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Bringing Chemistry to Life: What does it Mean to be Alive?

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Abstract

The definition of life has excited little interest among molecular biologists during the past half-century, and the enormous development in biology during that time has been largely based on an analytical approach in which all biological entities are studied in terms of their components, the process being extended to greater and greater detail without limit. The benefits of this reductionism are so obvious that they need no discussion, but there have been costs as well, and future advances, for example for creating artificial life or for taking biotechnology beyond the level of tinkering, will need more serious attention to be given to the question of what makes a living organism alive. According to Robert Rosen's theory of (*M,R*)-systems (metabolism-replacement systems), the central idea missing from molecular biology is that of metabolic circularity, most evident from the obvious but commonly ignored fact that proteins are not given from outside but are products of metabolism, and thus metabolites. Life can be embodied in a mathematical formalism that treats metabolism as a function able to act on an instance of itself to produce a new instance of itself.

Introduction

In his recent book, Budisa [1] discussed how the genetic code might be manipulated in order to produce novel proteins, and in his contribution to this Beilstein Workshop [2] he addresses the same topic. However, although his ultimate aim is clearly engineering he devotes a large amount of space to attempts to understand how the genetic code came to adopt the form that we see in nature, how it works in nature, and how coding changes such

as those seen in mitochondria came to evolve. The idea is that one needs to understand how a system fulfils its natural functions before one can modify it to do something else. This principle applies quite generally, and there can be no hope of creating new organisms, or fundamentally modifying existing ones, until the present tinkering approach to biotechnology is replaced by one that depends on a deep understanding of how natural organisms are able to stay alive.

In our contribution [3] to the Beilstein Workshop *Molecular Informatics: Confronting Complexity* we discussed whether the properties of living organisms could properly be called complex, or whether they were no more than complicated. The difference is important, because something may be beyond the reach of present-day computing techniques simply because not enough information is available and too much time would be needed to process it if it were available, not because it is *impossible in principle*. In the former case it is just a complicated problem that will eventually be soluble when enough information has been accumulated and computers have become fast enough, whereas in the latter case it is a complex problem in Rosen's sense [4] and will never be soluble. Some aspects of living organisms can be simulated with considerable accuracy, but that does not rule out the possibility that Rosen [4] may be right to argue that a complete description of a living system will always be beyond the reach of computation.

At present it is very difficult to answer, because we are far from an adequate understanding of what life is. Although Schrödinger's famous book *What is Life?* [5] was published more than half a century ago, and convinced some distinguished physicists that there were some interesting biological questions for them to study, most biologists during the era of molecular biology have taken life as a given, and largely ignore the question of what it is. The remark of Jacob [6] that "Today we no longer study Life in our laboratories" still represents the usual attitude, also expressed more recently by Atlan and Bousquet [7]. Unfortunately, however, although the reductionist approach has brought biology a very long way it cannot provide all the answers that will ever be needed; and the time has come to reopen the question of what life is. Certainly, at this time it would be difficult to argue that we understand life any better than Schrödinger did 60 years ago. Nonetheless, if manipulation of organisms for technological purposes is ever to move beyond the tinkering that characterizes present approaches to the design of drugs, pesticides, etc., then a better understanding of life will be needed. As Woese [8] has written, "Without an adequate technological advance the pathway of progress is blocked, and without an adequate guiding vision there is no pathway, there is no way ahead."

In the years since Schrödinger's book appeared, however, relatively few biologists have attempted to arrive at a theory of life [9 – 14]. Of these disparate approaches, we shall concentrate here on the theory of (*M,R*)-systems of Rosen [12], in part because it is one of the least well known, but also because it may offer the "guiding vision" that Woese [8] was asking for in the words quoted earlier. As discussed elsewhere [15], there are some points in common between autopoiesis [11] and (*M,R*)-systems, but autopoiesis puts the primary emphasis on the structural organization of organisms and the necessity to enclose them with membranes, whereas Rosen was more concerned with the logical organization in terms of

formal mathematics. This chapter can be regarded as a simplified version of our attempts [16, 17] to clarify Rosen's vision of life, especially his view of metabolic circularity, to provide examples of how this might work, and to define the limits of applicability of his ideas.

What is Metabolism?

Metabolism is usually regarded as a network of chemical reactions catalysed by enzymes that occur in a specific compartment defined by a membrane, the set of enzymes and other proteins constituting the *proteome*, and the set of *metabolites* the metabolome. The separation between proteome and metabolome is more artificial and misleading than it appears at first sight, because enzymes (and all other proteins) are not given from outside but are themselves products of metabolism. They are continuously degraded and synthesized, and to achieve this, the cell requires complex machineries involving numerous macromolecules (RNA, proteins) and regulatory mechanisms. However, as we have recently discussed Rosen's view of metabolism in the context of proteomics [18] we shall not go into more detail here.

Inadequacy of the Machine Analogy

There has always been a desire to understand living organisms in terms of machines and other artifacts of technology. This is certainly useful for understanding certain functions of organisms, in particular mechanical ones such as muscle action and blood flow. The function of the human heart, for example, can be understood in considerable detail in terms of a sum-of-the-parts model [19]. There is, however, a fundamental difference between machines and organisms that render this analogy much less general and useful than it may appear at first sight. All machines, at what ever level one defines the word “machine”, whether a simple tool like an axe, a more complex machine such as an aeroplane or a computer, or even a complete factory, require external agencies to construct them and to maintain them-determining when defective components need to be replaced or repaired, and carrying out the replacement or repair when necessary. Note that here the words “replace” and “repair” describe the same process, but emphasize different levels of a hierarchy: repairing a computer, for example, usually involves identifying and then replacing the defective component. In his writing Rosen always referred to “repair”, inviting confusion with better known and probably more appropriate uses of this term, such as DNA repair and chaperone function; we prefer the term “replacement”.

In an organism, however, replacement is an internal function, involving no help (before the advent of modern medicine, at least) from an external agency, and aging and death can be considered as a loss of this capacity. To a considerable degree even the construction of an organism is an internal function: a bacterium makes itself, but no machine does that, and at our present level of understanding we cannot even conceive of how a machine of the future might construct itself and maintain itself. Sophisticated modern instruments often incorporate some internal testing to detect faulty components and alert their operators to them, and

even to an extremely limited degree, to replace them. But what they can do is vastly less than what living organisms can do, as they need to monitor the state of all of their components and maintain them all of the time.

Rosen summarized the essential property of organisms that makes them different from machines in the phrase "organisms are closed to efficient causation". The reference here is to Aristotle's four categories of causation [20], whose term αφτφα is usually translated as *cause* (or just transliterated as aitia). The word *make* conveys the meaning better, however, as one may illustrate in relation to the herbicide Glyphosate (the active component of Roundup). If the question of what Glyphosate is made out of is answered by saying that it is made from glycine and phosphoric acid, this refers to its *material cause*. If the question asks what makes it an herbicide, or why we call it an herbicide, and the answer is that plants die if their foliage is sprayed with it, then this describes its *formal cause*. If the question is about who (or what) makes Glyphosate, with the answer that it is made by the Monsanto Company, then this is its *efficient cause*. If the question asks why Glyphosate is made, what is it made for, with the answer that it is made to destroy unwanted plants, then this is its *final cause*. Calling these four categories causes sounds a little strange to modern ears, because our ideas of causation are derived from the work of David Hume, and really it is only the efficient cause that corresponds to the usual way we talk about causes.

Modern biology treats final causes with suspicion, because living organisms are the product of evolution, not of design. Nonetheless, as Atkinson [21], for example, has argued, a generalized rejection of teleological explanations is often unhelpful, because so many properties of organisms can be understood *as if* they had been designed for a purpose that forbidding reference to function puts an unnecessary constraint on the discussion of metabolic organization: the absence of a designer does not imply the absence of design. Meléndez Hevia [22] has subsequently analysed several metabolic pathways to show that perfection of design extends even beyond Atkinson's conception, as indeed it extends beyond the design of pathways [23]. However, Atkinson was writing before the current resurgence of creationism, with its scientific pretensions under the guise of "intelligent design", had started to raise a serious threat to biological education, not only in the USA but throughout the world. In the present context we need to be completely clear that apparently teleological rationalizations are just a convenience, and that in reality there are no final causes.

The formal cause, though important, played little part in Rosen's thinking, and we shall not refer further to it here. The interplay between material and efficient causes, however, is central to the understanding of (*M,R*)-systems. Notice that there is no suggestion that organisms are closed to material causation: organisms certainly need molecules available in the external milieu for their internal functions; what they do not need are external catalysts to oversee them. We return to this point in a later section.

Algebraic Formulation of Metabolism

In metabolism each reaction is catalysed by an enzyme, as in the following example, in which hexokinase catalyses the phosphorylation of glucose by MgATP:

$$\text{glucose} + \text{MgATP} \rightarrow \text{glucose 6-phosphate} + \text{MgADP} \quad (1)$$

This can be formalized algebraically by viewing an enzyme as an operator, *M*, that transforms a set of molecules (input materials) into another one (output materials):

$$\overset{\text{M}}{a_1 + a_2 \rightarrow b_1 + b_2} \quad (2)$$

The catalyst *M* acts formally as a *mapping*, because it transforms some variables belonging to the admissible set of input materials into other variables belonging to the set of admissible output materials:

$$M((a_1, a_2...)) = (b_1, b_2..) \quad (3)$$

where the equation is written to indicate that although hexokinase catalyses a reaction with two substrates and two products, other enzymes may have more or less than two of each. Rosen generalized this mathematical model of a single metabolic reaction into one that takes account of an entire metabolic network. He interpreted the complete metabolism as a kind of generalized enzyme or operator (mapping), M_{met}, that transforms all of the input materials (all of the left-hand sides of all of the chemical equations) into all of the output materials. Moreover, enzymes are not totally specific, for example most hexokinases will accept other hexoses, such as mannose and fructose, as alternatives to glucose as substrates [26], or other nucleotides, such as MgITP (the magnesium salt of inosine triphosphate), as alternatives to MgATP, so a_1, a_2 etc. must be understood as sets of admissible metabolites rather than as individual substances, and Equation 1 would be more accurately written as follows:

$$\begin{matrix}\{\text{glucose} \\ \text{mannose-} \\ \text{fructose }\}\end{matrix} \quad \begin{matrix}+ \{\text{MgATP} \\ \text{MgITP }\}\end{matrix} \quad \rightarrow \quad \begin{matrix}\{\text{glucose 6-phosphate} \\ \text{mannose 6-phosphate} \\ \text{fructose 6-phosphate }\}\end{matrix} \quad \begin{matrix}+ \{\text{MgADP} \\ \text{MgIDP }\}\end{matrix} \quad (4)$$

Ultimately, therefore, we arrive at a formulation like:

$$\overset{\text{Mmet}}{\text{A} \rightarrow \text{B}} \quad (5)$$

as a summary of the whole of metabolism. As the enzymes represented here by M_{met} are being degraded (or damaged), they need to be continuously replaced in order for the living system to have more than a transient existence. There are, in fact two problems to be solved. The first is to achieve some permanence, but the second is to maintain an identity, that is to say to maintain an organization, even though many details may need to be continuously revised to take account of variations in the environment in which the organism has to live. How do living systems solve these problems? Rosen's view, illustrated in Fig. 1, is that a circular organization is needed, and this will be analysed in the next section.

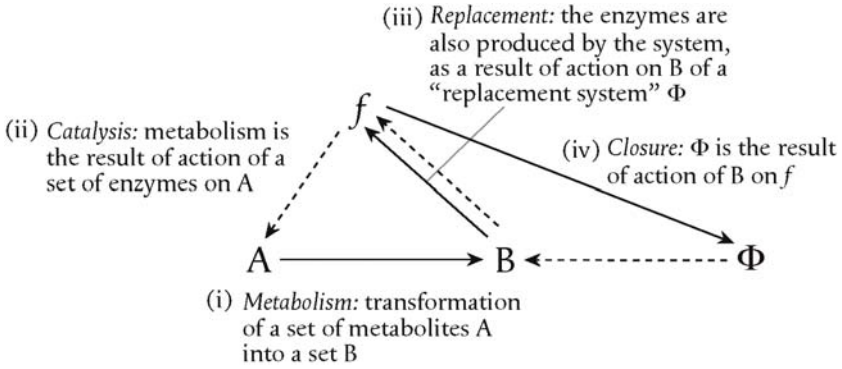

Figure 1. Rosen's view of metabolic circularity. The diagram illustrates the closed nature of metabolic systems, in which there are no external causes, and no final causes in Aristotle's sense. Material causes are represented by full arrows, efficient causes by broken arrows. The diagram is best understood in four stages: (i) metabolism; (ii) catalysis; (iii) replacement ("repair" in Rosen's terminology); (iv) closure.

Metabolic Circularity

The considerations discussed in the previous section lead to the idea that metabolism is part of a *metabolism-replacement system*, or *metabolism-repair system* in Rosen's terminology, both conveniently represented by the same shorthand as (*M,R*)-*system*. Such a system is closed ("organisms are closed to efficient causation"), and the way in which closure is achieved is explained in terms of a diagram of the type shown in Fig. 1. The essential point is that the enzymes that catalyse metabolism are themselves products of metabolism. This idea has led us to regard metabolism as a mathematical function (or mapping), that acts on an instance of itself to produce another instance of itself [16, 17]:

$$f(f)=f \tag{6}$$

This is an application to metabolism of an equation [24] related to the concept of fixed-point combinators in the theory of computer languages [25]. It defines a remarkable property in which the value of a function at a single point defines the whole function. Ordinary mathematical functions do not behave like this, and, in particular, operations on

sets do not behave like this. Trying to understand metabolic circularity can thus be formalized as the search for functions that can be regarded as solutions of Equation 6. Writing the equation as shown underlines its unusual mathematical character, but it is open to the objection that the three occurrences of *f* in it are not exactly equivalent in meaning, and one might prefer to write it as follows:

$$\beta(f)=\Phi \qquad (7)$$

emphasizing that the operator β on the left-hand side of the equation is regarded as a property of the product B of metabolism, and that the result Φ on the right-hand side is the replacement system.

The major conceptual difficulty in circular organization is implicit in the last step: how does B, the product of metabolism, induce the system to maintain the primary replacement system Φ? There are, in fact two difficulties here: is it possible, even in a formal mathematical sense, for a generalized operation on sets to be inverted? Even if it is, how does the system "know" how it is organized? How does knowledge of B imply knowledge of Φ? The first of these difficulties was raised by Landauer and Bellman [27], who went as far as to claim that "unfortunately, the mathematics [of Rosen's analysis] is incorrect, and the assertions remain unproven (and some of them are simply false)". Fortunately this conclusion is incorrect in formal mathematics [17]. Although it is unusual for operations on sets to be invertible, it is not impossible. For example, for any set *i* that is a sub-set of the set {0, 1, 2, 3 . .. 11} the operation $j = i \times 7 \bmod 12$ is invertible, i.e. it is possible to deduce *i* from *j*; for example, the equation $\{3, 7, 9\} = i \times 7 \bmod 12$ has the unique solution $i = \{1, 3, 9\}$, and there is a unique solution regardless of the set on the left-hand side of the equation [17]. This argument is of course too abstract to carry much weight in a biological discussion; the essential point is that it disposes of the main claim of Landauer and Bellman [27].

The second question is more difficult to answer in satisfactory biological terms, and for the moment needs to be left open. In more precise mathematical terms, it means that the system must be able to invert the evaluation map at B, with a unique Φ such that Φ(B)= f. However, we were able to define the limits within which Rosen's conclusions could be valid, i.e. that a unique Φ could exist [17]. The biological example of an (*M,R*)- system that we shall give in the next section goes only part of the way towards explaining how knowledge of the organization of a system can be coded within the system itself.

Organizational Circularity, Not Thermodynamic Circularity

We have mentioned already that the claim that organisms are closed to efficient causation does not in any way imply that they are closed to material causation. However, we need to return to this point, first because it can generate serious misconceptions, and second because the idea that organisms are open systems in the thermodynamic sense is in a different way just as fundamental for understanding life. There is, however, no incompat-

ibility between the two statements, which address different causes. To understand the thermodynamic nature of living organisms it is essential to recognize, as Schrödinger [5] did with his discussion of "negentropy", that organisms convert external sources of energy into energy for their own use, and that all of metabolism is driven by this: this is a statement about material causation. However, it is also true, as we discussed in the preceding section, that organisms must organize themselves without external help, that is to say that they must manufacture all of the specific catalysts that they need: this is a statement about efficient causation, and in no way contradicts the previous one.

An Example of an (M,R)-System

Figure 2 shows a naive attempt to illustrate the metabolism of an organism the entire metabolic activity of which consists of producing a single molecule ST by the action of a catalyst *M* on two molecules S and T available from its environment. It fails to be a satisfactory model, because it treats the catalyst as given; however, even if this happened to be available at the beginning it would have an inevitable tendency to become degraded, and therefore the process illustrated in the figure could exist only transiently.

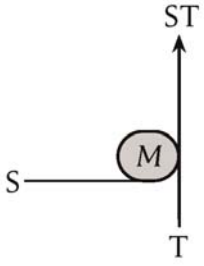

Figure 2. A naive view of metabolism. In this simple example the only metabolic function of the organism is to produce a single metabolite ST from precursors S and T available in its environment. This requires a catalyst, the molecule *M,* but the illustration makes no allowance for the fact that *M* will have an inevitable tendency to become degraded, and that the system can therefore have only a transient existence. It is in that sense that we call it naive, and a more realistic view is shown in Fig. 3.

An improvement on this example is provided by the complete (*M,R*)-system shown in Fig. 3a. The entire metabolic activity again consists of producing a single metabolite ST from molecules S and T, but now the model allows for the catalyst *M* to be replaced by the action of a second catalyst *R* on the product ST together with a third molecule U available from the environment. As *R* is likewise subject to decay it also needs to be replaced, and this is achieved by supposing that *M* can accept U as an alternative substrate that leads to production of *R*. Stoicheiometric considerations suggest that *M* and *R* have the structures STU and SU respectively, and in Fig. 3b these identifications are made explicitly; in addition, each catalytic process is represented as a cycle of three chemical reactions. Thus

although Figs 3a and 3b represent the same model, Fig. 3b does so in a way that makes its chemical nature explicit. The example is based on a slightly simpler one proposed by Morán *et al.* [28].

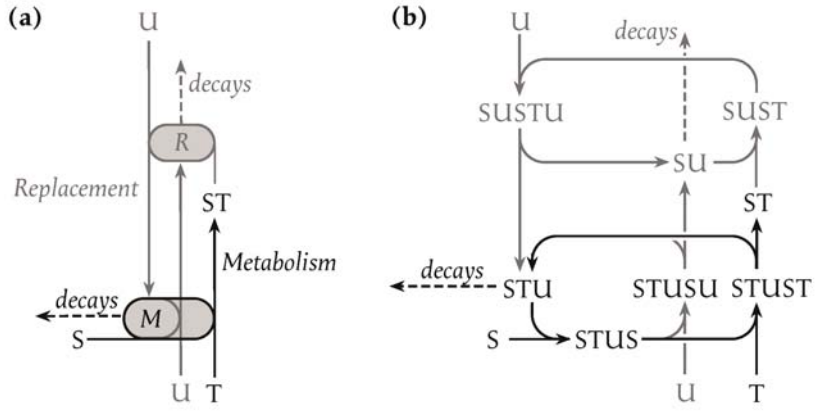

Figure 3. A metabolic example of an (*M,R*)-system. **(a)** The model of Fig. 2 is extended to allow for decay of the catalyst *M* and the need for it to be replaced by action of a second catalyst *R* acting on a third external molecule U, *R* being itself replaced in a secondary activity of *M* acting on U as an alternative substrate to S. The replacement module is shown in grey. **(b)** The catalysts are represented by the structures STU and SU, and each catalytic process is represented as a cycle of three reactions, for example STU→STUS→STUST, so as to make the chemical nature of the catalysis explicit.

Despite the trivially minimal nature of the metabolism achieved by this system, with just one metabolite produced from two precursors, the system as a whole appears remarkably complicated, especially as shown in Fig. 3b. The version in Fig. 3a may appear a little simpler, but this just disguises the complication; it does not eliminate it. The reason for making the example so complicated is that the catalyst is not directly available from the environment and it has an unavoidable tendency to decay with time. So even if STU happened to exist initially it would not exist indefinitely unless replaced. However, as SU is just as liable to decay as STU, its existence implies the existence of another replacement process. In principle, each enzyme requires a replacement system, but as each replacement system is itself an enzyme it requires its own replacement system, and an infinite regress, or combinatorial explosion, seems inevitable. To escape from this, the example suggests that SU is produced from S and U in a secondary activity of the first catalyst STU. The result is a complete (*M,R*)-system, closed to efficient causation. Note that the need for one catalyst to have more than one function emerges automatically from the need to escape from infinite regress. This implies that the multifunctionality of proteins that is being increasingly observed is more than just an interesting fact about living systems; it is an absolute necessity for life.

Complicated though it is, Fig. 3 only partly satisfies the need for an example of an (*M,R*)-system, because although it is indeed an (*M,R*)-system it does not have organizational invariance: it does not contain the information needed for the arrows B →> f → Φ of Fig. 1, and is thus not guaranteed to maintain its identity indefinitely. In effect, the missing information is the knowledge of which of the three metabolites STU, SU and ST are the enzymes, i.e. which intermediate in the network acts to catalyse each reaction. Although the illustration assumes that STU catalyses two reactions and SU catalyses the third, this is not inevitable: in principle, with three metabolites and three reactions one could conceive of 3^3 different assignments, even in this minimally small system, so there is not the unique solution that would be needed for organizational invariance. In a model of more reasonable size (though still very small compared with a real organism), such as a recent stoicheiometric model of *Escherichia coli* metabolism [29, 30], which contained 89 metabolites and 110 reactions, the corresponding numbers become huge, 89^{110}, or more than 10^{214}, in this case. In the simple example of Fig. 3 we [17] offered some arguments about how the total of 3^3 possibilities might be decreased, but it is clear that further study will be needed to understand how organizational invariance can be achieved in a living organism.

Reproduction and Evolution

According to Dobzhansky [31], nothing in biology makes sense except in the light of evolution, a sentiment with which nearly all modern biologists would agree. Yet equation 6 as an expression of metabolic circularity apparently says nothing about evolution or about reproduction, an essential part of evolution: why $f(f)=f$ rather than, say, $f(f)=2f$? Before answering this apparent criticism it is interesting to note both that Dobzhansky was addressing his remarks to teachers of biology in the USA, and that he was answering an attack on the Copernican view of the solar system from an Islamic fundamentalist point of view. Writing in 1973, he perhaps did not foresee the great harm that the revival in the influence of the Christian fundamentalist denial of evolution would represent for science teaching in the USA in the subsequent 30 years, but he may have foreseen the rise in Islamic fundamentalism that is beginning to offer the most serious challenge that biology teaching now faces in Western Europe.

The point here is that staying alive was the problem that needed to be solved first: the early organisms could not begin to reproduce or evolve until they had learned how to stay alive, maintaining organizational invariance in the face of changing conditions. Rosen's theory of (*M,R*)-systems does not in its present form solve all of the problems of how that is possible, or of what constitutes a living system, but it represents a major step. In particular, it addresses the right questions, questions that have been overlooked in nearly all studies of modern biology, when the essential nature of life has been set aside as having little interest or importance [6, 7]. The idea that the proteome is not a separate entity from the metabolome, but a part of it, is obvious once pointed out, but is easily ignored.

Its importance is in recognizing that enzymes and indeed all proteins are not given from outside but are themselves products of metabolism, and hence metabolites, like any other. The ideas that we have tried to analyse are abstract, and it may be many years before they

can be translated into practical applications, but such applications will be necessary before there can be significant progress towards creating artificial life, and they will also be very useful if modifying existing organisms for biotechnological purposes is to move beyond tinkering, and useful also for defining criteria for recognizing whether candidates for living systems that may eventually be found elsewhere in the universe are truly living or not. A complete theory of life is unlikely to be based solely on (*M,R*)-systems: it is also likely to incorporate ideas from autopoiesis [11] and autocatalytic sets [13], for example, especially as the important role of the membrane for defining the limits of an organism needs to be taken into account.

Kováč [32] recently remarked that "biologists should keep in mind that life is written in the language of chemistry." True, but they should also keep in mind Galileo's original version, that nature is written in the language of mathematics, because a fully satisfying theory of life will require not only all of the biological detail, with obedience to the laws of chemistry and thermodynamics, but also the sort of mathematical formalism that Rosen tried to develop.

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