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PUTTING THE SYSTEMS BACK INTO SYSTEMS BIOLOGY

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ABSTRACT In recent years the term “systems biology” has become widespread in the biological literature, but most of the papers in which these words appear have surprisingly little to do with older notions of biological systems: they often seem to imply little more than reductionist biology applied on a large scale, with a little attention to interactions between some of the components, but with minimal attention to the kinetic properties of enzymes, which supplied much of the reductionist foundation of biochemistry. A systemic approach to biology ought to put the emphasis on the entire system; insofar as it is concerned with components at all, it is to explain their roles in meeting the needs of the system as a whole. Genuinely systemic thinking allows us to understand how biochemical systems are regulated, and why clumsy attempts to manipulate them for biotechnological purposes may fail. At a more abstract level, it is necessary for understanding the nature of life, because as long as an organism is treated as no more than a collection of components, one cannot ask the right questions, and certainly cannot answer them.

Now after all this that hath been said, I cannot but hope that Those many False and Ignorant Outcries against the Lute will be laid aside, and deem'd (as indeed they are) False.

I will here Name some of Them;

First, That it is the Hardest Instrument in the World.

Secondly, That it will take up the time of an Apprenticeship to play well upon It.

Thirdly, that it makes Young People grow awry.

Fourthly, That it is a very Chargeable Instrument to keep; so that one has as good keep a Horse as a Lute, for Cost.

Fifthly, that it is a Womans Instrument.

Sixthly, and Lastly, (which is the most Childish of all the rest) It is out of Fashion.

I will give here a short (but True) Answer to each of These Aspersions.

—Thomas Mace (1676)

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WRITING IN 1676, THOMAS MACE was concerned with the playing of the lute, not the kinetic study of enzymes, but most of the objections that he lists (apart from the tendency to make young people grow awry) have their parallels in the modern habit of thinking that biochemistry can be taught and practiced without giving serious attention to metabolism and enzymology. The study of kinetics has always been regarded as difficult; there are those who think that it takes too much student time to be taught properly; and in recent years it has come to be unfashionable. Mace described the idea that the lute is difficult to play as “that *Flim-Flam-Ignorant saying of the Vulgar*”; anyone who has seriously studied the kinetic behavior of enzymes will feel, similarly, that the difficulties are greatly exaggerated. In any case, one can hardly expect to make significant advances in any domain of knowledge if its classical foundations are ignored. Over the past decades the effects of ignoring classical enzymology in the stampede into molecular biology can be seen in the failure to achieve the promised benefits of biotechnology as easily and as rapidly as many early enthusiasts had expected.

GENOME STUDIES AND BIOTECHNOLOGY

The disappointing results of efforts in biotechnology can be attributed to their having been based on a false premise, and the main purpose of this essay will be to discuss this false premise. First, however, I should mention the failure of genome sequencing to provide benefits commensurate with the enormous financial investment. Although it might be argued that there has not been enough time to see the benefits, this argument must eventually wear thin. In any case, a long list of gene sequences is barely more useful in itself than a list of all the telephone numbers in a city would be if it contained no information about which telephone corresponded to any given number. A telephone directory that contained no information at all about the telephones corresponding to half of the numbers, together with plausible and mainly correct guesses about the other half, would be better than nothing, but it would be far from an ideal tool for making the best use of a telephone. This analogy describes quite well the genome data for organisms that have hardly been studied at all in biochemical terms, such as *Treponema pallidum*, the organism responsible for syphilis. The functions of perhaps half of the genes of *T. pallidum* can be plausibly guessed by comparing their sequences with those of genes identified in other bacteria that have been more thoroughly studied from a biochemical point of view, such as *Escherichia coli*. This is useful, of course, but not as useful as it may appear at first sight, because it tells us more about the similarities between *T. pallidum* and *E. coli* than it does about their differences, whereas it is the differences that make *T. pallidum* worthy of sequencing in the first place—after all, it causes syphilis, and *E. coli* does not.

The point here is that when we want to understand why two systems behave differently we need to study the differences between them, but the futility of try-

ing to understand differences by looking at similarities is perhaps better illustrated with a kinetic example. A major point of interest of hexokinase D, the predominant isoenzyme of hexokinase in the mammalian liver, is that its kinetic properties are very different from the isoenzymes characteristic of brain, muscle, and other tissues: it is insensitive to its product glucose 6-phosphate at physiological concentrations, and instead of being saturated at physiological concentrations of glucose, it responds with kinetic cooperativity to variations in the physiological range (Cárdenas 1995). These properties suit it well for its role as a glucose sensor and also for regulating the rate of glycogen synthesis in the liver according to supply, unlike many biosynthetic processes, which are typically regulated according to demand for the end-product. These properties also explain why mutations of the relevant gene are dominant in maturity-onset diabetes of the young, in contrast to the usual recessive character of most genes for the enzymes of primary metabolism. Until recently, however, the only information about the three-dimensional structure of hexokinase D came from studies of yeast hexokinase, an enzyme with completely different kinetic properties. Such comparisons were useful for indicating that the chemical mechanism of phospho-transfer is essentially the same in all hexokinases—something worth knowing, certainly, but not in any way surprising—but they explained none of the properties that made hexokinase D interesting in the first place.

Returning to genome sequences, the problem is not so much that they contain no phenotypic information, but that we do not have reliable methods for undertaking all of the steps involved in deducing a phenotype from them (Cornish-Bowden and Cárdenas 2000). Present methods of sequence analysis allow a genome sequence to be converted into a list of genes without much difficulty, but transforming the list of genes into a list of enzymes has a high failure rate, perhaps 50%. In any case, however, this is only the beginning of the process. A list of putative gene products, or even a list of putative enzymes, is not a phenotype, and converting it into a phenotype requires construction of a plausible metabolic map, which then needs further work to convert it into a possible phenotype. Finally, the possible phenotype can only become a real phenotype when all relevant kinetic and regulatory properties are taken into account, together with information about how all the components are organized into a three-dimensional whole—even a four-dimensional whole, given that the times when different components are made may be just as important as where they are placed.

Current methods of sequence analysis are useful for beginning this process, and knowledge of classical biochemistry is useful for finishing it, but there are several intermediate stages that present more difficulty. This is especially true when dealing with an organism for which no classical biochemical measurements have been made, so that kinetic modeling is virtually impossible. This problem has led to the development of methods of stoichiometric analysis that seek to deduce as much as possible from the structure of a metabolic map alone, making no use of kinetic or regulatory information. In an application of this

approach, Stefan Schuster, David Fell, and Thomas Dandekar (2000) noted that the genome of *T. pallidum* contains genes for two different kinds of transketolase, an enzyme needed for energy management in many organisms, but no recognizable gene for a related enzyme known as transaldolase. This could be taken as evidence that the bacterium does not use the second kind of enzyme, except that the two reactions always occur together in other organisms, so that in terms of known metabolism neither can have any role in the absence of the other. This led Schuster and colleagues to conclude that the failure to identify genes for both enzymes in *T. pallidum* reflected incomplete information and not a genuinely missing enzymic activity.

THE FALSE PREMISE OF BIOTECHNOLOGY

But one thing is certain: to understand the whole you must look at the whole.

—**Henrik Kacser (1987)**

The forms of hexokinase that predominate in mammalian muscle and brain are among the enzymes that are often asserted in textbooks and even in review articles to “control glycolysis.” An even more popular candidate for the enzyme that supposedly fulfills this role is phosphofructokinase, but in any case the claim that any single enzyme controls a major process is largely a illusion, though it is one that underlies much of the effort in biotechnology over past decades. The idea, dating from the earliest studies of metabolic regulation in the 1950s and 1960s, is that any metabolic pathway has a definite “rate-determining step” catalyzed by a “key enzyme,” and that once this enzyme has been identified the techniques of genetic engineering allow it to be overexpressed, thereby increasing the metabolic flux through the pathway in question to any desired extent.

The theoretical reasons why this approach does not work have been known for 30 years (Kacser and Burns 1973), but even biotechnologists who are impatient with theoretical arguments may pay attention to the experimental observations that it does not work in practice, even in fermenting yeast. There has been widespread agreement in the biochemical literature that the enzyme that controls ethanol production in fermentation is phosphofructokinase. What could be more clear, then, that the rate of ethanol product could be increased by overexpressing phosphofructokinase? Clear or not, the appropriate experiments were first published two decades ago, however, and showed that there is now no justification for continuing to believe that the naive expectation is the correct one: a 3.5-fold increase in the phosphofructokinase activity in fermenting yeast not only does not lead to a 3.5-fold increase in the rate of ethanol production, it does not have any detectable effect at all on this rate (Heinisch 1986). This result might just reflect some peculiarity of yeast, except that essentially the same observations have now been made in several very different organisms, including potatoes, mice, and bacteria.

Part of the reason why overexpressing a supposedly rate-determining enzyme fails is that organisms have feedback regulatory mechanisms to ensure that metabolic fluxes are suited to the needs of the organism, not to those of an external agency, such as a biotechnologist. This immediately suggests a different way of making organisms satisfy biotechnological ends: using genetic manipulation to eliminate the feedback loops. However, this also fails to have the desired effects, for reasons discussed later.

Yeast also illustrates why another promise of the genomic revolution has proved to be false. It was realized, of course, that sequencing genomes would reveal the existence of many previously unknown genes—this was, after all, a major reason for undertaking the sequencing in the first place. It was widely assumed, however, that the function of a previously unknown gene could be identified, or at least suggested, by doing appropriate genetic experiments to delete it from the genome and seeing what happened. In practice this rarely works, as a high proportion, maybe as many as 80%, of genes in yeast are “silent”: when one of them is deleted the organism grows and multiplies quite normally, at the normal rate, and the rates of any metabolic processes that are measured usually prove to be normal as well (Cornish-Bowden and Cárdenas 2001a).

Escaping the false line of reasoning requires a different way of looking at biological systems that incorporates the idea that they are more than just collections of components. The major conceptual advance was made by Henrik Kacser and Jim Burns (1973) in Edinburgh, with important contributions a little later from Reinhart Heinrich and Tom Rapoport (1974) in Berlin, with similar ideas developed in parallel by Michael Savageau (1976) in Ann Arbor. Instead of a single enzyme that all by itself regulates the flux of metabolites through a pathway, flux control is a property that is shared unequally among all the enzymes in the metabolic system.¹ As a typical cell contains thousands of different enzymes, this implies that the average share held by any enzyme is less than 0.1% of the total. This in turn suggests that varying the activity of a randomly selected enzyme (or the expression level of its gene) by a small amount should typically have no detectable effect on any flux that we measure. Even a large increase in activity will typically have no measurable effect (as with the 3.5-fold increase in phosphofructokinase activity in fermenting yeast mentioned earlier). These considerations do not make it impossible for all control to be concentrated in one enzyme, at least over a limited range of its activity, but they imply that this will be exceptional.

A minor complication needs to be mentioned. By considering the average

¹In the context of multi-enzyme systems, the word *flux* is commonly used for the system property that corresponds to the rate of reaction of a single enzyme. In an unbranched section of a pathway in steady state, the flux through each reaction is, of course, the same as the rate of the same reaction, but it is still useful to use different words to make it clear whether the reference is to a property of the system as a whole or to a property of a particular enzyme within it.

share of control, we are implicitly assuming that all the shares have positive values, because if large negative shares are allowed there can be more than one enzyme with a large positive share. More detailed analysis shows that although negative shares are not impossible, they are usually not large or numerous enough to destroy the argument: they just make it a qualitative argument that is not quantitatively exact.

The effects of large decreases in enzyme activity are more complicated, as there are various points to take into account. Typically the share of flux control held by any enzyme increases when the enzyme activity decreases, and may approach 100% when the activity approaches zero. If this happens, a particular metabolic activity will be eliminated if the enzyme activity is eliminated, but that does not necessarily imply that the organism will not continue to grow normally, because organisms have alternative ways of achieving their needs. If the flux in question is just the rate of growth, the proportion of absolutely essential genes is low; hence the large number of silent genes in yeast. With more specific fluxes, such as the rate of producing a pigment that is desirable but not essential for life, eliminating a gene necessary for producing it may have no observable effect on growth rates, but it will still usually have an easily observable effect on the appearance of the organism. Even for essential genes, large decreases in expression levels may have little or no observable effects. In diploid organisms, for example, the expression level of the protein coded by the unique copy of a gene in a heterozygote will typically be about half of that in the normal homozygote, but fluxes that depend on the protein may be so little changed that they appear to be normal. According to Kacser and Burns (1981), that is why so many mutations are observed to be recessive. Their argument provides a far simpler interpretation of genetic recessivity than the ideas of Ronald Fisher (1928a, b) in terms of “modifier genes,” which were universally accepted as correct for many years, and which are still taught in genetics courses even today, if only for their historical importance. Fisher considered that dominance and recessivity were evolved properties that were needed to avoid what would otherwise be the deleterious effects of having low doses of gene products.

Reviewing the history today (Cornish-Bowden and Nanjundiah 2006), it is hard to escape the feeling that Fisher was catering to people’s need for a difficult and complicated explanation, even though Sewall Wright (1929, 1934) was already offering one much closer to that of Kacser and Burns. Even earlier, George Shull (1909), who helped to revolutionize 20th-century agriculture with the introduction of hybrid corn, was describing the phenomenon in the following words: “when there is complete dominance of presence over absence, it may mean that already the presence of the one unit A of the heterozygote is sufficient to result in the maximum reaction, in which case the doubled factor AA of the positive homozygote can do no more.”

THE BIRTH OF “SYSTEMS BIOLOGY”

If someone were to analyze current notions and fashionable catchwords, he would find “systems” high on the list.

—Ludwig von Bertalanffy (1969)

After a slow start, the ideas of Kacser and Burns (1973) are gradually coming to be accepted as the appropriate basis for analyzing the kinetic behavior of multienzyme systems. What, however, does this acceptance of one kind of systemic view of biology have in common with the sudden vogue for “systems biology” on the one hand, or with the older ideas of systems theory advocated by Ludwig von Bertalanffy and Robert Rosen on the other?² At the moment it would seem that there is remarkably little connection with either. Bertalanffy (1969) in particular laid great stress on the need to see systems as wholes, and he was critical of what he saw of the ever-increasing specialization of modern science, “necessitated by the enormous amount of data,” yet the very popularity of “systems biology” is itself a product of the amount of data generated by genome sequencing—enormous beyond anything that Bertalanffy encountered. Bertalanffy’s major concern was to emphasize the essential Unity of Science (his capitals), which he thought was granted “not by a utopian reduction of all sciences to physics and chemistry, but by the structural uniformities of the different levels of reality.” He was skeptical of reductionist ideas, and these played little part in his thinking. In practice it has proved difficult for others to build on his work.

When I started thinking about this essay in 2003, I was already thinking that “systems biology” was a vogue term, but the vogue that existed then was as nothing compared to what it has become. More than half of the 600 or so publications one can find by searching for this combination of words at the end of 2005 date from 2005, and much more than half of the remainder from 2004. This sudden apparent enthusiasm for systemic ideas after many years in the wilderness must, however, offer little comfort to those who have thought in terms of systems for a long time, because “systems biology” in current practice is not easy to distinguish from old-style reductionist biochemistry applied on an ever-larger scale. The main novelty is the recognition that many aspects of cell biology have to be understood in terms of interactions between two or more proteins, or two or more other entities: this is certainly an advance on the way of thinking that characterized much of classical biochemistry, but it is still far from an appreciation of systems as systems.

A simple example of the importance of system-level thinking is provided by feedback inhibition of a biosynthetic pathway by its end-product, a type of metabolic regulation described in any textbook of biochemistry. For example, aspartokinase (which in some organisms is a mixture of two or more isoenzymes

²The quotation marks are to stress that much of the work that currently goes under the name of “systems biology” hardly merits this name.

with different regulatory properties) catalyzes the first step in the interconversion of the amino acids aspartate and lysine, and it is inhibited by lysine. As drawn in most textbooks, the pathway ends there, at lysine, but that representation leaves much unclear, because it fails to indicate why the cell needs to make lysine in the first place, or what purpose is served by having the first enzyme in the pathway inhibited by lysine. The point that is left unstated, and that some will consider too obvious to need stating, is that lysine is needed for protein synthesis; more generally, the “end-product” in almost any biosynthetic pathway is not the end of anything, but just the link between two processes. People who claim that that is obvious may then go on to claim that it is also obvious that feedback inhibition of aspartokinase is needed to ensure that lysine is synthesized at the rate needed for protein synthesis, neither faster nor slower. This second point is not only not obvious, it is not even true, because if regulation of rates were the only thing needed, it could be perfectly well satisfied by the ordinary product inhibition backwards through the pathway that arises from the everyday properties of virtually all enzymes. As long as rates are the only concern, no feedback loop is needed, but it becomes absolutely necessary when one recognizes that metabolite concentrations need to be taken into account as well: without feedback inhibition (and other classical regulatory mechanisms), flux control would be achieved at the expense of huge uncontrolled variations in the concentrations of intermediates (Cornish-Bowden and Cárdenas 2001b); these would normally be very harmful, even lethal, to the organism, and explain why suppressing regulatory mechanisms is not normally a useful way of achieving biotechnological aims. All of this can be summarized by saying that the regulation of most biosynthetic metabolic pathways can be understood in terms of ideas of supply and demand, with the benefit that in general economic laws work much better in metabolism than they do in economics (Hofmeyr and Cornish-Bowden 2000).

GENERAL SYSTEMS THEORY

What about the influence of Bertalanffy’s view of systems on Kacser and Burns and their more recent followers? As long as one confines oneself to general remarks there may seem to be some influence, but when one examines the major modern systemic approaches in detail they prove to be entirely mechanistic. Carl Woese (2004) makes a useful distinction between what he calls “empirical reductionism” and “fundamentalist reductionism,” the former being purely methodological, a way of analyzing systems into their component parts in order to understand them better. Reductionist ideas played little part in Bertalanffy’s thinking, but Kacser and Burns, as well as Heinrich, and Rapoport, and Savageau, are empirical reductionists in Woese’s sense, as they explain the properties of systems essentially as the sums of the properties of their components. Savageau (1990), however, makes the valuable point that “any respectable reductionist is also a reconstructionist”: it is not enough to show how a system can be reduced

to its components, one should also show that the components can be put together again to make a functional whole. He adds, however, that “the problem is that the reconstructionist phase of this program is seldom carried out.”

By contrast, fundamentalist reductionism is essentially metaphysical, a belief about the nature of that living organisms, that they can be completely understood in terms of the properties of their components. Anything else may be, and has been, derided as a return to vitalism, but Robert Rosen in particular has argued very strongly that there is a third way of interpreting life that is neither vitalism nor exclusively mechanism, but is complexity. Before discussing Rosen’s view, however, a detour is necessary to outline Erwin Schrödinger’s question about the nature of life, and his efforts to answer it.

“WHAT IS LIFE?”

We must therefore not be discouraged by the difficulty of interpreting life by the ordinary laws of physics. For that is just what is to be expected from the knowledge we have gained of the structure of living matter. We must also be prepared to find a new type of physical law prevailing in it. Or are we to term it a non-physical, not to say a super-physical, law?

—Erwin Schrödinger (1944)

Schrödinger’s book *What Is Life?* (1944) was enormously influential in persuading physical scientists such as Max Delbrück that biology had something to attract their interest and attention. Others, such as Linus Pauling and Max Perutz, were far more skeptical about its value, at least when they came to look back on it long afterwards. Pauling (1987), for example, gave the highest praise to Schrödinger for supplying the theoretical basis for all of chemistry, but answered the question of whether he had contributed anything to our understanding of life by saying that “it is my opinion that he did not make any contribution whatever.”

Anyone coming to Schrödinger’s book for the first time more than half a century after it was written is likely to find it a mixture of the obvious, the familiar, and the bizarre. His main points illustrate this: that biological systems obey the laws of physics has been accepted by almost everyone since vitalism was overthrown by Eduard Buchner (1897) at the end of the 19th century; regarding genes as aperiodic crystals seems an unhelpful way of looking at something that is now understood in great detail, and understanding of inheritance was not much illuminated by this description even in 1944; that organisms feed on negative entropy seems an unnecessarily poetic way of saying that organisms are open systems that maintain themselves far from equilibrium by exporting their entropy production to the environment.

In 2006, however, we cannot put ourselves into the minds of Schrödinger’s audience in the Dublin of 1944; in any case, he has another point that may well be more important than any of those that I have so far mentioned, though it has sometimes been shrugged off by biochemists as an embarrassing excursion into

vitalism. As well as arguing that biological systems obey the laws of physics, the part that most everyone would now regard as too obvious to be worth saying, Schrödinger also suggested that new laws of physics, beyond those needed for physics itself, might be needed to explain biology. Seeing no difference between this and vitalism is failing to distinguish between necessary and sufficient conditions: it would certainly be vitalism to say that adherence to the laws of physics is not necessary for a biological system; it is much less clear that the known laws of physics are all that we need for understanding biology, or that admitting this amounts to vitalism.

ROBERT ROSEN AND “LIFE ITSELF”

Quite early in my professional life, a colleague said to me in exasperation, “The trouble with you, Rosen, is that you keep trying to answer questions nobody wants to ask.” This is doubtless true. But I have no option in this; and in any event, the questions themselves are real, and will not go away by virtue of not being addressed. This attitude, I know, has estranged me from many of my colleagues in the scientific enterprise, and has put me far from today’s “main stream.”

—Attributed to Robert Rosen by Judith Rosen (2004)

Although Schrödinger asks what life is, he does not try to answer his question in any depth. This task was taken up by Robert Rosen, who defined a living organism by saying that “a material system is an organism if and only if it is closed to efficient causation”; this, unfortunately, will seem obscure enough to readers well versed in Aristotelian categories of causation, and utterly unintelligible to everyone else. In his illuminating review of Rosen’s book *Life Itself* (1991), Cliff Joslyn (1993) explains that it means that no production rule in an organism is given from outside: all must be generated from within the organism, as anything else will result in an infinite regress. In biochemical terms, a “production rule” can be equated with an enzyme that determines what reactions a given metabolite can undergo, so Rosen can be understood as saying that all of the catalysts necessary for an organism to live need to be produced by the organism itself.

The essential difference between an organism and a machine can be explained without resorting to mathematics or philosophy. Although usually ignored in analogies between machines and organisms, there is a gulf between the two that is so wide and so far from being bridged by any existing or currently conceivable machine that it is not absurd to regard it as unbridgeable. This point was taken up recently by Woese (2004), but it has had little impact on biology in general, which continues to develop as if it were a branch of engineering. Both organisms and machines consist of components with finite lifetimes that need to be repaired or replaced when they wear out. The lifetime of a modern throw-away machine may be the same as that of its weakest component, but for more classical machines, and for all machines in principle, if not in practice, the lifetime of the machine is longer, often very much longer, than the lifetimes of some

of its components. Although some very advanced modern machines (such as the computer on which I am typing this essay) may include some internal checking to alert their operators to faulty parts that need to be replaced, the actual maintenance requires external intervention, and in any case no machine keeps track of the state of repair of all of its parts.

Yet that is what a living organism does. Not only does it make itself (something no existing machine can do), but it also monitors the working state of all of its parts, and replaces those that need replacing, all of this being done from within. As we know, in good conditions a human being typically lives for about 70 years (even without the intervention of modern medicine, which is, of course, external), while containing necessary components with lifetimes in the range of minutes. This is a discrepancy of well over six orders of magnitude in the lifetime of the organism compared with that of its parts. Proteins vary considerably in their lability, and in many cases they are degraded as a result of specific catalyzed processes and not just by being worn out, but, even if this is ignored, none survive completely unchanged for decades. Some proteins, such as the crystallins of the vertebrate eye, are never replaced, and remain in use for 90 years or more, but this does not mean that they remain in perfect condition and suffer no damage. On the contrary, cataract is just the most obvious indication that crystallins do not survive unchanged for decades. The difference for crystallins is that the organism can survive without repairing the damage, whereas the overwhelmingly more usual case is that damage needs to be repaired at a rate essentially the same as the rate at which it occurs.

Understanding how this maintenance is achieved is a huge problem, and even if we restrict attention to the purely chemical part of what it means to be alive, that is to say to metabolism, it is still a huge problem. The chemical reactions that constitute metabolism require enzymes to catalyze them, and these enzymes survive for periods that are several orders of magnitude shorter than the period in which the metabolism continues to function normally. They therefore need to be replaced. (Rosen referred to “repair” rather than replacement, but that was an unfortunate choice of term, especially now that we know of many examples of genuine repair of nucleic acids, and a few examples of repair of proteins.) The enzymes themselves must therefore be regarded as metabolites, in other words, as products of metabolism, and other enzymes are needed to catalyze the replacement process. However, these other enzymes also have finite lifetimes, and also need to be replaced, in processes catalyzed by yet other enzymes, which that also need to be replaced, and so on for ever unless there is a way to close the circle. We therefore need a way of conceiving that the organization of metabolism is circular, so that at no point do we need to rely on any external help. This statement of the problem is probably clearer and easier to understand than the proposal of circular organization as a solution to it.

For almost all modern organisms a small amount of external help does exist, in the sense that apart from strict chemotrophs, we are all parasites, as we need

some of the products left by other organisms in order to survive. However, this dependence on other organisms solves only a tiny part of the problem, even for the most thoroughly parasitic of organisms, and it cannot even have solved a tiny part for the first organisms, which needed to survive in a world with no others to parasitize. Even the first organisms, of course, required some inorganic nutrients, just as all modern organisms do, so of course no organism is closed to material causation (and Rosen did not suggest that they were).

Having presented the problem without reference to Aristotle's four causes, we can now return to it to see how machines and organisms differ in terms of them.³ As Joslyn (1993) explains, we choose different categories of causation according to the answers we give to "why" questions. If we ask why a car engine produces water and carbon dioxide, an answer in terms of fuel and oxygen provides the material cause of the water and carbon dioxide. However, that is not the only possible correct answer: they are also caused by mixing and sparking the starting materials in the carburetor, and this is now an efficient cause. If we ask why the engine mixes fuel and oxygen and then ignites them, then answering that it is to provide power to drive the car forward appeals to a final cause, which for a machine, and indeed for everything else in classical Aristotelean philosophy, is always something outside the machine itself. The final cause remains essential for discussing engineering, but it has largely been banished from the modern scientist's view of the natural world, which has no room for an external designer with definite intentions. The fourth category is the formal cause, which concerns the essential nature of the process; it plays little role in Rosen's analysis.

If we ask similar questions about metabolism—for example, why glucose 6-phosphate is produced by most organisms in glycolysis (catalyzed by the enzyme hexokinase mentioned earlier)—then initially the argument runs in parallel to that for machines: glucose and ATP are the material cause of glucose 6-phosphate production, and hexokinase is the efficient cause. So far so good, but when we ask where the hexokinase comes from there is no final cause: it certainly does not come from outside the organism, so it must be produced from within. We can trace back material and efficient causes for that, but we never reach a final cause. This is what it means to say that organisms are closed to efficient causation. In admitting it to be true, however, we are stepping outside everything that we know about machines, and everything we can derive from classical philosophy.

³Aristotle's word αἰτία is usually translated as *cause*, but it is sometimes simply transliterated as *aitia*, to avoid confusion with the present-day idea of a cause, which corresponds more with the efficient cause than with the other three. This word is the origin of the English word *etiology*.

CONCLUSION

In an animal the fitness of the organization, and the motion wherein life consists, begin together, the motion coming from within; but in machines the force coming sensibly from without, is often away when the organ is in order, and well fitted to receive it.

—John Locke (1689)

We are still far from full and satisfying answers to the questions that Rosen asks about what makes a living organism alive. In general terms, Rosen's view of the essential difference between a machine and an organism was foreshadowed by John Locke more than three centuries ago, but his detailed analysis is mathematically demanding. It has been followed up by few other authors, and asserting that the organization is circular is not the same as explaining exactly how the circularity is achieved. The essential point is to recognize that there is a real problem—as he said, the questions are real, and will not go away by virtue of not being addressed—so it is not sufficient to say that we do not like Rosen's answer. If we regard it as unsatisfactory or just wrong, we need to propose an alternative; we cannot pretend that there is no question to be answered. In summary, the classical reductionist approach to science can be understood as a way of understanding the functioning of a whole system in terms of the properties of its parts, but now we must learn to understand the parts in terms of the whole (Cornish-Bowden et al. 2004). To make Rosen's ideas more easily intelligible to biologists, they will need to be put in the context of current knowledge of biology, and the limits within which his interpretation of the circular organization of living organisms can apply need to be specified (Letelier et al. 2006).

I have concentrated here on Robert Rosen's ideas, because of all the current approaches to our understanding of the nature of life they appear to me to be the most promising. Being also among the least well known, they are also more in need of development by other researchers than, for example, the self-reproducing automata of John von Neumann (1966), the autopoiesis of Humberto Maturana and Francisco Varela (1980), or the autocatalytic networks of Stuart Kauffman (1993). Autopoiesis is currently receiving much attention, especially from neuroscientists, and a possible synthesis of Rosen's and von Neumann's ideas may be found in an article by Jan-Hendrik Hofmeyr (2006). Rosen often seemed to be so anti-reductionist that he ignored the known facts of the structure and physical organization of biological organisms altogether: these aspects are far more prominent in Kauffman's approach.

A problem that worries some biologists is the lack of any mention of reproduction and evolution in Rosen's approach. Surely, they argue, these are the fundamental facts of life as we know it? Theodosius Dobzhansky's (1973) famous statement that "nothing in biology makes sense except in the light of evolution" is often quoted, because it expresses an idea that is central to modern biological thinking. Rosen's omission therefore appears serious at first sight, but until our early ancestors had solved the problem of staying alive—existing as organisms in

Rosen's sense—questions of reproduction and evolution could not arise. Dobzhansky's statement, important though it is, refers to what has happened since the first organism managed to stay alive.

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