}\cdot(U_k^*U_kM\vec{q}) - U_k^*U_k(\vec{\nabla}\cdot M\vec{q}) - U_kM\vec{q}\cdot\vec{\nabla}U_k^*\right]$$

$$= i\int_S U_k^*U_kM\vec{q}\cdot\vec{n}\,ds - i\int MU_k\vec{q}\cdot\vec{\nabla}U_k^*$$

$$= -i\int MU_k\vec{q}\cdot\vec{\nabla}U_k^*$$

$$= \lambda_k^* .$$

Here, S is the surface of the unit sphere in $\mathcal{P}$ space, $\vec{n}$ is the normal to S, and ds is an incremental area on S. We have imposed the boundary condition $U_k = 0$ along S. Since λ_k is equal to its complex conjugate, it is a real quantity.

2. <u>The Liouville equation</u> -- The equation $\partial D/\partial t + \vec{q}\cdot\vec{\nabla}D = 0$ leads to an equation for the time development of U_k. For simplicity, assume $\vec{\nabla}\cdot\vec{q} = 0$ so that $M = 1$. Then

$$0 = U^*\left[\frac{\partial U}{\partial t} + \vec{q}\cdot\vec{\nabla}U\right] + U\left[\frac{\partial U^*}{\partial t} + \vec{q}\cdot\vec{\nabla}U^*\right] .$$

Set $U = \mathrm{Re}\,U + i\,\mathrm{Im}\,U$, $U^* = \mathrm{Re}\,U - i\,\mathrm{Im}\,U$. Then

$$0 = 2\,\mathrm{Re}\,U\left[\frac{\partial}{\partial t}\mathrm{Re}\,U + \vec{q}\cdot\vec{\nabla}\mathrm{Re}\,U\right] + 2\,\mathrm{Im}\,U\left[\frac{\partial}{\partial t}\mathrm{Im}\,U + \vec{q}\cdot\vec{\nabla}\mathrm{Im}U\right].$$

In general, Re U and Im U are independent and non-zero, so that we must have

$$\frac{\partial}{\partial t} \text{Re } U + \vec{Q} \cdot \vec{\nabla} \text{Re } U = 0$$

and

$$\frac{\partial}{\partial t} \text{Im } U + \vec{Q} \cdot \vec{\nabla} \text{Im } U = 0 .$$

Thus, $\partial U/\partial t + \vec{Q} \cdot \vec{\nabla} U = 0$. Finally, we have the Liouville equation for U:

$$i \frac{\partial U}{\partial t} + L U = 0 .$$

D. Determination of the Functions $U_k(p,r,t)$

The time dependent behavior of the $U_k(p,r,t)$'s is governed by the Liouville equation; the time independent behavior is determined by the eigenvalue equation. To determine the functions U_k we assume that the variables are partially separable so that $U_k(p,r,t) = \vartheta_k(t) u_k(p,r)$ for some functions of time $\vartheta_k(t)$ and some functions of p and r, $u_k(p,r)$.

1. Time dependent part -- Using $U_k = \vartheta_k u_k$ and the Liouville equation we find that $\vartheta_k(t)$ must satisfy the ordinary differential equation

$$i \frac{d}{dt} \vartheta_k(t) + \vartheta_k(t) \lambda_k = 0 . \qquad (11)$$

The solution to Equation (11) is $\vartheta_k(t) = e^{i\lambda_k t}$. λ_k must have the dimensions of $(\text{time})^{-1}$.

Two important results follow directly from the form of $\vartheta_k(t)$.

(a) We have

$$\frac{\partial}{\partial t} U_k(p,r,t) = u_k(p,r) \frac{d}{dt}\vartheta_k(t) = ikU_k(p,r,t).$$

Thus, unless $u_k(p,r)$ is identically zero, $\partial U_k(p,r,t)/\partial t = 0$ if and only if $k = 0$. In this case, the eigenvalue equation becomes just $Lu_k = 0$, which is satisfied by $u_k(p,r) = \text{constant}$. In particular, we may set $u_k(p,r) = \exp(\text{constant})$, or

$$u_k(p,r) = U_k(p,r,t) = \exp(G - \Psi/\Theta)$$

where G is the talandic total energy, Ψ is the talandic free energy, and Θ is the talandic temperature. Thus, the Goodwin theory is restricted to the special case in which $k = 0$ and is not equipped to discuss the N-1 other cases in which $k \neq 0$.

(b) The functions U_k form an orthonormal set. To verify this property, we introduce a constant of normalization, N_k. Consider the integral

$$N_{k'}N_k \int U^*_{k'}U_k = N_{k'}N_k \int e^{i\lambda_{k'}t} u^*_{k'} e^{-i\lambda_k t} u_k .$$

There are two cases: (1) if $\lambda_k = \lambda_{k'}$, we can have

$$N_k^2 \int U_k^* U_k = 1$$

if we set $N_k^2 = \left[\int U_k^* U_k\right]^{-1}$.

(2) If $\lambda_k \neq \lambda_{k'}$, then

$$N_{k'}N_k \int U_{k'}^* U_k = N_{k'}N_k \int e^{i(\lambda_{k'} - \lambda_k)t} u_{k'}^* u_k$$

$$= \lim_{T\to\infty} \frac{1}{T} N_{k'}N_k \int_0^T e^{i(\lambda_{k'} - \lambda_k)t} u_{k'}^* u_k \, dt,$$

by the Ergodic theorem (see Khinchin, 1949). This integral is easily evaluated:

$$\frac{N_{k'}N_k u_{k'}^* u_k}{-i(\lambda_{k'} - \lambda_k)} \lim_{T\to\infty} \frac{1}{T}\left[e^{-i(\lambda_{k'} - \lambda_k)T} - 1\right] = 0 .$$

Thus, $N_{k'}N_k \int U_{k'}^* U_k = \delta_{k'k}$; the functions U_k, when normalized, form an orthonormal set.

2. Time independent part -- $u_k(p,r)$ must satisfy the eigenvalue equation; written explicitly, this is

$$(12) \qquad i \sum_{j=1}^{n} R_j(p_1,\ldots,p_n,r_1,\ldots,r_n)\frac{\partial u_k(p_1,\ldots,r_n)}{\partial p_j} +$$

$$P_j(p_1,\ldots,r_n)\frac{\partial u_k(p_1,\ldots,r_n)}{\partial r_j} = \lambda_k u_k(p_1,\ldots,r_n).$$

Equation (12) is a linear, first order, partial differential equation in 2n variables. The solutions of such an equation is usually effected by reducing it to a system of ordinary differential equations[23].

For the purposes of this section only, we use p and r to designate just two variables out of the complete set of 2n variables of S (i.e. $p = p_j$, $r = r_j$, for some j). Thus, Equation (12) becomes

$$(13) \qquad R(p,r)\frac{\partial u_k(p,r)}{\partial p} + P(p,r)\frac{\partial u_k(p,r)}{\partial r} = -i\lambda_k u_k(p,r).$$

We now introduce a three dimensional space with coordinates given by p,r, and $u_k = u_k(p,r)$; and note that u_k is just a surface in this space (Figure 7). A general function in p,r,u_k- space is represented by $\varphi(p,r,u_k)$. For example, we might have

$$\varphi(p,r,u_k) = 0 = (p-a)^2 + (r-a)^2 + (u_k-a)^2 - b^2 ,$$

which is the sphere in p,r,u_k-space. At any given point, (p,r,u_k), the normal to the surface $\varphi(p,r,u_k)$ is given by

$$(14) \qquad \vec{n} = \vec{\nabla}\varphi(p,r,u_k) = \frac{\partial\varphi}{\partial p}\vec{e}_1 + \frac{\partial\varphi}{\partial r}\vec{e}_2 + \frac{\partial\varphi}{\partial u_k}\vec{e}_3,$$

where we designate the unit vectors of p,r,u_k-space by $\vec{e}_1,\vec{e}_2,\vec{e}_3$.

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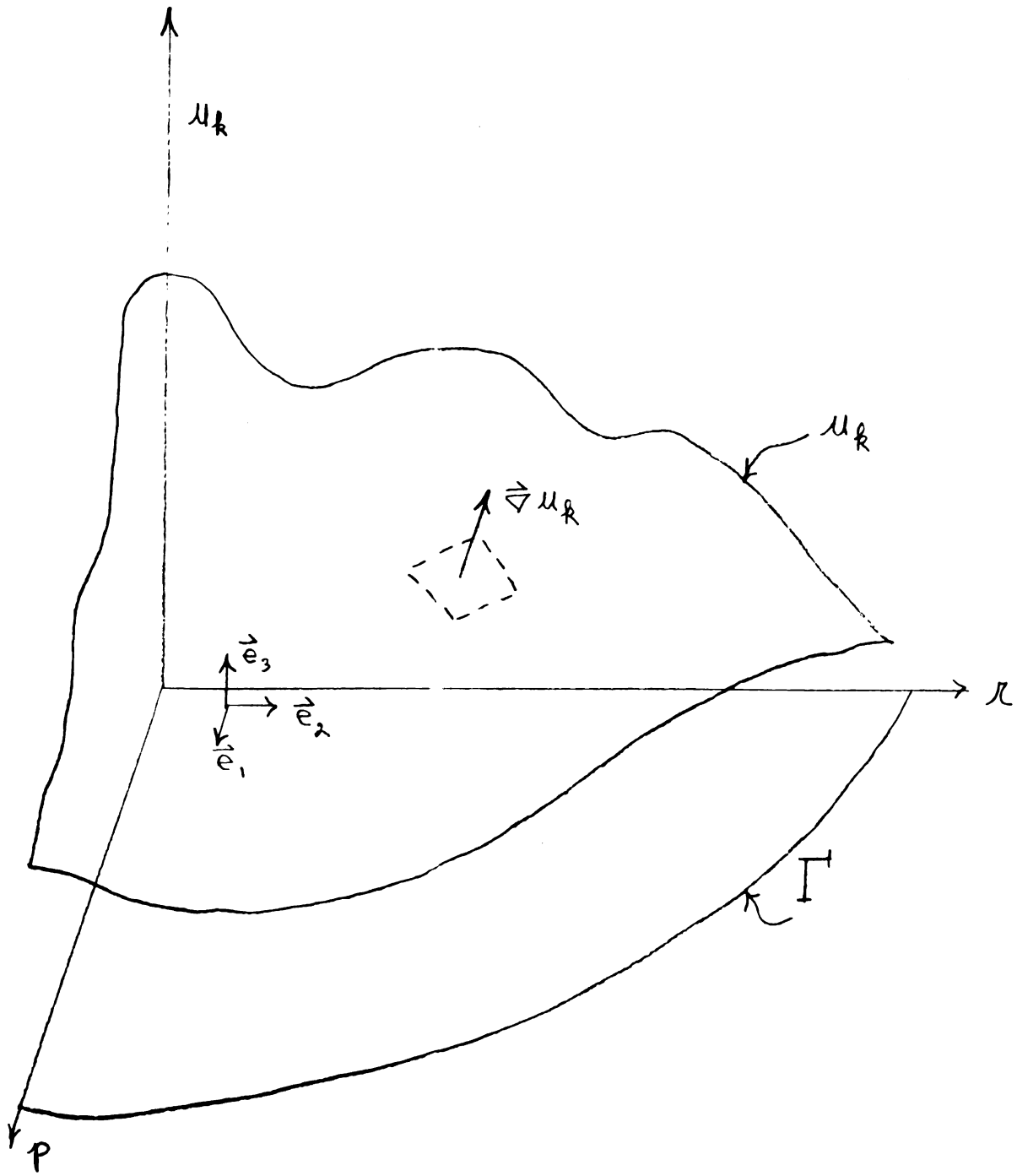
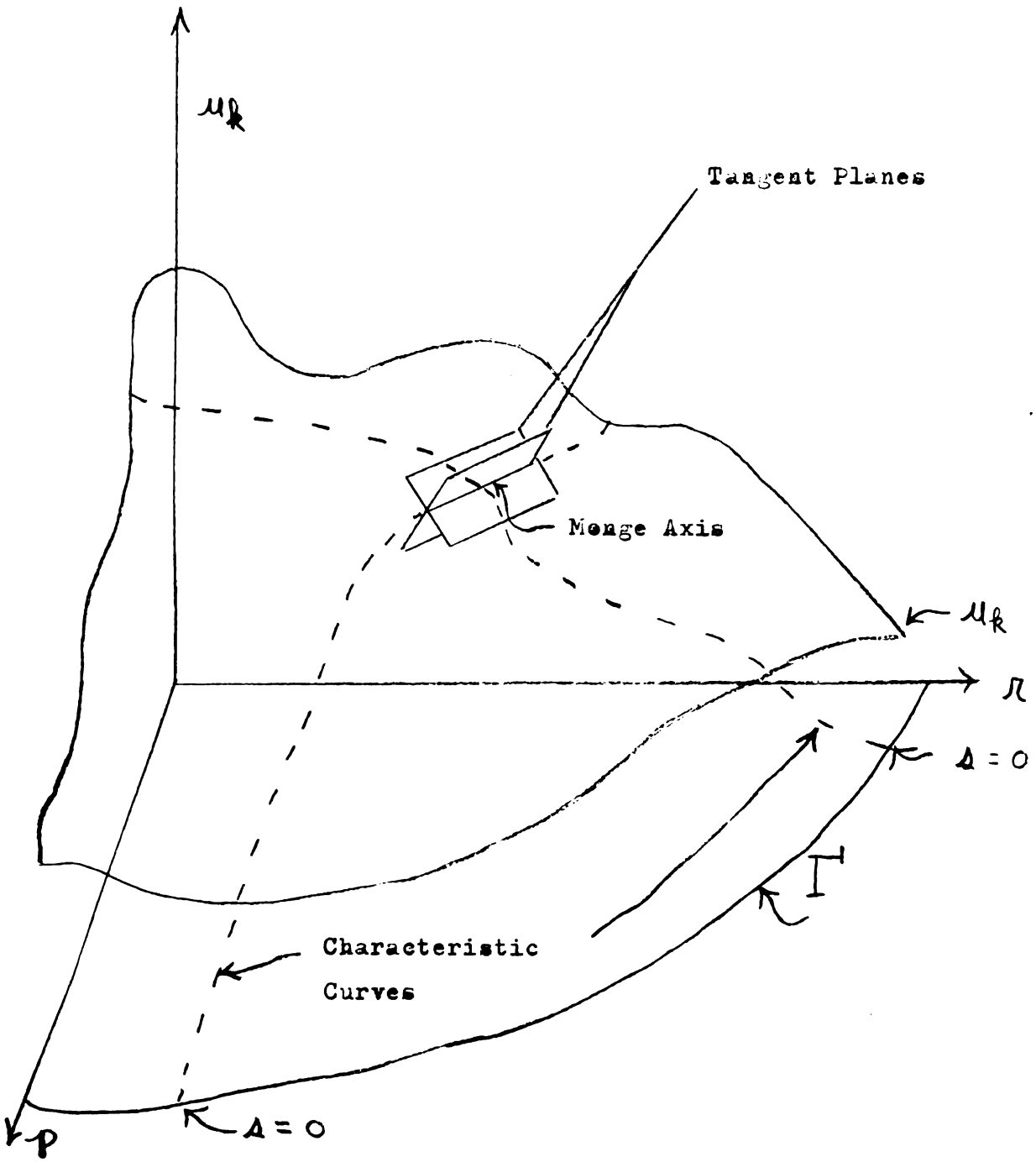
[23] This method is discussed in detail by Courant and Hilbert (1962), Chap. II of vol. 2. We will only outline the proceedure here. Our choice of boundary conditions guarantees that a unique solution to Equation (12) will always exist.

Figure 7. The eigenfunction u_k as a surface in p,r,u_k-space.



Now consider the special case in which a $\varphi(p,r,u_k)$ is a solution to Equation (13). Later, we will specify a set of boundary conditions which determine a unique solution; but, for the moment, we must consider $\varphi(p,r,u_k)$ as a family of surfaces in p,r,u_k-space, each of which satisfies Equation (13) and a given boundary condition. Since it is to be a solution of Equation (13), we have $u_k = \varphi(p,r,u_k)$. We seek a set of ordinary differential equations which generate the family of surfaces $u_k(p,r)$. To find them, we use $\varphi(p,r,u_k) = u_k(p,r)$ in equation (14) and note that the normal to any solution of Equation (13) must have the direction numbers $\partial u_k/\partial p$, $\partial u_k/\partial r$, and 1. Since the third number is the same for all of the solutions, the normals to all of the solutions at any given point will be distributed about a point in a certain plane (Figure 8). This means that the planes tangent at a given point to all of the solutions of Equation (13) will form a pencil of planes; the axis of this pencil is called the Monge axis after the French mathematician Gaspard Monge. The characteristic curves of Equation (13) are defined to be a set of curves in p,r,u_k-space such that each curve is tangent at every point to one of the components of the Monge axis at that point (Figure 9). It is possible to show (Courant and Hilbert (1962)) that there is an equivalence between a set of characteristic curves and a given first order, linear, partial differential equation. Thus, we may take the differential equations of the characteristic curves as our desired ordinary differential equations. If we introduce the parameter s which measures the distance along a given characteristic curve, we see that the differential equations of the charac-

Figure 8. Characteristic curves in p,r,u_k-space.



teristic curves are

$$(15) \qquad \frac{dp}{ds} = R(p,r), \ \frac{dr}{ds} = P(p,r), \ \frac{du_k}{ds} = -i\lambda_k u_k(p,r) .$$

Equations (15) are called the characteristic equations of Equation (13).

We can single out one of the family of sets of characteristic curves which generate solutions of Equation (13) by requiring that the characteristic curves of interest intersect a curve Γ in p,r,u_k-space. We consider Γ to be described parametrically in terms of a parameter η; and, for our purposes, we specify that Γ is not itself a characteristic curve of the partial differential equation. If the solutions of the Equations (15) are given by $p = p(s)$, $r = r(s)$, and $u_k = u_k(s)$, we specify that $s = 0$ along Γ and determine $p = p(s)$ and $r = r(s)$ so that they satisfy the initial value conditions $r = g(\eta)$ and $p = h(\eta)$ when $s = 0$. This gives

$$(16) \qquad p = p(s,\eta), \ r = r(s,\eta), \text{ and } u_k(s) = e^{-i\lambda_k s} .$$

Integration of Equation (13) is then accomplished by eliminating η from the equations (16), solving the resulting system of equations for s, and substituting the value of s into the expression $u_k(p,r) = \exp\left[-i\lambda_k s(p,r)\right]$. $u_k(p,r)$ is a solution of Equation (13) which satisfies the desired boundary conditions along Γ. For boundary conditions, we will always take $s = 0$ at the curve Γ in p,r,u_k-space and require that $u_k = 0$ at Γ, i.e. $u_k(0) = 0$. We choose to

define Γ by the equations

(17) $$p = \cos\eta \ , \ r = \sin\eta$$

in terms of the parameter η. That is, we require that the probability of finding a cell with the concentration of its jth protein or mRNA such that $p_j = 1$ or $r_j = 1$, be zero. This discussion is readily generalized to the case of 2n variables.

E. An Example

To illustrate the theory developed in this Chapter, we will study a simple example. Consider a system, S, described by the kinetic equations

(18)
$$\frac{dp}{dt} = ap + br + \epsilon$$
$$\frac{dr}{dt} = cp + dr + \epsilon$$

where a,b,c, and d are constants and $\epsilon \ll 1$. We could allow ϵ to be any arbitrary constant, but assumming that it is small considerably simplifies the calculations. No real biological system is apt to be describable by such a simple set of equations, but Equations (18) are complex enough to illustrate a general principle which is of biological interest.

p and r have the dimensions of molecules/cell, say; the constants have dimensions of reciprocal time units. Since this sys-

tem is unrealistic, no attempt will be made to assign values to the constants. Goodwin (1963) discusses in detail how this can be done.

The idea of the Example is as follows. The kinetic equations (18) are taken to describe the biochemical system of some cell of imaginary simplicity. The problem is to predict the possible morpholo ical states of the cell on the basis of these equations. Two steps are involved: (1) the determination of the probability density function $D_k(p,r,t)$, and (2) the evaluation of the expectation values $\lambda_k, \bar{p}$, and $\bar{r}$.

1. Determination of D -- The probability density function is given by $D_k = MU_k^* U_k$. U_k satisfies the eigenvalue equation $LU_k = \lambda_k U_k$, or more explicitly

$$(ap + br + \epsilon)\frac{\partial}{\partial p} u_k + (cp + dr + \epsilon)\frac{\partial}{\partial r} u_k = -i\lambda_k u_k.$$

The time dependent part of U_k is $\vartheta_k(t) = e^{-i\lambda_k t}$ as above.

The associated characteristic equations are

$$\frac{dp}{ds} = ap + br + \epsilon \ , \ \frac{dr}{ds} = cp + dr + \epsilon \ , \ \frac{du_k}{dt} = -i\lambda_k u_k.$$

It is easily determined by elementary methods that the solutions to these equations are

$$u_k = e^{-i\lambda_k s}$$

$$(19) \qquad p = \frac{1}{h_2-h_1}\left[h_2C_1e^{(a+h_1c)s} - h_1C_2e^{(a+h_2c)s} + \frac{\epsilon(d-b)}{bc-ad}\right]$$

$$r = \frac{1}{h_2-h_1}\left[C_2e^{(a+h_2c)s} - C_1e^{(a+h_1c)s} + \frac{\epsilon(a-c)}{bc-ad}\right]$$

where h_1 and h_2 are given by

$$h_1 = \frac{1}{2c}\left[(d-a) + \sqrt{(a-d)^2+4bc}\right]$$

$$h_2 = \frac{1}{2c}\left[(d-a) - \sqrt{(a-d)^2+4bc}\right]$$

and C_1 and C_2 are constant of integration.

Since the characteristic equations are linear, the h_1-terms and the h_2-terms are separately solutions. If the problem be worked through using just the h_1-terms, it is found that u_k is irregular at the origin. Thus, we set $C_1 = 0$. C_2 is to be determined so that Equations (19) satisfy the boundary conditions

$$p = \sin\eta \ , \ r = \cos\eta \ \text{ at } s = 0 \ .$$

C_2 is given, in parametric form, by

$$C_2 = \frac{-(h_2-h_1)}{h_1}\left[\sin\eta - \frac{\epsilon(d-b)}{bc-ad}\right]$$

$$c_2 = (h_2 - h_1)\left[\cos\eta - \frac{\epsilon(a-c)}{bc-ad}\right].$$

If we set

$$\alpha = p - \frac{\epsilon(d-b)}{(bc-ad)(h_2-h_1)}$$

$$\beta = r - \frac{\epsilon(a-c)}{(bc-ad)(h_2-h_1)}$$

$$\gamma = \frac{(d-b)}{bc-ad}, \quad \delta = \frac{(a-c)}{bc-ad}, \text{ and } x = e^{-(a+h_2c)s},$$

Equations (19) become $\alpha x + \gamma = \sin\eta$ and $\beta x + \delta = \cos\eta$.

Whence

$$(\alpha^2 + \beta^2)x^2 + (2\alpha\gamma + 2\beta\delta)x + (\gamma^2 + \delta^2 - 1) = 0.$$

If we neglect terms quadratic in ϵ:

$$\alpha^2 = p^2 - \frac{2\epsilon p(d-b)}{bc-ad}, \quad \beta^2 = r^2 - \frac{2\epsilon r(a-c)}{bc-ad}$$

$$\alpha\gamma = \frac{\epsilon p(d-b)}{bc-ad}, \quad \beta\delta = \frac{\epsilon r(a-c)}{bc-ad}, \quad \gamma^2 + \delta^2 - 1 \approx -1.$$

Then,

$$s = \frac{-1}{a + h_2 c} \ln\left\{ \frac{\sqrt{\alpha^2 + \beta^2} - \frac{\epsilon}{2c - d}\left[2(a-b) - r(a-c)\right]}{\alpha^2 + \beta^2} \right\}$$

Thus, u_k is given by $u_k = T^{\frac{i\lambda_k}{a + h_2 c}}$, where the expression in the curly brackets has been set equal to T. The transition probability density function is

$$u_k^* u_k = T^{\frac{i\lambda_k c(h_2^* - h_2)}{(a + h_2 c)(a + h_2^* c)}}.$$

For the kinetic equations (16) we have $\vec{\nabla}\cdot\vec{r}_t = a + d$. Thus,

$$N = \exp\left[- \int (a + d)\, dt \right] = e^{-(a + d)t}.$$

i is determined as a function of ρ and r analogously to the determination of c:

$$t = \frac{-1}{a + h_2 c} \ln T .$$

t may be a complex function of ρ and r for some values of the constants, so we must use $D_k = (N^* N)^{\frac{1}{2}} u_k^* u_k$. We find that $D_k = T^{\Sigma}$ where

$$(20) \qquad E = \frac{(a + d)}{2}\left[2a + c(h_2^* + h_2)\right] + i\lambda_k c(h_2^* - h_2) \Big/ (a + h_2^* c)(a + h_2 c) .$$

Equation (20) is valid for all values of the constant. However, E must be a real quantity if the expectation values of p and r are to be real (see below). Thus, we assume that $(a - d)^2 + 4bc < 0$ and agree to read $\left[\left|(a - d)^2 + 4bc\right|\right]^{1/2}$ for $\left[(a - d)^2 + 4bc\right]^{1/2}$, so that $h_2 = 1/2c \left[(d - a) - i\sqrt{(a - d)^2 + 4bc}\right]$.

Making these assumptions, we find that

$$E = \frac{(a + d)^2 - 2\lambda_k\sqrt{(a - d)^2 + 4bc}}{a^2 + d^2 + 2bc} .$$

Note that if $a = d = 0$ and we take the limit $\epsilon \to 0$, Equations (18) become equivalent to the harmonic oscillator

$$\frac{d^2r}{dt^2} - cbr = 0 .$$

Under these conditions, we have $M = 1$, $T = (p^2 + r^2)^{-1}$, and $E = -2\lambda_k/\sqrt{bc}$. Thus, the corrected probability density function for the harmonic oscillator is

$$D_k(p,r) = (p^2 + r^2)^{\frac{2\lambda_k}{\sqrt{bc}}} .$$

It is readily verified that D_k for the harmonic oscillator has no maxima; it simply increases from $D_k(0,0) = 0$ to $D_k(1,1) = 1$ for all values of the index k.

On the other hand, the original case gives

$$\frac{\partial}{\partial p} D_k = E\ T^{E-1} \frac{\partial T}{\partial p} .$$

$\partial D_k/\partial p = 0$ if (a) $E = 0$, or (b) $\partial T/\partial p = 0$. In the first case we find that $\partial^2 D_k/\partial p^2 = 0$. In the second case, we find that $\partial D_k/\partial p\Big|_{r=0} = 0$ leads to an extremum at

$$p \approx \frac{bc - ad}{d - b} + 2 .$$

It is not difficult to show that if this value of p is within the range of $0 \leq p \leq 1$, then the extremum is a maximum. Thus, $D_k(p,r)$ increases from $D_k(0,0) = 0$ to

$$D_k(1,1) = \left[\frac{\sqrt{2}}{2} - \frac{\epsilon}{2(bc - ad)} (d - b + a - c)\right]^E$$

with a maximum at $p = (bc - ad)/\epsilon (d - b) + 2$, $r = (bc - ad)/\epsilon (a-c) + 2$.

2. Evaluation of expectation values -- To evaluate the various expectation values, we set $p = A \sin\theta$ and $r = A \cos\theta$, where A and θ are variables such that $0 \leq A \leq 1$ and $0 \leq \theta \leq \pi/2$. We neglect the ϵ-terms in α and β as compared to p and r so that $\alpha \approx p$, $\beta \approx r$, and set

$$\phi = \frac{-\epsilon}{bc - ad}\left[(d - b)\sin\theta + (a - c)\cos\theta\right].$$

Then $D_k \approx (1 + \phi)^{E_A - E}$.

(a) We require that $N_k^2 \int_0^1 \int_0^1 D_k \, dp\,dr = 1$ where N_k is a norma-

lization constant. In terms of A and θ, the integral is

$$N_k^2 \int_0^{\pi/2} \int_0^1 (1 + \phi)^{E_A - E_A} \, dA \, d\theta = \frac{N_k^2}{1 - E} \int_0^{\pi/2} (1 + E\phi) \, d\theta$$

where we have expanded the binomial and dropped the terms in ϕ^2, ϕ^3, etc. The integration is easily executed, and

$$N_k^2 = \frac{1 - E}{\frac{\pi}{2} - \frac{\epsilon E(d + a - b - c)}{bc - ad}} .$$

(b) The expectation values of the morphological state of S is given by

$$\bar{L} = N_k^2 \int_0^1 \int_0^1 (M^*M)^{1/2} U_k^* L U_k \, dpdr .$$

We will calculate $\bar{L}$ for the harmonic oscillator. In this case

$$N_k^2 = \frac{4(\lambda_k + \sqrt{bc})}{\pi \sqrt{bc}}$$

and M = 1. We have

$$\bar{L} = N_k^2 \int_0^1 \int_0^1 u_k^* \, i(br \frac{\partial}{\partial p} + cp \frac{\partial}{\partial r}) \, u_k \, dpdr .$$

By differentiating the characteristic equations:

$$\frac{\partial \rho}{\partial \xi} = -i\sqrt{bc}\ \sin\eta\ e^{-i\sqrt{bc}\,\xi} = -i\sqrt{bc}\ \rho$$

$$\frac{\partial r}{\partial \xi} = -i\sqrt{bc}\ \cos\eta\ e^{-i\sqrt{bc}\,\xi} = -i\sqrt{bc}\ r.$$

Thus, $\dot{r} = -i\sqrt{bc}\ \rho$ and $\dot{\rho} = -i\sqrt{bc}\ r$. And

$$\bar{L} = N_k^2 \int_0^1 \int_0^1 u_k^* \sqrt{bc}\ \left(\rho \frac{\partial}{\partial \rho} + r \frac{\partial}{\partial r}\right) (\rho^2 + r^2)^{\frac{\lambda_k}{2\sqrt{bc}}} \rho\, d\rho\, dr$$

$$= N_k^2 \int_0^1 \int_0^1 \lambda_k u_k^* u_k\, \rho\, d\rho\, dr$$

$$= \lambda_k .$$

This result is not entirely satisfactory because it was asserted that morphological cell types are discrete, and up to this point there is no reason to suspect that the λ_k's are discrete. However, they can be made discrete by assuming that $U_k(\rho,r,t + t') = U_k(\rho, r,t)$ for some period t'. This condition implies that $\exp(-i\lambda_k t') = 1$, and $\lambda_k = 0,1,2,\ldots$ Thus, we could set $\lambda_k = k$ and have a discrete set. There is some experimental evidence that protein concentrations oscillate <u>in vivo</u> (see Goodwin (1963,1964a)). A theorem, due to Volterra, which suggests that most of the variables in a complicated system will oscillate about their mean values has

already been mentioned (Chapter II, Section A.2.). But, there is no justification for accepting this as the general rule in integrated biological systems without further evidence.

(c)

$$\bar{p} = N_k^2 \int_0^1 \int_0^1 (M^*M)^{1/2} u_k^* \, p \, u_k \, dp dr$$

$$= N_k^2 \int_0^{\pi/2} \int_0^1 (1+\phi)^E A^{-E} A \sin\theta \, A \, dA \, d\theta$$

$$= \frac{N_k^2}{2-E} \left\{ 1 - \frac{E \epsilon}{bc - ad} \left[\frac{\pi}{4}(d-b) + \frac{(a-c)}{2} \right] \right\}.$$

Similarily

$$\bar{r} = \frac{N_k^2}{2-E} \left\{ -1 + \frac{\epsilon E}{bc - ad} \left[\frac{(d-b)}{2} - \frac{\pi(a-c)}{4} \right] \right\}.$$

In the case of the harmonic oscillator,

$$\bar{p} = \bar{r} = \frac{4(k + \sqrt{bc})}{\pi(2k + 3\sqrt{bc})} .$$

3. Switching states and organization states -- Of special interest are eigenstates in which one or more genes are "shut off" so that $\bar{r}_j = 0$ for one or more values of j. In the case of the harmonic oscillator, we find, by evaluating $\bar{r}$ for $k = 0$ and $k \to \infty$, that $0 < 4/3\pi < \bar{r} < 2/\pi$. That is, none of the morphological states of this system result from the gene locus being "shut off". In the more general system described by the kinetic equations (1c) we

find that $\bar{r} = 0$ if

$$k = \frac{(ad - bc)(a^2 + d^2 + 2bc)}{\epsilon\sqrt{(a - d)^2 + 4bd}\left[\frac{(a - b)}{2} - \frac{\pi(a - c)}{4}\right]} .$$

In that case,

$$\bar{p} = \frac{(d - b)(2 + \pi/4) + (a - c)(1 - \pi/4)}{(d - b)(\pi/4 + 2) + (a - c)(2 - \pi/16)} .$$

$\bar{p} > 0$ if $d > b$ and $a > c$. That is, in this system <u>one</u> morphological state results from the gene being shut off while there are an <u>infinity</u> of different morphological states possible with the gene active.

We will say that two cells are in different <u>switching states</u> if they have different morphological types (i.e. different λ_k's) and have different configurations of inactive genes. We will say that two cells are in different <u>organization states</u> if they have different morphological types and have the same configuration of inactive genes. If the <u>jth</u> gene is inactive, then $\bar{r}_j = 0$. For example, suppose a cell contains three genes. It will then have a potency of being in six switching states and an indefinite number of organization states. One possibility is:

State	Gene Configuration	Morphological State
1	$r_1 = 0 \quad r_2 = 0 \quad r_3 > 0$	1
2	$r_1 = 0 \quad r_2 > 0 \quad r_3 > 0$	2
3	$r_1 > 0 \quad r_2 = 0 \quad r_3 > 0$	3

State	Gene Configuration	Morphological State
4	$r_1 > 0 \quad r_2 > 0 \; r_3 = 0$	4
5	$r_1 = 0 \quad r_2 = 0 \; r_3 = 0$	5
6	$r_1 > 0 \quad r_2 > 0 \; r_3 > 0$	6
7	$r_1 > 0 \quad r_2 > 0 \; r_3 > 0$	7

States 1,2,3,4,5, and 6 are different switching states while states 6 and 7 are the same switching state but different organization states.

F. The Relationship between Morphological and Biochemical Cellular States

The problem of the Thesis is to discover the relationship of the biochemical system of a single cell to the morphological type of the cell. The biochemical state of a cell was defined in two ways: On the one hand, it was defined as a certain eigenvector U_k. In this case, the morphological state of S is the corresponding eigenvalue, λ_k. These definitions suffice to specify the concrescence because, given U_k one can always find λ_k by forming the scalar produdt $\int U_k^* L U_k \, d\tau$. On the other hand, the biochemical state of S was defined as a point in the space $\mathcal{P}$. From the experimental point of view, this definition is dependent on the first definition because the experimentally useful values p and r can be predicted only from a knowledge of U_k. The relationship between the biochemical state of a cell and the morphological state of a cell, thus, may be described as the relationship between an eigenvector and an eigenvalue. That this is, in fact, the nature

of the relationship follows directly from the stability of cell type. This result is valid regardless of what form we assume for $L(p,r)$.

CHAPTER IV. THE NATURE AND LOCUS OF CELLULAR STABILITY

> "The more concrete the biochemical studies of the self-reproduction of living things, the more obvious it becomes that the process is not just bound up with this or that particular substance or a single molecule of it, but is determined by the whole system or organization of the living body ... which is flowing in nature and is in no way to be compared with a stamping machine with an unchanging matrix."
>
> -- A. I. Oparin

The previous Chapter, arguing on purely formal grounds, suggested that morphological cellular states may be considered as eigenstates of the biochemical system of the cell. This Chapter will correlate the initial assertions of Chapter III to some of the biological properties of cellular states and will compare the concept of cellular eigenstates to some of the control mechanisms thought to figure in the manifestation of cellular characteristics. Because the topic of cellular states is a very general one, encompassing aspects of a great majority of living systems, it would be impossible to discuss all the literature pertinent to the assertions of Chapter III. Instead, we shall proceed by using a few examples to delineate the bounds inside of which the assertions are

probably true and outside of which they are probably false.

Part 1: Assertions

A. The Relationship between Morphological and Biochemical Cellular States

The second assertion of Chapter III was that there is an isomorphism between the morphological state of a cell and its biochemical state. Biologically this means that distinct patterns of biochemicals are associated with morphologically distinct cells. In some cases this assertion is unquestionably accurate: erythrocytes are associated with large amounts of hemoglobin, muscle cells with large amounts of myosin, and chondrocytes are associated with large amounts of chondroitin sulfate. In other cellular lineages such as some varieties of leucocytes and the various nervous system constituents both the morphological and biochemical differences between cell types are subtle enough to make the validity of the assertion uncertain. As an example, we shall review some of the literature on the differentiation of rodent cerebral cortex and argue that the cells of both the adult and fetal cortex are associated with distinct enzymic patterns.

1. <u>The histogenesis of the cerebral cortex</u> -- (a) The development of the cerebral hemisphere follows a pattern common to most of the regions of the central nervous system (CNS)[24]. When it is originally formed, the neural tube consists of a layer of pseudostratified epithelial cells known as the neuroepithelium. As the development of the CNS proceeds, a more peripheral mantle layer is formed by the centrifugal migration of neuroblasts and an

[24] Reviews: P. Weiss (1955), R. L. Watterson (1965).

outermost acellular layer called the marginal layer becomes apparent. Neuroblasts may move out from the neuroepithelium in a series of distinct migrations so that an embryonic CNS may, at some stages, show a number of strata known as transition zones. As cells move peripherally they either differentiate into recognizable neurons in one of the transition zones or differentiate after they reach the marginal zone.

(b) The development of the cerebral cortex in the albino rat has been described by Sugita (1917) who distinguished five layers in the adult cortex. To facilitate comparison with the human cortex these layers are numbered in analogy with Brodmann's description of the adult human cortex. Layer I, the most peripheral, is the lamina zonalis and contains only scattered glial cells. The second Brodmann layer, the lamina granularis externa, is not distinguishable in the rat. Layer III is the lamina pyramidalis which contains deep-staining, closely spaced pyramidal cells. Layer IV is the lamina granularis interna consisting of crowded and deep-staining granules which resemble glial cells. The lamina ganglionaris, made up of large and loosely packed pyramidal cells, is Layer V. The innermost layer VI is the lamina multiformis which contains polymorphous cells.

By weaning (which occurs on the 21st day after birth) the cortex has taken on adult characteristics. Although the cortex of the neonate rat is roughly like that of the adult, two important differences can be observed. First, the adult layers are not as easily distinguished, the lamina pyramidalis appearing to have been invaded by cells from the lamina zonalis and the lamina granularis

internal being indemonstrable. The cells of the [illegible] layer have an immature spindle shape. Secondly, two transition layers are to be seen between the [illegible] multiforme and the ventricular wall. The cells of these layers are presumably migrating to form the cortex; the layers disappear during the 3rd and 4th postnatal days.

Supita recognised three phases in the transition between the neonate and the adult cortex. The first phase lasts from birth until the 10th day and is characterized by the proliferation of neuroblasts and their centrifugal migration. During the first phase the rate of increase in the thickness of the cortex is six times as great as during the third phase. The second phase lasts from the 11th to the 20 day and is characterized by the enlargement and differentiation of cortical cells. The cortex receives few new cells during the second phase and its rate of increase of thickness is only 7 times as great as that of the third phase. The third phase lasts from the 21st to the 50th day and is characterized by the myelination of neuronal processes. Supita (1961) has shown that the same pattern of development occurs in the guinea pig; the end of the first phase, however, occurs at the 41st day of gestation[25].

Supita's data is supported by data from other workers. Peters and Flexner (1950) studied the morphogenesis of the frontal cortex of the fetal guinea pig and verified that the differentiation of this region occurs rapidly during the period between 41 and 49 days,

[25] The gestation period of the guinea pig is 65 days. The body of evidence would suggest that the rat at the 10th postnatal day is well developed as the guinea pig is at its 41st day of gestation.

during the "critical period", the cortex increases in thickness and the cortical layers characteristic of the first two-thirds of metathesis change into the five layers found in the adult cortex. Although the total volume of the cells continues to increase after 45 days, the volume of the cells continues to increase after 45 days, the volume of the cell nuclei increases rapidly at 30 days and levels off to their adult value at about 40 days. Between 41 and 45 days, the Nissl substance appears and the number and sizes of neuronal processes increases. Using the Feulgen technique, La Velle (1951) has correlated the transformation of the nucleoli from a densely staining type typical of early developmental stages to a vacuolated type typical of the adult guinea pig.

(c) In addition to anatomical changes, the electrolyte and water balance of the cellular and the extracellular phases is changing during the critical period. Flexner and Flexner (1936) found that the intracellular water content decreases from 35 to 45 days and then increases to a constant value at 45 days while the extracellular water is increasing and then decreasing to a constant value during this period. Also, before 45 days, the concentration of sodium and chloride ions in the extracellular phase is equal; at 45 days, the extracellular sodium concentration increases markedly (Flexner and Flexner, 1949). Flexner interprets this change to the establishment of a selective permeability which is to be related to the onset of electrical activity. However, it is not clear that the data actually support this interpretation (cf. the discussion in Flexner (1950)).

Jasper et al. (1937) found that the first electrical recon-

tials appeared in the brain of the fetal guinea pig between 48 and 56 days. Flexner, Tyler, and Gallant (1950), however, were able to record cortical potentials on the 46th day but not on the 45th day. Crain (1952) using neonate rats obtained spontaneous but irregular and intermittent electrical activity during the first week; during this period he could also elicit strychnine potentials. By the tenth day, the regularity and rhythmicity of the potentials was adult in character. Kimel and Kavaler (1951) were first able to induce muscular responses to electrical stimulation of the motor areas of the guinea pig cortex between 42 and 46 days. Kavaler and Kimel (1952) found that the level of acetylcholinesterase activity begins before this at about 35 days.

(d) In both the rat and the guinea pig, the cerebral cortex seems to acquire its adult characteristics during the second phase of development. The histological arrangement of the cortex has been attained by this time; during the first phase cells are moving into their final positions and during the third phase they are finishing their differentiation into adult neurons. Neuroblasts differentiate during the second phase into immature neurons: they develop an adult configuration of axons and dendrites, they obtain Nissl substance, and they undergo nuclear and nucleolar changes which give them adult characteristics. The electrolyte difference between the cell and its environment may be established at this time, and the newly formed neurons seem to begin functioning during the second phase. In terms of cellular eigenstates, a neuroblast during the first phase would be in a given eigenstate of the morphological operator, call it the kth eigenstate. That is, if it

were possible to actually measure the value of the morphological function L(p,r) for such a neuroblast, the expectation value for a series of measurements would be λ_k. During the second phase, the neuroblast undergoes a transition to a different eigenstate, k', so that measurements of L(p,r) would yield $\lambda_{k'}$, as the expectation value. In general, we would expect that the expectation values of each of the cellular proteins would be different in the neuroblast and in the neuron; if there is an isomorphism between morphological and biochemical states, the neuroblasts of the first phase should have an enzyme pattern different from the enzyme pattern of the neurons of the second phase.

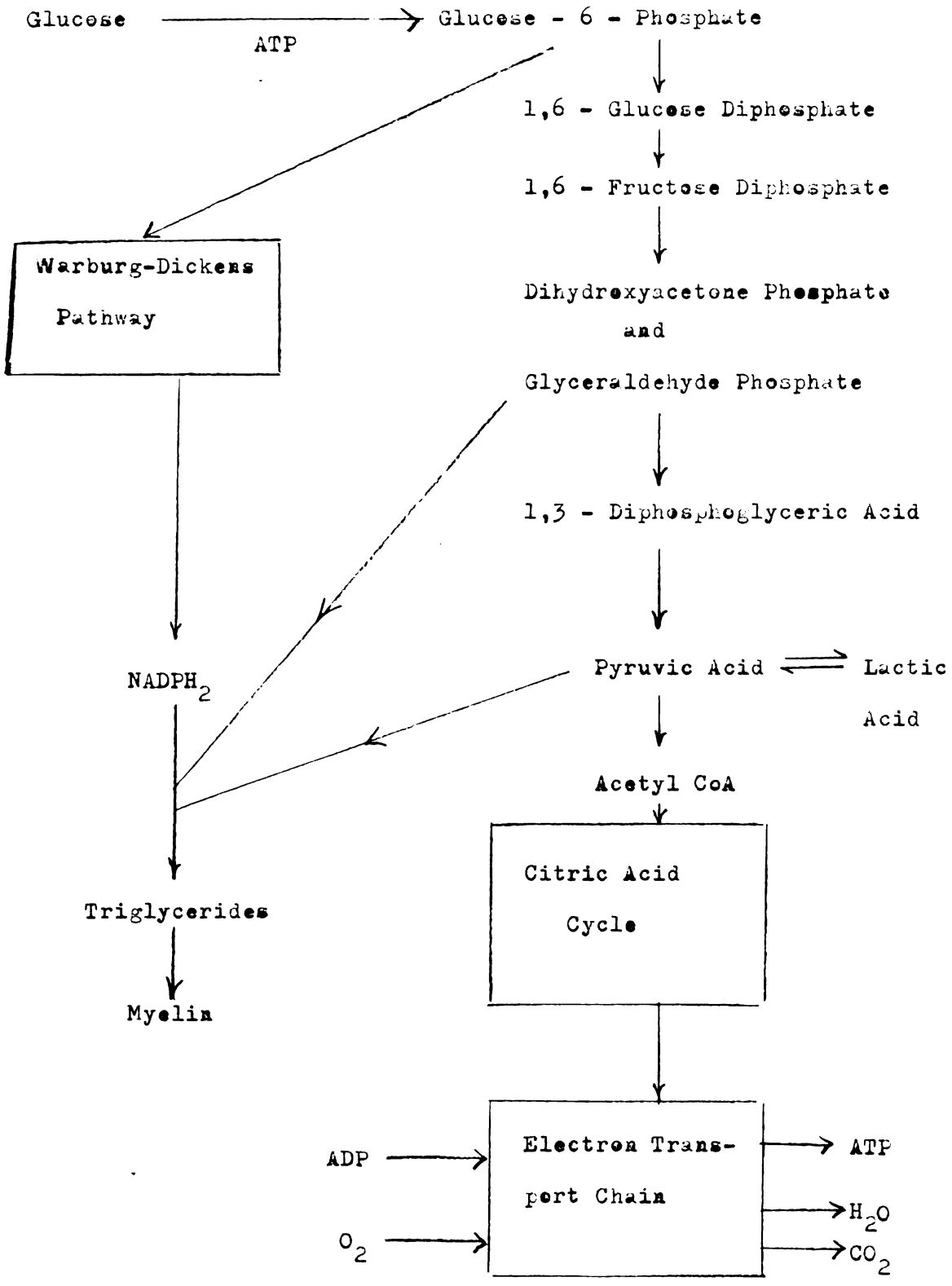
2. Chemical ontogeny of the cerebral cortex -- Louis and Josefa Flexner have approached the relationship between biochemical and morphological states by studying the enzymes in the frontal cortex of the rat, pig, and guinea pig during the first and the second phases (Reviews: Sperry (1962), Flexner (1952,1955a, 1955b,1950)). When the information obtained by the Flexners is combined with the data of other workers, a fairly detailed picture of the biochemical differentiation of the cortex can be obtained. To provide a perspective for a review of these studies it will be helpful to outline the general nature of brain metabolism.

(a) (Reviews: Richter (1955), Balazs and Richter (1961)). Brain is a unique tissue in that it depends almost entirely for its nutrition on the metabolism of glucose, a peculiarity imposed by the "blood-brain" barrier which restricts the entrance of almost everything except glucose into the brain from the blood. In the brain, part of the glucose is utilized for the synthesis of amino

acids (e.g. Roberts et al. (1959)) and other compounds, part is degraded via the Warburg-Dickens pathway to provide $NADPH_2$ for the metabolism of fatty acids and a source of five carbon sugars, but most of the glucose is used in the Embden-Myerhoff scheme which degrades glucose to pyruvic acid. This compound has two principal fates: in the presence of oxygen, it is used in the citric acid cycle and the electron transport system of mitochondria to provide 38 moles of ATP per mole of glucose (aerobic glycolysis). In the absence of oxygen, it is converted to lactic acid and two moles of ATP (anerobic glycolysis). The relationship between these metabolic pathways is diagrammed in Figure 9.

Adult and fetal brain tissue differ in their modes of carbohydrate metabolism. Fetal brain tissue almost exclusively uses pyruvic acid to form lactic acid. Since anerobic glycolysis does not require molecular oxygen, fetal brain is remarkably insensitive to anoxia (Fazeka et al. (1941)). In adult brain, however, anerobic glycolysis prevails. Most tissues retain the compounds needed for anerobic glycolysis so that they can function, albeit less efficiently, under anerobic conditions. Adult brain tissue is peculiar in having seemingly lost the capacity to metabolize glucose anerobically and is notoriously sensitive to anoxia. If there is a biochemical-morphological isomorphism, it should be possible to show that the components of the citric acid cycle and the electron transport chain become active during the second phase of development when the cortical neurons are differentiating. In addition, some mechanism responsible for the adult sensitivity to anoxia should become operative.

Figure 9. Schematic diagram of the interactions between metabolic pathways important in cerebral ontogenesis.



(b) The data on the biochemistry of differentiation will be discussed under four headings: (1) metabolism of phosphocompounds, (2) Embden-Myerhoff enzymes, (3) citric acid cycle and electron transport components, and (4) lactic dehydrogenases.

(1) Flexner and Flexner (1950b) found that the total phosphorous content per kilogram of the cortical cellular phase fluctuates about a mean during the last half of gestation. The individual phosphorous containing fractions vary, however: between the 40th and the 45th days, the concentration of phospholipids increases, presumably in conjunction with the synthesis of myelin. Previous to this period, the content of pentose nucleic acids had been increasing, but during this period it decreases so that the total phosphorous content is approximately constant (Flexner and Flexner (1951)). Flexner and Flexner (1948) found that the concentrations of adenylpyrophosphatase increases sharply at 42 days. These changes suggest an increase in protein synthesis during the critical period (Flexner et al. (1951)).

(2) The degradation of glucose begins by two phosphorylations and an isomerization to produce diphosphofructose which is split into one molecule of dihydroxyacetone phosphate and one molecule of glyceraldehyde phosphate by aldolase. Flexner et al. (1956) report that the activity of aldolase is approximately constant in the cortex of the guinea pig until about the 33rd day when it begins to increase slightly up to the 35th day; then it increases to its adult value at term.

Dihydroxyacetone phosphate is readily isomerized to glyceraldehyde phosphate so that the degradation of one molecule of di-

phospho fructose results in the formation of two molecules of the aldehyde. Glyceraldehyde phosphate has two metabolic fates of interest: (a) it may be reduced by glycerol phosphate dehydrogenase to glycerol phosphat which combines with fatty acids to form the lipids needed for myelination, and (b) it may be oxidized by glyceraldehyde phosphate dehydrogenase to phosphoglyceric acid which is metabolized to pyruvic acid. Laetsch (1962) found that the rat forebrain showed a 20% increase in glyceraldehyde phosphate dehydrogenase activity between the 4th and 21st days post partum and a fivefold increase in glycerol phosphate dehydrogenase activity to reach the adult level between the 21st and 33rd days.

(3) Succinic dehydrogenase is one of the enzymes of the citric acid cycle. Flexner and Flexner (1946) have shown that in the cortex of the fetal pig the activity of succinic dehydrogenase is constant at 35% of the adult value until between the 68th and the 75th days when it begins to increase, reaching the adult value by birth. In the guinea pig, the activity of succinic dehydrogenase begins to increase from a low level between the 40th and 45th days and reaches the adult level ten days before term (Flexner et al. (1953)). There is some evidence that attainment of the adult level of succinic dehydrogenase is a reversible process, for the transection of the anterior nerve roots in adult monkeys led to a 12% reduction in activity of that enzyme within 31 to 54 days (Howe and Flexner (1947)). In rats, thyroidectomy leads to a decrease in the succinic dehydrogenase activity with little effect on several enzymes. This too is a reversible process: a normal level of activity can be restored if hormone therapy is initiated

before the 10th day, but no restitution of activity can be achieved if treatment is delayed until the 15th day (Hamburgh and Flexner (1957)).

Malic dehydrogenase, another citric acid cycle enzyme, retains a constant level of activity in whole brain homogenates of rats until the 7th postnatal day when it begins to increase fourfold to the adult level. Between the 10th day and maturity, malic dehydrogenase activity increases in the outer layers of the cerebral cortex and decreases in the inner layers (Kuhlman and Lowry (1956)).

Flexner et al. (1941) have studied the development of some of the components of the electron transport system in the parietal cortex of the fetal pig and identified two critical periods: at about 60 days, the concentration of cytochrome c increases slightly from a low level to a new level. At about 100 days, the concentration of cytochrome oxidase increases markedly, so that the development of the cytochrome-cytochrome oxidase system is biphasic. During the first critical period the neuroblasts take on the size and form of neurons, the Nissl substance appears and the initial fissuration of the neocortex occurs; during the second critical period, differentiation is completed and there occurs a great proliferation of the cell processes and the neuropil. However, in the guinea pig the cytochrome system develops during the morphological critical period, the activity of cytochrome oxidase increasing fivefold to the adult value at about the 43rd day.

(4) Lactic dehydrogenase catalyzes the interconversion of pyruvate and lactate requiring NAD^+ and $NADH_2$ as a coenzyme. Lactic dehydrogenase is actually a system of five "isozymes", each of

which has basically the same catalytic properties but differs from the others in physical and chemical details. Each isozyme is a tetramer made up of two kinds of genetically determined subunits called M and H. The pattern of isozymes varies with type of tissue and species; in a given tissue, the pattern may change during development.

Flexner et al. (1960,1962) have identified four of the five isozymes in the lactic dehydrogenase of mouse and guinea pig cortex. They could be distinguished by the use of cellulose ion exchange and starch gel electrophoresis as well as by their different rates of reduction of NAD^+. In the mouse, the lactic dehydrogenase level is constant until the 10th day when it begins to increase fivefold to reach its adult level. In the neonate cortex two isozymes could be identified, but in the adult all four components could be found. Bonavita, Ponte, and Amore (1964) found that there is a progressive dominance of the H subunit during development in the neonate rat. Plageman et al. (1960) have shown that the H subunit is inhibited by an excess of pyruvate while the M subunit is not. In contrast to the cortex, the retina is highly resistant to anoxia (Noell (1958)) and has a predominance of the M subunit (Bonavita, Ponte, and Amore (1963)).

(c) The biochemical differentiation of the cortex proceeds concomitantly with morphogenesis. The enzymes of the Embden-Myerhoff patheway are present during all three phases, as is lactic dehydrogenase. The citric acid cycle and electron transport components studied, however, increase during the second phase in step with differentiation of adult neurons. The concentration of lactic

dehydrogenase also increases during the second phase, but there is a relative increase of the H subunit. Thus, as development proceeds the lactic dehydrogenase system will be less and less able to sustain the brain when anoxia and a build up of pyruvate occurs. The cytological differentiation of cortical neuroblasts, then, accompanies their acquisition of the equipment needed for anerobic glycolysis and the development of the adult sensitivity to anoxia.

3. Other examples -- A correspondence between biochemical and morphological development is exhibited by a number of other systems. For example, in the development of epidermal structures in the chick embryo (Review: Bell (1966)) the determination of the prospective epidermis seems correlated with the acquisition of three immunologically detectable proteins, and the final appearance of the adult x-ray diffraction pattern of feather β-keratin is concommitant with the appearance of a protein with high cystine content. In the fetal lung, the form of the bronchial tree is laid down by a group of rapidly dividing and relatively undifferentiated cells. Histochemical data (Review: Sorokin (1966)) suggest that this process is accompanied by glycogen storage and reliance upon anerobic glycolysis for metabolic needs. The subsequent period of differentiation and low mitotic rate is accompanied by an increase of citric acid cycle enzymes, glucose-6-phosphate dehydrogenase, and the components of the cytochrome system. In both heart and skeletal muscle, contractions first appear in the embryo shortly after the appearance of the second of the two contractile proteins (Ogawa (1958a, 1958b, 1961, 1962), Ebert (1952, 1953, 1955, 1956)).

Greenstein (1954) has argued that a similar biochemical-morphological isomorphism is important in the anaplastic transformations diagnostic of cancerous growth. He attempts to summarize the changes in chemical patterns that accompany the morphological transition from restrained growth and relative cellular specialization to unrestrained growth, loss of the tendency towards segregation by cellular type, and less of cellular differentiation in five general conclusions[26]: (a) Each normal tissue is characterized by the posession of an individual pattern of enzymatic activity which may serve to distinguish it from all other tissues., (b) No enzyme peculiar to tumors has ever been found so that tumors must be assumed to have enzymes qualitatively the same as those of normal cells., (c) The enzymes pattern of a tumor appears to be constant even though the environment of the tumor may change. The enzyme pattern of a tumor will not change if the tumor be explanted to a culture medium or transplanted to a host of different genetic composition or just allowed to grow in a constant environment., (d) The range of enzyme activity or concentrations of small molecules in cancerous cells is narrow relative to the great variations shown by normal cells. Cancerous cells, thus, tend to approximate a uniform cell type distinct from normal cells., (e) Specific functional activities tend to become reduced or entirely lost in tumor cells. Liver cells, for instance, are characterized by high arginase, catalase, and cystine desulfurase activities; in hepatomas, all of these activities are reduced or absent.

[26] The literature of cancer biochemistry is extremely voluminous, and the present author is not competent to judge Greenstein's thesis. His peers seem, generally, to accept the interpretation; e.g. Reid (1965) and LeBreton and Moulé (1961).

4. Qualifications -- The embryogenesis of the cerebral cortex and the examples of developing and metaplastic systems cited above support the assertion of a biochemical-morphological isomorphism. However, at least three kinds of considerations suggest that it is not an accurate generalization.

(a) The cellular slime mold Dictyostelium discoideum demonstrates two morphological forms. During favorable conditions, the organism exists as unicellular myxoamoebae. But when starvation conditions obtain, the myxoamoebae aggregate and differentiate into an organism consisting of a stalk and a sorocarp or spore case. An important aspect of this differentiation is the formation of the glycogen and cellulose cell wall constituents from endogenous protein. Wright (1963,1966) has studied the metabolism of glutamic acid in Dictyostelium. This compound is metabolized via its oxidation by glutamic dehydrogenase to α-ketoglutaric acid, a citric acid cycle intermediate, to CO_2. This sequence, along with others, provides energy for the synthesis of cell wall carbohydrates. The concentration of glutamic dehydrogenase is approximately constant throughout differentiation. However, the rate of in vivo evolution of CO_2, measured by C^{14} tracer studies, increases sevenfold during this period.

This paradox is resolvable by taking into account the concentration of glutamic acid. The Michaelis-Menten equation is

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

where v is the velocity of an enzyme reaction — in this case, the glutamic dehydrogenase reaction — K_m is a constant, V_{max} is the theoretical maximal velocity of the reaction, and $[S]$ is the concentration of substrate — glutamic acid, here. V_{max} is given by $V_{max} = k_3 [E]$; k_3 a rate constant and $[E]$ the enzyme concentration. This equation can be rewritten as

$$v = \frac{k_3 [E]}{K_m / [S] + 1} .$$

The reaction velocity can be increased in two ways. First, the concentration of E can be increased. But, Wright's data show that $[E]$ is constant throughout differentiation. Secondly, the substrate concentration could increase, making v larger. Wright has measured the ratio $K_m / [S]$ and found that it decreases during differentiation at the right rate to account for the increase of the rate of CO_2 evolution. Thus, in this instance, the substrate concentration is limiting and the concentration of an enzyme, alone, was not an adequate index of biochemical differentiation.

(b) Rudnick and Waelsch (1955) have studied the ontogeny of glutamotransferase, an enzyme which catalyzes the formation of glutamohydroxamic acid from glutamine and hydroxylamine. In the chick embryo nervous system, the level of glutamotransferase activity increases slowly from the 9th day of incubation to a peak eight weeks after hatching. Except for minor variations, the level of activity in the optic lobes, the cerebellum, the diencephalon, the medulla, the cerebral hemispheres, and the spinal cord follow the same trend. In the retina, however, the level of glu-

tamotransferase activity is low and shows no rise until the 17th day. On that day it increases twentyfold. On the 15th day, the retina begins a critical period which results in the final differentiation of the rods and cones (Coulombre (1955)). Between the 18th and the 20th days, the level of acetylcholine increases sharply and the retina becomes functional as judged by the appearance of the pupillary constrictor reflex. But, it is not clear what role glutamotransferase could play in these anatomical and physiological changes.

(c) R. L. Friede (1959a) has studied the distribution of succinic dehydrogenase in frozen slices of rat brain by histochemical means throughout postnatal development. He reports a general increase of succinic dehydrogenase activity (SDA) in the cerebral cortex from birth to weaning. There is a general caudal-cranial gradient in the time at which SDA first develops. In general, enzyme activity develops in cell bodies before it does in the neuropil. Distinct lamina of SDA develop in the layers of some cortical areas, and some areas show signs of temporary hyperactivity (nucleus coeruleus) which might reflect special events such as melanin deposition. In other papers (1959b, 1959c, 1960), Friede has extended his techniques to the adult guinea pig and constructed an atlas depicting regions of high and low SDA throughout the medulla, the midbrain, and the cortex. He finds that the distribution of SDA closely follows the general morphology of the brain. Thus, he (1961) was able to distinguish on the basis of SDA several histologically defined nuclei of the reticular system. His data also suggest that functionally related systems exhibit similar

values of SDA. For instance, he was able to associate gradations in various thalamic nuclei with parallel gradations in SDA in their cortical projections. In a general way he suggests a relation between cellular function and intracellular SDA distribution: cells in regions of integrative or receptive function show high enzyme activity in the neuropil (e.g. olfactory bulb) while cells in regions of effector or relay function show high SDA in the perikarya (e.g. nucleus of the mesencephalic root of the trigeminal nerve).

Thus, in some instances, it may not be possible to assume an isomorphism between biochemical and morphological states in the way we have done in Chapter III. It may be necessary to explicitly take into account more biochemical parameters than we have done or might care to do. It may turn out for some systems that there simply is no correspondence. Or, it may turn out that the correspondence is not apparent when looking at a single cell because it involves gradients of characteristics spread over many cells or it involves characteristics localized within the cell. Nevertheless, if these and similar difficulties are kept in mind it seems legitimate to accept the biochemical-morphological isomorphism as a first approximation which is applicable to at least some interesting cases.

B. Cellular Stability and Discreteness

The first assertion of Chapter III was that cellular morphological states are discrete and are stable. A cell was said to be stable with respect to a given variable if the variance of measurements of that variable is zero. The quote from C. H. Waddington in

Chapter II (Page 27) is a statement of this assertion. Like the second assertion, it is valid only within certain limits. We will try to establish these limits by means of an example.

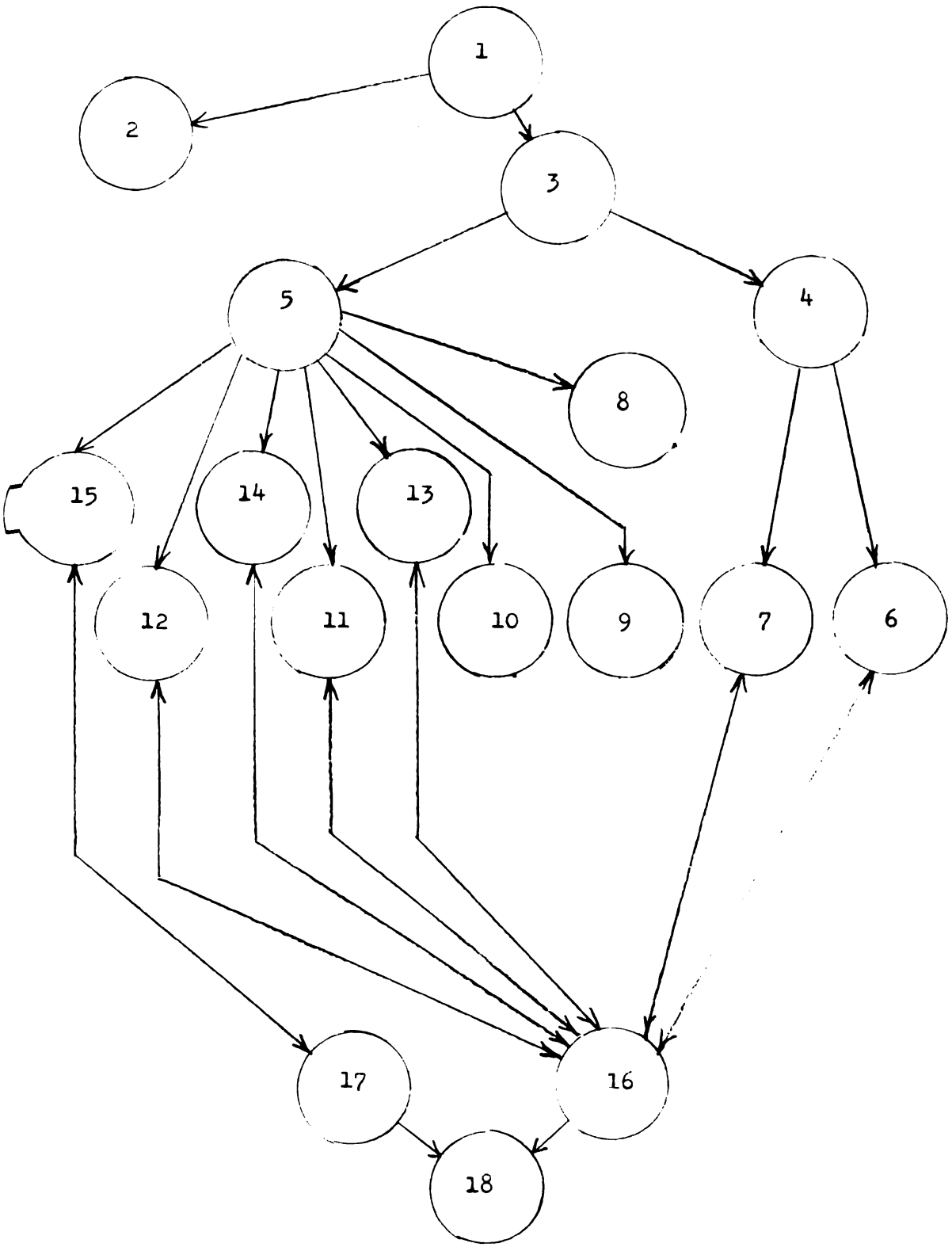
1. The embryology of the neural crest -- (a) As the neural tube of a vertebrate embryo forms, a certain amount of neural plate material is excluded from the tube itself and comes to rest on top of the developing CNS. This group of cells is known as the neural crest. The pertinent literature has been reviewed by Horstadius (1950); a recent corroboration of the classical work by radioactive tracers is by Weston (1963). References to the primary literature will be found in these sources. The histological descriptions used here are from Bloom and Fawcett (1962).

The propensity of a given type of cell to become transformed into another type of cell is described by its transition vector V_{mk} (see Chapter III Section B.8.), where the original cell starts in the mth morphological state and changes to the kth morphological state. If $V_{mk} = 0$, the transition $m \rightarrow k$ is not possible; if $V_{mk} = 1$, the transition $m \rightarrow k$ will definitely occur. If cells are stable with respect to morphological type m, V_{mk} should equal zero or be very small for most values of k. To gain some idea of the nature of cellular stability, we will outline the potencies of a single neural crest cell. Figure 10 represents the possible morphological transformations of a neural crest cell. Each circle represents a morphological state; (k) is the state with eigenvalue λ_k. Of course, these assignments of eigenvalues are entirely arbitrary. Arrows between circles represent possible transformations; if there is no arrow between two states, a transformation is not possible

Figure 10. (Description of Figure)

State	Description
1.	Undifferentiated ectoderm
2.	Neural plate (presumptive CNS)
3.	General neural crest
4.	Head crest
5.	Trunk crest
6.	Gill arch cartilage
7.	Cartilage of anterior trabeculae
8.	Dorsal spinal ganglia
9.	Sympathetic nervous system
10.	Schwann cells
11.	Meninges
12.	Dentin of teeth
13.	Corium of skin
14.	Connective tissue of dorsal fin
15.	Pigment cells (melanocytes)
16.	Mechanocytes
17.	Amoebocytes
18.	Generalized dedifferentiated cell

Figure 10. Transition diagram for prospective fates of neural crest line cells. Numbers represent cellular morphological states. Arrows represent possible transitions.



(i.e. it has never been observed).

(b) The possible transformations are: $V_{1,2}$ and $V_{1,3}$: The neural crest cells form on the lateral margins of the neural plate from the ectoderm covering of the embryo. Like the neural plate cells proper, the neural crest cells form only when they have been in contact with the roof of the archenteron which lies below the neural plate in the embryo. The medial portions of the archenteron roof induce central nervous system while the lateral portions induce neural crest.

$V_{3,4}$: The anterior neural crest has the potency to form cartilage. The fate of these cells is determined quite suddenly at the end of the yolk plug stage. Before this stage they are fairly totipotent. Even after determination, however, anterior neural crest material can form cartilage only when it has been in contact with another tissue, such as pharynx. Cartilage cells are spheroidal in shape and characteristically produce a matrix of glycoproteins containing chondroitin sulfate.

$V_{4,6}$: The posterior part of the anterior neural crest forms the cartilage of the gill arches, the hyoid arch, and Meckel's cartilage (lower portion of the lower jaw). It has the capability of forming trabeculae when transplanted rostrally.

$V_{4,7}$: The anterior part of the anterior neural crest forms the cartilaginous trabeculae of the chondrocranium which, after ossification, form the nasal portion of the skull. The major portion of the skull is of endomesodermal origin. This part of the anterior neural crest does not have the ability to form visceral arch cartilage.

$V_{3,5}$: The posterior portion of the neural crest material is totipotent until the late yolk plug stage when it attains the ability to autonomously differentiate into a number of cell types and looses the ability to form cartilage.

$V_{5,8}$: Neural crest cells migrate down between the myotomes to form the dorsal ganglia of the spinal nerves. A given ganglion is formed in part by cells from the ipsolateral side and in part by cells which cross over from the contralateral side. Although the ganglia differentiate autonomously, the regularity of their spacing is dependent on the spacing of the myotomes. The neurons of the dorsal ganglia are typically T-shaped with a process which extends from the cell body and divides into an axon and a dendrite. They are myelinated and surrounded by small, flat, satellite cells.

$V_{5,9}$: The neural crest cells contribute to the formation of the sympathetic ganglia. Material from the ventral part of the spinal cord also contributes. The neurons of the sympathetic ganglia are usually small, polymorphous multipolar cells.

$V_{5,10}$: Schwann cells are considered as resulting, at least in part, from neural crest cells. Schwann cells are long, flat cells which spiral about neurons in a large number of laminae and constitute the myelin sheath of the neuron. Myelin is a complex of lipids and mucopolysaccharides.

$V_{5,11}$: The majority of the pia and the arachnoid matters are derived from neural crest cells. The dura is of endomesodermal origin. The arachnoid and the pia are composed of intertwining collagenous bundles interspersed with fibroblasts, macrophages, mast cells, and other connective tissue elements. The surfaces of

both meninges are covered with a layer of squamous mesenchymal epithelium.

$V_{5,12}$: The dentin of the teeth is derived from neural crest cells. Dentin is a yellowish semitransparent substance, similar in structure to bone, which contains a network of tubules. Dentin contains both inorganic materials and a glycoprotein.

$V_{5,13}$: The innermost layer of the skin, the corium, is derived from neural crest material. This layer is a webwork of collagenous and elastic fibers.

$V_{5,14}$: In fishes, the connective tissue of the dorsal fin is of neural crest origin.

$V_{5,15}$: The melanocytes or pigment cells are derivative from the neural crest. These are long cells with irregular outgrowths; they can elaborate melanin.

(c) The cell types 6 through 15 are found in adult vertebrates, and, in situ, they tend to maintain their identity as long as they are alive eventhough they may be constantly changing their shape or the character of their metabolism. It is this preservation of identity that we refer to as cellular stability. Cells of the neural crest lineage demonstrate some remarkable diversities. Gill arch chondrocytes and dorsal ganglionic neurons represent extremely different morphological types. At the same time, there is a considerable overlap between some states: the meninges and the corium are basically similar in histology and both contain pigment cells.

(d) When a cell in one of the states 6 through 15 is removed from its normal environment, as in tissue or cell culture, it can

undergo further transitions. When animal cells are cultured in lumps of tissue or in a mass of dissociated cells they undergo transformations known as dedifferentiation. Willmer (1960) has classified dedifferentiated cells into three main groups in terms of their morphological characteristics, their locomotory behavior, and their biochemical and physiological properties. Epitheliocytes characteristically grow in vitro as an intact sheet of cells. They tend to grow on the surfaces of culture flasks and move by a gliding movement of the sheet followed by extensions of the peripheral cells. They may produce carcinomas. They store mucosubstances in the cytoplasm and may form keratin. Mechanocytes grow as a reticulum or network of cells which ataches itself to the coverslip only at its extremities. The individual cells move with a definite polarity. They may form sarcomas. They exude mucosubstances and may form collagen. Amoebocytes grow as isolated cells which move in an amoeboid fashion without a definite polarity. They may produce leucemias. They actively pinocytose and collect mucosubstances in their cytoplasm. More detailed descriptions of these classes of cells can be found in Willmer's book. These three classes seem to account for most dedifferentiated cells of all of the vertebrates and at least some of the invertebrates.

Cells derived from neural crest material show three types of transitions in tissue culture:

$V_{8,k}$,$V_{9,k}$,$V_{10,k}$: Spinal ganglion and sympathetic ganglion cells retain their basic characteristics in vitro. That is, they do not dedifferentiate. They may temporarily loose their Nissl substance or develop axonal swellings. Schwann cells seem to retain their

characteristics, also.

$V_{6,16}$,$V_{7,16}$,$V_{11,16}$,$V_{12,16}$,$V_{13,16}$,$V_{14,16}$: Chondrocytes and connective tissue cells become mechanocytes in tissue culture.

$V_{15,17}$: Melanocytes and other pigment cells become amoebocytes in tissue culture.

It is not entirely certain whether or not dedifferentiation is reversible. There is a good deal of evidence suggesting that cells in tissue cultures may revert to their original types under appropriate conditions. On the other hand, isolated cells in cell culture seem to permanently loose their identities and become "generalized dedifferentiated cells". (Review: Grobstein (1959)).

(e) The "adult" cell states appear to be discrete in that they have different characteristics (shape, size, biochemistry, potencies, etc.) and in that they do not appear to become directly transformed between themselves. A chondrocyte, however, could conceivably become a mechanocyte and then become a corium cell. The adult states are stable in that they tend not to become transformed as long as they are in their normal environment. They are clearly not totally stable for some of them may dedifferentiate. Since this is quite arbitrary, the proper use of the first assertion should be as a hypothesis in need of verification.

2. <u>Transition rules for developing systems</u> -- The transition diagram (Figure 10) for neural crest cells can serve as the basis for a formal description of the behavior of developing systems. Verbal descriptions of such systems are, of course, well known, and in effecting a symbolic description we are contributing nothing new to the science. (A recent "axiomatization" of developing systems

in a slightly different context has been done by Apter (1966).) This is an important point because scientific mythology fosters the fallacy that a discussion is necessarilly rendered more accurate or otherwise more "scientific" by the introduction of symbols. The reason for formally describing embryological "rules" is that a picture of cells in terms of stable states is not very useful without a way of calculating the transition vectors V_{mk}. An appropriate description of the constraints placed by the cell on transitions may allow the development of a method for calculating these vectors. The hope is that the successful completion of this project would contribute something new to embryology.

Figure 10 suggests four rules governing transitions between morphological states. (a) Transitions are dependent on the state in which the cell is at the start of the transition. In our notation this is clear because each transition vector, V_{mk}, depends on the index m. Thus, if we are given an undifferentiated ectoderm cell, we could not confidently predict that it will become a chondrocyte until it makes the transitions described by V_{13} and V_{34}. This kind of behavior is to be contrasted with that of some physical systems in which the development of the system depends only on the initial state of the system and not on intermediate states.

(b) Transitions are dependent on the environment of the cell; cells remain in a stable state unless they are perturbed by an external stimulus. The embryologist calls such stimuli "inductions". The transition $1 \rightarrow 2$, is known to depend upon such an induction; it has already been mentioned that the transition $4 \rightarrow 7$ depends upon the inductive influence of mesodermal tissue. This rule is some-

thing of an overstatement, for although a great many transitions are known to depend on inductions, there is nothing to rule out the possibility that some cells may spontaneously initiate transitions.

(c) Most transitions are impossible. That is, $V_{mk} = 0$ for most values of m and k. In Figure 10 there are 18 states and, thus, 324 transitions. But only 30 of these are possible. This kind of behavior is to be contrasted with the behavior of some physical systems in experential space for which all transitions are possible. It has already been noted (Chapter II, Section D.2.) that this difference between integrated biological systems and physical systems suggests that they cannot be described by the same set of laws.

(d) Most transitions are irreversible. That is, $V_{mk} \neq V_{km}$ for most values of m and k. This rule does not apply to plants and it is not clear whether or not it applies to animals. However, it is generally clear that most cases of "dedifferentiation" actually invclve transitions to new states. Thus, if $V_{mk} > 0$, it is most likely that $V_{km} = 0$.

Part 2: Cellular States

C. The Locus of Cellular Stability

The realization that cells show the kind of "stable state" behavior we have just sketched leads one naturally to inquire what agents are responsible for maintaining a cell in its current stable state, guiding it to its next stable state, and determining which states it can change to. Historically, this question has been approached by supposing that some "thing", some cellular subunit perhaps, serves to effect cellular stability. The results of Chapter

III, however, suggest that some kinds of cellular stability reflect the total cellular organization rather than the action of any cellular subentity. To set the stage for this concept, we will briefly outline the current dogma on the loci of cellular stability.

1. Ideas of cellular control -- (a) The early ideas about cellular control centered about the notion that a cell's phenotype was determined by the presence or absence of one of several kinds of "determinants" in the cell. Weissmann argued that the determinants were actually fragments of the chromosomes. During embryogenesis, he suggested, the chromosomes undergo a fragmentation at each cleavage so that different blastomeres get different chromosome fragments. If the determinants are located on these fragments, each cell would get a different determinant and have a different phenotype. The somatic line cells in Ascaris actually undergo this kind of fragmentation. However, the karyotypes of almost every other species show that most somatic cells retain intact chromosomal complements, so that Weissmann's thesis is generally without support.

A more viable application of the same idea is that cytoplasmic substances act as determinants. The mollusc Dentalium or the oligochaete Tubifex, for example, have eggs with different kinds of cytoplasm. In Dentalium, a mass of clear cytoplasm localized in the lower half of the zygote ends up in just one of the blastomeres in the four cell stage. This "polar lobe" can be shown to be necessary for the formation of the mesodermal components of the embryo. No other part of the egg can form mesoderm. Similar situations occur in a variety of groups of animals (e.g. see Balinsky

(1960)). It is difficult to generalize the role of cytoplasmic determinants; but in most animals, the cytoplasmic organization of the egg seems to play an important part in guiding development up to about the gastrula stage.

(b) After gastrulation, development is thought to become progressively more controlled by the genes. If one rejects Weismann's thesis, it becomes necessary to explain how the expression of genes can be regulated to allow eye pigment genes to direct the production of eye pigments only in eye cells, to allow keratin genes to direct the synthesis of keratin only in skin cells, etc. The picture which has been developed in the past twenty years is that differentiation is the result of a highly synchronized sequence of gene "activations" and "inactivations" with the result that each differentiated cell type has the genes appropriate to its phenotype in the "active" state while the inappropriate genes are in the "inactive" state. In terms of Chapter III, different types of cells are in different switching states. (Review: Ursprung (1966)). On the molecular level, these events are currently interpreted as analogous to the repression of gene loci known to occur in the bacteria (Jacob and Monod (1961)) although there is little direct evidence to warrant the generalization. A totipotent zygote has a full complement of "active", non-repressed genes. During differentiation various loci are repressed, accounting for the loss of potencies which accompanies development. There is some evidence that the repressor substance is a histone protein (Review: Bonner and T'so (1964)). A finer level of control is known to be effected by the inactivations of enzymes by substrates and small molecules

(Changeux (1965)).

2. Cellular eigenstates -- A model of this picture of cellular differentiation has been presented by Simon (1965). A system of two loci is considered (Figure 11); G_1^+ represents an active gene locus and G_1^- represents the inactive allele. R_1 and R_2 represent the concentrations of two repressor substances. The system is described by the stoichiometric equations

$$G_1^+ + R_2 \underset{X_1^-}{\overset{X_1^+}{\rightleftharpoons}} G_1^-$$

$$G_2^+ + R_1 \underset{X_2^-}{\overset{X_2^+}{\rightleftharpoons}} G_2^- .$$

If we assume that $G_1^+ + G_1^- = 1$ and that $dG_1^-/dt = 0$, we find that

$$G_1^- = \frac{X_1^+ R_2}{X_1^- + X_1^+ R_2}$$

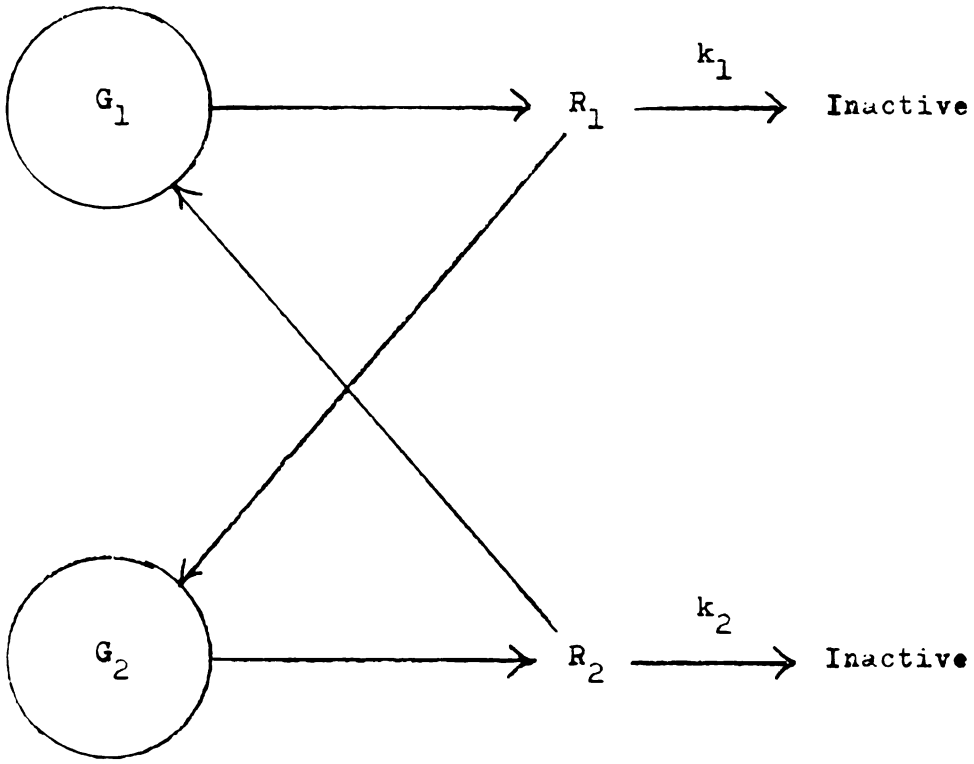
$$G_1^+ = \frac{X_1^-}{X_1^- + X_1^+ R_2} .$$

We may take the kinetic equations of the system to be

$$\frac{dR_1}{dt} = -k_1 R_1 + v_1 G_1^+$$

$$\frac{dR_2}{dt} = -k_2 R_2 + v_2 G_2^+$$

Figure 11. A model for cellular differentiation in a system with two gene loci. G_1^+ and G_2^+ are active gene loci; G_1^- and G_2^- are inactive gene loci. R_1 and R_2 are repressors. k_1 and k_2 are constants.



where v_1 and v_2 are constants. At steady state

$$R_1 = \frac{v_1 X_1^-}{k_1(X_1^- + X_1^+ R_2)}$$

(1)

$$R_2 = \frac{v_2 X_2^-}{k_2(X_2^- + X_2^+ R_1)} \quad .$$

The system has three distinct states:

$$S_1 \begin{cases} R_1 = v_1/k_1 \\ R_2 = 0 \end{cases} \qquad S_2 \begin{cases} R_1 = 0 \\ R_2 = v_2/k_2 \end{cases} \qquad S_3 \begin{cases} R_1 > 0 \\ R_2 > 0 \end{cases} \quad .$$

For S_3 the values of R_1 and R_2 are determined by solving Equations (1). Simon's claim is that states S_1 and S_2 are more generally important than state S_3. If it be assumed that a gene must be active a minimum period of time for the synthesis of an mRNA molecule, he argues that the system will respond to external inducers by either retaining its current state (either S_1 or S_2) or "switching" to the other state, depending on the concentration and the nature of the inducer. However, his analysis does not seem to exclude the possibility of an external inducer causing a transition from S_1 or S_2 to S_3.

To see the relation between Simon's model and the theory of Chapter III, assume that the units are arranged such that $R_1 \ll 1$ and $R_2 \ll 1$. Then, we can expand G_1^+ and G_2^+ in a MacLaurin series and retain only the first order terms:

$$G_1^+ \approx 1 + \frac{X_1^+}{X_1^-} R_2$$

$$G_2^+ \approx 1 + \frac{X_2^+}{X_2^-} R_1 .$$

Then, the kinetic equations become

$$\frac{dR_1}{dt} = -k_1 R_1 + \frac{v_1 X_1^+}{X_1^-} R_2 + v_1$$

$$\frac{dR_2}{dt} = -k_2 R_2 + \frac{v_2 X_2^+}{X_2^-} R_1 + v_2 .$$

These equations are of the same form as the kinetic equations used in the Example of Chapter III except that $\epsilon \longrightarrow v_1, v_2$. Although the formulae of the Example may not be applicable if v_1 and v_2 are too large, the general results about the nature of the morphological states is relevant to Simon's system. Simon's states S_1 and S_2 correspond to the switching states of the Example in which $\bar{p} = 0$ and $\bar{r} = 0$; his state S_3 corresponds to one of the organization states of the Example in which $\bar{p} > 0$ and $\bar{r} > 0$. However, Simon's analysis misses the N-3 other states included in the results of the Example. This omission arises because he does not include states for which $dR_1/dt \neq 0$ and $dR_2/dt \neq 0$ most of the time but for which R_1 = constant > 0 and R_2 = constant > 0.

The point is that in many cases it may be meaningless to assign a locus to cellular stability. The stability of cell type may be a result of the total organization of the cell rather than of the inactivation of part of the cell's genome. The traditional picture of gene action contains a non sequitor: The facts that the entire genome is retained in each cell of an organism and that these cells differ biochemically and morphologically do not necessarilly imply that genes are inactivated during differentiation. It is possible, in fact more likely, that two different types of cells have exactly the same configuration of active and inactive genes, their differences arising because they are in two distinct organization states. It must be emphasized that switching states and organization states are not incompatible so that some differences in cell type may arise from genic inactivation while others result from cell-environment interactions maintaining two cells with the same active genome in different morphological states. The possibility of organization states follows from the structure of cellular biochemical systems, but this does not mean that they actually occur in living cells. Only an experimental approach can establish this.

D. Testability

It is obvious that the theory of cellular states developed in Chapter III cannot, in its present form, be readily used to design experiments. The only way to distinguish switching states and organization states in a living cell, for example, would be to measure the mRNA concentration of all species of mRNA. This is not

technically feasible. Nevertheless, it should be possible to put yhe theory into a more practical form without making any modifications in the arguments. This would involve the following steps.

1. The theory is applicable to any organism with any finite number of cellular types. However, it needlessly complicates calculations to deal with metazoa or metaphyta which may have hundreds of different cellular states. Equally interesting problems can be undertaken using organisms with two or three cellular states. The slime molds, for instance, undergo a transition from an amoeboid state to a sporophyte state. The relationship between this morphological transformation and tne underlying biochemical transformation is within the grasp of current biochemical methods (see Section A.3.a.). The protozoan Naegleria has two distinct cellular states, a flagellate state and an amoeboid state. Transitions between these states can be effected through changes in the cell's environment (Willmer (1956a, 1956b)).

2. The exact relationships between gene loci and enzymes are known in only a very few instances, so that it is not possible to write authoritative kinetic equations in terms of the p_j's and the r_j's. Even if it were possible, a prohibitively large number of loci would have to be considered. It is more practical, therefore, to represent the biochemical system of a cell by its enzymes and substrates. Thus, the Goodwin control loops of Figure 3 would be replaced by the metabolic loops of Figure 9. In many instances, these pathways are known in some detail. Also, some important pathways of, say, carbohydrate metabolism can be considered as independent of other pathways to a first approximation. Thus, rea-

listic and interesting problems could be stated in terms of kinetic equations involving between 20 and 40 independent variables.

3. The most important extension of the theory itself which is necessary (to some extent) for practical applications is a method of calculating the transition vectors V_{mk}. This would allow predictions of the effects of controlled perturbations on cellular states. It is not obvious how to calculate these vectors.

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APPENDIX. THE EQUIVALENCE BETWEEN LINEAR DIFFERENTIAL OPERATORS AND MATRICES

The assertion that there is an equivalence between linear differential operators and square matrices is mathematically identical to asserting the equivalence of the Schrödinger and the Heisenberg pictures of quantum mechanics, and it can be proven in a variety of ways. Schrödinger (1926) established the equivalence of the two systems by arguing from the fact that the commutation relationships between the variables of a mechanical system are the same whether the variables are represented as operators on configuration space or as matrices in Hilbert space. Dirac (1926) established a unification of the two pictures in his "transformation" theory by showing that the Schrödinger wave functions correspond to similarity transformations of the Heisenberg matrices. However, von Neumann (1955) criticized Dirac's theory because it relied on the use of the "improper" delta functions, and offered, instead, a more rigorous discussion of the equivalence. More recently, such functions have been placed on a better mathematical foundation[27]. The demonstration given here is based on von Neumann's discussion of the Dirac theory. It is not intended to be rigorous.

We denote the elements of $A(p,r)_{Ma}$ by a_{kk}, $k = 1,2,\ldots,N$, and consider the vectors U_k as functions of the discrete variable, k.

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[27] The theory of such improper functions or "distributions" is beyond the scope of this work. A readable discussion is to be found in Streater and Wightman (1964).

The eigenvalue equation is just a linear transformation in $\mathcal{R}_N$ which can be written as

$$\sum_{k'} a_{kk'}U_{k'} \longrightarrow a_{kk}U_k \ .$$

The problem is how to define an analogous linear transformation on $\mathcal{P}$. To do this, we must replace the discrete variable k by the continuous variables p and r; thus, we also replace the summation by the integral

$$\int (\ldots)\, d\tau'$$

where $d\tau' = dp'dr'$. Similarly, we replace the matrix elements $a_{kk'}$ by a function $h(p,r,p',r')$. Then, the desired transformation is

$$\int h(p,r,p',r')\, U_k(p',r')\, d\tau' \longrightarrow a_{kk}U_k(p,r) \ .$$

But, this means that $A(p,r)_{Op}$ must be an <u>integral operator</u> defined by

$$A(p,r)_{Op}U_k(p,r,t) = \int h(p,r,p',r')U_k(p',r',t)\, d\tau'$$

where $h(p,r,p',r')$ is the kernel of the operator. This is a peculiar circumstance, for we have asserted that $A(p,r)_{Op}$ should be a

differential operator. To complete the discussion of the correspondence we must answer three questions: (a) Do there exist on a set of operators which are at once differential and integral operators?, (b) If such operators do exist, how does one proceed to find a particular operator on $\mathcal{P}$ which corresponds to a given matrix in $\mathcal{R}_N$?, (c) Conversely, given such an operator on $\mathcal{P}$, how does one proceed to find a corresponding matrix in $\mathcal{R}_N$?

(a) To show the existence of such an operator, $A(p,r)_{Op}$, it is sufficient to show the existence of the corresponding kernel. First, suppose that $A(p,r)_{Op}$ is just the identity operator, i.e.

$$(1) \qquad U_k(p,r,t) = \int h(p,r,p',r')U_k(p',r',t)\, d\tau'.$$

Equation (1) is satisfied if we set $h(p,r,p',r') = \delta(p - p')\delta(r - r')$ where $\delta(p - p')$ is the well known delta function.

The properties of these functions are well known[28]. In particular, for a function, f(x), of x:

$$\int \delta^n(x - x')\, f(x')\, dx' = (-1)^n\, d^n f(x)/dx^n$$

where $\delta^n(x - x')$ is the nth derivative of the delta function. Thus, for any linear differential operator, we can construct a kernel in terms of delta functions and the derivatives of delta functions. For example, if

[28] For example, see Messiah (1964), vol 1, p 468 et seq.

$$A(p,r)_{Op} = 1 + \partial^2/\partial p^2 ,$$

then we take $h(p,r,p',r') = \delta(p - p')\delta(r - r') + \delta^2(p - p')\delta(r - r')$.

(b) In this work, we will take an operator as the starting point of the analysis, so we may ignore the problem of constructing operators on $\mathcal{P}$ which correspond to given matrices on $\mathcal{R}_N$.

(c) Instead, we consider the problem of constructing a matrix on $\mathcal{R}_N$ which corresponds to a given operator on $\mathcal{P}$. This is easily done if we recall that the N elements of the matrix $A(p,r)_{Ma}$ are just the expectation values of the function $A(p,r)$ in the N stable states of S. Then, if $A(p,r)_{Op}$ is considered as a function on $\mathcal{P}$, it has as its kth expectation value

$$\overline{A_k(p,r)_{Op}} = \int U_k^*(p,r,t) A(p,r)_{Op} U_k(p,r,t)\, d\mathcal{T}.$$

Thus, we have the required matrix if we set

$$a_{kk} = \overline{A_k(p,r)_{Op}}.$$