Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 5126–5129, August 1984
Cell Biology

Outline of a theory of cellular heterogeneity

(cell biology/theoretical biology)

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Contributed by Walter M. Elsasser, January 23, 1984

ABSTRACT **In the effort to advance toward a theory of cellular function that is not completely mechanistic, I have encountered a variable whose significance is often overlooked. This is the degree of heterogeneity of organic tissue, which may vary from highly homogeneous conditions to quite large degrees of heterogeneity, primarily with respect to chemical bonding. For several decades, I have dealt with the theoretical analysis of this type of problem, and here I give a condensed outline of the conceptual changes to which such an analysis is likely to lead. I believe the time is ripe to compare these theoretical arguments with observations. The data that occasion this note are those of Rubin [Rubin, H. (1984) *Proc. Natl. Acad. Sci. USA* 81, 5121–5125]; they and numerous similar observations suggest the possibility of an advance toward a nonreductionist model of cellular function along lines that are here indicated; the theoretical model is thought of as operating entirely within the framework of quantum mechanics.**

This paper forms part of a long-extended effort at applying epistemological analysis to biological subject matter. This type of analysis, introduced into physics a century ago by Ernst Mach, has been eminently successful there: One can say with certainty that the two great theoretical edifices that dominate modern physics, the theory of relativity and quantum mechanics, could not have come into existence without thorough epistemological analysis preceding them. The idea that a similar analysis can be applied to matters biological is natural; it has preoccupied me for several decades (1–3; also 4–6).

Obviously, the concepts to which epistemological analysis is to be applied are different in biology from what they are in physics. Here, to show the use and meaning of such analysis, I introduce a particularly significant type of concept, namely, the pair homogeneity–heterogeneity. This is occasioned by comparison with Rubin's experimental results about the heterogeneity of cancer cells (7).

There are good reasons to suspect that heterogeneity (i.e., variability within any given set of samples) is an *essential* characteristic of organic life. This idea differs widely from the traditional view that heterogeneity is only a nuisance that is to be circumvented or otherwise eliminated. Hence, controversies and mutual misunderstandings of two groups adhering to these diametrically opposed viewpoints are just about inevitable.

Rubin's results indicate that there may be heterogeneity in the observable behavior of cells in addition to heterogeneity of molecular structure. In this note, I shall confine myself to "molecular" heterogeneity; the problem is too large to do more here than to evaluate one special case. But the fact that, in the view presented here, heterogeneity is a constructive element that requires a considerable amount of rethinking of the basics of biology must not be forgotten.

Definitions

It is advantageous to first define homogeneity because it is so closely tied in with the phenomena of chemistry. In quantum chemical theory, bonding depends on the interchangeability of the variables in the mathematical solution of the Schrödinger equations for electrons that represent chemical bonds. It is therefore generally recognized that (disregarding now the phenomenon of isotopes) atoms, ions, radicals, or (small) molecules of the same kind have the same physical characteristics (masses, charges, magnetic moments, spectral lines, and so on) for measurements going to as many decimal places as one wishes to reach.

Cells contain numerous chemical mechanisms that, from the present viewpoint, can be characterized as near-homogeneous processes. That is to say, there is present a small number of different species of molecules, so that all molecules of the same species traverse the same reaction path. This statement may be taken as the definition of a chemical mechanism. From here, one can readily advance to the definition of a chemically heterogeneous system as one in which many molecular species are intermingled.

At this point, let me emphasize the fact that all the methods of chemical or physical analysis contain a powerful bias in favor of homogeneous systems or processes. This arises simply from the circumstance that molecules are small, so that only very large numbers of molecules of one species can be reliably identified. Thus, even in the most sensitive tests, on the order of a million molecules are required to permit positive identification of a molecular species by chemical means. It is therefore not to be wondered at if past biological research has been so strongly centered on mechanistic models that require near-homogeneous sets of molecules. For our purposes, so as to put heterogeneity in its proper focus, let us conceive of a cell (and for that matter of any organism) as a system of intrinsic complexity in which homogeneous and heterogeneous aspects are mixed in such a way that these aspects cannot be fully separated from each other by any operational means.

Duality of Models

We require a model of the living cell that reflects its intrinsic complexity. To obtain this, we follow a mode of thought due to Niels Bohr (8). Bohr is one of the fathers of the wave–particle duality that appears in quantum mechanics, which is mathematically expressed as complementarity: The more precisely one elaborates, say, the particle model, the more diffuse and meaningless the wave model becomes and vice versa. Bohr, in dealing with biology, has created the term "generalized complementarity," which implies a dual model such that a sharpening of one aspect involves a weakening of the other. My own work has convinced me that, although the dual character of models can be transferred successfully from quantum mechanics to biological theory, the concept of complementarity cannot. The two models describe two aspects of biological reality that do not exclude each other (which would be the case of complementarity) but supplement each other instead. These two models will be designated as the *mechanistic* model and the *holistic* model, respectively. (The suitability and precise meaning of the term

"holistic" remains to be clarified as we proceed.) A main distinction is that in the mechanistic model near-homogeneous chemical mechanisms as defined above do prevail. The holistic model, on the other hand, will here be based on maximum heterogeneity, for the sake of clarity. The traditional methodological bias in favor of homogeneity has had the consequence that biologists use homogeneous models almost exclusively, thinking of them as "the" model. Therefore, I shall confine myself in this note to a discussion of some salient features of an extreme holistic model in which heterogeneity dominates.

The Holistic Model

Our holistic model is a limiting case, as is the mechanistic model; it is meant to maximize the aspects of heterogeneity of which a cell is capable. As emphasized already, chemical heterogeneity is an aspect of organic tissue that exists entirely *within* physical theory (quantum mechanics) and has nothing to do with imagined violations of that theory sometimes invoked by careless speculation.

Texts of biochemistry (9) tell us that 99% of organic tissue consists of only four elements; they are C, H, O, and N. Since we are interested in the biological effects of heterogeneity and not in any specific mechanistic devices, we shall assume that our model contains only these four elements. Let us next assume that a cell contains on the order of 10^{12} atoms. Of these, most will form inert material (e.g., water). A very small fraction, estimated as 10^6–10^8 atoms, may be assumed to constitute specific "living" material, that is, material in which metabolic transformations are taking place at any given moment.

Conceive now of the atoms of these four elements as represented by little spheres thrown together in a container. On schematizing to the utmost the chemical structures that can exist in such an assembly of atoms, we shall assume that two neighboring spheres that touch each other can be in one of three possible relationships: The spheres can merely touch each other without chemical interaction; there can be a single bond between neighbors; or there can be a double bond.

It is clear that with a number of atoms of the order of some millions any computation of its actual dynamics is quite out of the question. This was recognized more than a century ago by the founders of statistical mechanics, Maxwell and Boltzmann, who therefore replaced dynamics by the much simpler task of *counting* the number of different states that can arise under a given set of conditions, and this has long since become the standard method for dealing with such large assemblies. The methods of counting are the subject of a specialized branch of mathematics known as *combinatorics*.

A quite general result of the application of combinatorics to the determination of the number of different possible states is that this number is extraordinarily large. We rewrite this number of states, N, therefore as a power of 10.

$$N = 10^k,$$

where k, then, is the logarithm of N to base 10. One finds even by a very crude, order-of-magnitude estimate, that k is an extremely large number. Thus, for a cell having, say, a million biologically active atoms, k may well be of order of several ten or hundred thousand; this result holds independently of the detailed characteristics of the model assumed. It is convenient to have a terminology for such extremely large numbers: We shall call a number N "immense" if the exponent, k, is a large number. Specifically, we shall somewhat arbitrarily call a number immense whenever the exponent, k, equals or exceeds 100. The calculations of combinatorics indicate that, for a system such as a cell, the number k is quite large, of order of many thousands at least; we shall speak of "very immense" numbers, in analogy with very large numbers.

Scientists are not accustomed to dealing with immense numbers because such numbers do not occur in ordinary life or even in ordinary scientific practice. This may be illustrated by the fact that astronomers estimate the number of protons in our universe as of order 10^{80}, a number that, although very large, is by our definition not quite immense. Now it should not be thought that such an immense number of states of a cell can actually be realized; the overwhelming majority of states so defined will not represent biologically viable structures. We can only expect a small subset of these possible patterns to represent a biologically meaningful cell.

The essential value of this exercise in combinatorics is not found in its arithmetical results; its value is *conceptual*. It shows that the holistic model, the alternative to the mechanistic model, points away from physical causality toward a condition that in ordinary language is described as *indeterminacy*. That is to say, since an immense number of states is compatible with the rules of quantum mechanics, there is no need to assume that the transition of the molecular system from one state to the next-following state is uniquely determined by a causality derived from the laws of physics. Instead, what we just have called indeterminacy may give rise to an independent or semi-independent order of the succession of states that can occur in organisms. We can say therefore that the hypothesis of there being only one kind of order—namely, one derived entirely from physics—appears too narrow. It is to be replaced by an as-yet none-too-well defined type of ordering relationship. For now this can only mean a search for such relationships, described as "holistic," a term to be filled with more specific content below.

Ensembles

I shall try to be as simple as possible in speaking about the different kinds of order that may be encountered in a transition from quantum physics to biology. The main distinguishing feature of quantum mechanics is its statistical character. This results in our being able to interpret the results of individual measurements only in special cases. In the general case, we must deal with sets of measurements that have a statistical scatter. A broad, general theory of complex systems that is required by these conditions was given long ago by the mathematician von Neumann (10). He pointed out that the natural method of describing complex systems with statistical aspects is by the Gibbs "ensemble." The ensemble is a collective representation of a set of systems, where all systems are molecular entities of the same "structure" (which structure includes gross composition as well as the pattern of intramolecular bonding; i.e., two isomers are different structures). The properties that vary from one member of the ensemble to the next are those that are modified by a change of thermal energy (e.g., vibrational states, states of very loosely bound electrons). von Neumann (10) succeeded in proving some very powerful theorems about the behavior of ensembles. He showed that any kind of dynamical quantity if averaged over the ensemble gives rise to a number that is a function of time. The time dependence is described by a set of simple differential equations to which one can ascribe the following two properties: (*i*) the equations imply "causal" behavior of the averages and (*ii*) the equations can be derived by mathematical deduction from the principles of quantum mechanics.

From von Neumann's analysis there results a set of propositions that are all but indistinguishable from those usually described as "reductionism," although the arguments used are of a totally different kind: In the case of reductionist conclusions, one starts from numbers of observed data and

generalizes these by means of induction—as opposed to the wholly deductive method used by von Neumann. The results obtained by the two methods are practically indistinguishable. But von Neumann's method has one great advantage: In using mathematics, he forces us to follow the argument step by step; this makes it so much easier to see where its limitations lie.

It is readily seen now that if one feels impelled to go beyond the powerful arguments just mentioned, one must question the validity of the ensemble as a representation of reality to be used in biology. But the ensemble is the prototype of a homogeneous class (set) of objects, because in it each individual member of the ensemble has the same chemical structure as any other member. Thus we find ourselves confronted with a clear-cut alternative that arises from the fact that ensembles represent by definition chemically homogeneous systems for which chemical causality in a reductionist sense has been defined above (*Definitions*). If one wants to go beyond this form of description, one must give up the idea of the homogeneity of living matter and accept its heterogeneity. A theory of heterogeneous systems is obviously most difficult. (I am here using the term "holistic" to describe such a theory for reasons that can be explained only in a much longer paper.)

The Heterogeneous Model

In this model, also called the holistic model, one starts out with an image of the cell as containing many atoms; we assumed some millions of biologically active atoms of four elements. Then something new and quite unexpected happens: The number of possible chemical patterns of the cell vastly exceeds the number of cells of any one type in the real world, and it does so on a logarithmic scale (scale of k). In this way, we make constructive use of the large size of a cell compared with simple molecules! One can readily estimate that the number of actually existing cells of any one type, while exceedingly large, will be less than immense (as the term immense is defined above). Under these conditions, the formation of averages that makes indeterminacy ineffectual in the case of straightforward quantum mechanics becomes meaningless.

Now, as already mentioned, we need not assume that all of the molecular patterns of a cell play a role in such a theoretical scheme. This number is very immense. I shall base the theoretical model of holistic behavior on the assumption that the states accessible to the cell form a subset of the set of all possible molecular configurations, where this subset is either immense or large enough to approach immensity. The mechanism whereby such states are "played into" the structure of the cell can be reasonably well understood on the basis of quantum mechanics (6): First, the valence electrons rearrange themselves, a process that requires negligible energy; this leads to the making and breaking of chemical bonds; eventually thermal motion will separate the newly formed molecules.

One must now recognize that the existence of an immense "reservoir" of potential states is the ground on which a holistic model of the organism is based. We have thus made it plausible that a theoretical biology that is indeterministic from a purely physical (quantum mechanical) viewpoint—that such a theory is possible: It rests on the mathematical fact that the probability of the appearance of any one state is the reciprocal of the number of states available, hence immensely (or very) small. Then, no averaging is possible, nor is any other mathematical process that would imply predictability based on physical law.

Just as quantum mechanics gave rise to the appearance of an entirely new concept, that of probability that has no counterpart whatever in classical mechanics, the holistic model gives rise to a concept that has no counterpart in purely physical science; it is that of "creativity." I have spoken of *creative selection* (4), since what happens is that among the many states that the living system could assume according to the laws of quantum mechanics there is one it does assume. This concept would be pointless without the choice of states that is brought in by the heterogeneity of living matter. One may speak of a two-step transition from classical physics and chemistry to life science: first, there comes quantum mechanics leading to the introduction of probabilities as essential components of description; second, there appears holistic theory that introduces creativity—that is, the appearance in organisms of novel chemical structures (sometimes of considerable size and complexity) whose causal origin cannot be traced backward because of two basic obstacles: the statistical scatter due to quantum mechanics, *plus* the fact that the number of molecular patterns that can be realized by a cell of any one type is immense. The implications of this concept will be reported elsewhere; here, we must limit ourselves to the more immediate implications of heterogeneity.

Relation to Observations

I should next like to draw attention to the radical consequences that such a view must have on the interpretation of biological morphology. On the mechanistic model, morphology clearly appears as representing only a set of chemical mechanisms, where many molecules are assumed to follow the same reaction path. On the holistic model, insofar as creative innovation can occur, there must be a certain dissociation between morphology and mechanisms. Just how far this dissociation will go is difficult to foresee, but there can be little doubt about the challenge that is offered to many traditional concepts by the holistic model in this respect, implying a great deal of critical work that remains for the future.

According to the holistic model, an immense set of potential molecular structures can play a role in the dynamics of the organism. The structural heterogeneity of living tissue is the observational counterpart of these theoretically conceived circumstances. But owing to the extensive preoccupation of observers' minds in favor of homogeneous models, the amount of data available for testing holistic models is as yet small. One such instance is the paper of Rubin (7), which has given rise to the present note; it deals with the properties of growth and reproduction of cancerous cells, essentially with properties of a pathological type of tissue. Another, older and rather extensive body of information related to heterogeneity was developed by Williams (11). The subject matter is clearly indicated in the title of Williams' book: "Biochemical Individuality." The main tool is an assay of the concentration of various naturally occurring compounds in different tissues. I have reported elsewhere (4, 5) on these results. Thus, for instance, Williams says (ref. 11, p. 77), "Interindividual variations in enzyme efficiencies in normal individuals, insofar as they have been determined, are not of the order of 20 to 50 percent, but are more often at least 3- to 4-fold. Differences of 10- to 50-fold(!) have been observed in a substantial number of cases. . . ." Concerning the effort that had to go into observation of these effects, Williams has this to say (at the same place): "Interindividual differences related to metabolism come to light only when detailed items are compared. When two individuals of the same height and weight yield total metabolism values that are about the same, it is easy to conclude that their metabolisms are substantially identical. The evidence presented here, however, indicates that the details of metabolism in two such individuals may be very different indeed."

In the case of Rubin's experiments, cancerous growth seems to be a process that amplifies interindividual distinctions. As the example of Williams' results shows, such distinctions do not go away but grow in importance as one descends to structural and dynamical details. Now it is exactly the difference or else similarity between large-scale and small-scale behavior that allows us to discriminate between biological models of the homogeneous and heterogeneous variety. In the homogeneous case, the small-scale variations are just fluctuations whose mathematical theory is well worked out and thoroughly understood. In a theory of heterogeneity, things are not that simple. Following the ideas of Williams quoted above, we are led to think that in a theory based on molecular heterogeneity *there is no identity between mechanisms and biological morphology*. Now although this statement is vague, it may well be able to serve as a starting point in our program toward a theory of heterogeneity, designated before as a holistic theory.

In this note, I have been unable to justify the term "holistic" as describing properties of systems that cannot be combined into "ensembles"; let it suffice to say that further pursuit of the ideas described here leads one to an understanding of the properties of objects that form classes in spite of the fact that they do not form ensembles. These concepts are in part indicated in refs. 1–6; in a larger part, they remain for the future.

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