Organizational Invariance in (*M*,*R*)-Systems

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Robert Rosen's concept of (*M*,*R*)-systems was a fundamental advance in our understanding of the essential nature of a living organism as a self-organizing system, one that is closed to efficient causation, synthesizing, and maintaining all of the catalysts necessary for sustained operation during the whole period of its lifetime. Although it is not difficult to construct a model metabolic system to represent an (*M*,*R*)-system, such a model system will typically appear to lack *organizational invariance*, an essential property of a living (*M*,*R*)-system. To have this property, an (*M*,*R*)-system must not only be closed to external causation, it must also have its organization coded within itself, *i.e.*, the knowledge of which components are needed for which functions must not be defined externally. In this paper, we discuss how organizational invariance may be achieved, and we argue that the apparent failure of previous models to be organizationally invariant is an artifact of the usual practice of treating catalytic cycles as 'black boxes'. If all of the steps in such a cycle are written as uncatalyzed chemical reactions, then it becomes clear that the organization of the system is fully defined by the chemical properties of the molecules that compose it.

Introduction. – *Robert Rosen* tried, essentially alone, to advance the understanding of the nature of life beyond the state in which *Schrödinger* [1] had left it. He made many important contributions, among these the initial papers on his *relational theory of biological systems* [2–4], later developed into his *(*M,R*)-systems* [5–8] or *metabolism-replacement systems*[1]). *Rosen* summarized his ideas in several books, especially in '*Life Itself*' [10] and '*Essays on Life Itself*' [11].

Crediting *Rosen* as the major contributor to our understanding of life is not intended to undervalue other work, but, in most cases, we regard this as less fundamental. The standard idea of the modern biologist that reproduction and evolution are the defining characteristics of a living organism is not entirely false, but it presupposes that the problem of staying alive is already solved, whereas a real theory of life needs to explain how organisms stay alive before it can begin to discuss how they reproduce or evolve. Reproduction and evolution are by no means irrelevant to the study of life, but they are not *defining* characteristics of life. In other words, although *Dobzhansky*'s often-quoted statement that nothing in biology makes sense except in the light of evolution [12], describes a necessary condition for understanding life as it is now, it is not a sufficient condition.

At a somewhat more fundamental level than evolution, *hypercycles* [13] and *sysers* (or *systems of self-reproduction*) [14] are valuable contributions to understanding

[1]) *Rosen* himself took the '*R*' to stand for 'repair', but the intended meaning is 'replacement' or 'resynthesis' [9], not 'repair' as understood in other biological contexts.

replication of complex organisms, but they are already too complex to be useful models of the simplest organisms that must have existed at the origin of life. In our view, therefore, the major competing ideas are those of *autopoiesis* [15] and *autocatalytic sets* [16]. Both of these incorporate major insights that are not much developed in *Rosen*'s work: for example, fundamental to autopoiesis is the idea that membranes or cell walls are needed to define the limits of an organism, which cannot diffuse freely in a mixture with other organisms, exchanging material and catalysts without restriction.

In an earlier study [9], we modified a suggestion by *Morán* and co-workers [17] to propose the model of an (*M*,*R*)-system shown in *Fig. 1*,*a*. Both metabolism and replacement modules are present, because the two different molecules that catalyze the three chemical reactions are by themselves produced and maintained by the system itself. They are also complete, because the model includes a mechanism for replacing the catalyst *R* needed for replacing *M*, a mechanism that does not depend on the existence of any molecule not already present. However, at least as presented in *Fig. 1*,*a*, this model fails to be *organizationally invariant*[2]), because it fails to encode within itself any information about which molecule catalyzes which reaction. With three reactions (S + T → ST, ST + U → *M*, and S + U → *R*) and three candidate catalysts (ST, *M*, and *R*), there are apparently $3^3 = 27$ ways of assigning catalysts to reactions: even though there are only three products (ST, *M*, and *R*), there is not enough

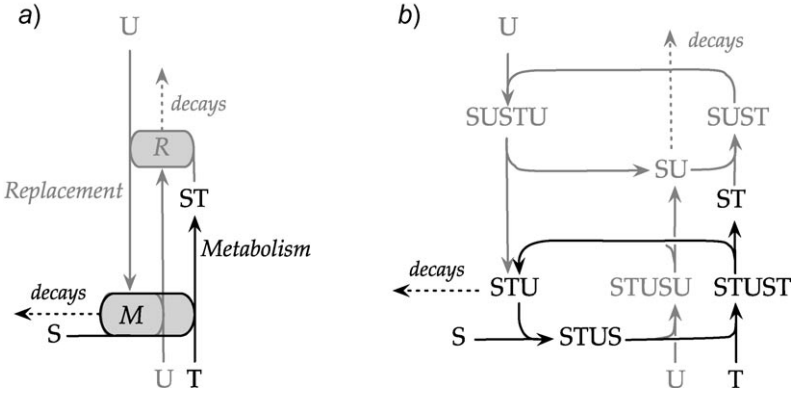

Fig. 1. *A metabolic example of an (*M,R*)-system*. *a*) This model was suggested previously [9]: it allows for decay of the primary catalyst, *M*, which catalyzes formation of the molecule ST from the precursors S and T, and for its replacement by action of a second catalyst, *R*, acting on a third external molecule, U, *R* itself being replaced in a secondary activity of *M* acting on U as an alternative substrate to S. The replacement module is shown in gray. *b*) The catalysts are represented by the structures STU and SU, and each catalytic process is represented as a cycle of three reactions (*e.g.*, STU → STUS → STUST), so as to make the chemical nature of catalysis explicit. Later in the paper, we shall see that ornithine plays the same role in the urea cycle as STU plays here, though ornithine is usually regarded as a metabolite, whereas STU is said to be a catalyst.

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[2]) *Rosen* referred to *replicative* (*M*,*R*)-systems rather than to (*M*,*R*)-systems with organizational invariance, but his use of this term can be confusing because it is not related to conventional ideas of biological replication, such as DNA replication.

information to allow the system to 'know' which molecules need to be synthesized in order for the catalytic requirements to be satisfied. Even with the introduction of restrictions, such as not allowing a molecule to catalyze its own formation or removal[3]), this total of 27 possibilities cannot be decreased to one by any reasonable argument, and the combinatorial explosion gets much worse if one tries to build a more elaborate model.

However, in an effort to explain this model to a more general audience, we have recently represented it in the more explicit form shown in *Fig. 1,b* [18]. This representation includes exactly the same chemical transformations with the same catalysts, but now each catalytic process is shown as a cycle of three reactions. Although we originally devised this representation as no more than a way of making *Fig. 1,a* more understandable, it can be maintained that the reverse transformation, from *Fig. 1,b* to *Fig. 1,a* involves *loss of information*, and that the information lost is exactly the information needed to make the model organizationally invariant. In other words, one can claim that *Fig. 1,b* does represent an (*M*,*R*)-system with organizational invariance, and that is the main argument that we shall develop in this paper.

Looking for Principles of Metabolic Organization: the Nature of Catalysis. – The idea of catalysis (though not the word, which was suggested a generation later by *Berzelius* [20], who is usually also credited with the idea) was first proposed more than two centuries ago by *Fulhame* [21]. She considered that all chemical reactions are catalyzed by water, that the catalysis occurs because a reaction occurs in several steps, and that any water consumed in one step is regenerated in a later one. This is now almost universal as an interpretation of how enzymes and other catalysts work, though in the older literature on enzymes there were occasional suggestions that an enzyme might act just by virtue of its presence [22], perhaps by emitting some sort of radiation [23]. Although this idea is not given any overt credibility today, it still perhaps underlies a false dichotomy by which the molecules of metabolism are divided into *enzymes* (or catalysts) on the one hand, and *metabolites* (or reactants) on the other.

To understand why this dichotomy may be misleading, it is useful to examine the urea cycle, which is commonly presented in textbooks of biochemistry in the form shown in *Fig. 2,a*. The representation can be made more 'anonymous' by replacing names with symbols, as in *Fig. 2,b*, but the classification of reactants into enzymes and metabolites is still clearly implied. Moreover, writing enzyme names or symbols alongside the reactions they catalyze implies that they are somehow external to these reactions.

To correct the misleading impression given by *Fig. 2*, we need to draw it in a way that plainly shows the enzymes as participants in these reactions. This is done in

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[3]) We used this prohibition of autocatalysis previously [9] as a way of reducing the 27 possibilities to 4, but it is a restriction of questionable validity, because autocatalytic processes do occur in real organisms: for example, the stomach enzyme pepsin catalyzes its own formation from an inactive precursor, pepsinogen [19]. This is an extracellular process, and so it does not strictly occur 'in' the organism, but it is still necessary for the survival of the organism, so it is certainly a process essential for life. Even at the origin of life, we do not consider that one can deny the possibility of autocatalytic reactions.

Fig. 3, *a*. When the reactants are made even more anonymous, as in *Fig. 3*, *b*, it becomes evident that there is nothing in the structure of the cycle that justifies a distinction between enzymes and metabolites: there is no way to deduce from this representation that A, for example, is a 'metabolite', whereas B is an 'enzyme'. In fact, both have exactly the same role in the system: each is consumed in one step and regenerated in another.

If the stoichiometry of this system were analyzed with a computer program such as Metatool [24] that was provided with no information about the roles of the different elements, it would, indeed, classify them into two groups, but these would *not* correspond to the conventional classification into enzymes and metabolites; instead it would find that three of them, C, D, and I, have non-zero stoichiometric coefficients in

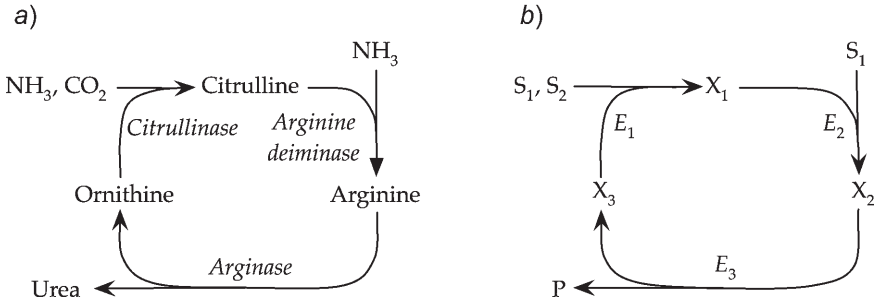

Fig. 2. *The urea cycle. a*) The cycle appears in the form commonly given in textbooks of biochemistry, with explicit names for the metabolites and enzymes. *b*) Here the names are replaced by symbols, but the implied distinction between metabolites and enzymes is maintained, and catalysts are implied to be external to the reactions they catalyze.

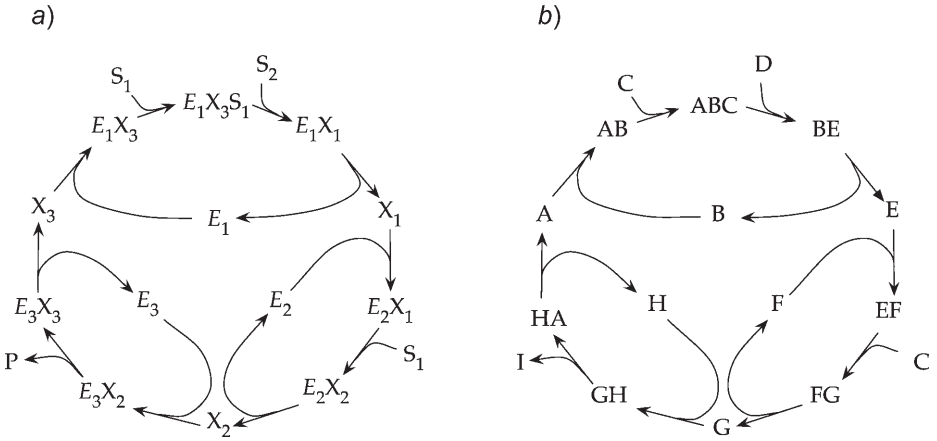

Fig. 3. *The urea cycle as a sequence of chemical reactions. a*) Substrates are represented as S_1 and S_2, the product as P, metabolic intermediates as X_1, X_2 and X_3, and enzymes as E_1, E_2, and E_3. *b*) The cycle is redrawn with all 'interpretation' of the roles of the different elements eliminated.

the overall process, whereas each of the others has a stoichiometric coefficient of zero. The net effect of the cycle can, therefore, be written as $2\,C + D \rightarrow I$, or, in the terminology of *Fig. 2*, *a*, as $2\,NH_3 + CO_2 \rightarrow$ urea. It may be objected that this equation does not balance, but this is simply because we are ignoring the production of H_2O. Allowing for this is more complicated than just including H_2O in the mechanism (and more complicated than is useful for this discussion), because H_2O is not produced as a discrete molecule, but as the net effect of several H^+ transfers; and, in any case, we are ignoring the true states of NH_3 and CO_2, which both exist as mixtures of different ionic states.

There is thus no rational basis for a classification that puts citrullinase, arginine deiminase, and arginase in a different class from ornithine, citrulline, and arginine: the first three are catalysts, and the last three are three different states of a fourth catalyst. It could perhaps be argued that, even if there is no qualitative difference between these two sets of three, there is a quantitative difference, because the three 'enzymes' participate in three small cycles of three steps each, whereas the three 'metabolites' participate in one grand cycle of ten steps. However, quite apart from the arbitrary nature of this distinction, it does not correspond to biochemical reality, because some enzymes catalyze reactions with very large numbers of substrates, and participate in cycles of many steps; cyclosporin synthetase, for instance, is a single molecule that participates in a catalytic cycle of at least 40 steps[4]) [25][26].

Even with proteins, there is more arbitrariness than is usually recognized about which are considered enzymes and which are not. The cytochromes, for example, are not normally regarded as enzymes, because the reactions in which they are oxidized are traditionally regarded as separate from the reactions in which they are reduced; however, in reality, these just supply two halves needed to complete cycles: ferrocytochrome c and ferricytochrome c together constitute 'an enzyme' that is just as real as the pyridoxal phosphate and pyridoxamine phosphate forms of a protein that constitute the two states of a transaminase. Any enzyme following a substituted-enzyme ('ping pong') mechanism – and if we accept the view of *Spector* [27], this means any enzyme at all – can be regarded as an acceptor–donor pair, or as a pair of metabolites, with just as much justification as is used to exclude the cytochromes from the class of enzymes.

A More Complicated (*M*,*R*)-System. – *Fig. 3*, *b*, as it stands does not constitute an (*M*,*R*)-system, because it makes no allowance for the finite lifetimes of the catalysts. Even if (returning to the initial idea that this represents the urea cycle) we postulate that ornithine, citrulline, and arginine are stable for indefinite periods, we cannot reasonably suppose this for the other three. Thus, we must allow for possible decay of the catalysts, which implies that we need to incorporate replacement mechanisms. This is done in *Fig. 4*, with the assumption that only one new external metabolite (J) is needed. We make no pretense that this model is a realistic model of a real organism: we give this example only to show that the approach that allowed construction of the much

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[4]) Arguably, there are many more than 40 steps, because each of the 'partial reaction steps' that *Lawen* and *Zocher* [25] list is a composite process involving several of what we would call 'steps' in the context of this discussion.

simpler model of *Fig. 1* can be applied to more complicated cases. We also make no pretense that there was anything inevitable about the particular additions that are made to *Fig. 3*,*b* to convert it into *Fig. 4*: it could have been done without introducing any new external metabolite, or by introducing more than one, and there were many arbitrary decisions as to which metabolites participate in which cycles. The point is that once a particular chemical process has been specified, the implication is that all of the reactions shown are nothing more or less than the chemical properties of the molecules included.

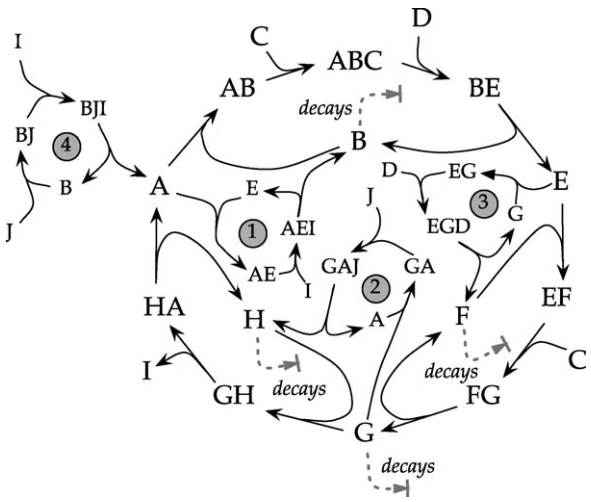

Fig. 4. *A more complicated (*M,R*)-system*. The model of *Fig. 3*,*b* is extended by the inclusion of one new external metabolite, J, four decay reactions to take account of the finite lifetimes of the catalysts, and four additional cycles labeled kB = 1 to kB = 4, to allow for replacement of the catalysts. With these changes, the model of *Fig. 3* becomes an (*M*,*R*)-system. As A, E, and G are different states of the same catalyst, the decay arrow leading from G is sufficient to take account of the decay of all three.

The essential characteristics of the models in *Figs. 1* and *4* are that a finite number of external metabolites can be used to construct a system in which all intermediates are allowed to decay, or otherwise to disappear from the system, and all can be replaced. In Aristotelean terms, the system requires only a limited set of material causes, but, although it thus is *open to material causation* [18], it is *closed to efficient causation*. The distinction here is important, because a system that is closed to material causation cannot satisfy the thermodynamic requirements for the bioenergetic functions of a living organism, whereas a system that is open to efficient causation cannot be organizationally invariant. Just as in the simpler example, closure was made possible by assuming that at least some of the catalysts fulfill more than one role: A, for example, is required not only for the main ('metabolic') cycle, but also for the replacement of the catalyst B. The phenomenon of 'moonlighting' is noteworthy in this connection: many proteins have functions in addition to those that are regarded as their primary roles [28][29]. It has been known for many years, for example, that several of the proteins

that constitute the lens of the vertebrate eye are none other than glycolytic enzymes, and the number of such examples known is continually increasing. We consider that this is more than just an interesting fact about living organisms, because it is an *essential* property if they are to achieve closure. Even though the specific cycles that we propose in *Fig. 4* are arbitrary (and could not be justified in terms of the known chemistry of the participants in the urea cycle), the fact that they involve multifunctionality is not.

Organizational Invariance? – The point that must be considered now is whether either of these models can be considered to be organizationally invariant. We do not need to study all the intricacies of *Fig. 4* to address this question, because the much simpler model of *Fig. 1* is sufficient. The point of introducing *Figs. 2–4* was primarily to use a real biochemical example, the urea cycle, to establish the argument that the usual distinction between enzymes and metabolites (and, in the modern fashion, between *proteome* and *metabolome*) is arbitrary, and that the real distinction is between a relatively small number of external material causes and a large number of internal metabolites that fulfill (in many cases) catalytic functions in addition to any others they may have. Ornithine as a catalyst for the formation of urea is not unique, but is just one of many possible examples in metabolism; another is oxaloacetate, which, as a participant in the tricarboxylate cycle, catalyzes the oxidation of pyruvate.

Most 'metabolites' are, thus, in some sense 'enzymes', and a classification that is useful in one context should not be unthinkingly extended beyond its proper domain. To take another example, it is useful in physiology to distinguish metabolites that participate in many different reactions, such as NAD and pyridoxal phosphate, from ones that participate in very few, such as ethanol. This kind of classification is the basis of the 'small-world' view of metabolism [30], but it has no meaning in the context of a single enzyme, and to say that ethanol is the 'substrate' of alcohol dehydrogenase, whereas oxidized NAD is its 'coenzyme', is positively misleading if one is simply concerned with mechanistic or kinetic studies of the enzyme: both are substrates; neither is more fundamental than the other, and both are required for the reaction to take place [31]. It is not important for this purpose to know whether all enzyme mechanisms involve covalent changes to the enzyme molecule [27]: even if one allows that an enzyme may sometimes simply provide a surface on which the chemical changes to the reactants can occur without any covalent changes to the enzyme molecule itself, it is still true that mere binding of a reactant to the active site reflects a chemical property of the enzyme molecule. Isolation of a covalent enzyme–substrate complex, as was done long ago for threonyl-tRNA synthetase [32], for example, is an important step in understanding an enzyme mechanism, but it is less important for a general discussion of metabolic organization.

We therefore need to ask whether 'catalysis' is a fundamental property of nature, or whether it is just a convenient shorthand way of summarizing the net effects of cycles of reactions. It is simply a fact of chemistry that ornithine is capable of reacting with NH_3 and CO_2 to produce citrulline, that citrulline is capable of reacting with NH_3 to produce arginine, and that arginine is capable of decomposing to ornithine and urea. No catalysts, apart from the ornithine–citrulline–arginine trio itself, are necessary, though admittedly the cycle would proceed very slowly without any. No laws of chemistry are violated, therefore, by drawing the urea cycle as shown in *Fig. 5*. In this particular case,

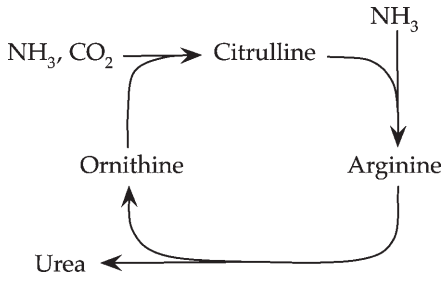

Fig. 5. *The urea cycle without enzymes.* This is the same as *Fig. 2,a*, except that the enzymes are omitted. The cycle is chemically possible (and would operate at a low rate) without the presence of any proteins.

we are not aware of experimental measurements of how fast the three reactions would proceed in the absence of protein catalysts, but there are others in biochemistry where the principle has been thoroughly established. For example, many reactions commonly attributed to enzymes that use pyridoxal phosphate as cofactor proceed at readily measurable rates in the presence of pyridoxal phosphate without any protein [33].

However, if we accept that the capacity of arginine to catalyze the conversion of NH_3 and CO_2 to urea is just a chemical property of arginine, why should we treat the molecule named 'arginase' in *Fig. 2,a* any differently? We know that in modern organisms this molecule is a protein, but in a primordial organism, it could have been something much simpler; and in any case, even if it is a protein, its capacity to promote the decomposition of arginine is explained in the same way, as a capacity to participate in a cycle of reactions that regenerate it unchanged, while converting arginine into ornithine and urea.

Fig. 1,b is, therefore, not just a more explicit version of *Fig. 1,a*. It contains additional information. Thus, redrawing *Fig. 1,b* as *Fig. 1,a* implies *loss of information*, and the information that is lost is precisely the information that defines how the system is organized. As long as the model is presented only in the summary form there are uncertainties about which molecule catalyzes which reaction: with three reactions and three candidate catalysts, there are 3^3 possibilities and no unambiguous way of choosing between them, as we discussed in the *Introduction*. However, in the complete form of the model, with all steps shown in chemically explicit form and with no catalysts, there is only one possibility, and the system is seen to be organizationally invariant. The extent to which this argument eliminates possibilities increases extremely steeply (much more than exponentially), as the size of the model increases: for example, if the model of *Fig. 4* is considered in summary form (*Fig. 6*), there are potentially $7^6 =$ 117,649 assignments of catalysts to reactions, and this large number is negligible in comparison with the number of possibilities that would potentially apply to whole organisms.

Discussion. – The argument that we have tried to defend in this paper is that the apparent difficulty of achieving organizational invariance in an (*M*,*R*)-system is an artifactual consequence of treating catalytic effects as 'black boxes'. As long as catalytic

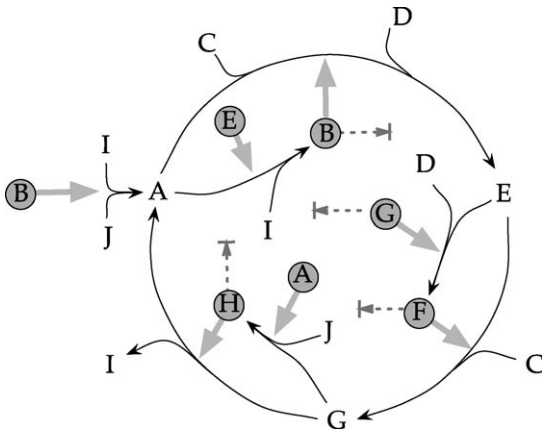

Fig. 6. *Summary of the more complicated (*M,R*)-system*. Here the model of *Fig. 4* is drawn with the replacement cycles shown as catalyzed reactions, the catalytic effects being represented by thick gray arrows, and decay reactions indicated by dashed gray arrows. There are seven reactions to be catalyzed, and six potential catalysts, so, if the fuller information given in *Fig. 4* is not available, the particular arrangement of catalysts appears to be an arbitrary choice out of $7^6 = 117{,}649$ possibilities.

effects are treated as they really are, as cycles of chemical reactions, then there is no mystery and no problem to be solved. In *Figs. 1*,*b* and *4* there are no catalysts, and, therefore no decisions need to be made about which molecules catalyze which reactions. Discussing metabolism in terms of catalytic effects of enzymes is convenient as a way of summarizing large amounts of information, of course, but it would be perfectly possible to draw any of the standard metabolic charts without invoking catalysis at all, and, indeed, the first attempts to model metabolic systems with a computer [34] were done in just that way; it was cumbersome, and it involved a great deal of unnecessary work for both operator and machine, but it worked.

Whether or not the particular way of achieving organizational invariance that we suggest in this paper is accepted, the question is an important one to address. *Rosen*'s conception of the organizationally invariant (*M*,*R*)-system was a major intellectual achievement, but before it will be accepted by biologists as a credible picture of the living organism, it will have to be shown in chemical terms how it might work, as abstract mathematical arguments carry little weight with experimental biologists. In our earlier studies [9][18], we made some progress in explaining what an (*M*,*R*)-system might look like, but we did not succeed in showing how it could be organizationally invariant.

We have suggested that multifunctionality is an essential part of achieving metabolic closure, or avoiding the infinite regress that is otherwise implicit in the need for every replacement module to have in turn its own replacement module. Examination of *Fig. 4* suggests that multifunctionality must occur on a vast scale: it would not be possible to achieve closure with just one or two molecules fulfilling two functions each. Seen in this light, the number of 'moonlighting' proteins known [28][29] is rather small and falls far short of what is actually needed to create an (*M*,*R*)-system of significant size. How do real organisms overcome this problem? One way is that the

protein-synthesizing apparatus of any organism attributes a large number of functions to a small number of molecules. In a bacterial cell, all of the genetic information is stored in a single molecule, and a modest number of enzymes is sufficient to catalyze the synthesis of all of the proteins the cell needs, including the enzymes needed for protein synthesis themselves. This can be regarded as multifunctionality on a very large scale.

REFERENCES

[1] E. Schrödinger, 'What is Life?', Cambridge University Press, Cambridge, 1944.
[2] R. Rosen, *Bull. Math. Biophys.* **1958**, *20*, 245.
[3] R. Rosen, *Bull. Math. Biophys.* **1958**, *20*, 317.
[4] R. Rosen, *Bull. Math. Biophys.* **1959**, *21*, 109.
[5] R. Rosen, *Bull. Math. Biophys.* **1966**, *28*, 149.
[6] R. Rosen, *Bull. Math. Biophys.* **1967**, *29*, 91.
[7] R. Rosen, *Bull. Math. Biophys.* **1971**, *33*, 303.
[8] R. Rosen, in 'Foundations of Mathematical Biology', Ed. R. Rosen, Academic Press, New York, 1972.
[9] J.-C. Letelier, J. Soto-Andrade, F. Guíñez Abarzúa, A. Cornish-Bowden, M. L. Cárdenas, *J. Theor. Biol.* **2006**, *238*, 949.
[10] R. Rosen, 'Life Itself: A Comprehensive Inquiry into the Nature, Origin and Fabrication of Life', Columbia University Press, New York, 1991.
[11] R. Rosen, 'Essays on Life Itself', Columbia University Press, New York, 2000.
[12] T. Dobzhansky, *Amer. Biol. Teacher* **1973**, *35*, 125.
[13] M. Eigen, P. Schuster, *Naturwissenschaften* **1977**, *64*, 541.
[14] V. Ratner, A. A. Zharkikh, N. Kolchanov, S. N. Rodin, V. V. Solovyov, A. S. Antonov, 'Molecular Evolution', Springer, Berlin, 1996.
[15] H. R. Maturana, F. J. Varela, 'Autopoiesis and Cognition: the Realisation of the Living', D. Reidel Publishing Co., Dordrecht, The Netherlands, 1980.
[16] S. Kauffman, 'The Origins of Order: Self-Organization and Selection in Evolution', Oxford University Press, Oxford, 1993.
[17] F. Morán, A. Moreno, E. Minch, F. Montero, in 'Artificial Life V: Proceedings of the Fifth International Workshop on the Synthesis and Simulation of Living Systems', Eds. C. G. Langton, K. Shimohara, MIT Press, Cambridge MA, 1996, p. 255.
[18] A. Cornish-Bowden, M. L. Cárdenas, J.-C. Letelier, J. Soto-Andrade, *Proteomics* **2007**, *7*, 839.
[19] J. Al-Janabi, J. A. Hartsuck, J. Tang, *J. Biol. Chem.* **1972**, *247*, 4628.
[20] J.-J. Berzelius, *Ann. Chim. Phys.* **1836**, *61*, 146.
[21] E. Fulhame, 'An Essay on Combustion, with a View to a New Art of Dying and Painting, wherein the Phlogistic and Antiphlogistic Hypotheses are Proved Erroneous', published by the author, London, 1794.
[22] V. Henri, 'Lois Générales de l'Action des Diastases', Hermann, Paris, 1903.
[23] H. P. Barendrecht, *Biochem. J.* **1913**, *7*, 549.
[24] T. Pfeiffer, I. Sánchez-Valdenebro, J. C. Nuño, F. Montero, S. Schuster, *Bioinformatics* **1999**, *15*, 251.
[25] A. Lawen, R. Zocher, *J. Biol. Chem.* **1990**, *265*, 11355.
[26] H. Kleinkauf, H. von Döhren, *Eur. J. Biochem.* **1996**, *236*, 335.
[27] L. Spector, 'Covalent Catalysis by Enzymes', Springer-Verlag, New York, 1982.
[28] K. F. Tipton, M. I. O'Sullivan, G. P. Davey, J. O'Sullivan, *Biochem. Soc. Trans.* **2003**, *31*, 711.
[29] G. Sriram, J. A. Martinez, E. R. B. McCabe, J. C. Liao, K. M. Dipple, *Am. J. Hum. Genet.* **2005**, *76*, 911.
[30] A. Wagner, D. Fell, *Proc. R. Soc. London, Ser. B* **2001**, *280*, 1803.
[31] A. Cornish-Bowden, 'Fundamentals of Enzyme Kinetics', 3rd edn., Portland Press, London, 2004, p. 158.

[32] J. E. Allende, C. C. Allende, M. Gatica, M. Matamala, *Biochem. Biophys. Res. Commun.* **1964**, *16*, 342.
[33] E. E. Snell, S. J. Di Mari, in 'The Enzymes', 2nd edn., Ed. P. D. Boyer, Academic Press, New York, 1970, Vol. 2, p. 335.
[34] D. Garfinkel, B. Hess, *J. Biol. Chem.* **1964**, *239*, 971.

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