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Review article

Contrasting theories of life: Historical context, current theories. In search of an ideal theory

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ABSTRACT

Most attempts to define life have concentrated on individual theories, mentioning others hardly at all, but here we compare all of the major current theories. We begin by asking how we know that an entity is alive, and continue by describing the contributions of La Mettrie, Burke, Leduc, Herrera, Bahadur, D'Arcy Thompson and, especially, Schrödinger, whose book *What is Life?* is a vital starting point. We then briefly describe and discuss (*M, R*) systems, the hypercycle, the chemoton, autopoiesis and autocatalytic sets. All of these incorporate the idea of *circularity* to some extent, but all of them fail to take account of mechanisms of metabolic regulation, which we regard as crucial if an organism is to avoid collapsing into a mass of unregulated reactions. In a final section we study the extent to which each of the current theories can aid in the search for a more complete theory of life, and explain the characteristics of metabolic control analysis that make it essential for an adequate understanding of organisms.

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1. General introduction

1.1. Definitions of life

Numerous books (Eigen and Schuster, 1979; Maturana and Varela, 1980; Rosen, 1991; Kauffman, 1993; Varela, 2000; Gánti, 2003, and others) present the various current theories of life. However, each of these concentrates on just one or two, largely to the exclusion of others, and there are no books known to us that compare the various theories and their relationships to one another.[1] Nor do they discuss what is missing from all of them.

In this section we describe older attempts to define life, including those of La Mettrie, Burke, Leduc, Herrera, D'Arcy Thompson, and especially Schrödinger, who set much of the agenda for later work. In Section 2 we summarize the principal current theories: (*M, R*) systems (Rosen, 1958b, 1991); the hypercycle (Eigen, 1971; Eigen and Schuster, 1977, 1978a,b); the chemoton (Gánti, 1971, 2003); autopoiesis (Maturana and Varela, 1973, 1980); and autocatalytic sets (Dyson, 1982; Kauffman, 1986, 1993; Friston, 2013). In Section 3 we compare them and discuss them in relation to what we think an ideal theory should contain, emphasizing that they all ignore the need for metabolic regulation.

1.2. The living state

Most modern biological scientists pay almost no attention to life as such, and, before describing investigation of life, Harold (2001) saw this as something to be regretted:

> As a subject for serious inquiry, the category "life" has all but vanished from the scientific literature; it is the particulars of life, not its nature, that fill the numberless pages of scientific journals.

Others, such as Atlan and Bousquet (1994), regarded the decline of interest in life as a natural development of biology and not something to be regretted:

> The basis of biology is physical chemistry. From the moment that one works in biochemistry and biophysics, and understands the physico-chemical mechanisms that account for the properties of living beings, life vanishes! Today molecular biologists have no need to use the word "life" in their work.

If biologists do not study life, what do they study? As Harold pointed out, biologists study, almost exclusively, details of living organisms, not life itself. The *Journal of Biological Chemistry* published 20 307 pages in 2018,[2] each of them packed with information, but virtually all of them concerned with small details of living organisms, not with living organisms as such, and none of them asking the question of what life is. The *Journal of Biological Chemistry* is the largest journal in its field, but it is far from being the only one: all the biochemistry journals put together would account for more than 60 000 pages per year, and all the biology journals together many more.

As long as ninety years ago, Woodger (1929) thought that "life" had become irrelevant to biology:

> It does not seem necessary to stop at the word "life" because this term can be eliminated from the scientific vocabulary since it is an undefinable abstraction and we can get on perfectly well with "living organism" which is an entity that can be "speculatively demonstrated" i.e. pointed out.[3]

Much later, Woodger (1962) seemed to have softened his view of the usefulness of discussing the nature of life:

> One of the most striking and basic characteristics of life is its dynamic nature. Life, while essentially closely related to complex structures, is basically a process.[4]

Most modern biologists regard evolution and reproduction as the most important characteristics of living organisms. They are, of course, fundamental properties that set living organisms apart from everything else: organisms reproduce, and they evolve, but they are less fundamental than another characteristic that is often forgotten or ignored: a living organism needs to be capable of staying alive. Before the first organisms at the origin of life were capable of staying alive for a significant time there was no question of either reproducing or evolving: each "attempt" at living was a completely new start, independent of all the previous attempts. All modern scientific efforts to understand what this means involve the idea of maintaining a constant internal organization without guidance from outside,[5] and in the face of environmental changes that the organism cannot control or avoid.

We all think we can recognize a living organism when we see one, but it is not so easy to give a *definition* of "living" that includes all the entities we consider to be alive, and excludes the ones we do not. Table 1 shows an example by Luisi (2003), based on a discussion

[1] There are, however, some papers and book chapters, such as those of Moreno Bergareche and Ruiz-Mirazo (1999), Hofmeyr (2007), Bechtel (2007), that discuss them more broadly, though they do not discuss the hypercycle.

[2] This is a considerable drop from the more than 30 000 of a few years ago, but it is still an impressive total.

[3] It is not clear to us what Woodger meant by the term "speculatively demonstrated", which is in quotation marks in the original, even with the help of his own gloss of "pointed out".

[4] This idea of life as a process is reinforced in autopoiesis (Section 2.5).

[5] We do not regard "intelligent design" (Behe, 1998), invoked by some creationists (Section 1.6.3), as scientific.

Table 1
Which are alive?

Living	Non-living
Fly	Radio
Tree	Car
Mule	Virus
Baby	Crystal
Mushroom	The Moon
Amoeba	Computer

by Varela (2000), who asked how one would answer an intelligent extraterrestrial who visited the Earth and wanted to know how to recognize a living creature. Everyone would agree that the entities in the left-hand column are living, whereas those in the right-hand column are, with one exception, non-living.[6] The exception is the virus[7]: some experts, such as Forterre (2010), insist that a virus is a living organism; others, just as expert, such as Moreira and López-García (2009), insist that it is not, and they had harsh words for some of the efforts to redefine life so that it would include viruses (López-García and Moreira, 2012):

> Defining an entity (a virus) in terms of itself plus a portion of another entity (a cell) is alien to logic and can be viewed as epistemological cheating.

This hybrid "organism" would be a *chimera* in the sense of Rosen (2000, p. 272):

> A metaphor I use to motivate the study of this biological encyclopedia in technological contexts is that of the chimera. In biology, this term connotes a single organism possessing more than the usual number of parents—e.g., whose cells arise from genetically diverse sources. The chimera is in fact a point of departure from biology into technological considerations, and this in many ways. Our civilization has become replete with man–machine chimeras, and even machine–machine chimeras, which manifest emergent functions their constituents do not possess. Social structures, and even ecosystems, are chimerical in this sense.

As much as 90 years ago the nature of viral reproduction was well understood, for example by J. B. S. Haldane (1929) when he addressed the question of whether bacteriophage could be regarded as alive:

> The bacteriophage is like a book or a work of art, which is constantly being copied by living beings, and is therefore only metaphorically alive, its real life being in its copiers.

Maureen O'Malley (2014, pp. 55–57, 208–213) has discussed in more detail the question of whether viruses can be considered organisms. She refrained from taking a dogmatic position of her own, but left it as a discussion that philosophers should consider deeply when choosing between the following arguments about the nature of viruses:

1. Viruses are the precursors of cells (Koonin et al., 2009);
2. Viruses are life forms in their own right (Raoult and Forterre, 2008)[8];
3. Viruses are not living organisms at all (López-García and Moreira, 2012).

In Varela's example of the visiting extraterrestrial, a farmer consulted proposed various definitions of life, such as capacity to reproduce, motility, and so on, but the extraterrestrial rejected all of these. Varela concluded that the crucial definition was as follows:

> A system can be said to be living if it is able to transfer external matter/energy into an internal process of self-maintenance and production of its own components.

We shall see in Section 2.5 how Varela applied his criteria to various entities. The inclusion of the mule in Table 1 is important, because if mules are alive then definitions of life that require a capacity to reproduce and evolve must be discarded. Nonetheless, Joyce (1994), for example, defined life as follows:

> Life is a self-sustained chemical system capable of undergoing Darwinian evolution.

Taking the minority view that mules cannot be regarded as alive does not solve the problem, because many people, including many distinguished biologists, have passed the age when they can reproduce, but would dispute any claim that they are not alive. Joyce's definition does not stand up to examination, therefore. It has become well known, however, because it has been adopted by NASA, but it is just one of many proposed definitions. Koh and Ling (2013) listed 135 of these, from the 19th to the 21st century. About 120 were written after Schrödinger (1944) set out his ideas.

In the 19th century the poet Samuel Taylor Coleridge (1888) criticized theories that say too much or too little[9]:

> Without further preface or apology, therefore, I shall state at once my objections to all the definitions that have hitherto been given of Life, as meaning too much or too little, with an exception, however, in favour of those that mean nothing at all.

Much more recent definitions range from the utterly obscure to the absurdly precise. As an example of an utterly obscure definition, Argyle (1977) offered the following:

> Life on earth today is a highly degenerate process in that there are millions of different gene strings (species) that spell the one word "life".

Another definition by Jibu et al. (1997) will appeal more to physicists than to biologists:

> The existence of the dynamically ordered region of water realizing a boson condensation of evanescent photons inside and outside the cell can be regarded as the definition of life.

Barbieri (2003, p. 262) attributed to Sidney Fox (1996) this absurdly precise definition:

> Life consists of proteinaceous bodies formed of one or more cells containing membranes that permit it to communicate with its environment via transfer of information by electrical impulse or chemical substance, and is capable of morphological evolution by self-organization of precursors, and displays attributes of metabolism, growth, and reproduction. This definition embraces both protolife and modern life.

[6] Many robots are motile, and some are claimed to show intelligence, but they do not appear in Table 1. However, there is little doubt that both Varela and Luisi would have put them in the right-hand column, and would not have considered them to be alive.

[7] Viruses do not appear in either of the two columns of a more recent version of the table given by Luisi (2006, p. 24). He gave no reason for the omission, but later in the book (p. 159) he made it clear that he did not consider viruses to be autopoietic systems.

[8] Raoult and Forterre's opening words leave no doubt as to their own view: "Viruses are the most abundant living entities".

[9] Medawar and Medawar (1985, pp. 26–29) considered Coleridge to show "inspired insights" about life that they thought were nowhere to be found in Aristotle's writing.

Daniel Koshland (2002) proposed a definition that he called PICERAS, which has been widely cited. It consists of a list of properties that he considered necessary for a living organism to have: information storage, ability to cope with changes in the environment, boundaries between individuals, obedience to thermodynamic laws, energy management, and specificity.

Most of his ideas can be found in one form or another in the various theories we shall discuss, though one is conspicuously missing, both from Koshland's list and from the principal theories, or present only by implication, the need for metabolic reactions to be *regulated*: no reaction can be allowed to proceed as fast as its catalysts permit, but only as fast as necessary to satisfy the demand for its product.[10] In view of the major contribution that Koshland et al. (1966) made to the theory of metabolic regulation it is remarkable that he did not mention it here.

Several of the proposed definitions of life consider reproduction and capacity for natural selection (rather than just staying alive) as essential, but although these are certainly characteristic of life as we know it today we do not see them as part of the definition of life. Rosen (1991, p. 255), for example, wrote as follows:

> To me it is easy to conceive of life, and hence biology, without evolution. But not of evolution without life. Thus, evolution is a corollary of the living.

Similar views have been expressed explicitly by Maturana and Varela, and also by Gánti, who did, however, think that living systems should be "capable of undergoing Darwinian evolution": he regarded this as a potential rather than as an essential property of life.

Today the definition of life is primarily the preserve of philosophers such as Ruiz-Mirazo et al. (2004) and O'Malley (2014, pp. 208–213), but it is also bound up with studies of the *origin* of life (Section 1.4) and efforts to create artificial life. Oparin (1924, 1961) emphasized that

> the problem of defining life is tightly intertwined with the problem of its origin.

but there are actually two problems here that should not be confused:

1. Can we study the origin of life without a definition of what it is?
2. Can we study the definition of life without any knowledge of its origin?

Despite the contrary opinion expressed by Szostak (2012) we believe that the answer to the first question must be no: one cannot study how life began without a conception of what it is that began, and one can hardly understand how life arrived where it is today without some notion of how it began. Likewise it is futile to try to create artificial life without a definition of what is to be created. Not surprisingly, therefore, the main scientific communities today that show much interest in the definition of life are those concerned with the origin of life and with artificial life. In some fields a definition of life is absolutely necessary: astrobiologists, for example, search for extraterrestrial life (Mariscal et al., 2019), but how will they recognize it when they find it if they have no definition of what they are searching for? However, these are small minorities among biological scientists as a whole, and it is mainly philosophers and historians of science that still take the question seriously.

On the other hand we agree with Rosen (2000, p. 40) that the answer to the second question must be yes:

> Here, irreversibilities mean, among other things, that such reductions are not invertible; they are not the inverses of syntheses (at least, not in any predicative sense). That is, ultimately, why the origin-of-life problem is so hard. From this perspective, rummaging through a rubble of reductionistic fragments tells us little about either how the organism from which they came actually worked, or how it came to be; the "analysis" that produced those fragments cannot be inverted in either sense (again, not in any purely predicative context).

If extraterrestrial life is ever convincingly demonstrated, it must, we believe, obey the same organizational principles that we discuss in this review. However, it may be morphologically very different from any living organism that can be found on the Earth, and it may have different biochemistry. This raises the question of whether such morphologically and biochemically alien forms of life may exist on Earth but have not been discovered. Cleland and Copley (2005) argue that the absence of evidence for alien life does not prove that it does not exist.[11]

Cleland discusses the possibility of alien life more fully in her recent book (Cleland, 2019, pp. 195–216). Similarly, Davies and Lineweaver (2005) concluded that "it is difficult to rule out the possibility of extant alien life".

In 2005 the journal *Science* celebrated 125 years of its existence by listing 125 questions selected by its editors as "the most compelling puzzles and questions facing scientists today" (Kennedy and Norman, 2005). About two-thirds of the questions were in the general area of biology:

2. What is the biological basis of consciousness?
3. Why do humans have so few genes?
6. How much can human life span be extended?
9. How does a single somatic cell become a whole plant?
12. How and where did life on earth arise?
60. How do proteins find their partners?
110. How will ecosystems respond to global warming?

and many others. Some, such as the origin of life, are of genuine philosophical interest; others, such as determining how proteins find their partners, are surely just a matter of doing the necessary research; at least one, the response of ecosystems to global warming, will answer itself in a few more years; another, the extension of human life, seems more suitable for a popular magazine than for a serious journal like *Science*. One question was conspicuously missing:

- What is life?

This seems to be such an obvious question that we may wonder why it was omitted. Perhaps, with Atlan and Bousquet (1994), the editors of *Science* thought it was not interesting or "compelling", or perhaps they thought the answer had already been provided by Schrödinger (1944) or by one of the authors quoted by Koh and Ling (2013).

The appearance of *systems biology*, apparently a new scientific discipline, at the end of the 1980s, and its explosive growth since 2000, might seem to indicate a resurgence of interest in the study of organisms as complete systems, but this is in reality a change of name more than a change of heart, as we discuss in Section 4.2.2.

1.3. Why does it matter?

In answering a question often asked by non-biologists, Medawar and Medawar (1985, pp. 66–67) wrote as follows:

[10] The claim that triose phosphate isomerase (Knowles and Albery, 1977) and other enzymes are "perfect" overlooks this point. It may be advantageous for enzymes involved in detoxication to operate as fast as chemically possible, but that argument does not apply to enzymes in intermediary metabolism. Bar-Even et al. (2015) point out that only about ten enzymes are known that approach perfection in the sense of Knowles and Albery (1977).

[11] "Absence of evidence is not evidence of absence" (Altman and Bland, 1996), or, as Wang (1969) put it, "We cannot prove that the platypus does not lay eggs with photographs showing a platypus NOT laying eggs".

> "What is the *true* meaning of the word 'life'?" they ask. There is no true meaning. There is a *usage* that serves the purposes of working biologists well enough, and it is not the subject of altercation or dispute.

If few biologists are interested in the nature of life, we need to ask whether a definition of life is really needed, and if so why. Tibor Gánti (1997) gave a very clear answer to this question:

> The extent of which this subject has decreased from the focus of today's scientific interests is notable. In the last decades, practically only Varela has been examining this problem (Varela, 1979, 1994; Varela et al., 1974). It is most interesting, as the question: "what is the essence of life?" is not only the basic problem in biology as a science, and is not only an inevitable condition for the interpretation of the biogenetic process, but it is also indispensable in the ethical and legal clearing up of social-religious conflicts such as the problem of abortion, organ transplantation or euthanasia. Thus, studies on the merits concerning the real nature of life originate from times when there was not an exact answer to this question due to the lack of appropriate scientific (mainly molecular biological) knowledge.

Gánti was mistaken in thinking that, apart from himself, interest in this question was confined to Francisco Varela and his colleagues, but he was right to emphasize the importance of understanding the essence of life for addressing many questions of human importance.

We now describe some of the earlier theories of life, from Julien Jean Offray de La Mettrie in the 18th century to Erwin Schrödinger in the middle of the 20th. Apart from those that we call obsolete (Section 1.6), all make useful contributions to present understanding.

1.4. Origin of life

We are primarily concerned in this review with the essence of life rather than with its origins, but the two topics are clearly related: we cannot usefully discuss the origin of life without a definition of what it is.

For many years the dominant hypothesis for the origin of life was that of Oparin (1924, 1961), which suggested that the first life occurred in *coacervate droplets* in two co-existing liquid phases: a dense, polymer-rich phase (coacervate phase or coacervate droplets) and a very dilute, polymer-deficient phase (dilute phase). Haldane (1929) independently proposed similar ideas in a few words in a popular magazine[12]:

> When ultra-violet light acts on a mixture of water, carbon dioxide, and ammonia, a vast variety of organic substances are made, including sugars and apparently some of the materials from which proteins are built up. This fact has been demonstrated in the laboratory by Baly[13] of Liverpool and his colleagues. In this present world, such substances, if left about, decay—that is to say, they are destroyed by micro-organisms. But before the origin of life they must have accumulated till the primitive oceans reached the consistency of hot dilute soup.

Haldane's last word has caught the imagination of numerous scientists, and his and Oparin's theory are often referred to in terms of the "primeval soup", "primordial soup" or "prebiotic soup". However, arguments advanced by Michael Russell, William Martin and their colleagues (Martin and Russell, 2007; Martin et al., 2008; Russell et al., 2010) make it very unlikely that a compartment as large as the ocean could concentrate organic molecules to anything resembling soup. Furthermore, many originators of theories of life ignore the need for an osmotic barrier to permit the production of a gradient necessary to allow management of energy. Russell and Martin prefer to argue that life originated in small mineral compartments produced by "serpentinization" close to deep-ocean hydrothermal vents (which continue to support thriving colonies of living organisms today), and are reviving interest in the work of Stéphane Leduc (Section 1.9.2) with experiments to investigate the viability of this approach (Barge et al., 2011).

1.5. La Mettrie (1748): Man a Machine

The book *L'Homme Machine* (de La Mettrie, 1748), or *Man a Machine*[14] (de La Mettrie and Bussey, 1912), can be regarded as the starting point for modern ideas about life. His view of life includes some ideas that are still relevant today. Vitalism (Section 1.6.1) was a widely accepted idea in his time, but he rejected it, arguing that living organisms required no vital spark but were simply machines. He recognized, far sooner than anyone else apparently did, that organisms must be understood as systems[15]: not just collections of components, but collections of components that operate in harmony, so that a global action results from local effects because of interactions between them:

> The human body is a machine which winds its own springs. It is the living image of perpetual movement. Nourishment keeps up the movement which fever excites. Without food, the soul pines away, goes mad, and dies exhausted.

This may be the first statement of a crucial insight into the nature of the living state: the idea of *circularity*. We emphasize that we do not mean material circularity: food enters and waste exits from every organism, so there is no circularity there, and nor can there be, because every organism needs to generate energy, and it can only do this by extracting chemical energy from nutrients (or, in photosynthetic organisms, physical energy from light). However, there is a circular organization: an organism must itself make every enzyme that it needs. In this sense, of course, an organism is emphatically *not* a machine, because no machine that we can currently construct is able to maintain itself without external help. Monod (1972, pp. 110–111) took the opposite view in relation to living cells, just as emphatically:

> Through its properties, by the microscopic clockwork function that establishes between DNA and protein, as between organism and medium, an entirely one-way relationship, this system obviously defies any "dialectical" description. It is not Hegelian at all, but thoroughly Cartesian: the cell is indeed a *machine*.[16]

Alberts (1998) likewise regarded cells as collections of protein machines. Nicholson (2019) has recently discussed the question in depth, including Monod's and Alberts's points of view.

1.6. Obsolete theories

In this section we consider three notions of life that form no part of modern science. Of these, *vitalism* (Section 1.6.1) is still found in the writings of some philosophers and, in particular, as the basis of *naturopathy*; *spontaneous generation* (Section 1.6.2) is no longer discussed except in a historical context; and *creationism* (Section 1.6.3) continues to be invoked by religiously motivated authors.

[12] He apparently never developed them in an academic publication.

[13] Haldane did not specify the research he had in mind, but he was probably referring to Baly et al. (1927).

[14] The most scandalous passages, dealing with human sexuality, are left untranslated.

[15] Others, such as René Descartes (1668, p. 62), had already commented on the similarity of animal bodies to machines.

[16] Italics in the original.

1.6.1. Vitalism

Ideas about life had a very muddled and confusing history until the end of the 19th century (Friedmann, 1997b), much of it dominated by *vitalism*, the claim that life is fundamentally different from other processes, driven by a so-called *vital force* that does not exist in non-living systems. La Mettrie did not accept this idea, and later Reil (1795) rejected it also, arguing that life is just chemistry:

> Darwin[17] is of the opinion that growth and the maintenance of living beings occurs not via chemical affinities, but via animal appetites. Every part, he says, has its own appetite.... However, can one possibly think of an appetite without any supposition? If, indeed, we remove those suppositions from Darwin's animal appetites, what remains? In fact nothing remains but chemical attraction, unless we wish to denote one thing with two types of words.... [The] phenomena of [animal] bodies are activities and properties of their matter.

However, vitalism survived as a widely accepted scientific theory for another century. Opinions were expressed on both sides, with some distinguished scientists, such as Liebig, rejecting vitalism for invalid reasons,[18] and others, such as Pasteur, accepting vitalism, but with cogent arguments.[19] Vitalism was not overthrown until Buchner (1897)[20] showed that a cell-free extract of yeast could catalyse alcoholic fermentation. Kornberg (1997) called this discovery the birth of modern biochemistry, as it set off the great development of biochemistry in the 20th century. So far as most scientists, including nearly all biochemists,[21] were concerned, vitalism was dead, and today one of the worst insults that one can throw at biologists is to accuse them of vitalism (Section 1.12). It still has some adherents among philosophers today, influenced by the concept of *élan vital* ("life force") of Bergson (2013, 1st edition 1907), still taught in some schools in France.[22] It is also very much alive as the basis of some varieties of quack medicine, most notably naturopathy (Jagtenberg et al., 2006).

For Jacques Monod (1972, p. 26), Bergson had an "engaging style and a metaphorical dialectic bare of logic but not of poetry". Even during his lifetime his book was strongly criticized. Bertrand Russell (1928, p. 50), for example, wrote as follows:

> His great reputation began with *L'Évolution Créatrice*, published in 1907—not that this book was better than the others, but that it contained less argument and more rhetoric. This book contains, from beginning to end, no argument, and therefore no bad argument; it contains merely a poetical picture appealing to the fancy.

Francis Crick (1967, p. 99) and Monod (1972, pp. 27–29) were both worried that suggestions of vitalism were still current among scientists. Crick thought that vitalism was the last refuge of cranks, and Monod objected to Elsasser's "biotonic laws" (Section 1.11.2). Robert Rosen (2000, p. 8, see also p. 85) thought that this was also directed at Schrödinger's suggestion that biology might need some laws of physics not needed for physics itself, discussed in Section 1.11.2):

> Monod did not dare to attack Schrödinger personally, but he freely condemned anyone else who suggested there might be "new physics" wrapped up in organism, or in life, in the harshest possible way; he called them vitalists, outside the pale of science.

1.6.2. Spontaneous generation

The origin of life was not always regarded as a difficult problem, as Haldane (1929) pointed out:

> Until about 150 years ago it was generally believed that living beings were constantly arising out of dead matter. Maggots were supposed to be generated spontaneously in decaying meat.

Ever since Aristotle propounded his theory of *spontaneous generation* (Deichmann, 2012b; Panayides, 2013), most authorities believed that living organisms could arise spontaneously. Van Helmont was a distinguished scientist in his time, with important work on gases to his credit (including the introduction of the word *gas*), but he thought that basil could be transformed into scorpions or wheat into mice. Pasteur (1864) referred to his ideas as follows:

> The purest fountain water, said Van Helmont, placed in a vase impregnated with a ferment, goes mouldy and engenders worms. The odours that rise from the bottoms of marshes produce frogs, slugs, leaches, grasses.... Make a hole in a brick, introduce some crushed basil, put a second brick on top of the first in such a way that the hole is perfectly covered, expose the two bricks to the sun, and after several days the odour of the basil, acting as a ferment, will change the plant into veritable scorpions.

Pasteur then went on to show that no scorpions or mice would appear in a properly controlled experiment.[23]

Spontaneous generation, a process that is supposed to be occurring continuously, including today, should not be confused with *abiogenesis* and the *origin of life*, which may have occurred only once, as a rare and improbable event billions of years ago. Explanations of the origin of life remain controversial (Peretó, 2012, 2019), with many opinions based on mainly sound chemistry, but no agreement. However, there is no doubt that it happened, at least once. It may well have happened more than once, and may still be happening, but in today's competitive world any "new" life form would be rapidly eliminated by the far more evolved life forms that we know.

The experiments of Burke, Leduc, Herrera and Bahadur (Sections 1.9.1–1.9.4) can be regarded as attempts to demonstrate the appearance of lifelike structures in abiotic processes. However, none of them thought that scorpions and so on were continuously appearing in such ways.

1.6.3. Creationism

Conjure up demons from the main,
Bid Ocean howl and Nature weep,
Storms upon storms indigent heap,
Till the Creator blush to see
How horrible his world can be:
While I will glory to blaspheme,
And make joys of hell my theme.

[Robert Merry, *To Anna Matilda*]

[17] The reference is to Erasmus Darwin, the grandfather of Charles Darwin.

[18] Liebig did not believe that yeast was alive, and he used his prestige as editor of a major journal to publish a scurrilous and anonymous attack on those, such as the much younger Theodor Schwann, who had the temerity to think otherwise (Wöhler and Liebig, 1839). This was illustrated with a drawing intended to ridicule the idea that yeast could be alive. See Schlenk (1985).

[19] Pasteur had carried out experiments similar to those later carried out by Buchner, but they failed. Harden (1914, p. 25) later showed that the difference was due to the fact that Pasteur used a top-fermenting yeast whereas Buchner used a bottom-fermenting yeast.

[20] Available in an English translation by Friedmann (1997a).

[21] The only contrary views that we know of have been expressed by Dix (1968, 1983). The first of these was entitled "A defense of vitalism", but Ozonoff (1969) argued that "it is not really a defense, nor does it concern vitalism (as this term is usually understood)".

[22] See, for example, https://tinyurl.com/yapeutjc.

[23] Even in the second half of the 19th century, Pasteur, stimulated by the book *Hétérogénie* (Pouchet, 1859) that promoted spontaneous generation, still thought that he needed to demonstrate that living organisms could not arise in this way. By then biologists were coming to accept the view of Robert Remak (1852) that "cells always come from division of other cells", popularized as the doctrine *Omnis cellula e cellula* (every cell comes from a cell: Virchow, 1859, p. 25).

At one time nearly all biologists believed that living creatures were created by God, and even Charles Darwin believed this before his discoveries made such a belief impossible to maintain. Today the notion that life is the work of a divine creator can hardly be regarded as a scientific theory, but if it were one it would be the simplest of all and would make most of the discussion in this article unnecessary. Nonetheless, it is an approach that biologists, especially those involved in education, need to be aware of. The danger is well known in the USA, but it exists in many other parts of the world (Cornish-Bowden and Cárdenas, 2007b). It is very rare to find creationist papers in the mainstream scientific literature, but such papers (e.g. Umer, 2018) do occasionally slip through the net of the reviewing and editing processes that would normally filter them out.

Not all scientists consider religious beliefs to be incompatible with serious science, and even the great evolutionary biologist Theodosius Dobzhansky (1973) described himself as a creationist:

> I am a creationist and an evolutionist. Evolution is God's, or Nature's, method of creation. Creation is not an event that happened in 4004 BC; it is a process that began some 10 billion years ago and is still under way.

However, Dobzhansky did not allow his belief as a practising Russian Orthodox Christian to interfere with his scientific work, and today, most religious scientists have no difficulty practising their work in scientifically valid ways. Examples include Francisco Ayala (2007), Francis Collins (2006), Kenneth Miller (1999) and Rafael Vicuña (Vicuña et al., 2012).

1.7. Final cause

The *final cause* is the ultimate reason for something. As such it has usually been interpreted as *teleological*, a call to God, asking *why* God did things in a particular way, and has long been banished from scientific discourse, at least since the time of David Hume (1739, Section 1.iii.14). Even before Hume, Descartes (1644) had objected that we do not know the purposes for which the world was created, and that we should be satisfied with efficient causes:

> We should not examine why God did everything, but only by what means He wanted it to be produced.... We shall completely reject the search for the final causes from our philosophy.

The term "final cause" has largely been banished from scientific discourse in the biological sciences, but this needs to be reconsidered in the light of the fact that an organism fabricates itself. Of the authors discussed in this review, Rosen (1991) rehabilitated the final cause without invoking an external creator, regarding it as a necessary consequence of closure to efficient causation (Section 2.2).

In any case, terms such as "purpose" and "function" that are virtually equivalent to the final cause are commonplace in the biological literature. For example, one of us (Cornish-Bowden, 2013b) wrote as follows in a commentary about supply and demand in bacteria[24]:

> Metabolic regulation is most easily analysed in economic terms of supply and demand, especially given that the primary function of feedback inhibition is to regulate metabolite concentrations, rather than fluxes.

The word "function" also appeared several times in the same sense in the article that was the subject of the commentary (Reaves et al., 2013). It could have been written as "final cause" with very little change in meaning but a large change in how it is perceived.

It is, in fact, virtually impossible to discuss physiological phenomena without mentioning their functions. Despite Monod's vehement hostility to anything he regarded as vitalism (Section 1.6.1), Monod et al. (1963) used the words "function" and "functional" many times in their paper; sometimes it had the sense of a mathematical function but it was also used in the sense relevant here, that of physiological function. Likewise, there are numerous instances in Monod's paper on cooperativity (Monod et al., 1965):

> It has become clear, especially during the past few years, that, in bacteria as well as in higher organisms, many enzymes are electively[25] endowed with specific functions of metabolic regulation. A systematic, comparative, analysis of the properties of these proteins has led to the conclusion that in most, if not all, of them, *indirect* interactions between *distinct* specific binding-sites (allosteric effects) are responsible for the performance of their regulatory function.[26]

Monod's colleague and coauthor François Jacob (1970, p. 17) expressed the ambivalence that most biologists feel about teleology:

> The biologist has long regarded teleology as he does a woman that he cannot do without but does not want to be seen with in public. The concept of a programme now gives a legal status to this hidden liaison.[27]

Jacob's *programme* was based on genetic determinism, and his teleology was quite different from Rosen's rehabilitation of the final cause that will be described in Section 2.2.

Biological functions and the appearance of design require no explanation beyond natural selection. We can still refer to "function", and, cautiously, "design", as long as it is clear that the only designer is natural selection, operating over vast stretches of time. Many biologists and philosophers[28] have no problem with the idea of purpose in Nature.

1.8. Nineteenth century

In a little known letter to *The Athenæum*, a gentlemen's magazine,[29] Darwin (1863) discussed whether life originated from inorganic materials:

> A mass of mud with matter decaying and undergoing complex chemical changes is a fine hiding-place for obscurity of ideas. But let us face the problem boldly. He who believes that organic beings have been produced during each geological period from dead matter must believe that the first being thus arose. There must have been a time when inorganic elements alone existed on our planet: let any assumptions be made, such as that the reeking atmosphere was charged with carbonic acid, nitrogenized compounds, phosphorus, &c. Now is there a fact, or a shadow of a fact, supporting the belief that these elements, without the presence of any organic compounds, and acted on only by known forces, could produce a living creature? At present it is to us a result absolutely inconceivable.

His later letter to his friend Joseph Hooker (Darwin, 1871) is much better known:

[24] We choose this example out of many available to avoiding suggestion of singling out particular authors for criticism.

[25] The word seems to imply conscious design, but Monod would have been horrified at such a suggestion, and presumably it means something else.

[26] Italics in the original.

[27] The book was published half a century ago, when this sort of comparison raised fewer eyebrows than it would today. Numerous authors, including Hull (1974) and Mayr (1974), attribute an earlier statement along the same lines to J. B. S. Haldane: "Teleology is like a mistress to a biologist: he cannot live without her but he's unwilling to be seen with her in public", but we have not found a primary source.

[28] See, for example, Allen et al. (1998).

[29] Peretó et al. (2009) quote this letter more fully than we do here. It is sometimes mistakenly stated, for example by one of us (Cornish-Bowden, 2016), that Darwin never published his view on the origin of life.

> It is often said that all the conditions for the first production of a living organism are now present, which could ever have been present. But if (and oh! what a big if!) we could conceive in some warm little pond, with all sorts of ammonia and phosphoric salts, light, heat, electricity, &c., present, that a proteine compound was chemically formed ready to undergo still more complex changes, at the present day such matter w^d be instantly absorbed, which would not have been the case before living creatures were found.

This reference to instant absorption corresponds to the point in Section 1.6.2 that any "new" life form could not compete with modern organisms.

Darwin's conception of a "warm little pond" as the environment in which life may have originated is often quoted, and is sometimes presented, especially by creationists (Section 1.6.3) such as Mastropaolo (1999), as a central part of his view of evolution, but nothing could be further from the truth, because it played no part at all in his development of the theory of natural selection.[30] The two quotations represent the sum total of all Darwin's writings on the subject, one in a letter to a magazine and the other in a private letter not originally intended for publication. The very tentative mode of expression—"But if (and oh! what a big if!)"—makes it clear that Darwin was not setting out a fully developed theory of the origin of life but simply trying out an idea on his friend. He did not attempt to define life but assumed that Hooker would know what it was.

Although various expressions of what life is were made in the 19th century, it is hard to find one that seems very illuminating today. Perhaps the closest to satisfying us was the comment of Lamarck, quoted by Packard (2007), who understood that the fundamental problem to be solved was that of staying alive:

> Life is an order and a state of things in the parts of every body possessing it, which permits or renders possible in it the execution of organic movement, and which, so long as it exists, is effectively opposed to death. Derange this order and this state of things to the point of preventing the execution of organic movement, or the possibility of its reestablishment, then you cause death.

1.9. Creating lifelike particles

Here we discuss 20th century efforts to show how lifelike particles could be generated from non-living starting materials. For recent reviews, see Peretó (2016, 2019), and for an older but important one, see Fox (1968), who pointed out that Herrera's experiments (Section 1.9.3) did not rely on polymers from living organisms, and compared them favourably with the coacervate droplets of Oparin (1961) described in Section 1.4.

1.9.1. John Burke (1906)

Although it is almost forgotten today, the theory proposed by John Benjamin Butler Burke (1906) in his book *The Origin of Life: its Physical Basis and Definition* should be mentioned. He studied the effects of radium salts[31] on sterilized gelatin and other substances, and observed the appearance of bodies that he called *radiobes* (Fig. 1), a name derived from radium and from the perceived resemblance to microbes. He described them (p. 112) as "possessing the elements of vitality in a primitive and most undeveloped state".

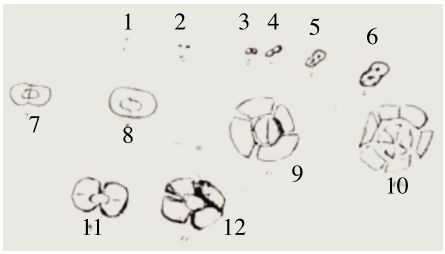


Fig. 1. Radiobes found by John Burke (1906). Twelve stages in the appearance of lifelike structures after treatment of sterilized gelatin "bouillon" with radium bromide. As the original numbers from 1 to 12 are virtually illegible they have been added.

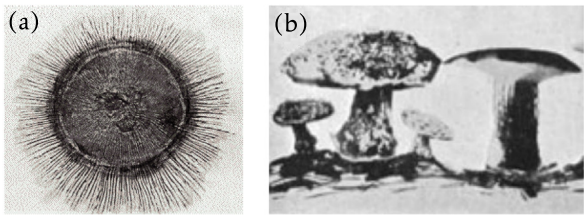


Fig. 2. (a) An inorganic "cell", a structure obtained by adding a drop of a mixture of Na_2CO_3 and Na_2HPO_4 to a solution of NaCl containing a trace of $CaCl_2$ (Leduc's Fig. 11). Leduc thought that the similarity of appearance of this to a living cell shed some light on the nature of life. (b) Inorganic "fungi" (part of his Fig. 41). Leduc did not specify the composition of the inorganic mixture needed to produce these.

1.9.2. Stéphane Leduc (1910, 1912)

The next serious attempts to investigate the nature of life were described in the books *Théorie physico-chimique de la vie* and *La Biologie Synthétique* of Leduc (1910, 1912). He illustrated his theory with many photographs of inorganic "cells" (Fig. 2a) and "fungi" (Fig. 2b) and other kinds of "vegetation" obtained from inorganic crystals placed in a purely inorganic medium. It is difficult to judge to what extent Leduc considered his inorganic structures to be alive, but it is clear that he regarded their similarity to organisms as more than superficial. For example, in discussing the "fungi" in Fig. 2b he wrote as follows:

> The feet of the osmotic mushrooms are fibrous, the surfaces of the caps are smooth like those on the right, or covered with little scabs like those on the left, and the whole surface is layered or perforated. This similarity, compared with natural mushrooms, of general form, of details and of structure, is extremely worthy of attention.

Deichmann (2012b) has commented on Leduc's failure to distinguish unambiguously between synthetic life and resemblance to living organisms:

> While Leduc used terms like "imitating life" or "analogous to life", the border between synthesizing artificial life, that is something that imitates life, and artificially synthesizing life, are blurred throughout his *La biologie synthétique*.

1.9.3. Alfonso Herrera (1904, 1924)

In the same period Herrera (1904, 1924) developed his theory of *plasmogeny*. He reasoned that as life was the result of purely physicochemical phenomena, it should be possible to create a structure with similar properties to natural protoplasm out of relatively simple organic and inorganic compounds in the laboratory. He expanded the range of inorganic reactions that could produce lifelike structures (Fig. 3).

[30] This is, of course, a favourite, and probably deliberate, confusion on the part of some creationists, who try to insist that continuing uncertainty about the origin of life means that nothing is known about the mechanisms of evolution. As one of many possible examples, consider the book title *Evolution Impossible: 12 Reasons why Evolution cannot explain the Origin of Life on Earth* (*Ashton, 2012*).

[31] The dates are interesting: radium had been discovered (Curie et al., 1898) only a few years before Burke's book was published, but already it was available for study in laboratories other than the Curies'. Around the same time, Maud Menten, better known for her later work with Leonor Michaelis on enzyme kinetics (Michaelis and Menten, 1913), worked with Simon Flexner at the Rockefeller Institute on the effects of radium bromide on rat tumours (Stock and Carpenter, 1961; Deichmann et al., 2014, Part 2, pp. 448–451) and Stéphane Leduc was one of the first in France to use radiotherapy in the treatment of cancer (Drouin et al., 2014).

Fig. 3. Lifelike structures created by Herrera (1924).

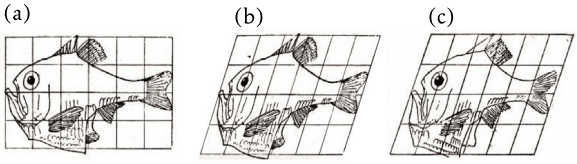


Fig. 4. Transforming one fish into another. The oceanic ray-finned fish *Argyropelecus olfersi* (a) is easily distinguishable from (c) *Sternoptyx diaphana*, another oceanic ray-finned fish from a different genus, but if the coordinate system of *Argyropelecus olfersi* is stretched somewhat in the vertical direction and skewed, the resulting form (b) is very similar to that of *Sternoptyx diaphana*. The outer illustrations are from Figs. 517 and 518 of d'Arcy Thompson's book.

Like Leduc, he considered growths based on silicates, but in addition he examined the results of evaporating solutions of salts. For example, he found that evaporating a solution of sodium stearate in petrol produced what he called imperfect crystals that resemble cells. In general his work can be seen as parallel to Leduc's.[32]

Leduc's and Herrera's illustrations are impressive, but we do not find them helpful for understanding present-day life. However, they may still be useful for shedding light on how life originated. In particular, a major requirement for any living system is the capacity to obtain energy from the environment, and in all organisms known to us this requires different concentrations of ions, most obviously H^+ ions, on the two sides of a membrane or other barrier. Osmotic structures such as those in Fig. 2 have the appearance of being soft and flexible, but in reality they are brittle, like many inorganic materials. They can form hollow tubes that can separate an inner solution from its external environment, thus in principle allowing ion gradients. This type of osmotic structure is currently being studied with a view to determining whether it can support an ion gradient capable of driving chemical reactions (Barge et al., 2011).

1.9.4. Krishna Bahadur (1950s)

In much more recent times Bahadur continued the tradition of Burke, Leduc and Herrera in studying the *Jeewanu*,[33] a lifelike structure obtained by the effect of molybdenum oxide and light on various mixtures of chemicals. His original publications are almost unobtainable, but have been reviewed by Grote (2011). They have led to almost no subsequent research, but are worth mentioning as they are discussed favourably by Gánti (2003, pp. 144–145).

1.10. Physical and mechanical constraints

D'Arcy Wentworth Thompson (1945, 1st edition 1917) did not reject natural selection as a mechanism of evolution, but he believed that physical and mechanical constraints were at least as important for determining the form and structure of living organisms,[34] and he pointed out that mathematical transformations could account for the differences in form between related organisms. His ideas are also very relevant to embryogenesis.

The fish *Argyropelecus olfersi* can be "transformed" into *Sternoptyx diaphana* by stretching it slightly in the vertical direction and skewing the whole grid (Fig. 4). Other examples require more complicated transformations, with the introduction of curvature as well as simple linear distortions, but the point is the same: the shapes of many related species are related by mathematical transformations. D'Arcy Thompson's principal point in these examples was to emphasize that many of the changes that occur during evolution are constrained by engineering considerations, and so changes along different dimensions cannot be selected independently of one another. That is certainly correct, but although some of his illustrations are widely reproduced,[35] his underlying ideas have not survived much better than Leduc's, though we may note a recent use of them to explain formation of hexagonal packing in the plant *Persea americana* (Gabarayeva et al., 2010), and Abzhanov (2017) has thoroughly reviewed D'Arcy Thompson's legacy. Nonetheless, few researchers today consider that his work is fundamental for understanding the nature of life.

For reasons different from D'Arcy Thompson's, Kauffman (2008) also doubts that natural selection explains all of evolution:

> Self-organization may require that we rethink all of evolutionary theory, for the order seen in evolution may not be the sole result of natural selection but of some new marriage of contingency, selection, and self-organization. New biological laws may hide in this union.

Many biochemists, including Moran et al. (2012) and ourselves (Cornish-Bowden et al., 2014b), consider that the neutral theory of evolution (Kimura, 1983) is just as important as natural selection for driving evolution; indeed, in the case of protein sequence evolution, it is much more important.

1.11. Erwin Schrödinger (1944)

> If a man never contradicts himself it will be because he never says anything.[36]
>
> [Miguel de Unamuno (1934)]

1.11.1. Negative entropy, codescript

The modern study of life begins with the book *What is Life?* (Schrödinger, 1944) based on lectures given in 1943 to a general audience in Dublin. A case could be made that it started a decade earlier with a lecture on "Light and life" by another distinguished physicist, Niels Bohr (1933). However, although this lecture stimulated Max Delbrück's interest in biology (see Domondon, 2006),[37] in general it was much less influential than Schrödinger's book, which convinced physicists such as Francis Crick that biology offered questions that they could find interesting. Schrödinger tried to answer three main questions:

[32] With today's technical capacities far beyond anything available to Leduc, one can repeat his experiments to produce colour pictures even more impressive than his (see https://tinyurl.com/y56fjcvp), but they still tell us nothing useful about the organization of living systems.

[33] Jeewanu is an invented word based on Sanskrit roots meaning "particle of life".

[34] Constraints of this kind provide at least a partial explanation of the similarities that Leduc noted between the "fungi" in Fig. 2b and natural mushrooms.

[35] The famous illustrations come near the end of a long book, as might be guessed from the figure numbers, 517 and 518. The earlier chapters are rarely discussed.

[36] Si un hombre nunca se contradice, será porque nunca dice nada. Spoken orally to Erwin Schrödinger at the International University of Santander in 1934, and quoted by Schrödinger (1944) in *What is Life?*

[37] Max Delbrück was thus already interested in the nature of gene structure and mutations long before the publication of *What is Life?* Chapter 5 of Schrödinger's book is devoted to a discussion of Delbrück's work on that subject (Timoféeff-Ressovsky et al., 1935). By 1944 he had started the work on bacteriophage for which he became well known (Ellis and Delbrück, 1939).

1. How can organisms maintain their organization in the face of a continuous production of entropy as a consequence of the second law of thermodynamics?
2. What is the nature of the hereditary material?
3. Can biology be fully understood (even in principle) in terms of the known laws of physics?

He answered the thermodynamic question with the statement that "what an organism feeds on is negative entropy". This may seem an unnecessarily poetic way of expressing an idea that is well understood today, that the inevitable production of entropy by an organism is compensated for by the ingestion of low-entropy food and excretion of higher-entropy waste.[38] However, this was not well understood at all at the time he was writing, and his statement undoubtedly cleared away some confusion.

Schrödinger suggested that the hereditary material must be a sort of "aperiodic crystal", a substance with a high degree of repetitive regularity, as in a crystal, coupled with non-repetitive elements whose structures did not interfere with the general regularity, but whose irregularity allowed them to act as a "codescript". After the tremendous increase in knowledge of molecular genetics that has occurred in the half-century that followed his lectures we can recognize this as a prediction of the nature of DNA, whose structure appears completely regular when viewed from a distance, but completely irregular when viewed with enough resolution for the individual bases to be identified.

Both Delbrück and Schrödinger considered that to have the necessary stability the genetic material must be a molecule. Today this seems obvious, but in the first half of the 20th century almost nothing was known about the structure of genes, and it was widely thought that macromolecules such as proteins and DNA were amorphous colloids (Deichmann, 2012a).

Most modern biologists would probably consider that these first two of Schrödinger's three points cover the whole story. Thermodynamic analysis of organisms as open systems explains their energy management and information storage in DNA explains heredity, and that is all there is to it. However, neither of these considerations explains how organisms maintain their organization, virtually indefinitely, without external help, in the face of frequent and sometimes large changes in their environments. Schrödinger's third question was the least well understood when he was writing, and remains the least well understood (and the most controversial) today.

Critics such as Pauling (1987) and Perutz (1987) considered that Schrödinger had contributed nothing of value to the understanding of life, but they appear to have missed the point that matters that were obvious to them in 1987 were not equally obvious to Schrödinger's audience in Dublin in 1943, and to the readers of his book.

1.11.2. Biology and the laws of physics

To answer the third question Schrödinger (1944) suggested that the known laws of physics might not be sufficient to explain biological systems:

> What I wish to make clear in this last chapter is, in short, that from all that we have learnt about the structure of living matter, we must be prepared to find it working in a manner that cannot be reduced to the ordinary laws of physics.

This suggestion has been widely misunderstood. It was not a return to the vitalism (Section 1.6.1) that characterized earlier efforts to understand living systems until it was swept away by the discovery that a cell-free extract of yeast could catalyse fermentation, the conversion of glucose into ethanol and CO_2 (Buchner, 1897). No one today doubts that an organism must obey all the laws of physics; in particular, organisms are not exempt from the laws of thermodynamics. The known laws of physics are *necessary*, therefore, for understanding biological systems, but that does not mean that they are *sufficient*. The point, strongly emphasized by Rosen (1991, pp. 34–38) and (in conversation) by Varela, is that the universe of biology is vastly larger than the universe of physics. It is perfectly possible that there may be physical laws necessary for understanding biology that cannot be revealed by studying physics alone, because the world that physicists study is too limited.[39]

For the moment we do not know if Schrödinger's conjecture is true, and, if any new laws of physics are needed for understanding biology, they have not been discovered yet. Before leaving this topic we should mention that the distinguished physicist Walter Elsasser (1961) took seriously the possibility of biological laws that cannot be reduced to physics, which he called *biotonic laws*. In a valuable review of his work Gatherer (2008) accepted that these biotonic laws exist:

> Although Elsasser drew some conclusions from his epistemology that are not justifiable in the light of subsequent research, his insistence on the existence of biotonic phenomena in biology, irreducible (either at present, or in principle) to physics, is correct. Ironically, the most significant biotonic principle is one which Elsasser largely ignored in his own work, that of Natural Selection.

We remain somewhat sceptical about this, in part because it is hard to regard natural selection as a "law", crucial as it undoubtedly is. Rosen (1991, p. 12) discussed Elsasser's ideas briefly:

> His argument was, roughly, that anything rare disappears completely when one takes averages; since physicists are always taking averages in their quest for what is generally true, organisms sink completely from physical sight. His conclusion was that, in a material sense, organisms are governed by their own laws ("biotonic laws"), which do not contradict physical universals but are simply not derivable from them.[40]

However, he continued by doubting whether Elsasser's work had much influence:

> Ironically, ideas like Elsasser's have not had much currency with either physicist or biologist, although one might have thought they would please both.

Eigen (1971) discussed the question of "new physics" in his paper introducing the hypercycle (Section 2.3), without mentioning Schrödinger's and Elsasser's suggestions in that context, though he discussed Schrödinger's book elsewhere in the paper. He took "new physics" to mean

> the abandonment of the general validity of previously accepted fundamental principles required by experimental facts which, although obtained under clear and defined conditions, are in disagreement with the conclusions of theory.

[38] Bauer (1935) discussed thermodynamics and energy management very thoroughly in a little-known book, and foresaw some of Schrödinger's points.

[39] Schrödinger's suggestion may have seemed less startling at the time of his lectures than it does today. The major advances in physics, the sort that physics textbooks for undergraduates mention—Bohr's hydrogen atom, Einstein's general relativity, and Schrödinger's own quantum mechanics—had all been made within living memory for the first readers of *What is Life?* Theoretical physics was still in a ferment, and 70 years later these advances are in the history books.

[40] Francis Galton (1894, p. 62) made a similar remark: "It is difficult to understand why statisticians commonly limit their inquiries to Averages, and do not revel in more comprehensive views. Their souls seem as dull to the charm of varieties as that of a native of one of our flat English counties, whose retrospect of Switzerland was that, if its mountains could be thrown into its lakes, two nuisances could be got rid of at once. An Average is just a solitary fact, where if a single other fact be added to it, and entire Normal Scheme, which nearly corresponds to the observed one, starts potentially into existence".

but he continued by making it clear that he did not regard the hypercycle as new physics in Schrödinger's sense:

> On the other hand, the second kind of "new concept" does not invalidate any principle so far accepted; it deals only with a new aspect and may be derived from known principles.

and later:

> An understanding of the basic principles of evolution as selforganization at the molecular level does not require "new physics", but rather a derivable principle which correlates macroscopic phenomena with elementary dynamical behavior.

We find these comments puzzling, as there is no suggestion in either Schrödinger's or Elsasser's arguments of abandoning accepted fundamental principles. They accept that the laws of physics are *necessary* for understanding living systems, but argue only that they may not be *sufficient* for explaining new discoveries in biology.

1.12. Theories of life and molecular biology

Our understanding of life can be considered to have begun with the ideas of La Mettrie and others described in Sections 1.5–1.10. Afterwards, *What is Life?* (Schrödinger, 1944), the general theory of systems (Bertalanffy, 1969, summarizing work initiated in the 1920s), the logic of automata (Von Neumann, 1951) and cybernetics (Wiener, 1948) were important influences on its development.[41]

Meanwhile *molecular biology*, which dominates much of biology today, was growing very fast (Fig. 5). The first isolation of DNA (Miescher, 1871) and the discovery of chromosomes (Flemming, 1879) were followed by a long period of apparent inactivity before DNA was shown to be the genetic material (Avery et al., 1944), a discovery that was given little credence until Hershey and Chase (1952) showed that only the DNA enters the cell when the bacteriophage T2 infects *Escherichia coli*. The discovery that DNA was structured as a double helix came a little later (Watson and Crick, 1953) and set off the explosion of research that followed, including the concept of the operon (Jacob et al., 1960), elucidation of the genetic code, and many other discoveries, followed by the determination of many genome sequences, first that of a virus (Fiers et al., 1976), then those of bacteria and other organisms, culminating in that of the human.

Unfortunately the development of molecular biology essentially ignored the definition of life, and the people who proposed theories of life did not, in general, incorporate the developing ideas of molecular biology in their theories.

Monod's view of life as the properties of amino acid sequences in proteins was clearly expressed by Fantini, a historian of science, in his Preface to Monod (1988):

> In 1960 a note to the Academy of Sciences[42] introduced the concept of the operon, and a unit of expression that it coordinated. It introduced a new dimension of the organization of the genome.... As a result of profound theoretical innovations and important discontinuities in the traditions of research,... the appearance of a powerful theoretical edifice can be seen, one that changes the very foundation of biology and provides new definitions of life and evolution.

Does any of this matter? Does biology really need a theory of the living state in order to advance? This can be answered by quoting Woese (2004):

> 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.

1.13. Is it possible to define "life"?

Most of this review is concerned with determining whether the word "life" has a meaning, and if so what that meaning is. However, some philosophers (for example, Cleland and Chyba, 2002; Cleland, 2012) argue that the meaning of life cannot be fully captured by a definition, and that even as familiar a concept as "water" presents similar difficulties, partly because before its structure as H_2O was established one could not even say what it was, as the following remarks from Leonardo da Vinci, quoted by MacCurdy (1938) and Cleland (2019, p. 50), illustrate:

> And so [water] is sometimes sharp and sometimes strong, sometimes acid and sometimes bitter, sometimes sweet and sometimes thick or thin, sometimes it is seen bringing hurt or pestilence, sometimes health-giving, sometimes poisonous. So one would say that it suffers change into as many natures as are the different places through which it passes. And as the mirror changes with the colour of its object so it changes with the nature of the place through which it passes: health-giving, noisome, laxative, astringent, sulphurous, salt, incarnadined, mournful, raging, angry, red, yellow, green, black, blue, greasy, fat, thin....

Cleland (2019, pp. 55–59) also cited the more philosophical "twin earth" argument of Putnam (1975).[43] However, we are not philosophers, and like most of the biologists and chemists that we discuss we do believe that *something* can be said about the nature of life.

Bich and Green (2018) have recently asked a slightly different question, not whether defining life is possible, but whether it is pointless, as claimed by Szostak (2012) and Machery (2012). However, we consider that understanding the essence of life is essential for progress in the field.

O'Malley (2014, pp. 208–218) has given a useful discussion of philosophical aspects of life, with emphasis on microbiology, and Cleland (2019) also provides a thorough discussion of philosophical aspects of life in her recent book.

Ever since the days of Leduc (Section 1.9.2) there have been claims that this or that experiment represented a step towards the creation of artificial life. Recent examples are those of Gibson et al. (2010), illustrated in Fig. 6, and Fredens et al. (2019). We, in common with Bedau (2010), are sceptical of such claims, which normally pay little or no attention to the difficulty of the problem, and do not believe that they help us to understand life.

2. Current theories of life

2.1. General points

2.1.1. Closure

An important principle of life that is obvious once pointed out, but ignored in most accounts of life chemistry, is that of *closure*. No matter how one defines a *machine*, whether a very simple tool such as an axe, a more complicated machine such as an aeroplane, or even an entire factory, it remains true that the machine does not make itself or maintain itself: it needs the input of an external agency in order to do this. It follows that an aeroplane may be *complicated*, but it is not *complex*, because all details of its structure and their relationships to one another can be understood. Likewise, regardless of how difficult it may be to understand the functions of all the pipes just by looking

[41] The discovery of negative feedback as a control mechanism in engineering systems (Black, 1934) was later seen as the basis of feedback regulation in metabolism (Dische, 1940, 1976; Umbarger, 1956; Yates and Pardee, 1956; Stadtman, 1970), but we defer discussion of this to Section 4.1.2.

[42] Jacob et al. (1960).

[43] This examines the consequences of the existence of another planet identical to the Earth, *except* that instead of water, H_2O, it has a different substance with exactly the same properties but a different chemical structure. People who are not philosophers are unlikely to find this discussion very illuminating.

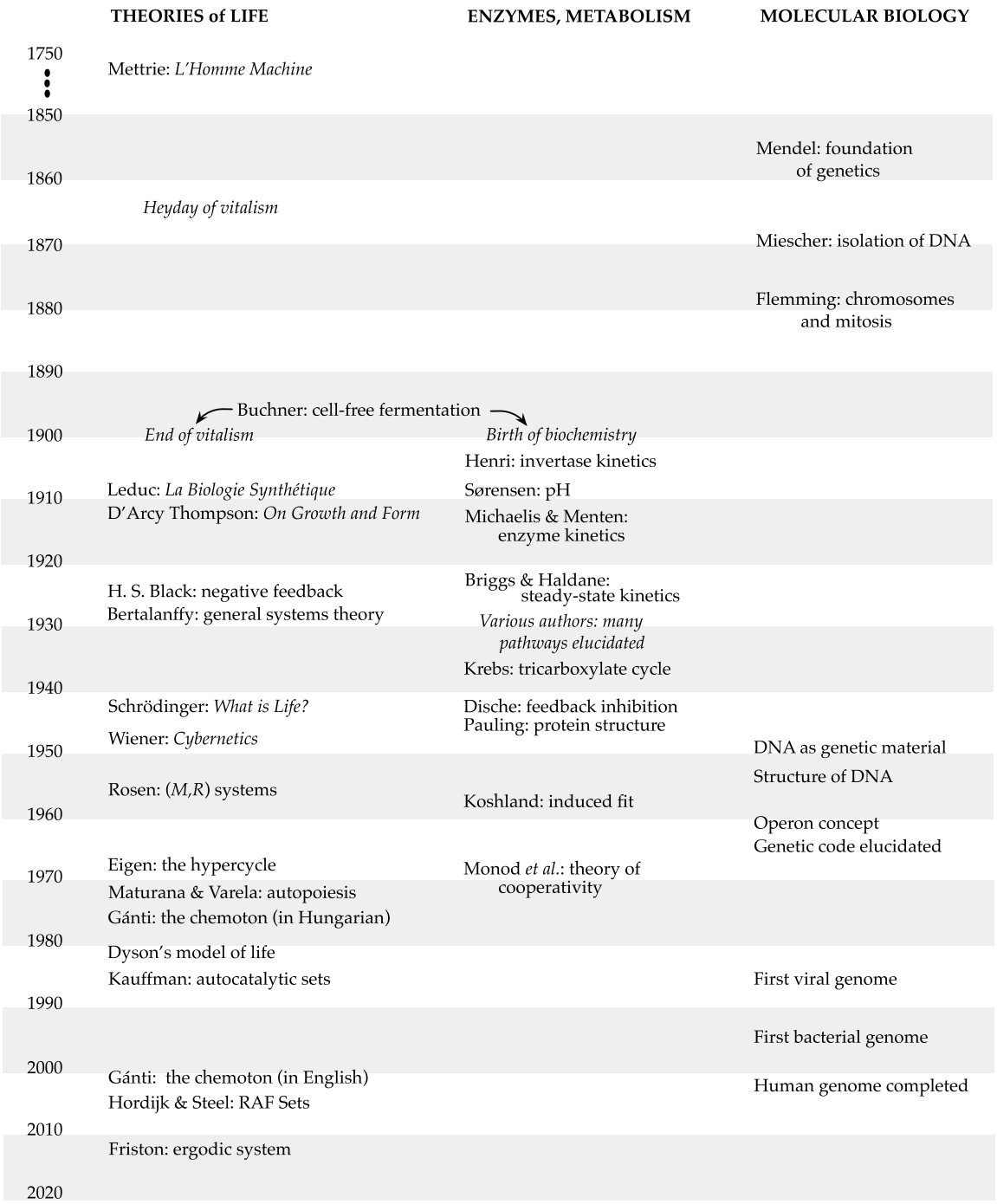


Fig. 5. Landmarks in the development of theories of life and of biochemistry and molecular biology. The explosive growth of molecular biology started with the recognition of the double-helix structure of DNA, and although it remains highly active to this day its crowning achievement may be regarded as the determination of the human genome. The same period corresponds approximately to the development of theories of life, though these have been largely ignored by molecular biologists.

at a picture of an oil refinery[44] (Fig. 7), we can be sure that they all have identifiable functions and so the arrangement is complicated, not complex.

In summary a system is *complicated* if it has many components that interact with one another in such a way that the whole acts as the sum of its parts. For example, computer models of metabolism require kinetic equations for many processes, but when these are all programmed into a computer they generate a model that behaves, to within experimental error, as the real system behaves. Voit and Ferreira (2000) and Mulquiney and Kuchel (2003) describe in general terms how this can be done, and examples include models of metabolism in the human erythrocytes (Mulquiney and Kuchel, 1999), glycolysis in the parasite *Trypanosoma brucei* (Bakker et al., 1997; Eisenthal and Cornish-Bowden, 1998), aspartate metabolism in *Arabidopsis*

[44] The refinery at the Étang de Berre was planned by Victor Henri, better known to biochemists as one of the fathers of enzymology, but was not completed until after he had moved to Liège as Professor of Physical Chemistry (Cornish-Bowden et al., 2014a).

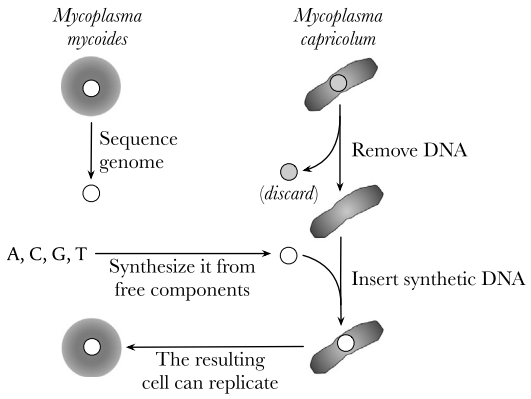


Fig. 6. Transfer of a genome from one bacterial species to another. Has a synthetic cell been created?.

Fig. 7. Complicatedness and Complexity. (a) Anyone who has flown into Marseilles will have passed close to the oil refinery at the Étang de Berre. It is tempting to call the arrangement complex, but it is only complicated: every pipe has an identifiable function. (b) Likewise, the tangle of telephone wires in Valparaíso appears completely chaotic, but every wire goes somewhere and was put there for a reason, in principle identifiable.

thaliana (Curien et al., 2009), and the threonine pathway of *Escherichia coli* (Chassagnole et al., 2001).

All of these are just complicated, because in every case the whole is fully understandable in terms of its parts. They would only be *complex* if they displayed *emergence*, so that they could not be modelled as the sums of their parts.

Living organisms are quite different from machines. They depend on their environments for material (food), and they release material (CO_2, water, other excreta) into their environments, so they are thermodynamically *open systems*, but they are organizationally *closed systems*. All of the catalysts that they need, apart from some metal ions,[45] they make themselves. In Rosen's terms they are *closed to efficient causation* (Section 2.2.2). This is a fundamental difference between organisms and machines, and although Von Neumann (1951) argued that machines that make exact copies of themselves can be designed, Rosen (1959a) detected a logical error in Von Neumann's argument, but Hofmeyr (2017) has shown that although Rosen was in part correct the paradox can be resolved without needing to reject Von Neumann's argument. More important, self-fabricating machines have yet to be built, and for the moment the gap between machines and organisms appears to be unbridgeable. Kauffman (2013) has also discussed this point.

2.1.2. Lack of communication between authors

The main current theories of life (Fig. 8) all embody the idea of closure, and overlap in other ways, but they differ in the emphasis they put on the various points. Rosen (1991) was especially concerned with the implications of closure to efficient causation for the possibility of making a model of a living organism (Section 2.2.3); Eigen (1971) with the need for specific proteins to catalyse production of molecules for storing information efficiently (Section 2.3); Gánti (1971) with the need to relate life chemistry to accepted principles of chemistry and chemical engineering (Section 2.4); Maturana and Varela (1973, 1980) with the need for an organism to be separated from its environment by a membrane or other barrier (Section 2.5); and Dyson (1982) and (independently) Kauffman (1986) with the possibility for life to arise naturally in mixtures of large numbers of components (Section 2.6).

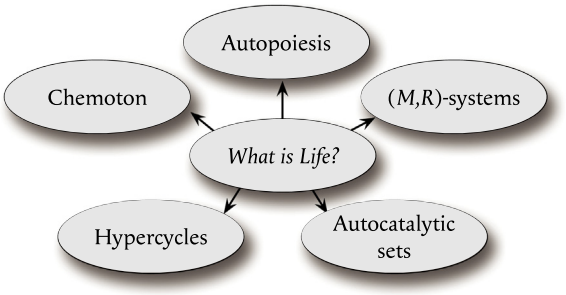


Fig. 8. Theories of life. Five of the best known theories of life are listed, which were developed essentially independently of one another, though all were influenced to some degree by Schrödinger's book *What is Life?*

The various theories had almost no influence on one another, because they were developed in total isolation from one another: none of the books written by any of these authors to explain their ideas refers to any of the others![46] Moreover, there is rather little overlap in the audiences reached by these various authors, and none of them has a large following close to the centre of current biology, none of them proving very easy for biologists to get to grips with. (*M, R*) systems are attracting increasing interest among mathematically minded biologists, but Rosen expressed himself in uncompromisingly mathematical terms, provided no examples to illustrate his points, not even mathematical examples, and made no concessions to any difficulties that his readers might have. Eigen and Schuster are the only ones to make a clear link between their theory and the known facts of molecular biology. Maturana and Varela presented their ideas in almost mystical terms with little to attract the attention of molecular biologists interested in studying the details of living systems; their theory has, however, become very well known to neuroscientists. Kauffman's discussion in terms of mixtures of very large numbers (typically of the order of 10^9) of different kinds of molecule assumes a far larger system than what is implied by efforts to express the main ideas of the others in small models, but in that sense it is probably realistic of what happened at the origin of life. Gánti is probably the most down-to-earth, but his ideas have only recently become accessible to most readers, as he published almost exclusively in Hungarian and his book has only become available in English in this century (Gánti, 2003).

The authors we have mentioned had very different backgrounds and training, and none of them were biochemists. Rosen considered himself to be a mathematical biologist, but many of his readers think of him as a mathematician; Schuster is a biophysicist, as was Eigen; Maturana is a neuroscientist interested in vision, as was Varela; Gánti was an industrial chemist, and like any engineer he was interested more in getting things to work than in deep philosophical questions; Dyson is a physicist, and Kauffman is a theoretical biologist. These diverse backgrounds explain in part why they owed so little to one another.

Some of the isolation of the various authors can probably be explained by their different backgrounds, but it seems unlikely that this is the whole explanation and that they were completely unaware of one

[45] Metal ions may have been all that were needed at the origin of life (Muchowska et al., 2017), but modern life depends on more specific catalysts.

[46] *The Principles of Life* (Gánti, 2003, pp. 76, 78, 157–168, 169–186) does include editorial notes about autopoiesis and the hypercycle, but these were written by the editors, not by Gánti.

another's work. As an anecdote, which we cannot support with a formal reference, we understand that Rosen and Varela once participated in the same meeting, but when they met they found that they had nothing to say to one another. In Rosen's case it may be that he preferred to be self-contained.

In this section we do not aim to present detailed descriptions of the theories we discuss. Most of them have required whole books for their description, which cannot be reduced to a few paragraphs. Instead we give pointers towards more complete accounts, in the hope of allowing them to be compared, with a view to arriving at a synthesis to be used as the basis of an improved theory of life.

2.2. (M, R) systems (1958 onwards)

> The history of science is replete with stories of unrecognized brilliance. Bob Rosen's story is among them. For those who have come to understand enough of his message, he is both a hero and a symbol.... He symbolizes the realization of an ideal, something usually expected to be approached but not reached.
>
> [Mikulecky (2001)]

Robert Rosen's theory of life tries to explain how a living organism could avoid *infinite regress*. It must synthesize all of the specific catalysts that it needs, and all of these are liable to be lost on account of degeneration, dilution by growth, and other mechanisms, and the catalysts responsible for the regeneration are also liable to be lost. How can the organism avoid piling up catalysts *ad infinitum*? He resolved this question in terms of *closure to efficient causation*, whereby there is a circular organization of effects with no beginning and no end, and that *final cause* was not an appeal to an external creator but a reference to the way in which an organism continuously recreates itself. He argued that this circularity meant that organisms are inevitably complex and that a model of an organism was impossible.

2.2.1. Infinite regress

Rosen defined organisms as *metabolism-repair systems*,[47] or (*M, R*) systems, in a long series of papers from 1958 onwards (Rosen, 1958a,b, 1959a,b, 1966, 1971, 1973, 1975, 1978, 1979, and others), which were summarized in his book *Life Itself* (Rosen, 1991). Like nearly all his publications, all of these were written without co-authors.[48] The lack of co-authors has the merit of indicating that he spoke with a single voice without needing to compromise with others, but it contributes to the great difficulties that most readers have for understanding his thinking, with almost no reference to the known facts of biochemistry. Without the need to explain everything to co-authors and convince them that his arguments were correct, Rosen lacked the corrective mechanism that most scientific authors face.

He was particularly anxious to avoid *infinite regress*, a problem that also applies to all theories of life, though not much emphasized in others: a solution to any problem often creates a new problem that is just as serious, and there is no obvious way to escape adding solutions that require their own solutions indefinitely. Nearly all of the catalysts (other than metal ions) needed by an organism must be produced by the organism itself, and replaced when they become degraded, or just diluted by growth. However, replacement itself requires catalysts, which must also be replaced for the same reasons, and so *ad infinitum*, as illustrated in Fig. 9. In the next section we describe Rosen's way of escaping infinite regress.

[47] We prefer *replacement* to *repair*, as it does not refer to repair as understood in modern biology. Louie (2009, p. 269) has pointed out that Rosen's use of *repair* and *replication* did not conflict with well established use of the terms in biology at the time when Rosen defined them. However, priority arguments are less important than being understood, and if we want to be understood we cannot use *repair* and *replication* in Rosen's senses. For convenient reference several differences between Rosen's usage and ours (Letelier et al., 2006) are listed in Table 2.

[48] Donald Mikulecky (2001) listed about 190 of Rosen's publications, of which all but two were published without co-authors.

Table 2
Rosen's terminology.

Rosen's terms	Our usage
Component	Catalyst (or enzyme)
Repair	Replacement
Replication	Organizational invariance
Transformable molecule	Metabolite

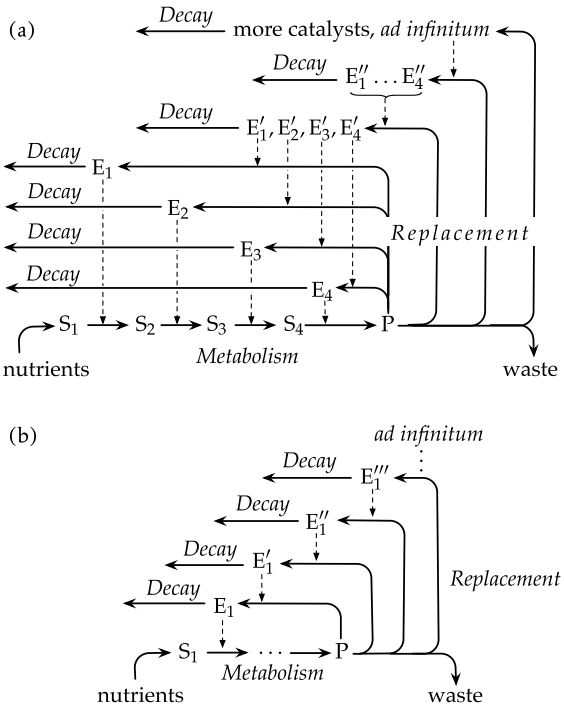


Fig. 9. Infinite regress. (a) Metabolism can be represented by a series of reactions: nutrients → S_1 → S_2 → S_3 → S_4 → P → waste. These are catalysed by a series of enzymes E_1, E_2, E_3, E_4 that must be produced by the metabolism. However, the enzymes are not indefinitely stable, or their concentrations are decreased by dilution as the organism grows. They must accordingly be maintained by another series of enzymes E'_1, E'_2, E'_3, E'_4. But these must also be maintained by another series $E''_1, E''_2, E''_3, E''_4$, and so *ad infinitum*. If each new level in the diagram needs another new level above it, and so on, the diagram is well on the way to an infinite regress. A major task of (*M, R*) systems, therefore, was to find away of escaping from the infinite regress, or achieving *replication* (in Rosen's terminology), or, more clearly, *organizational invariance*. (b) A simplified representation in which decay and replacement only of E_1 are shown, but the same considerations apply to all the enzymes in the system.

2.2.2. Closure to efficient causation

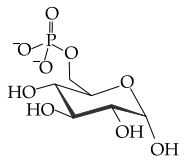
> Happy the Man, who, studying Nature's Laws,
>
> Thro' known Effects can trace the secret Cause.
>
> His Mind possessing, in a quiet state,
>
> Fearless of Fortune, and resign'd to Fate.[49]
>
> [Virgil, *Georgics II*, line 490.]

The solution to infinite regress that Rosen proposed in (*M, R*) systems was *closure to efficient causation*. Here the *efficient cause* is one of Aristotle's four causes. The *material, formal* and *efficient* causes have clear meanings in metabolism, as one might apply them to the metabolite glucose 6-phosphate (Table 3). The *final cause* was discussed in Section 1.7 as the ultimate reason for something. It was seen as an embarrassment implying an external creator. Nonetheless, an organism

[49] *Felix qui potuit rerum cognoscere causas, atque metus omnis et inexorabile fatum subiecit pedibus, strepitumque Acheronis avari.* (English version by John Dryden).

Table 3
Aristotle's four causes. They are best understood as answers to the different questions that can be asked to understand what something is: how was it *made* rather than what *caused* it (Cohen, 2016). Here we illustrate the four causes of glucose 6-phosphate in metabolism.

Aristotle's causes	Glucose 6-phosphate
Material cause: what is it made *from?*	Glucose and ATP
Formal cause: what is it made *as*?	A glycolytic intermediate
Efficient cause: what is it made *by*?	Hexokinase
Final cause: what is made *for?*	Harnessing energy



creates itself, and so the final cause does not imply God or another external creator. Rosen (1991) rehabilitated the final cause as a legitimate subject for scientific enquiry, and related it to his view that organisms are *anticipatory systems* (Section 2.2.6). He emphatically denied any relationship to teleology. See Kineman (2011) and Mossio and Bich (2017) for recent discussions of the four causes as Rosen saw them, and Hofmeyr (2018) for discussion of the formal cause in particular.

In metabolism the efficient causes are the catalysts, or enzymes. Closure to efficient causation thus means that all of the enzymes used by an organism need to be synthesized by the organism itself: none are given from outside. Nonetheless, Razeto-Barry (2012) has pointed out that even protein enzymes might in some circumstances be acceptable as exceptions:

> To my knowledge, no organisms have been reported to date that directly obtain a functional enzyme from its environment, but the requirement of cofactors and coenzymes have been widely described, which are necessary to catalysis processes. Anyway, it seems evident that if we discover that a kind of bacteria directly obtains some functional enzymes from its environment, that would not negate that they are living beings. Indeed, the bacterial production of enzymes was discovered much later than the discovering of bacteria, which were considered living beings from the very beginning.

Razeto-Barry seems to imply here that cofactors such as NAD can be harvested from the environment, but we know of no examples of this. The more important point concerns protein enzymes, and proteins such as green fluorescent protein can be transferred between cells of the bacteria *Desulfovibrio vulgaris* and *Clostridium acetobutylicum* (Benomar et al., 2015). One cannot exclude the possibility, therefore, that functional enzymes might be exchanged. Even if they are, however, very rare exceptions do not undermine the whole concept of closure to efficient causation.

Rosen's illustration of closure in Fig. 10 (especially Fig. 10b) exemplifies the folding back that he mentions in the following passage (Rosen, 2000, p. 137):

> The only other possibility[50] is to fold this infinite regress back on itself—i.e., to create an impredicativity.[51] That is, to suppose there is some stage N in this infinite regress that allows us to identify the system we require at that stage with one we have already specified at an earlier stage.

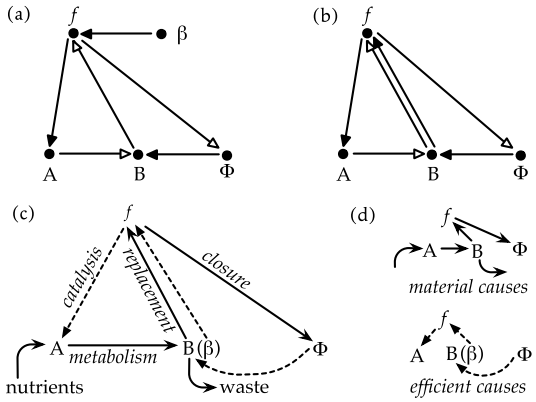


Fig. 10. Closure to efficient causation. (a) Rosen's diagram in Fig. 10C.5 of *Life Itself* to illustrate a series of alternating efficient and material causes: f, the efficient cause of metabolism, acts on A, the set of substrates of metabolic reactions, to produce B, the set of products; B is the source for forming f, under the action of Φ, the efficient cause of the regeneration of f (*repair* in Rosen's terminology). As drawn here it required an additional element β to catalyse the production of Φ from f, leaving the origin of β unexplained, so the system is on the threshold of infinite regress. (b) If β is represented as a property of B (not as the whole of B), then infinite regress is avoided. This is Fig. 10C.6 of *Life Itself*. Here the types of arrows in the two panels have been made consistent with one another, though they were used in opposite ways in the original figures. (c) An attempt to make it more intelligible (Letelier et al., 2011). The irreversible conversion of nutrients into waste, thermodynamically necessary, and explicit in *Essays on Life Itself* (Rosen, 2000, pp. 17–18), but only implicit in panels (a,b), is shown explicitly. (d) Definitions of the types of arrows in (c). In all these panels we have followed Rosen's practice of showing each catalyst as acting on the left-hand side of a transformation; the usual practice in biochemistry, however, is to treat a catalyst as acting on the transformation as a whole, not just on its left-hand side.

The identification of β with a property of B is a type of folding back.

Fig. 10b is no easier to understand than Rosen's text, and our redrawing (Letelier et al., 2011) in Fig. 10c is intended to be more easily intelligible, with the more explicit model shown in Fig. 11 as a more concrete example. The idea of a general efficient cause f catalysing the whole of metabolism, represented by conversion of A into B, is clear enough. However, catalysts are not indefinitely stable (and may be lost for other reasons, such as the dilution that results from growth), and need to be replaced, catalysed by another general efficient cause Φ, itself produced from f in a process catalysed by an entity β. Rosen (1991) did not make the nature of β clear: it is best thought of as a property of B, the set of products of metabolism.

One way to understand Fig. 10 is to say that metabolism is a function that acts on metabolism to produce metabolism:

$$\text{metabolism}(\text{metabolism}) = \text{metabolism} \tag{1}$$

which can also be written as follows (Letelier et al., 2005):

$$f(f) = f \tag{2}$$

in which f plays successively the roles of function, argument and result.[52]

2.2.3. Modelling and simulation

Rosen insisted that closure to efficient causation requires that an organism cannot have a model. To understand this it is important to realize that he made a crucial distinction between *modelling*, which he considered impossible, and *simulation*, which he considered possible. He did not regard simulation as the same as modelling, and to understand his theoretical ideas it is important to keep the two concepts separate,

[50] He meant other than resorting to an external agency, a solution that he rightly rejected.

[51] The concept of *impredicativity* was introduced by Bertrand Russell (1907) (as *non-predicativity*): put simply, it means defining something in terms of itself.

[52] Gutiérrez et al. (2011) call this the *Ouroboros equation*, from the dragon that nourishes itself by eating its own tail. However, the Ouroboros exemplifies material closure, not efficient closure.

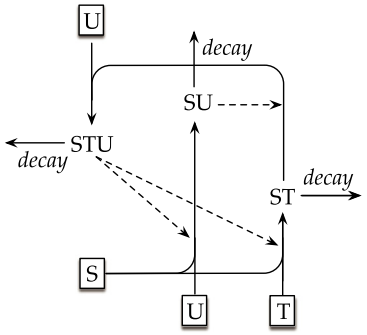


Fig. 11. A concrete example of an (*M, R*) system (Cornish-Bowden et al., 2007). S, T and U (shown in boxes) are molecules available from the environment. Of the three reactions, S + T → ST is catalysed by STU, ST + U → STU is catalysed by SU, and S + U → SU is catalysed by a second activity of STU. Chemical transformations are shown with full arrows and catalytic interactions with dashed arrows. To make the model susceptible to computer simulation (Cornish-Bowden et al., 2013) it is necessary to treat the catalysed reactions as cycles of chemical reactions. The model was developed from an earlier extension (Letelier et al., 2006) of a suggestion of Morán et al. (1996).

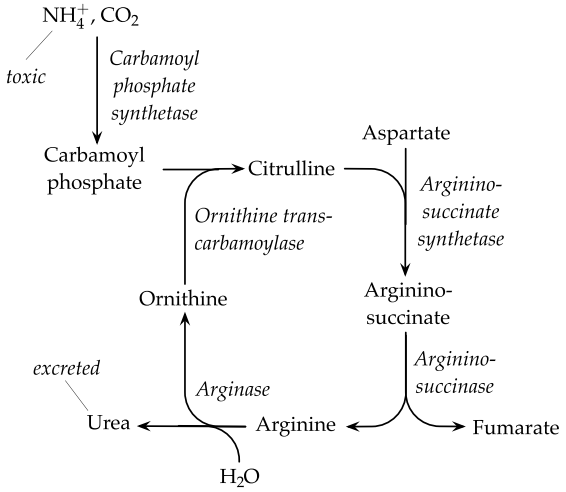


Fig. 12. The urea cycle. The cyclic portion is conventionally considered to be catalysed by arginase, ornithine transcarbamoylase, argininosuccinate synthetase and argininosuccinase. Ornithine is also a biological molecule, however, and it is regenerated at the end of the cycle, so it is a catalyst. If an enzyme is defined as a biological catalyst (a definition that some will find too loose, preferring to restrict the word to macromolecules) then ornithine fits as well as do the four proteins.

as we have tried to explain elsewhere (Cárdenas et al., 2010; Cornish-Bowden et al., 2013). For him a model of, for example, a machine incorporates understanding of how the machine works. A simulation just produces some of the same behaviour, without necessarily using any mechanisms that replicate what happens in the real machine. Rosen was not in general very interested in simulation, but he did not deny that it was possible, and gave some suggestions about how it might be done (Rosen, 1973). Seck and Honig (2012) have given a clear and informative explanation of what the modelling relation means.

The difference is evident in the different approaches used in numerical analysis and in statistics for fitting mathematical functions to experimental data. Numerical analysts will seek an equation that agrees with the observations as closely as possible, so that it can be used for predicting behaviour in the absence of experimental data for some conditions of interest. They use functions like splines and orthogonal polynomials that provide no insight into the reasons why a system behaves as it does. Statisticians, on the other hand, seek the best model that can explain a set of observations. Thus in Rosen's terms a model of a machine does not just mimic its behaviour; it incorporates an understanding of how it works.

Rosen's claim that an organism cannot have computable models has been contested by several authors, including Landauer and Bellman (2002), Wells (2006), Chemero and Turvey (2008), Chu and Ho (2007) and Mossio et al. (2009). Louie (2009) has argued the opposing case very strongly, and there is little doubt that at least some of the objections (though not those of Mossio et al. (2009), whose argument was based on the application of λ-calculus) are due to failure to understand Rosen's logic. Palmer et al. (2016) have proposed what may prove to be a resolution of the argument: their suggestion is that it may well be impossible for a single computer (more specifically, what they call a "finite state machine") to model an organism, a set of such computers that pass information to one another and act on information received from other computers, may be able to model an organism. It is too soon to know whether their interpretation will be accepted by others. For ourselves, we find it plausible.

2.2.4. Membranes and barriers

There is no membrane or other barrier in Figs. 10–11 to separate the system from its environment or from other similar systems, and no mention of membranes can be found in Rosen's writing. Nonetheless, he did consider that any living system had an inside and an outside environment, and he did discuss boundaries (Rosen, 1972). In any case, the system can be enclosed in a membrane without doing injury to his analysis (Cornish-Bowden and Cárdenas, 2008). We return to this question in Section 3.1.5. Meanwhile, simulation of the example illustrated in Fig. 11 showed that it was capable of reaching a steady state and exhibiting properties such as bistability (Piedrafita et al., 2010).

Rosen was, in fact, interested in systems in which two or more species share the same environment, and his last two books (Rosen, 1991, 2000) both appeared in a series on *Complexity in Ecological Systems*: in such systems the separation of self from not-self cannot be avoided. Although (*M, R*) systems have usually been discussed in relation to single species, there is no reason in principle why two or more (*M, R*) systems cannot occupy the same environment, each providing the other with metabolites that it needs but cannot produce itself (Benomar et al., 2015; Cárdenas et al., 2018). For this to work satisfactorily there needs to be a mechanism, which Xavier et al. (2011) have called *metabolic prudence*, to overcome "cheating" when organisms profit from resources provided by others without reciprocating.

2.2.5. Enzymes and metabolites

Catalytic closure has an important implication for the distinction usually made between enzymes and metabolites (Cornish-Bowden and Cárdenas, 2007a). If all enzymes are products of metabolism they are metabolites. Likewise, many metabolites behave like enzymes if they catalyse metabolic processes. Fig. 12 illustrates the urea cycle, a pathway for detoxication of NH_4^+ by converting it to urea. Ornithine binds carbamoyl phosphate, and is regenerated when urea is released: it is therefore a catalyst, and as it is a biological molecule it can also be regarded as an enzyme.[53] Thus in addition to the four protein enzymes in Fig. 12 there is a fifth biological catalyst, consisting of ornithine, citrulline, argininosuccinate and arginine together.

[53] Many definitions of *enzyme* include the word "macromolecule", and in the past, before catalytic RNA was discovered (Zaug and Cech, 1986), they often included the word "protein". If these words are included the small molecules in Fig. 12 are excluded, but we prefer to regard any biological molecule that acts as a catalyst as an enzyme. The definition given by *Encyclopædia Britannica* is even broader than ours: "*Enzyme*, a substance that acts as a catalyst in living organisms, regulating the rate at which chemical reactions proceed without itself being altered in the process" (https://www.britannica.com/science/enzyme). This would allow, for example, the Mg^{2+} ion to be called an enzyme.

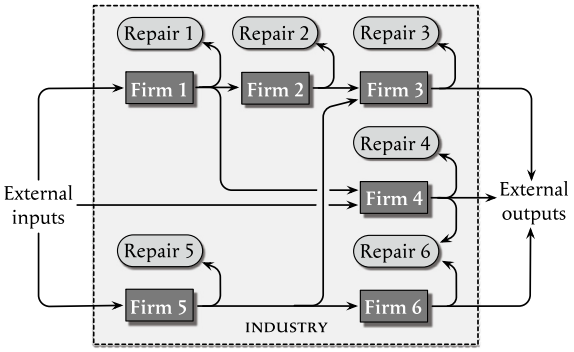


Fig. 13. Interaction between industrial firms. The set of firms in an industry can be regarded as a set of (*M, R*) systems that interact with one another and with the outside world. At least one, but usually more (Firms 1, 4 and 5 in this example), receives input from the exterior. Likewise at least one (Firms 3, 4 and 6) exports output to the exterior. Some (only Firm 2 in this example) interact only with other firms, not with the exterior. All of them must have self-maintenance systems, labelled here as *repair* in accordance with Rosen's terminology. In some cases one firm may aid the maintenance of another, for example Firm 4 connects to Repair 6: that is not obligatory in the model, and does not relieve the aided firm of responsibility for its own maintenance. The figure is based on Fig. 1 of Casti (1989).

In contrast to autopoiesis (Section 2.5.6) there have been almost no attempts to extend the concept of (*M, R*) systems beyond the domain of biology. One example, however, was an analysis of interactions between industrial companies (Casti, 1989), illustrated in Fig. 13.

2.2.6. Anticipatory systems

Rosen (1985) saw living organisms as *anticipatory systems*, a concept that underlay his thinking long before his book was published.[54] It means that organisms incorporate models of themselves that allow them to anticipate future changes and thus take action to cope with them. It is important to understand that they are responding to a *predicted* future, not to the *actual* future. As Mikulecky (2001) explained, the latter would be preposterous:

> In no way is a set of dynamics using information present only in the future. Rather, the system is making an "educated guess" about the future as it makes its responses in the present. The sorting out of these two very different aspects of system behavior, one which is preposterous and one which is so sensible as to be inescapable, is one of Rosen's greatest accomplishments, even though few have come to recognize its sweeping significance.

Rosen saw senescence as progressive decline in the capacity to anticipate correctly (Rosen, 1978). Several recent articles (Poli, 2014; Cevolini, 2016; Nasuto and Hayashi, 2016; Hofmeyr, 2017; Bettinger and Eastman, 2017; Gare, 2017) discuss anticipation, but none of them relate it to metabolic regulation, not even Hofmeyr (2017), despite the major contribution that he made to understanding of metabolic regulation. Rosen (1985) himself tried to do this, but he discussed it in terms of *feedforward activation*, which is very rare in demand-driven pathways, including nearly all biosynthetic pathways. The only example that we know of is the activation of pyruvate kinase by fructose 1,6-bisphosphate in erythrocyte glycolysis (Bali and Thomas, 2001), which agrees with Rosen's interpretation of the feedforward activation as a "warning" that there will be a need for the enzyme to be more active. However, this is a rare example in intermediary metabolism, as emphasized by the authors who described it. Feedforward activation does occur, however, in supply-driven pathways, such as glycogen synthesis in the liver (Cárdenas and Goldbeter, 1996), and in other detoxication processes.

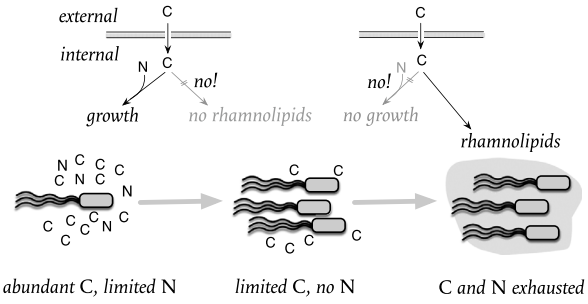


Fig. 14. Anticipating a need to swarm. When carbon is abundant and nitrogen is not, cells of *Pseudomonas aeruginosa* grow and multiply as individuals until the nitrogen is exhausted. They then use the remaining carbon to synthesize rhamnolipids, which, when present in large amounts, allow *swarming*, a form of collective surface motility that allows the cells to move as a colony to a region where nitrogen may be more available. No rhamnolipids are produced in conditions where the bacteria can grow.

The most likely explanation of why feedforward activation is almost unknown in demand-driven pathways is that most enzymes in such pathways have almost no control of the flux through their own reactions, so changing their activities is typically without noticeable effect. This is, of course, unintuitive,[55] and many biochemists have found it difficult to believe, but it is an important consequence of metabolic control analysis, discussed in Section 4.3.2.

However, anticipatory behaviour occurs in a much wider context than the biosynthetic pathways of intermediary metabolism, and it is unfortunate that Rosen (1985) chose such pathways, which are almost always regulated by feedback inhibition, not by feedforward activation, to illustrate his analysis. Walker et al. (2017) made the point that feedback regulation is not ideal in all circumstances, as also emphasized by Louie (2009):

> However, feedback control has significant limitations. First, it is reactive, and as such, can only respond to perturbations that have already occurred, even if these are detrimental to the controlled system. Thus, in the context of nutrition, the animal must experience a lack of nutrients, which can be detrimental to physiological systems, before mounting a regulatory response.

Even organisms as simple as bacteria have some anticipatory capacity, as illustrated for *Pseudomonas aeruginosa* in Fig. 14, based on results of Xavier et al. (2011). Likewise insects (Walker et al., 2017) can modify their behaviour to cope with expected needs. In *Drosophila melanogaster*, flies increase their intake of yeast, amino acids and sodium after mating, in order to ensure sufficient of these nutrients for egg production.

2.3. The hypercycle (1971 onwards)

Manfred Eigen was more conscious of the development of molecular biology in parallel with biochemistry than some of the authors discussed in this review. He started from the paradox whereby precisely defined nucleic acids could only be produced by highly specific enzymes, which themselves could only be produced with the use of precisely defined nucleic acids, and neither of these conditions could be fulfilled at the origin of life. To resolve the paradox, he proposed the *hypercycle* model, later developed with Peter Schuster, in which enzymes and nucleic acids are arranged in concentric cycles so that each enzyme acts to enhance the formation of the *next* nucleic acid in its cycle.

[54] The book itself was written in 1979, but published only six years later. Howard Pattee (2007) has described the evolution of Rosen's thought over the years until *Life Itself* (Rosen, 1991) appeared.

[55] To make it more intuitive, consider what happens when a rock is thrown into a stream: the levels of water around the rock may change, but the flow of water will not.

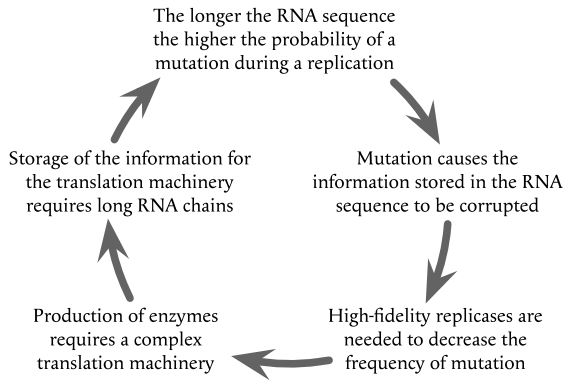


Fig. 15. Eigen's paradox. Following the arrows (starting anywhere) shows that a simple model in which the same kinds of molecules fulfil both functions cannot work: RNA does not have sufficient catalytic potential to provide the necessary specificity, and proteins alone cannot replicate. The scheme is based on Fig. 2 of Szostak et al. (2016).

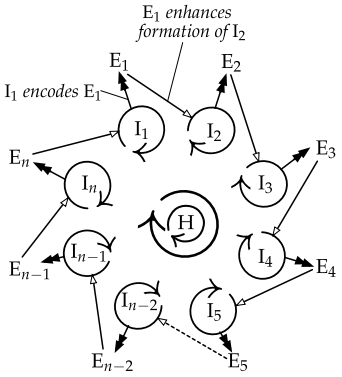


Fig. 16. The hypercycle: a cycle inside a cycle, based on Fig. 15 of Eigen (1971). The hypercycle can be regarded as a cycle of RNA fragments I_i surrounded by a cycle of proteins E_i. Each E_i acts to enhance formation of the *next* RNA fragment in the series I_{i+1}. The curved arrows around each I_i indicate that it is capable of self-replication, and the two such arrows around the central H indicate that the hypercycle as a whole consists of a cycle within a cycle.

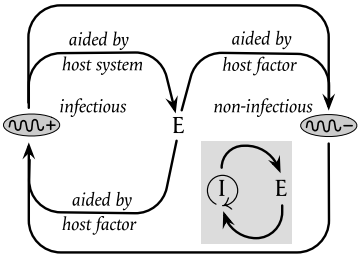


Fig. 17. Phage infection as an example of a hypercycle. The infectious + strand of the phage uses the translation machinery of the host cell to instruct it to synthesize a protein subunit E that associates itself with other proteins to form a phage-specific RNA replicase. This recognizes phenotypic features of the − strand as well as the + strand, and therefore replicates the + strand. The inset illustrates the hypercyclic nature of the process.

2.3.1. Eigen's (Woese's) paradox

Eigen (1971) originally proposed the hypercycle as a way to escape what Maynard Smith and Szathmáry (1995) later called *Eigen's paradox*[56]: there can be no efficient enzymes without accurate information storage, and no accurate information storage without efficient enzymes (Fig. 15). He presented the problem as follows:

[56] *Woese's paradox* might have been a better term, as Carl Woese (1968) was probably the first to discuss it.

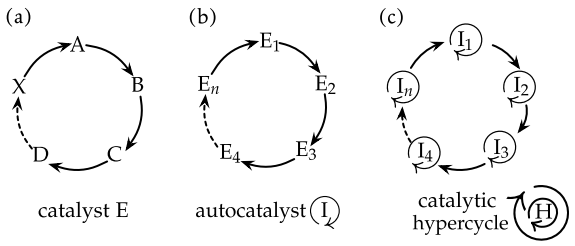


Fig. 18. Hierarchy of cycles. The hypercycle can be regarded as the highest level of a hierarchy of organization. (a) A cycle of metabolic reactions catalysed by an enzyme E. (b) A series of enzymes controlled by a self-replicating autocatalyst. (c) A catalytic hypercycle.

As a consequence of the exciting discoveries of "molecular biology",[57] a common version of the above question is: *Which came first, the protein or the nucleic acid?*—a modern variant of the old "chicken-and-the-egg" problem. The term "first" is usually meant to define a causal rather than a temporal relationship, and the words "protein" and "nucleic acid" may be substituted by "function" and "information". The question in this form, when applied to the interplay of nucleic acids and proteins as presently encountered in the living cell, leads ad absurdum, because "function" cannot occur in an organized manner unless "information" is present and this "information" only acquires its meaning via the "function" for which it is coding.

Maynard Smith and Szathmáry (1995, pp. 44–49) and Barbieri (2003, pp. 140–144) analysed Eigen's paradox and the *error catastrophe* that results, in which organisms cannot correct replication errors as fast as they arise. The essential point is that the RNA molecules of a replicating system without specific catalysts cannot reach a length of greater than (at most) 200 bases, as longer molecules will be overwhelmed by the error catastrophe.

2.3.2. The RNA world

Despite the problems that follow from Eigen's paradox, there continue to be adherents of the *RNA world*, the idea that in early life RNA fulfilled the functions of both DNA and proteins, probably because there are some obvious advantages to this view:

1. RNA can in principle encode protein sequences in the same way as DNA;
2. It can form base pairs and replicate in the same way as DNA;
3. It can fold into three-dimensional structures that would be very difficult for DNA,[58] but analogous to those of proteins;
4. It can recognize and interact specifically with other molecules;
5. It can act as a specific catalyst for chemical reactions, particularly ones involved in information processing (Zaug and Cech, 1986).

Nonetheless there are some serious difficulties with the concept of an RNA world, as Bregestovski (2015) has discussed in detail. Here we shall just mention two problems. Although RNA can indeed catalyse chemical reactions, RNA enzymes have in general much lower catalytic

[57] At the time Eigen was writing molecular biology was a sufficiently novel idea for the term to be placed in quotation marks. The remark of Chargaff (1963, p. 176) that "molecular biology is essentially the practice of biochemistry without a license" was still fairly recent, and many biochemists looked on molecular biology with some disdain.

[58] That was once thought to be impossible, but Shih et al. (2004) have succeeded in designing DNA sequences that fold spontaneously into preselected structures. However, it remains true that the range of possible three-dimensional structures possible for DNA is much smaller than for RNA or protein.

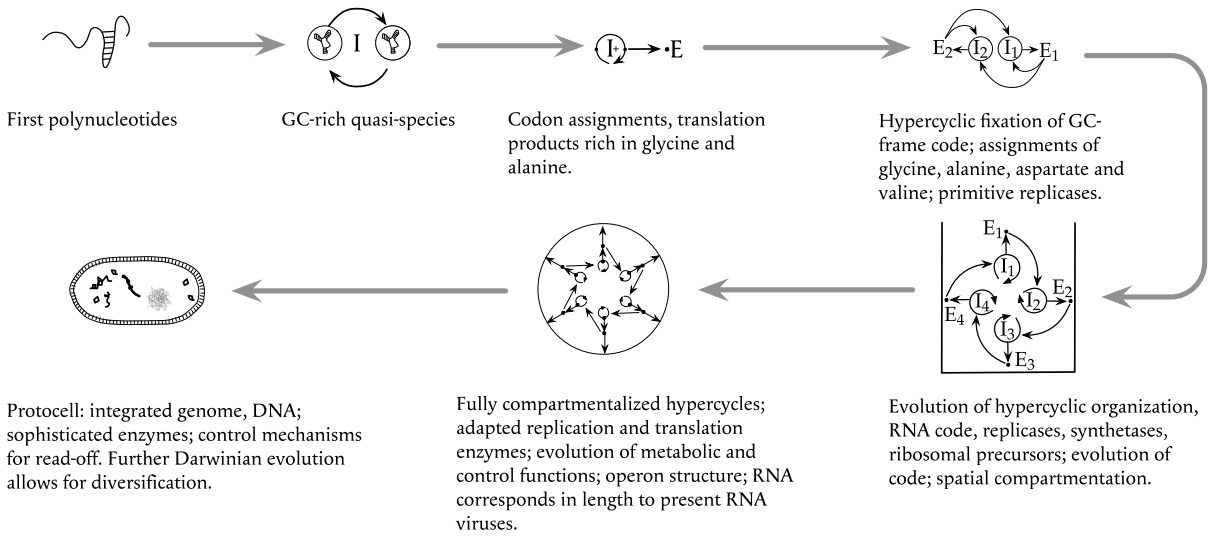


Fig. 19. Appearance of the first protocell. The scheme shows the sequence of events proposed by Eigen and Schuster (1978b) in their Fig. 63 to account for the development of a protocell from the first polynucleotides. There seems to be no indication of the source of the energy needed for the various processes. The last two drawings show what Eigen and Schuster (1978b) called "fully compartmentalized" structures, so they recognized the need for an enclosing boundary around a protocell.

activity than most protein enzymes. However, that is perhaps less important than it may appear, because in early life the degree of competition was much lower than we see today. More serious is the need for accurate replication: although RNA can form base pairs and replicate it does so with far less accuracy than is needed, and errors occur at an unacceptably high rate unless the sequence is very short. That brings us back to the error catastrophe noted by Eigen (1971).

2.3.3. The hypercycle

Eigen's ideas were later expanded into a series of papers written with Peter Schuster (Eigen and Schuster, 1977, 1978a,b), also collected as a book (Eigen and Schuster, 1979). The essential idea is illustrated in Fig. 16. It consists of a series of RNA fragments $I_1 \ldots I_n$, each of which codes for a protein in a corresponding series of proteins $E_1 \ldots E_n$. Each E_i acts to enhance formation not of its own RNA fragment I_i, but of the next one in the series, I_{i+1}.

The hypercycle model as drawn in Fig. 16 may seem too artificial to constitute a plausible model of a living system. However, Eigen and Schuster (1977) pointed out that viral infection of a bacterial cell provides a simple example of a real hypercycle, as illustrated in Fig. 17. They saw the hypercycle as being at the highest level of a hierarchy, as illustrated in Fig. 18.

2.3.4. Quasi-species

Eigen and Schuster introduced the important concept of the *quasi-species*. Evolution is usually thought of in terms of *evolution of species*, each species consisting of a population of individuals that are, if not genetically identical, at least extremely similar, and in particular similar enough to breed with one another. However, early in the development of life highly specific mechanisms for detecting and repairing replication and translation errors cannot have existed. Mutation frequencies must therefore have been high, leading to a much fuzzier kind of evolution and selection, in which the steady state is dominated not by the single fittest sequence but by a broad "cloud" of many sequences, all constantly mutating among a set of accessible sequences, with many genotypes differing to a greater or lesser extent from the average of the whole population, which defines the *wild type*. This cloud can be conceived of as a "quasi-species", and some authors see the situation at the time of LUCA (Section 2.6.1) in this light.[59] The idea of a quasi-species is particularly relevant to the evolution of rapidly evolving viruses with low fidelity (Domingo, 2002), and efforts are being made to find evidence for the existence of quasi-species in real organisms, not only in viruses but also in bacteria (Bertels et al., 2017).

A solid cancer fits the definition of quasi-species: it is genetically unstable, and replicates with very low fidelity, so that every cell is different from every other (Duesberg and Rasnick, 2000; Duesberg et al., 2011). It is not transmissible[60] or heritable (though susceptibility to particular forms of cancer may be), and thus proceeds from speciation to extinction within a fraction of the lifetime of its host.

Unlike most of the authors we discuss in this review, Eigen and Schuster were anxious not only to define the living state but also to suggest how it could have arisen from the first polynucleotides. At the end of their long series of papers they proposed the scenario illustrated schematically in Fig. 19.

Eigen and Schuster took replication of double-stranded DNA (in contrast to single-stranded RNA) as a "truly *self*-reproductive[61] example of a one-member catalytic cycle, i.e. both strands are copied concomitantly by the polymerase". Single-stranded RNA, in contrast, is not reproduced according to the same pattern.

2.3.5. Reaction cycles

Like Gánti (Section 2.4.3), Eigen and Schuster (1977) attached great importance to reaction cycles in metabolism. Unlike Gánti, however, they regarded the tricarboxylate cycle (Fig. 20) as a good example to illustrate their ideas.

2.3.6. Development of the hypercycle: Sysers

Sysers, "***sy***stems of ***se***lf-***r***eproduction", were explicitly proposed, independently by White (1980), Ratner and Shamin (1980), and Feistel (1983), as a development of hypercycles. The name was given by Ratner and Shamin, and they were intended to be more realistic and complete than hypercycles. A minimal model of a syser is illustrated in Fig. 21a, based on the analysis by Red'ko (1986, 1990).[62] It consists of an *information matrix* that is replicated under the influence of a *replication enzyme* E_1, and can be translated into E_1 and a *translation*

[59] Glansdorff et al. (2008) and Acevedo-Rocha et al. (2013) discuss this aspect of LUCA.

[60] The facial cancer of the Tasmanian Devil (Bender et al., 2014) may seem to be an exception, as it is transmitted from animal to animal, but the transmission is by biting, not by replication of the genome.

[61] The prefix *self-* is in italics in the original.

[62] Both papers are in Russian, and we thank Vladimir Red'ko for sending us an unpublished English manuscript combining the two. A more recent paper of Saakian and Red'ko (2018) discusses the adaptive syser in more detail.

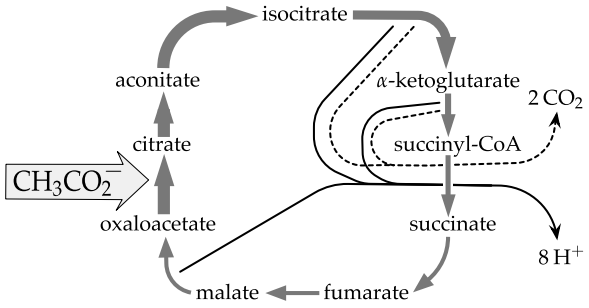


Fig. 20. Metabolic cycles. The tricarboxylate cycle (various coenzymes are not shown) is one of many cycles in metabolism that are more elaborate than individual enzyme-catalysed reactions. The overall reaction is catalytic, as oxaloacetate is regenerated at the end. Eigen and Schuster (1977) made the important point that all of the reactions are enzyme-catalysed, but they also noted that the necessary enzymes are not generated by the cycle, and so it is not closed to efficient causation. The scheme is based on their Fig. 3.

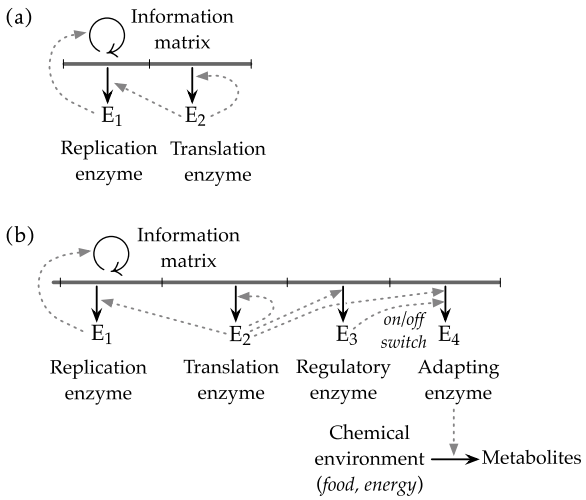


Fig. 21. Sysers. (a) A minimal model. A matrix molecule contains the information necessary for synthesizing two enzymes, a replication enzyme E_1, which catalyses replication of the matrix, and a translation enzyme E_2, which catalyses synthesis of both enzymes. (b) An adaptive syser. This was proposed as a way to overcome the problem that the minimal model was closed to material causation, and in consequence unable to grow or maintain itself. In addition to the enzymes E_1 and E_2 of the minimal model it also includes a regulatory enzyme E_3 that acts as an on/off switch for an adapting enzyme E_4 capable of catalysing reactions that convert molecules from the environment into usable molecules (metabolism). The diagram is based on Fig. 2b of an unpublished manuscript kindly provided by Dr. Vladimir Red'ko as an English version of two papers in Russian (Red'ko, 1986, 1990). He referred to "eatable food" rather than "metabolites", but we find this term a little misleading.

enzyme E_2, both translations catalysed by E_2. In this minimal form the scheme is closed to efficient causation, because all catalysts are products of the system itself. However, it is also closed to material causation, so it has no source of energy and cannot grow or maintain itself. Red'ko overcame this objection in the *adaptive syser* shown in Fig. 21b: this adds a *regulatory enzyme* E_3 that switches on or off synthesis of an *adapting enzyme* E_4 that catalyses the production of usable molecules from the chemical environment, in other words the metabolic component.

Notice also that E_2 is a "moonlighting" protein (Jeffery, 2003), as it catalyses at least two different processes, like the intermediate STU in Fig. 11. Moonlighting is an essential requirement for closure (Cornish-Bowden et al., 2007): if E_2 in Fig. 21 could only catalyse translation of the matrix into E_1, another enzyme would be needed for catalysing translation into E_2, and we should need to explain how this other enzyme is produced; unless at some point there was at least one enzyme with more than one function there would be infinite regress (Section 2.2.1). Increasing numbers of examples of moonlighting are being reported in biochemistry, for example in *Chlamydomonas reinhardtii* (Zhang et al., 2018).

2.4. The chemoton (1971 onwards)

> The intellectual seed of Gánti slowly delivers fruit, but the acknowledgement of the farmer is still lagging behind. This does not matter for him any longer, but it does matter for the content and moral of science: better late than never. I think that the time will come when his intellectual achievement will be regarded as outstanding.
>
> [Szathmáry (2015)]

Tibor Gánti was a chemical engineer, and developed his theory of life from his experience of managing a chemical factory. He saw life as an interaction between cycles of *metabolism* and *information* to produce an enclosing membrane. Unlike the authors of most of the other theories we discuss, other than the *hypercycle*, he emphasized the need for an organism to store and process information, and he regarded the enclosing membrane as essential. He was particularly interested in cycles of reactions, the formose reaction and others that are more elaborate.

2.4.1. History

Gánti (1971) proposed his theory of life in 1971, but it attracted very little attention outside Hungary until a collection of his work was translated into English by Eörs Szathmáry and published as a book (Gánti, 2003), with copious notes by Szathmáry and James Griesemer.

The essence of his model is shown in Fig. 22. As we shall see, the model of autopoiesis (Section 2.5) has some similarity to Gánti's, but with important differences.

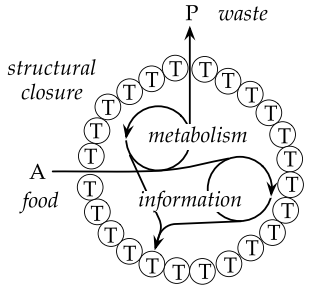


Fig. 22. The chemoton. It uses metabolism to convert food molecules A into waste, and uses the metabolic energy thereby provided to create an enclosing membrane. It does not define any specific catalysts for the individual reactions, but it recognizes that metabolism consists of an autocatalytic cycle, and also includes an information cycle. The food molecules A should be seen as a *set* of molecules rather than just as a single molecule.

2.4.2. Fluid machines

Gánti saw living systems as *fluid machines*, in contrast to the solid machines with pistons, wires and wheels that dominate our usual ideas of machines. As an example from pure (non-biological) chemistry to illustrate that liquids can act as machines he discussed the Belousov–Zhabotinsky reaction (Belousov, 1959, 1985; Zhabotinsky, 1964), which can generate oscillations both in space and in time (Figs. 23 and 24). He pointed out that the Belousov–Zhabotinsky reaction is a chemical analogue of the *predator–prey relationship* studied and analysed by Lotka (1910) and Volterra (1926) at the beginning of the 20th century. Gánti considered that fluid machines were vital for getting a better understanding of life, but he was interested in the possibility of a process that not only displays cyclic behaviour but also results in reproducing molecules.

In the chemoton, shown in Fig. 22, food molecules A enter a *metabolism* cycle in which they produce waste P, thereby driving the whole process, and also drive an *information* cycle. The two cycles

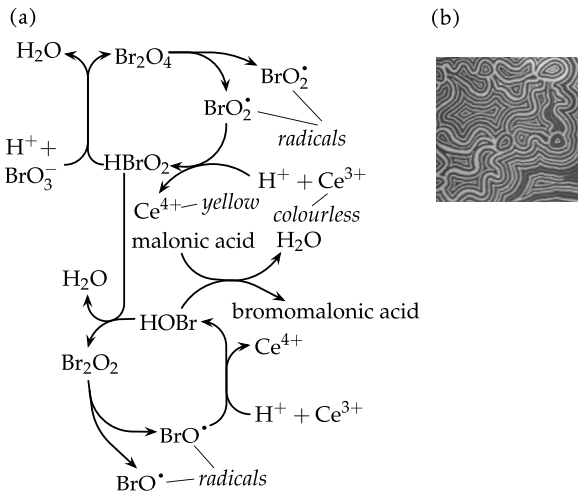


Fig. 23. The Belousov–Zhabotinsky reaction. (a) The oxidation of malonic acid by bromate in the presence of cerium salts follows a complex mechanism in which two cycles interact. The scheme is based on Fig. 2.3 of Gánti (2003). (b) This leads to oscillations in time and pattern formation in space. Belousov's original formulation was somewhat different from what is shown here. He used citric acid instead of malonic acid and the reaction gave oscillations in time, but no pattern formation in space.

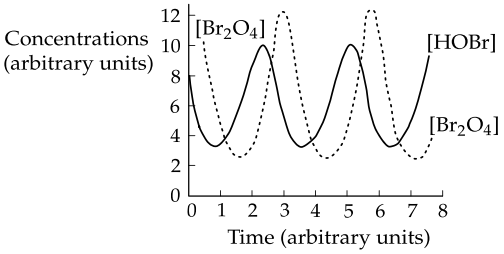


Fig. 24. Simulation of the Belousov–Zhabotinsky reaction. Gánti constructed a theoretical model based on the Belousov–Zhabotinsky reaction and calculated how it should behave, with results similar to those shown here. See Fig. 2.2 of Gánti (2003).

combine to produce molecules T that self-assemble to produce the enclosing membrane.

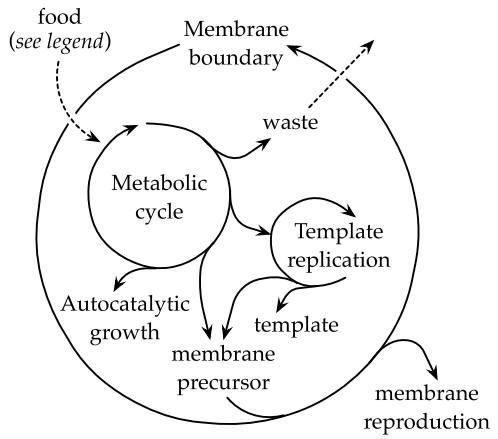


Fig. 26. A different way of showing the chemoton. This representation, based on one from Bich et al. (2016), gives a somewhat clearer idea of the working of the information cycle. Bich et al. labelled the input at top-left as "membrane precursor" rather than as "food" but we are treating that as an error.

As presented in Fig. 22, the way in which the information cycle actually encodes information is vague: the name "information cycle" seems to be little more than just a name, as Gánti's description includes no explanation of how information is coded, stored, and decoded. He did, however, provide an outline of how it might work (Fig. 25). Bich et al. (2016) have suggested a different way of representing it, shown in Fig. 26, that may make the functioning of the information cycle clearer.

The metabolic cycle is assumed to consist of several chemical reactions. As the component molecules are regenerated in each turn of the cycle, the cycle as a whole is autocatalytic, a characteristic that Gánti emphasized, but there is no mention of catalysts for the individual reactions.

2.4.3. *Reaction cycles in metabolism*

Like Eigen and Schuster (Section 2.3.5), Gánti gave great importance to the reaction cycles that play a major part in metabolism, such as the tricarboxylate cycle, the glyoxalate cycle and the reductive

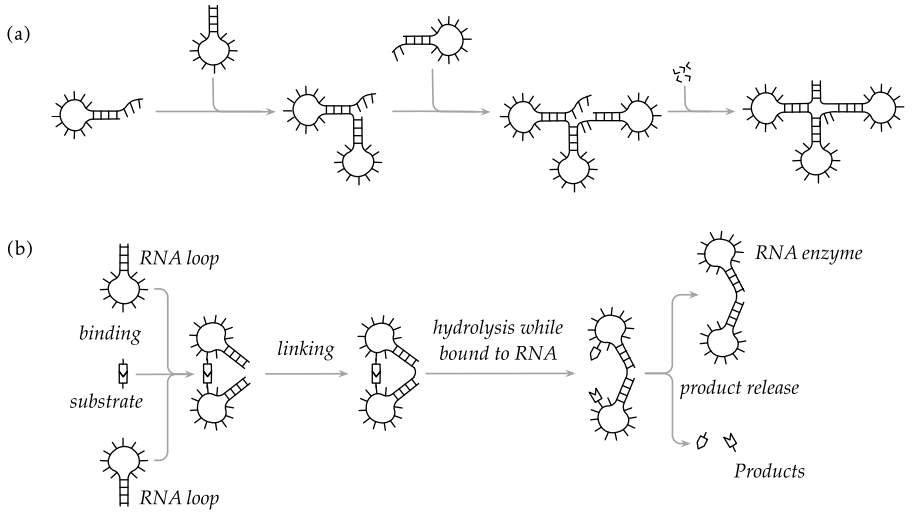


Fig. 25. Formation of RNA-based enzymes. (a) RNA molecules have a natural tendency to use base pairing to create larger structures similar to the clover-leaf structure of transfer-RNA as it exists today. (b) A plausible mechanism for the creation of an RNA enzyme. Two (different) RNA loops have the capacity to bind the two halves of what will become the substrate of a catalyst. This common affinity for the same small molecule brings them together to form a link that might otherwise be produced only by a chance encounter. Once formed the complex of two RNA loops can persist, leaving an RNA enzyme, after the substrate has been hydrolysed and the products released. The schemes are based on Figs. 2.7 and 2.8 of Gánti (2003, pp. 49–51).

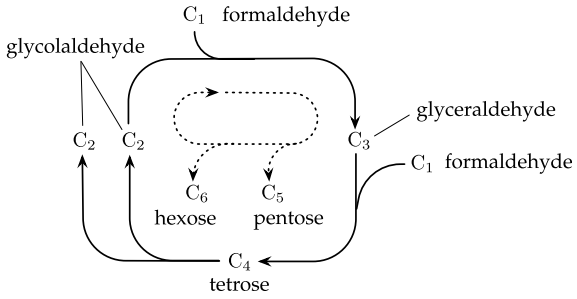


Fig. 27. The formose (Butlerov) reaction. Formaldehyde, a one-carbon molecule, C_1, can react with glycolaldehyde, a two-carbon molecule C_2, to produce glyceraldehyde, C_3, which reacts with a second C_1 to produce a four-carbon tetrose, C_4. This can then break down to two C_2, thereby allowing the cycle to continue, and so generate a second C_2 as product. The overall reaction is thus $C_1 + C_1 \rightarrow C_2$, and the catalytic cycle is $C_2 \rightarrow C_2$. In reality the process is more complicated than that, as indicated by the dashed lines, and also produces C_5 (pentose) and C_6 (hexose).

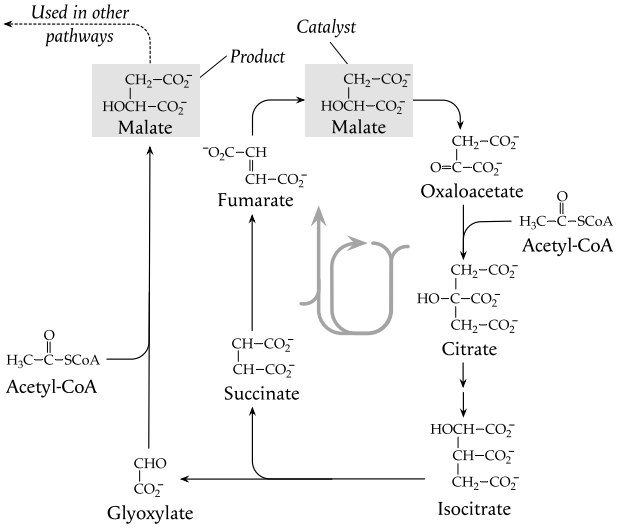


Fig. 28. The glyoxalate cycle (which Gánti called the "malic acid cycle"), in which malate acts not only as a catalyst, regenerated at the end of the cycle, but also, in the form of a second molecule, as a product that can be used in other metabolic processes, such as the malate–α-ketoglutarate antiporter.

pentose phosphate cycle,[63] and we have given the simple example of the urea cycle in Fig. 12. In all of these the catalytic molecule is also a product of the cycle. He also used the formose reaction (Boutlerow, 1861) as a simple and purely chemical cycle in which glycolaldehyde[64] is both catalyst and a product (Fig. 27): this was once thought a plausible model for the origin of life, as it can generate pentoses and hexoses from formaldehyde, a one-carbon molecule.

He also considered examples from real metabolism, such as the glyoxalate cycle (Fig. 28), in which the same metabolite, malate, acts both as catalyst and as product, because the overall process could be written as follows:

$$\text{malate} + 2\ \text{acetyl-CoA} \xrightarrow[\text{cycle}]{\text{Malate}} 2\ \text{malate}$$

[63] Gánti called these the Krebs cycle, the malic acid cycle and the Calvin cycle respectively.

[64] Glycolaldehyde, $HOCH_2CHO$, is a two-carbon carbohydrate but it is not regarded as a sugar by biochemists, though it is sometimes called a sugar by astrobiologists searching for evidence of extracellular life (Jørgensen et al., 2012). Formaldehyde, HCHO, is also a carbohydrate, but no one regards it as a sugar.

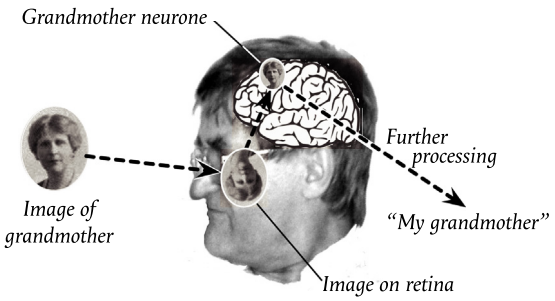


Fig. 29. Grandmother neurone. The positivist view of brain function supposes that an image of one's grandmother not only generates an image on the retina, but also stimulates a particular neurone, the *grandmother neurone*, to fire, and thereby stimulate further processing in the brain. This is the view that Maturana (1970) explicitly rejected.

He regarded this as a good model of the metabolic cycle in the chemoton; the reductive pentose phosphate cycle is a more complicated example. By contrast, he excluded the tricarboxylate cycle as a model, because oxaloacetate is regenerated but not released as a product:

> The Krebs cycle could only be a self-reproducing cycle if an oxaloacetic acid molecule passing through the constrained[65] path of the reactions, finally led to two oxaloacetic acid molecules.

2.5. Autopoiesis (1973 onwards)

Humberto Maturana and Francisco Varela developed autopoiesis from their view that the brain, and by extension the whole organism, is not a computer but must be analysed in terms of the interactions between its components. They gave very little attention to the detailed mechanisms that allow the organization. In this sense their ideas resemble Rosen's, but they did not take account of catalysis. Although their original description did not include a membrane or other enclosing barrier this was introduced early in the development and came to be regarded as essential.

2.5.1. History

During the 1960s, and still today (Kriegeskorte and Douglas, 2018), the principal metaphor for understanding the brain was the assumption that the nervous system is an information-processing device that decodes its sensory input, classifies it and then, according to the nature of the detected object, chooses a correct motor action. In other words the brain was seen as a computer, a metaphor often associated with John Von Neumann (1958), though his conception of the brain was far more subtle and carefully developed than that of some of his followers. This positivist viewpoint still dominates conceptual thinking in the field of neuroscience, and it seemed a natural way of thinking, at least initially. One interpretation based on this computer metaphor was that every *percept*[66] was coded (represented) by a specific neurone tuned to it, a *grandmother cell*[67] that only fires when it sees its own grandmother (Fig. 29).

[65] In Section 3.1.3 we shall consider the implications of Gánti's use of the word *constrained*.

[66] A percept is the thing perceived. If a person sees a flower, the image of a flower reconstructed in the person's brain is the percept of the flower. Humberto Maturana rejected this interpretation.

[67] This term is not only bizarre, but it is also misleading, because it does *not* imply the existence of a hierarchy from grandmother cell to cell: cell ← mother cell ← grandmother cell. It is a hypothetical neurone that fires when you recognize your grandmother or a picture of your grandmother. It was introduced informally by Jerome Lettvin (unpublished). Charles Gross (2002) has explained the history.

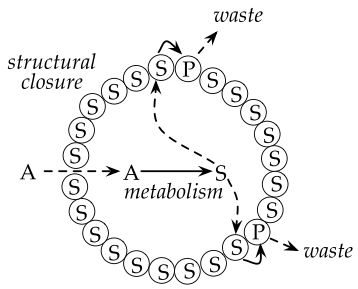


Fig. 30. An autopoietic system. The scheme is drawn to emphasize the similarity with the chemoton (Fig. 22). The starting material A should be seen as a *set* of molecules rather than just as a single molecule. The system is organized as a network of processes of synthesis and degradation of components, in such a way that these components are continuously generated, giving life to the network that produced them, and constitute a distinct unit in the domain where they exist. The definition of autopoiesis allows membrane synthesis to be incorporated (Varela, 2000).

Humberto Maturana (1970), already well known in 1963 as an author of a seminal paper in neurophysiology concerned with visual perception in the frog (Lettvin et al., 1959), challenged this representationist viewpoint on many grounds. His reflections led to a theory of life known as *autopoiesis*.[68] He proposed this with Francisco Varela, initially in a book in Spanish (Maturana and Varela, 1973), later translated into English (Maturana and Varela, 1980). Froese and Stewart (2010) see it as an extension of *cybernetics* as developed by Ashby (1956).

2.5.2. Metabolism and membrane formation

An autopoietic system is organized as a network of processes of synthesis and degradation of components, in such a way that these components are continuously generated, giving life to the network that produced them, and constitute a distinct unit in the domain where they exist. The definition of autopoiesis allows membrane synthesis to be incorporated (Varela, 2000).

The essential idea is illustrated in Fig. 30: a set of nutrient molecules A undergoes metabolism to products S, of which at least one can self-assemble into an enclosing membrane. S molecules in the membrane are degraded to waste products P that are exported. The process S → P provides the thermodynamic driving force for the whole system. In later work Maturana and Varela stressed the importance of the membrane for separating individuals from the environment and from one another. However, there is no provision for catalysis in their scheme, and, in particular, no provision for specific catalysis.

There is some similarity between autopoiesis and the chemoton (Section 2.4), and the two are drawn in Figs. 22 and 30 to emphasize the similarity. Both stress the importance of an enclosing membrane,[69] and both use the conversion of food molecules into waste as the source of the thermodynamic energy needed to drive the whole process. There are important differences, however.

Although the original formulation of autopoiesis made no mention of information, and there is no suggestion of nucleic acids in Fig. 30, Varela (2000) included a diagram similar to Fig. 31 that he described as a schematic illustration of the basic logic of cells.[70] He specifically mentioned DNA, RNA and proteins in the illustration, but he qualified this by insisting that the definition of autopoiesis specifies the general scheme of life without any reference to the structures of the components.[71]

[68] Greek αὐτό-ποίησις : self-creating.

[69] The membrane was not part of the original formulation of autopoiesis (Maturana and Varela, 1973). However, it appeared for the first time not long afterwards in the first attempt to model it (Varela et al., 1974), and has come to be regarded as a defining feature.

[70] Chapter 1 (pp. 21–40) of Varela's book is shown as a Spanish translation of Luisi et al. (1996). Our comments refer to Varela (2000).

[71] This recalls Rosen's presentation of his view of life in highly abstract terms with almost no mention of the molecules involved (Section 2.2).

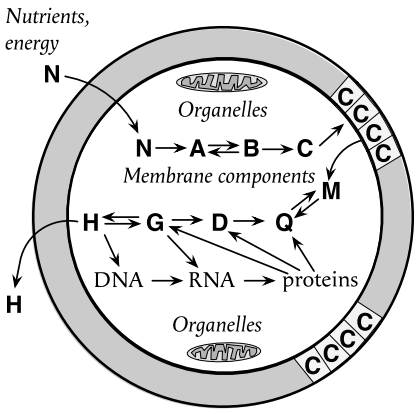


Fig. 31. The basic cellular logic. Varela (2000, p. 29, Fig. 2) used a figure similar to this to define a minimal form of life as the product of an emergent systemic organization (rather than of a specific structure or molecular reaction), linked to the notion of self-maintenance and self-regeneration of the components of the system. He added that the surrounding barrier was of vital importance to discriminate between self and not-self.

Varela (2000, p. 34) listed the following as the essential characteristics of an autopoietic system:

1. A semipermeable boundary;
2. A network of reactions;
3. Interdependence: the network of reactions is regenerated by the existence of the same boundary, so the boundary and the network depend on one another.

He went further, and drew two versions of the underlying reaction scheme, one of which (his Fig. 4a) made no reference to nucleic acids, and the other (his Fig. 4b) showed DNA, RNA and proteins as participating in the metabolic network. In the legend he described nucleic acids (his Fig. 4b) as regulating all the reactions in "our biological world": in other words he recognized that the biological world *as we know it* depends on nucleic acids, but these are not essential to the definition.

In the same chapter he revisited the question raised in Section 1.2 of how to decide if a particular entity is alive or not, applying it to a virus, a crystal, an amoeba, a mitochondrion and a stretch of DNA. As illustrated in Table 4, he concluded that only the amoeba satisfies all of the criteria. Notice the importance that he attached to circularity, that is to say to closure to efficient causation. Although in Table 4 we follow his classification of the mitochondrion as containing a network, this is problematic, because mitochondria in mammals (for example) code for very few proteins (Anderson et al., 1981), none of them directly involved in membrane formation or lipid production. *Amoebophrya ceratii*, a dinoflagellate, appears to have no mitochondrial genes at all (John et al., 2019). In such cases the network must be organized by the host cell, not by the mitochondrion.

2.5.3. Relationship to (M, R) systems

After a careful comparison of the relationship between autopoiesis and (*M, R*) systems (Section 2.2), Letelier et al. (2003) concluded that autopoietic systems could be regarded as a subset of (*M, R*) systems: all autopoietic systems are (*M, R*) systems, but not all (*M, R*) systems are autopoietic systems.[72] Their interpretation has been explicitly rejected by McMullin (2004) and by Razeto-Barry (2012), in part because it apparently rules out the possibility that autopoietic systems can be modelled by computer.

Razeto-Barry disagreed not only with Letelier et al. (2003) but with many others who tried to summarize the characteristics of autopoiesis, including Maturana and Varela (1973, 1980), Varela et al.

[72] Zaretzky and Letelier (2002) developed this idea further. Despite the years of publication, the paper of 2002 was written after that of 2003.

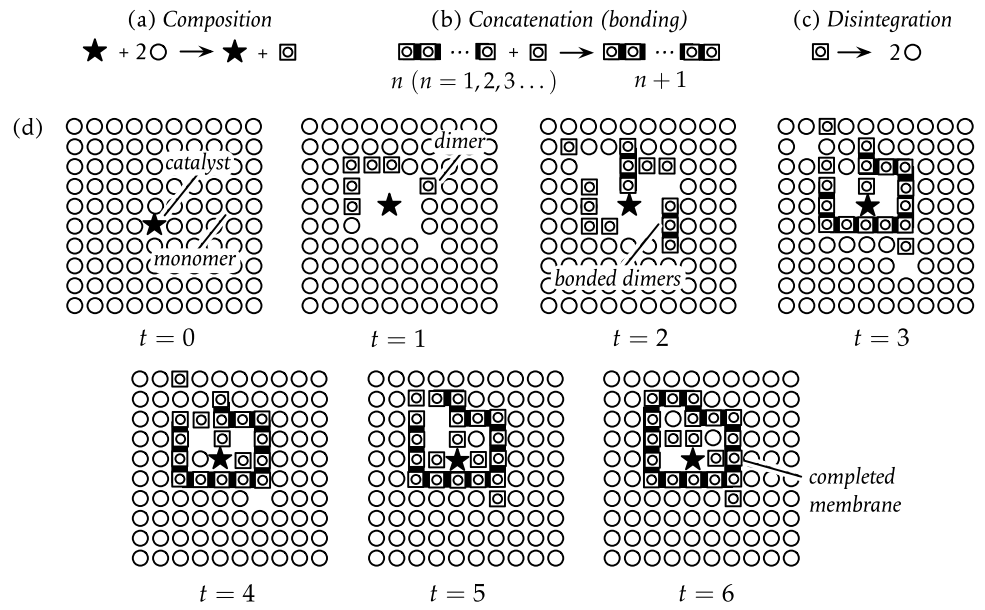


Fig. 32. Computer model of autopoiesis. Varela et al. (1974) set up a computer model in which an array of monomer units was influenced by a catalyst, with three processes, as shown at the top: (a) *composition*, catalyzed formation of dimers; (b) *concatenation*, addition of a dimer to an existing chain of *n* dimers; (c) *disintegration*, spontaneous conversion of a dimer to a pair of monomers. (d) After six iterations of one run of the program written to implement this scheme a closed cycle of dimer units appeared, illustrating the emergence of an autopoietic unit with limits. The figure is redrawn from Schema 1 and Fig. 1 of Varela et al. (1974).

Table 4
Detecting life: application of three criteria proposed by Varela (2000, p. 35, Table 2).

Entity	Is there a boundary?	Is there a network?	Is there circularity?	Is it alive?
Virus	Yes	No	No	No
Crystal	No	No	No	No
Amoeba	Yes	Yes	Yes	**YES**
Mitochondrion	Yes	Yes[a]	No	No
Stretch of DNA	No	No	No	No

[a]See the text.

(1974), Varela (1979), Luisi et al. (1996), and McMullin (2004). He objected to the idea of organizational invariance, because he considered that autopoietic systems do not maintain a constant organization. In addition, he thought infinite regress (Fig. 9, Section 2.2) was a "pseudo-problem", an opinion that we do not, of course, share.

2.5.4. Computational autopoiesis

Early in the development of autopoiesis, Varela et al. (1974) considered how an autopoietic system might be simulated computationally. They postulated the existence of three processes:

1. *Composition:* there was a two-dimensional array of monomer units ○ capable of forming dimers ◎ under the influence of a catalyst ★ (Fig. 32a);
2. *Concatenation*, or *bonding*: the dimers could spontaneously concatenate to form chains of arbitrary length (Fig. 32b);
3. *Disintegration*: concatenation was counteracted by a tendency of the dimers to break up to form monomers (Fig. 32c).

These operations were repeated until a closed cycle of dimers was achieved in the sixth iteration (Fig. 32d). The catalyst ★ was assumed to be capable of moving (as it did in the first iteration) as long as it was not blocked by the dimer chain. Once entirely enclosed in a chain, as at iteration 6, it could not move out of the enclosure. The monomer units ○, on the other hand, were allowed to diffuse across the membrane.

The computation illustrated in Fig. 32 was done in about 1971, a time of great political turbulence in Chile, and was published in 1974. The dates are important, because when Barry McMullin (2004) and Varela tried to repeat the computation in the early 1990s it proved very difficult to recover the details of how it had been done, and the computer program in FORTRAN IV seemed to have been definitively lost. However, a version of it was found unexpectedly in 1996 (though without any certainty that it was the version used for the original published results), and when McMullin (1997) studied it in detail he identified some inconsistencies in the original work. In the first place the catalyst ★ ought to have moved much more often than it did in Fig. 32. More important, the bonded dimers ◎◎ were unable to move once formed: this made it impossible for them to be used to fill gaps in the chain.

Analysis of the original program and the problems associated with it allowed McMullin (1997) to write a new program to implement the principles. With this application of the original theory McMullin and Varela (1997) were able to regenerate the original results of Varela et al. (1974). There remain problems with this and other attempts to simulate autopoiesis, however, as Cárdenas et al. (2010) have discussed in detail.

As the simulated system was not closed to efficient causation it tells us nothing about whether systems closed to efficient causation can have simulable models.

2.5.5. Experimental autopoiesis

Luisi (2003, 2006) has examined how autopoietic properties might be reproduced experimentally, and designed a system in which oleic aid, a surfactant capable of producing vesicles, was produced from oleic anhydride by alkaline hydrolysis, and was simultaneously oxidized to fragments that escaped into the medium, as shown in Fig. 33.

2.5.6. Autopoiesis in other fields

Autopoiesis has had comparatively little impact on mainstream biology, but it is well known in a wide variety of other fields, most notably sociology (Luhmann, 1988), but also such questions as whether buildings can think (Dollens, 2014). Most of these applications are too superficial to be useful, but a study of universities (Lenartowicz, 2015) may be an exception. In any case, none of them are pertinent to autopoiesis as a theory of life.

Maturana (2002) continued to insist that autopoiesis exists only in the molecular domain: he did not agree with the extension into

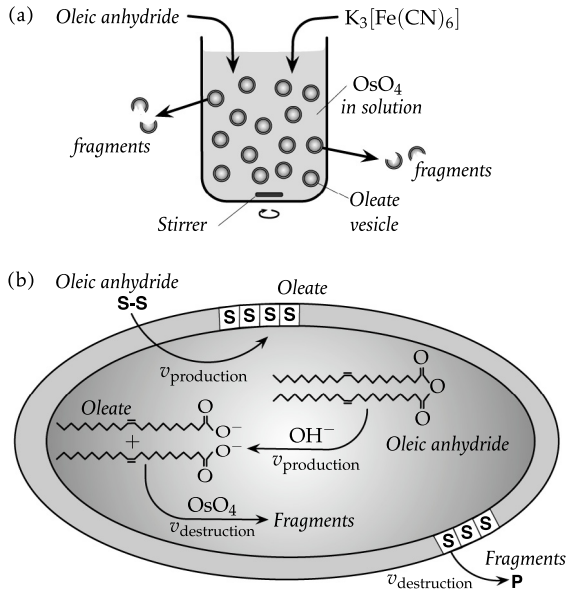


Fig. 33. (a) Oleic anhydride (precursor) and $K_3[Fe(CN)_6]$ are separately pipetted continuously into a reaction vessel containing a borate buffer at pH 8.8 and OsO_4. Alkaline hydrolysis converts the oleic anhydride (S–S) to the surfactant oleate (S), which forms vesicles. However, the oleate in the membrane is oxidized by the OsO_4 to fragments (P) that are released into the medium. The OsO_4 itself is regenerated by the $K_3[Fe(CN)_6]$. (b) Chemistry of the system. The figure is based in part on Fig. 8.5 of Luisi (2006).

sociology and other fields. The originator of a theory is not, of course, its owner and cannot dictate the directions it can take, but it is still helpful to consider his reasoning:

> A living system as a molecular system occurs as a closed dynamic molecular architecture that in its continuous transformation through thermal agitation continuously gives rise to itself.
>
> There is more, however. The molecular domain is the only domain of entities that through their interactions give rise to an open ended diversity of entities (with different dynamic architectures) of the same kind in a dynamic that can give rise to an open ended diversity of recursive processes that in their turn give rise to the composition of an open ended diversity of singular dynamic entities. Molecules through their interactions give rise to molecules and dynamic systems of molecular productions, in diffuse and localized processes that constitute discrete entities. I think that due to this peculiarity of the molecular domain this is the only domain in which autopoietic systems can take place as discrete singular systems that operate through thermal agitation and dynamic architecture.

He reasserted this view in a commentary on a paper of Cadenas and Arnold (2015, pp. 176–179).

2.6. Autocatalytic sets (1982 onwards)

A set of related ideas due to Freeman Dyson (1982), Stuart Kauffman (1986, 1993, pp. 298–341) and Karl Friston (2013) can be grouped under the heading of *autocatalytic sets*, though they differ in detail. What they have in common is an intention to show how self-organization could arise spontaneously in mixtures of chemical components. They are primarily theories of the origin of life rather than theories of the nature of life as it is now, unlike (*M, R*) systems, autopoiesis and the chemoton.[73]

[73] The hypercycle occupies a middle ground: it certainly concerns the origin of life, but it also sets out to be a model of life as it is.

2.6.1. Dyson's model of life

Dyson (1982) proposed a model that rests on the following postulates:

1. Molecular evolution occurs in small isolated populations, or "islands", which may be colloidal droplets, or solid particles. Each island exchanges molecular components with the surrounding medium, which supply the chemical energy needed to satisfy the laws of thermodynamics.
2. Evolution occurs by random drift (Kimura, 1983) only. Natural selection and Darwinian evolution begin only when the island populations begin to grow and compete for nutrients.
3. Each island contains a fixed number N of monomeric molecular units, some of which may be free (A, B, C...), others combined randomly into polymers (ABB, BABA...).
4. The polymers change by discrete mutations, one monomer at a time being added, subtracted or substituted in a polymer.
5. The multidimensional random walk of polymer mutations is mapped onto a one-dimensional walk by counting only the number of monomers that are "active" and "inactive": a monomer is active if it happens to be correctly placed as part of a structure that catalyses the synthesis of other catalytic structures. Otherwise it is inactive.
6. Each of the N monomers in an island mutates with equal probability $1/N$.
7. When a mutation occurs on an island with k active monomers the probability that the mutated unit be active is $\phi k/N$, where $\phi(x)$ is a function describing the autocatalytic capability of the whole assemblage of active monomers.
8. The function $\phi(x)$ is monotonically increasing on the interval $0 \le x \le 1$.
9. The equation $\phi(x) = x$ has three solutions, $x = \alpha, \beta, \gamma$, with $0 < \alpha < \beta < \gamma < 1$.

Dyson's paper did not include any figures, and in particular it did not include a diagram representing the system he described. He said that the crucial items in the list of assumptions were 7 and 9, adding that assumption 7 states that the effectiveness of active monomers in catalysing the placement of other active monomers depends only on the total number of active monomers present and not on their detailed placement. Assumption 9 states that there are three values of $x = (k/N)$, such that an island population with k active monomers is in a steady state.

Dyson ended his paper by raising seven questions that later research should address. Of these we shall just mention four:

1. Were the first living creatures composed of polypeptides or nucleic acids or a mixture of the two?
2. At what stage did random genetic drift give way to natural selection?
6. How late was the latest common ancestor of all living species?
7. Does there exist a concrete realization of the model, for example a population of a few thousand amino-acids forming an association of polypeptides which can catalyze each other's synthesis with 80% efficiency? Can such an association maintain itself in homeostatic equilibrium?

The first two of these questions are relevant to the hypercycle (Section 2.3), but they are ones that have been little addressed by authors other than Eigen. The sixth is one about which there is little agreement today. Some authors, such as Martin et al. (2016) place the last common ancestor very early, not long after the origin of life, or even before it (Di Giulio, 2011), whereas others, such as Tuller et al. (2010) and ourselves (Cornish-Bowden and Cárdenas, 2017), see it as resembling a bacterial cell, with a genome size similar to those of many extant organisms. Dyson (1982) expressed a similar view, rather tentatively: "It is therefore possible that the latest common ancestor

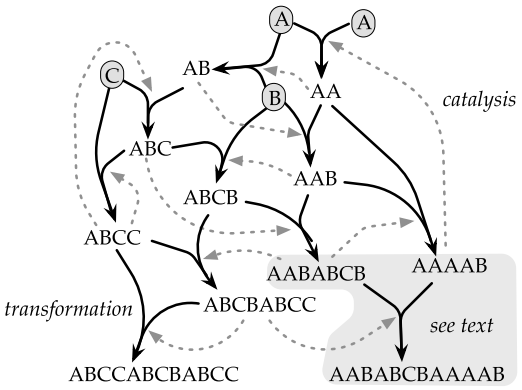


Fig. 34. An autocatalytic set as described by Kauffman (1986, 1993, pp. 298–341). The food molecules A, B and C shown in ovals are available in sufficient quantities from the environment. They can be amino acids, or RNA bases or other kinds of molecule that have some catalytic properties and are capable of polymerizing into chains of indefinite length. Full arrows represent chemical transformations, and broken grey arrows identify their catalysts. All of the intermediates can be generated from the food molecules by series of catalysed reactions. The shaded part of the diagram is discussed in the text.

came late in the history of life, perhaps as late as two-thirds of the way from the beginning". Dyson's seventh question is close to those that Kauffman (Section 2.6.2) and Friston (Section 2.6.4) addressed, as we now discuss. In a current review Peretó (2019, his Fig. 5.1.8) insists that "the origins of life and LUCA[74] are not the same". He sees LUCA as the only survivor of a long series of "attempts" at evolution and reproduction that occurred after the origin of life. His view is essentially the same as ours (Cornish-Bowden and Cárdenas, 2017, Fig. 4 therein).

2.6.2. Kauffman's autocatalytic sets

Kauffman (1986) proposed autocatalytic sets to see whether purely random collections of molecules with weak catalytic properties, such as peptides or RNA chains, could generate a self-organizing system without either natural selection or design. Rather than asking what properties were necessary for a system to be regarded as living, Kauffman, Dyson and, much later, Friston (Section 2.6.4) asked what sort of conditions might allow purely chance properties of sets of molecules to lead to self-organization. The most important part of Kauffman's definition of an autocatalytic set is the following:

> Catalytic "closure" must be achieved and maintained. Thus it must be the case that every member of the autocatalytic set has at least one of the possible last steps in its formation catalyzed by some member of the set, and that connected sequences of catalyzed reactions lead from the maintained food set to all members of the autocatalytic set.

The word *last* in the definition may be a source of misunderstanding. It does not mean that only the last step in each process needs to be catalysed: in fact *all* the steps in at least one route to a particular product need to be catalysed, but Kauffman is referring to the last step that produces the molecule in question from some other molecule in the set. Consider AABABCBAAAAB at the bottom-right of Fig. 34, for example: the last step in its formation involves ligation of AABABCB and AAAAB: we only need to worry about a catalyst for this step, because catalysts for the formation of AABABCB and AAAAB must exist.

The words *at least* in the definition mean that there is no requirement for every reaction to be catalysed but only that every member of the set must be reachable by a series of catalysed reactions. This does not exclude the possibility that alternative routes may exist that include uncatalysed reactions.

[74] The last universal common ancestor, also known as the *cenancestor*.

Fig. 34 illustrates a *small* autocatalytic set with only 11 members and three kinds of food molecule. This example is small enough to be used as an illustration, but far smaller than the sort of set that Kauffman initially thought would be needed, with around 10^9 molecules. As we shall see in Section 2.6.3, that was probably a gross overestimate. The untidy appearance of the illustration reflects the idea that it arose from purely chance properties of the component molecules.

Requiring every molecule to be reachable with a series of catalysed reactions is not the same as requiring every molecule to be a catalyst of some reaction, as in the following inversion of the definition: "a system is catalytically closed just in case every product of the system is also a catalyst in the system". Chemero and Turvey (2006) used this incorrect definition, in which the roles of catalysts and products are inverted, when trying to show that "catalytically closed systems are not closed to efficient causation", and claiming that the view of Robert Rosen that we explained in Section 2.2.2 was wrong.

Kauffman's aim was to study how autocatalytic sets could arise spontaneously, but very much smaller sets can be defined if they are specifically designed (Ashkenasy et al., 2004). In more recent work Vaidya et al. (2012) showed that a system of 16 catalytic RNA molecules could form an autocatalytic set. In both cases, however, the molecules had highly complicated structures that would be unlikely to form spontaneously, and in neither case were any external nutrients indicated.

In later books Kauffman (2008, 2016) extended his earlier ideas in important ways, and discussed those of Eigen and Gánti. However, for the sake of simplicity we have preferred to present them mainly in their original version, apart from describing RAF sets in the next section.

2.6.3. Hordijk and Steel's RAF sets

Hordijk and Steel (2004) introduced *RAF sets* ("***R***eflexive ***a***utocatalytic systems generated by a ***f****ood* ***set***") in an effort to construct a formalism for studying autocatalytic sets, so that they could be described and analysed in the computer. In a RAF set every reactant is either produced by the system or harvested from the environment, a definition that does not exclude the possibility that some catalysts are not produced internally.

The autocatalytic set $\mathcal{R}'$ is a subset of the set $\mathcal{R}$ of all possible reactions between reactants and products, together with X, the set of reactants in the autocatalytic set. X contains a subset F of molecules that are used in some of the reactions but not produced by them, these are the food molecules, assumed to be available from the environment. The meanings of the terms are as follows:

- *Reflexively catalytic:* Each reaction $r \in \mathcal{R}'$ is catalysed by at least one molecule in $\mathcal{R}'$;
- *Food-generated*: All reactants in $\mathcal{R}'$ can be created from the food set F by using a series of reactions that are all members of $\mathcal{R}'$ itself.

Each reaction r is represented as a sequence of elements (A, B), where $A, B \subset X, A \cup B = \emptyset$,[75] A is the set of reactants and B the set of products of reaction r. This formalism is similar to Rosen's treatment of enzymes as operators that transform sets of molecules into other sets.

We have applied the RAF set formalism of Hordijk and Steel (2004) to (M, R) systems (Jaramillo et al., 2010). Using RAF concepts we systematically explored the set of possible small idealized metabolic networks, searching for instances of (M, R) systems. This search showed that the central requirement of Rosen's framework, uniqueness of Φ, becomes harder and harder to obtain as the network grows in size. In addition, we gave expressions for the operators f, Φ and β in terms of RAF Sets.

[75] In words, A and B constitute a subset of a set X, and the intersection of A and B, the set of elements of A that are also elements of B, and vice versa, is the empty set $\emptyset$.

Hordijk, Steel and co-workers have shown that Kauffman's original estimates of how large an autocatalytic set needs to be were too pessimistic: first, Kauffman treated the probability P_n of catalysis by an intermediate of length n as constant, independent of n. However, Steel (2000) pointed out that that was unrealistic, and that we should expect that P_n should increase on average with n, as experience with amino acids, peptides and proteins would suggest. In addition, large autocatalytic sets can be decomposed into smaller subsets that themselves are autocatalytic, and these subsets can be identified and classified (Hordijk et al., 2012).

This means that an autocatalytic set can be much smaller than Kauffman's original calculations suggested (Hordijk and Steel, 2016), and that this structural decomposition of autocatalytic sets has important consequences for their potential evolvability, how they can enable their own growth and also the coming into existence of other autocatalytic (sub)sets, and how this can possibly give rise to higher-level, emergent structures.

2.6.4. *Friston's ergodic system with a Markov blanket*

Karl Friston (2013) is a recent newcomer to the field of theories of life. He argued that an *ergodic system* with a *Markov blanket* will inevitably result in life:

1. An *ergodic system* is a dynamic system in which the proportion of time that it spends in a particular state is the same as the probability that it will be found in that state at a random moment. This condition is not difficult to understand: coin tossing is ergodic if the proportion of tosses that result in a head is the same as the probability that a given toss results in a head.
2. A *Markov blanket* is the condition that all information about a variable in a Bayesian network is contained within the set of nodes composed of its parents (the set of states that influence it), children (the set of variables that are influenced by it), and other parents of its children.

Friston suggested that the surface of a cell might constitute a Markov blanket separating intracellular from extracellular states. This corresponds to the usual biochemical idea of *homeostasis* (Section 4.2.1), whereby the internal state is not affected instantaneously by changes in the external state, or *vice versa*. Friston contrasted this with a candle flame, which

> cannot possess a Markov blanket, because any pattern of molecular interactions is destroyed almost instantaneously by the flux of gas molecules from its surface.

The idea of a Markov blanket can be related to a property more familiar to biochemists, the rate of an enzyme-catalysed reaction in the middle of a metabolic pathway, as illustrated in Fig. 35. In the *local* Markov blanket around E_i the variables that influence E_i are the rate v_i and the concentrations of E_i, I_i and X_i. The variables that E_i influences are the concentrations of A_i, B_i, A_{i+1} and B_{i+1}. This is the system needed for defining the quantity known as the *elasticity* in *metabolic control analysis* (Section 4.3.4). However, it is part of a larger Markov blanket that includes the whole pathway that contains E_i. This is the "system" usually considered in metabolic control analysis, but it is itself part of a still larger Markov blanket consisting of the whole metabolism of the cell.

The different items in Fig. 35 are labelled with the terms used above in defining a Markov blanket:

1. *Variables in the network*: the concentrations of A_i, B_i, A_{i+1}, B_{i+1}, C_j and C_{j+1} are determined by the network.
2. *Their parents*: v_i, the rate at which A_i and B_i are supplied and A_{i+1} and B_{i+1} are consumed, and v_j, the rate at which C_j is supplied and C_{j+1} is consumed. These rates are determined externally and cannot be changed by the system. In addition, the concentrations of I_i and X_i are parents, as are also the concentrations and kinetic properties of E_i and E_j.

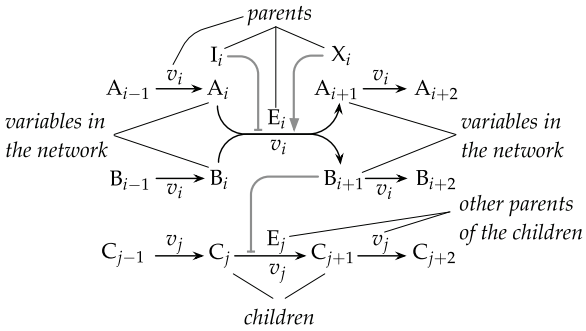


Fig. 35. Metabolism as a Markov blanket. An enzyme E_i, inhibited by one or more inhibitors I_i and activated by one or more activators X_i, catalyses a metabolic reaction $A_i + B_i \rightarrow A_{i+1} + B_{i+1}$. A second reaction $C_j \rightarrow C_{j+1}$ is catalysed by a different enzyme E_j that is inhibited by B_{i+1}, one of the products of the first reaction. If the whole system is in steady state all of the rates v_i must be equal, and E_i has no control of the rate of its reaction. Instead it must juggle the concentrations of its substrates and products so that they result in the necessary rate v_i. Likewise all of the rates v_j in the reaction catalysed by E_j must be equal. The labels refer to the definition of a Markov blanket (Friston, 2013); this is an attempt to illustrate a written definition, and is not based on one of Friston's own figures. These ideas are very important in the theory of metabolic control analysis (Section 4.3) and the scheme is used again in Fig. 44, relabelled to indicate the different degrees of isolation that need to be taken into account in analysing the properties of a metabolic system.

3. *Their children*: The concentrations of C_j and C_{j+1} are children, on account of the inhibition of E_j by B_{i+1}.
4. *Other parents of their children*: The other parents of the children are v_j and the concentration and kinetic properties of E_j.

An important part of Friston's argument is the *free energy principle* (Friston, 2012), which applies to any system that resists disorder:

> What are the basic principles that underwrite the self-organisation or self-assembly of biological systems like cells, plants and brains? This paper tries to address this question by asking how a biological system, exposed to random and unpredictable fluctuations in its external milieu, can restrict itself to occupying a limited number of states, and therefore survive in some recognisable form. The answer we entertain is based upon a variational free energy minimisation principle that has proved useful in accounting for many aspects of brain structure and function.

In other words the total Gibbs energy of a system can vary only within tight limits. We can understand this in relation to Fig. 35 by noting that any small perturbation, such as a small change in the concentration of A_i, will cause the system to return to exactly the same state as it was in before the perturbation. A somewhat larger perturbation, such as a change in the concentration of the inhibitor I_i, is usually resisted so that the new state is similar to the unperturbed state.

Friston (2013) maintained the tradition in the field of theories of life in making no reference in his paper of 2013 to any other modern theories apart from autopoiesis (Maturana and Varela, 1980), ignoring Rosen, Gánti, Eigen and Schuster, and, in particular, Kauffman. A more recent article (Ramstead et al., 2018) does mention Kauffman, as well as Eigen and Schuster, but does not discuss their work. More than 30 years ago, Kauffman (1986) said that it was probable that autocatalytic sets could arise spontaneously:

> The prebiotic emergence of reflexively autocatalytic sets of protein-like polymers may have been highly probable.

which can be regarded as a pre-echo of a much more recent statement by Friston (2013):

> Biological self-organization is not as remarkable as one might think—and is (almost) inevitable, given local interactions between the states of coupled dynamical systems.

The ergodic system with a Markov blanket can explain how a network can arise, but it is less clear how it explains Rosen's β, i.e. closure to efficient causation (Section 2.2.2).

3. Criticism of existing theories

> "The purpose of life", Munro said, "is to stay alive. Watch any animal in nature—all it tries to do is stay alive. It doesn't care about beliefs or philosophy. Whenever any animal's behavior puts it out of touch with the realities of its existence, it becomes extinct…"
>
> "Maybe there is a higher truth than merely staying alive", Ross said.
>
> "There isn't", Munro said.[76]
>
> [Crichton (1981)]

> The secret of man's being is not only to live but to have something to live for. Without a stable conception of the object of life, man would not consent to go on living, and would rather destroy himself than remain on earth, though he had bread in abundance.[77]
>
> [Fyodor Dostoevsky (1912, p. 268)]

3.1. Is there yet an ideal theory of life?

> The difficulty lies, not in the new ideas, but in escaping from the old ones.
>
> [Keynes (1936, p. ix)]

> The English … are said to be rigidly conventional, yet they behave with insouciance without parallel; and yet when you question them, they appear to have no definable theory of life.
>
> [Sayers and Walsh (1998, p. 1)]

Most of the theories we have mentioned seem very different from one another, but that is in part a consequence of their independent development by people with almost no contact with or knowledge of one another, not, in most cases, unbridgeable gaps between them. On closer examination only autocatalytic sets remain apparently rather different from the others, with the "untidy" appearance of Fig. 34 contrasting with the more "designed" appearance of the others, but this difference is rather subjective. Even there, catalytic closure is essential.

However, there is another respect in which autocatalytic sets, whether those of Dyson, Kauffman or Friston, differ from the others. (*M, R*) systems, autopoiesis and the chemoton were all conceived as descriptions of living systems, today and always, and none of them make strong appeals to the origin of life: although they can be seen as shedding light on it, that is not how their authors saw them. Hypercycles were introduced as a way of explaining the origin, but one can also interpret them as models of all life. However, the varieties of autocatalytic sets were all intended to explain how self-organizing systems first arose, and not how they are now.[78]

Probably on account of this separate and independent development of theories of life, there have been rather few attempts to compare and contrast them. We have tried to do this for a wide variety of theories (Letelier et al., 2011) and also in the specific case of (*M, R*) systems and the chemoton (Cornish-Bowden, 2015), and Moreno Bergareche and Ruiz-Mirazo (1999) gave brief summaries of some of them. Otherwise, Dennett (2011) asserted that two, autopoiesis and the chemoton, are "virtually synonymous", but that was a considerable exaggeration. Igamberdiev (2014) made a more profound comparison:

> Eigen's hypercycle is a formalized representation of the autopoietic system of Maturana and Varela or Rosen's (*M, R*) system.

As far as we are aware, this association between hypercycles and the two other theories mentioned, which Igamberdiev (2018) has extended more recently, has not been noticed by others.

Can any of the current theories be considered to be an ideal theory of life? To answer that we need to begin by listing the characteristics that an ideal theory ought to have. A living organism must then have the following characteristics:

1. It should be *thermodynamically open*, to allow a supply of energy to be harnessed.
2. It should incorporate *specific catalysts*, to ensure that its organization is not destroyed by unwanted parasitic reactions.
3. It should be *catalytically closed*, to be capable of maintaining its organization.
4. It should be *structurally closed*, with a barrier to separate self from not-self, and from its environment, and to allow concentration gradients across the barriers to be used for energy management.
5. It should have a mechanism for storing and reading *information*.
6. It should be capable of *controlled growth*.

We have not forgotten evolution and reproduction, but like Rosen (1991, p. 255), Varela (2000, p. 45), Maturana, commenting on a paper of Cadenas and Arnold (2015, pp. 176–179), and Gánti (2003, pp. 78–80), we regard them as consequences of life, not prerequisites. A self-organizing system in a constant environment could sustain itself for ever if it made no mistakes, even if it was unable to grow. However, no chemical reaction is 100% specific: "mistakes" always occur, in consequence the system evolves. If it grows it will inevitably reach a size where it needs to divide, and, because of "mistakes" the resultant entities will not be identical, and so reproduction implies a capacity for evolution.

In summary, staying alive is the fundamental necessity. Reproduction is not, because any organism unable to stay alive long enough cannot reproduce. Evolution is not even in third place, because an individual organism does not engage in evolution, and cannot. Organisms "want" to pass on their genes unchanged. The reason that they do not, and thus enable evolution, is simply that they cannot.[79] Gánti (2003, pp. 78–80) made the weaker condition that living systems should be *capable* of evolution, but he did not list this as an essential property. Our position is closer to Varela's, that the essential property is a capacity for staying alive, not necessarily for ever, as all organisms die, but for a period that is very long compared with the turnover times of its processes.

3.1.1. Checklist of theories against ideal requirements

Ruiz-Mirazo et al. (2004), despite being critical of the "checklist" approach to definitions of life, offered their own checklist, asserting that a definition of life should

[76] Michael Crichton's novel is not a reliable source of information about the biology of apes and their capacity for language, but the opinion expressed by Munro seems to us to be 90% correct—only 90%, because he ignores the amount of effort individuals put into mating, an activity that does not help them to remain alive, and, in the case of the males of many invertebrate species positively shortens their lifetimes.

[77] The statement spoken to Christ by the Grand Inquisitor in *The Brothers Karamazov* (Fyodor Dostoevsky (1912), translated by Constance Garnett), may apply to humans and the great apes, but the preceding quotation is more accurate for the overwhelming majority of organisms.

[78] Although Friston (2013) entitled his paper "Life as we know it", it is almost entirely concerned with the emergence of life, and says little or nothing about life as we know it.

[79] Errors are made inevitable by the fact that the interaction energies between partners are not infinite: for example, $tRNA^{Val}$ is sometimes charged with threonine (Fersht and Kaethner, 1976). Although editing mechanisms exist to recognize and correct errors, they are not perfectly reliable either, for the same reason. For a recent review of miscoding, see Westhof et al. (2019).

Table 5
Comparison of theories of life.

Property	(*M, R*) systems	Hypercycle	Chemoton	Autopoiesis	Autocatalytic sets[a]
Essential criteria according to Gánti (2003, pp. 78–80)					
Thermodynamically open	Implied	Implied	Yes	Yes	Implied
Specific catalysts	Yes	Yes	No	No	Yes
Catalytic closure	Yes	Yes	No	No	Yes
Structural closure	No	No	Yes	Yes[b]	No
Information coding	No	Yes	Claimed	No	No
Controlled growth	No	Unclear	Uncontrolled	Uncontrolled	Uncontrolled
Potential criteria according to Gánti (2003, pp. 78–80)					
Reproduction	No	Yes	Maybe	No	No
Evolvability	No	Yes	Maybe	No	No

[a]We consider the best-known version of autocatalytic sets, that of Kauffman (1986, 1993, pp. 298–341).
[b]Construction of an enclosing membrane produced by the system has been an important characteristic of an auto-poietic system since the paper of Varela et al. (1974), but the original presentation by Maturana and Varela (1973) did not include a membrane.

1. be fully coherent with current knowledge in biology, chemistry and physics[80];
2. avoid redundancies and be self-consistent;
3. possess conceptual elegance and deep explanatory power (i.e., it must provide a better understanding of the nature of life, guiding our search into its origins and its subsequent maintenance and development);
4. be universal (in the sense that it must discriminate the necessary from the contingent features of life, selecting just the former);
5. be minimal but specific enough (i.e., it should include just those elements that are common to all forms of life—not being, in principle, restricted to life on Earth—and, at the same time, it must put forward a clear operational criterion to tell the living from the inert, clarifying border-line cases, contributing to determine biomarkers, etc.).

Although these guidelines are useful to keep in mind when examining the theories, they are not easy to apply in practice: who is to judge whether a theory possesses "conceptual elegance and deep explanatory power"?

Rosen (2000, pp. 2–3) doubted whether a useful checklist had ever been produced, but he still regarded it as necessary for defining life:

> Despite the profound differences between those material systems that are alive and those that are not, these differences have never been expressible in the form of a list—an explicit set of conditions that formally demarcate those material systems that are organisms from those that are not. Without such a list, Schrödinger's question, and biology itself, become unanswerable at best, meaningless at worst.

Discussing the PICERAS definition of Koshland (2002), mentioned in Section 1.2, Ruiz-Mirazo et al. (2004) said:

> This author offers a definition that is not intended to be a mere list, but it ends up exhibiting very similar weaknesses. His suggestion of a set of seven "principles" or "pillars" of life (program, improvisation, compartmentalization, energy, regeneration, adaptability and seclusion) is not really satisfactory: it not only lacks elegance and explanatory power, but is clearly redundant.

That is not so clear to us. The items *improvisation* and *adaptability* do overlap to some degree, and *seclusion* may appear to overlap with *compartmentation*. However "seclusion" was perhaps not the best word for Koshland too have chosen to mean a high degree of catalytic specificity, which is not the same as compartmentation.

[80] However, one should not be too insistent that all current knowledge be satisfied, because some of our supposed knowledge may be misleading or even incorrect (Crick, 1988, pp. 59–60).

We now examine our own checklist, and Table 5 compares the various theories in relation to the properties we have described as essential. We also include capacity for reproduction and evolution in the lower part of the table: these are what Gánti (2003, pp. 78–80) called *potential life criteria*, as they are properties of the forms of life that we know of, but cannot be considered essential just for staying alive.

In the next sections we consider each criterion in turn to assess its importance for deciding the validity of each theory.

3.1.2. Thermodynamically open system

G. K. Chesterton (1925, Ch. VI) offered a "definition" of life that omitted many important points but recognized the need for energy management:

> A dead thing can go with the stream, but only a living thing can go against it. A dead dog can be lifted on the leaping water with all the swiftness of a living hound, but only a live dog can swim backwards.

The chemoton and autopoiesis explicitly include an overall irreversible process, but for the others we must take it as implicit. In the version of autocatalytic sets due to Dyson (1982) he clearly stated the need for molecular components to supply the chemical energy for satisfying thermodynamic requirements. However, the better known version of this approach is that of Kauffman (1986, 1993, pp. 298–341), in which there is no explicit mention of waste, but, as long as the supply of food molecules is large and stable enough to be treated as infinite, irreversibility must follow. Much the same applies to RAF sets (Hordijk and Steel, 2004), but in extending the theory of King (1977a,b, 1982) Fernando (2005) explicitly allowed for waste. Thus in general we do not consider that there is any real conflict between autocatalytic sets and thermodynamic requirements.

If we represent (*M, R*) systems as in Fig. 10c then the irreversible conversion of food into waste is explicit. However, we must remember that this represented our *interpretation* of (*M, R*) systems, and we do not know whether Rosen would have recognized it. His own diagram is in Fig. 10b: we were more explicit than he was himself, adding the arrows from nutrients and to waste that he did not show in his highly abstract version. However, in his last book Rosen (2000, pp. 17–18) stated explicitly that living systems must be thermodynamically open:

> The entire process of order from order that [Schrödinger] envisioned, and indeed the entire Mendelian process that it represented, cannot work in a (thermodynamically) closed system at all.

In the hypercycle the need to satisfy thermodynamic constraints was clearly taken into account in, for example, Fig. 12 of Eigen (1971), but no mention of this was made in his description of the hypercycle itself,

and it is not evident in Fig. 19 of this review, which is based on Fig. 63 of Eigen and Schuster (1978a).

In summary, none of the theories that we consider clearly violates the laws of thermodynamics. We suspect that authors who did not mention it would argue that it was not necessary because it was obvious that thermodynamic laws must be obeyed. However, energy management is so important that leaving it implicit cannot be regarded as satisfactory, especially at the origin of life. In this connection Russell and Nitschke (2017) wrote as follows:

> Our general conclusion is that what drove life's emergence was not merely speeding up of chemistry or geochemistry (toward biochemistry) as sometimes assumed. Key to the emergence and the maintenance of all life are specific enzymes, many of which are effectively disequilibria-converting engines. These are turnstile-like engines, often housed in membranes that, for example, couple strong redox and pH gradients to drive endergonic reactions.

3.1.3. Specific catalysts

The absence of specific catalysts from the descriptions of autopoiesis and the chemoton is a more serious problem. The usual presentation of autopoiesis overlooks the need for catalysis completely, probably because for Maturana and Varela the crucial point was that an organism was a network of processes, and the mechanism of how this was achieved was secondary. Importation of nutrient, all the reactions of "metabolism", assembly of the membrane, and decay of the membrane components, are all represented as spontaneous. With the exception of metabolism, a case can be made that they could indeed be spontaneous, as all of those processes do sometimes occur spontaneously in living organisms. However, that cannot be said of metabolic reactions, which are specifically catalysed,[81] and must be, if collapse into a mass of parasitic reactions is to be avoided. For example, methylglyoxal is produced in uncatalysed parasitic reactions, and enzymes are necessary for its detoxication.

Emphasis on an absolute requirement for specific catalysts is vital, as it ensures kinetic autonomy by lifting the metabolic network onto a faster timescale than the underlying network of spontaneous mass-action chemical transformations, providing the "seclusion" that Koshland (2002) considered necessary.

Although Gánti (2003, pp. 85–94) referred to the "constrained path" of the reactions of the tricarboxylate cycle (Section 2.4.3) and other cycles, he did not define the origin of the constraints other than the chemical reactivities of the reactants. In the presence of all the reactants of the cycle, but no specific catalysts, the cycle can operate, but one might expect it to be swamped into invisibility by the mass of unwanted parasitic reactions at every step. So, the necessary constraints can only exist in the presence of specific catalysts, which need to be products of the system if it is to be closed to efficient causation (Sections 2.2.2, 3.1.4) in Rosen's terminology. There is no way to add these specific catalysts to Gánti's scheme without introducing a combinatorial explosion, or infinite regress.

Monod (1972, p. 145) considered that "the universe was not pregnant with life," and, more recently, Orgel (2000) in particular was sceptical:

> To postulate one fortuitously catalyzed reaction, perhaps catalyzed by a metal ion, might be reasonable, but to postulate a suite of them is to appeal to magic.

The argument loses some of its force in the light of recent studies of the reactions of the reverse tricarboxylate cycle catalysed by Zn^{2+}, Cr^{3+} and Fe^0 (Muchowska et al., 2017): although parasitic reactions do occur they are less devastating than Orgel's argument suggested. Fe^0 exists on the Earth's surface today only as a product of human industry, so during the long period before the beginning of the iron age, around 3000 years ago, it was not present in significant amounts. However, the bombardment of the Earth more than 4×10^9 years ago by planetesimals of the order of 3000 km in diameter (approaching half the diameter of Mars) caused large amounts of Fe^0 to be raised to the upper atmosphere, which rained down for some 10^7 years (Genda et al., 2017; Marchi et al., 2018). Thus Fe^0 may well have been available as a catalyst at the origin of life, an idea supported by other work (Springsteen et al., 2018; Islam and Powner, 2018; Muchowska et al., 2019).

The question of parasitic reactions concerns autopoiesis and the chemoton in particular, but it also arises with (*M, R*) systems. A modern organism clearly requires a large suite of specific reactions, but the model in Fig. 11 contains just two specific catalysts, and can escape Orgel's objections. Autocatalytic sets as defined by Kauffman (1986) also escape them, as he explicitly took account of the low probability that any randomly chosen peptide might be a specific catalyst. In a posthumous paper Orgel (2008) discussed Kauffman's analysis in some depth and appeared to accept its validity, though he concluded that "It is unlikely … that Kauffman's theory describes any system relevant to the origin of life".

In summary, the lack of specific catalysts represents a problem for autopoiesis and the chemoton, and it is not obvious how to incorporate them without making the theories vastly more complicated.

3.1.4. Catalytic closure

The need for catalytic closure is stronger than the need for specific catalysts, because one could imagine a theory that required a set of catalysts but did not specify that they must be products of metabolism. In practice, however, the theories that lack catalytic closure, autopoiesis and the chemoton, are the same ones that fail to include specific catalysts, and the ones that include specific catalysts also allow for catalytic closure. This is very clear in what Rosen (1991) called *closure to efficient causation*, the need for all catalysts to be products of the system itself, as illustrated in Fig. 10.

3.1.5. Structural closure

Structural closure obviously needs to be considered for the theories that lack it. For the hypercycle, it can easily be corrected by supposing that some of the reactions catalysed by the enzymes E_i result in the production of a membrane that encloses the whole system. In discussing their Fig. 63 (Fig. 19 in this review), which includes a boundary in the last two stages, Eigen and Schuster (1978b) mention the need for compartmentation, but do not explain how it arises. The possibility that several cycles can coexist and compete with one another for resources clearly implies a barrier to separate self from not-self. Similar considerations apply to (*M, R*) systems (Cornish-Bowden and Cárdenas, 2008)[82] and autocatalytic sets.

It is more arguable whether the first living systems *needed* barriers that they themselves had created. All modern cells have such barriers, but at the origin of life they could have depended on naturally existing compartments in rocks, such as those found in "serpentinized" rocks such as mackinawite (Branscomb and Russell, 2018a,b). On the other hand, the widely invoked theories (Haldane, 1929; Bernal, 1951; Oparin, 1953) that see the origin of life in a *prebiotic soup* in the ocean do require membranes or other barriers, because individuals could not be distinguished from one another in a homogeneous environment,

[81] This does not, however, exclude the possibility that some reactions may occur without enzyme catalysis, either uncatalysed or catalysed by small molecules (Keller et al., 2015). Even a molecule as elaborate as fructose 1,6-bisphosphate can be produced in ice by non-enzymatic condensation of glyceraldehyde 3-phosphate with dihydroxyacetone phosphate (Messner et al., 2017).

[82] Nonetheless, Zaretzky and Letelier (2002) said that "every (*M, R*) system has a physical boundary". However, they did not describe how this boundary would be produced.

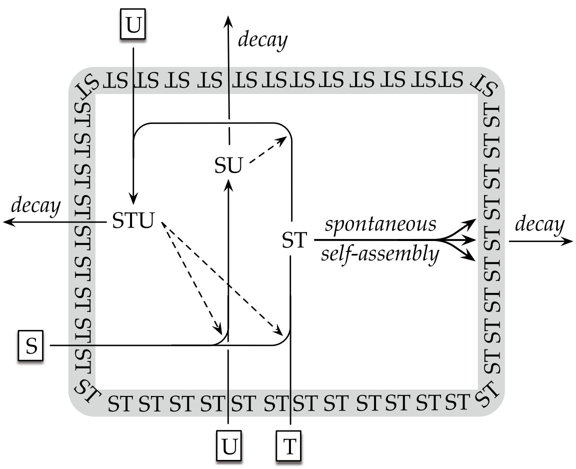


Fig. 36. Construction of a membrane by an (*M, R*) system. The product ST of the model in Fig. 11 is assumed to be capable of self-assembling to an enclosing membrane.

and could not use an ion or other gradient across the barrier to generate energy. Accordingly, Martin and Russell (2007), and more recently Branscomb and Russell (2018a,b), argue strongly against such theories on various grounds, but most notably this thermodynamic objection. We find their arguments persuasive, and accordingly do not regard the lack of membranes fabricated internally by (*M, R*) systems and autocatalytic sets as an overwhelming objection to them, as long as alternative natural compartments are available. In any case, there is no great difficulty in supposing that the product of an (*M, R*) system, or one of the components of an autocatalytic set, is capable of self-assembly to produce a membrane (Fig. 36), and lipids are certainly known that can do this. This was essentially the strategy used by Varela et al. (1974) for adding a membrane to the membraneless autopoietic system described initially (Maturana and Varela, 1973).

We studied a membrane-bound (*M, R*) system to determine the extent to which realistic prebiotic compartments, such as fatty acid vesicles, could constrain the chemical network dynamics necessary for a minimal form of metabolism (Piedrafita et al., 2012a,b). We did not assume that the vesicle was produced by the system, but there was no reason why it could not have been.

3.1.6. Size matters

We noted in Section 2.2.4 that the model of Fig. 11 could maintain itself, and if necessary restoring, a steady state (Piedrafita et al., 2010). However, this was done in terms of concentrations, but for organisms of very small size, such as bacteria, statistical fluctuations in the numbers of molecules must be taken into account, i.e. a stochastic simulation is needed. This indicated, for reasonable assumptions about the values of the rate constants, that the cell volume needed to be greater than about 10^{-18} L for the system to be capable of arriving at and maintaining a quasi-stable steady state (Piedrafita et al., 2012a).

Bacterial cells are typically much larger than that, for example *Escherichia coli* has a cell size of the order of 10^{-15} L (Milo and Phillips, 2016, pp. 9–12), and although some bacteria have smaller cells they are all larger than 10^{-18} L: for example, *Mycoplasma pneumoniae*, one of the smallest bacteria, has a cell volume of about 1.5×10^{-17} L (Milo and Phillips, 2016, p. 107). This is, of course, consistent with our knowledge that bacteria are capable of self-organization. Many viruses, however, are smaller than 10^{-18} L. For example, polio virus has a volume of the order of 10^{-26} L (Milo and Phillips, 2016, p. 5–9) and would not be capable of self-organization even if it had the enzymes needed for metabolism.

3.1.7. Information coding

Of the theories we are considering only the hypercycle incorporates a clear account of how information can be stored and used. The description of the chemoton includes an "information cycle", but, in the absence of any explanation of how this cycle actually stores information and allows it to be recovered and used, this must be regarded as a claim rather than a fully worked-out theory. The others, autocatalytic sets, (*M, R*) systems and autopoiesis, make no claim to process information.

How serious is this omission? For a theory of life as we know it today, which in all known and conceivable[83] cases includes information storage by nucleic acids, it certainly needs to be incorporated, but we do not see it as a major problem at the origin of life.

3.1.8. Controlled growth

A supply of energy alone cannot account for a living organism (Schwartz, 2007; Benner, 2009; Benner et al., 2012). Without a means of regulating the consumption of energy, heating an organic sample never results in growth, but instead results in formation of asphalt, "gunk", or tar. Living organisms do not normally produce anything resembling tar.[84]

Unfortunately, however, none of the authors of the theories we are discussing appear to have been aware of the problem of tar production,[85] and the lack of a provision for controlled growth is a weak point in all of them.[86] (*M, R*) systems take no account of growth at all: it is not clear how an (*M, R*) system could grow. The hypercycle does consider growth, but it is unclear what mechanisms prevent uncontrolled growth until the whole system becomes a tarry mess, and that is certainly what we should expect in autocatalytic sets, autopoiesis and the chemoton.

In addition to the authors of general theories of life that we have been considering, others who put most of their emphasis on energy management and thermodynamics, such as Russell et al. (2014), tend to ignore the problem of tar production:

> There is an advantage to be gained from examining the transition from geochemistry to biochemistry from the bottom up, that is, to "look under the hood" at life's first free energy-converting nanoengines or "mechanocatalysts". Such an approach encourages us to see life as one of the last in a vast hierarchical cascade of emergent, disequilibria-converting entropy-generating engines in the Universe.

These points are important, but excessive emphasis on thermodynamics can be misleading: it is not enough to have the capacity to make molecules; it is also necessary to have organization, and regulation to maintain it.

Lack of awareness of the need for controlled growth suggests a lack of awareness of the principles of metabolic control and regulation. Most biochemists today have some knowledge of the mechanisms of regulation: feedback inhibition (Section 4.1.2), allosteric interactions (Monod et al., 1963), cooperativity (Monod et al., 1965; Koshland et al., 1966; Cornish-Bowden, 2014) and so forth, but very little of metabolic control, often thinking of it as the same thing as metabolic regulation (Section 4.1.1). However, none of the originators of the main theories were biochemists, and seem to have known very little of either regulation or control. Before developing the theory of (*M,*

[83] Cleland (2019) would probably disagree with "conceivable" here.

[84] In a living organism uncontrolled growth results in cancer, which is not asphalt, but it is no more to be welcomed.

[85] It is especially baffling that Gánti ignored this: mathematicians and neurobiologists might not know about tar production in organic synthesis, but how could an industrial chemist be unaware of it?

[86] Hofmeyr (2007) was interested in developing a theory of self-fabrication, a necessary but not a sufficient condition for life; he was of course well aware of the importance of metabolic control analysis and metabolic regulation, but it was not part of his objective. Recent papers of de la Escosura et al. (2015), and Bich et al. (2016) have discussed how to reconcile knowledge of metabolic regulation with understanding life.

R) systems, Rosen (1979, 1985) described organisms as *anticipatory systems*, and his description of how anticipation might be achieved included some rudimentary notions of metabolic regulation, but not enough to constitute an adequate account. For this reason, and the importance of tar production, it is essential to give an account here, as we shall do shortly (Section 4.1). First, however, we need to consider the last of the criteria listed in Table 5.

3.1.9. Reproduction and evolution

We consider these together because reproduction with errors implies a capacity to evolve. As no chemical reaction is perfectly specific (though replication of DNA comes much closer to it than any technological process) errors are inevitable, and a capacity to reproduce inevitably means that evolution must occur. Not surprisingly, the rows in Table 5 for reproduction and evolution were the same: only the theory of the hypercycle deals profoundly with these criteria, and of the others only that of the chemoton discusses it at all.[87] This could be taken as a serious shortcoming of all the other theories, but, as Gánti (2003, pp. 78–80) pointed out, these are *potential* rather than *real* or *absolute* criteria of life, as most of the theories are concerned with what it means to be alive, rather than with trying to relate a definition of life to the characteristics that we observe in living organisms today. Varela (2000, pp. 55–56) considered reproduction as an additional complexity superimposed on a more basic identity, the autopoietic unit. However, he thought that reproduction was essential for long-term viability, but that a unit can only reproduce when it has an identity.

4. The need for metabolic regulation: a major omission from the existing theories

In this section we are concerned with the regulation of metabolism in present-day organisms, i.e. as a characteristic of life as we know it. Regulation may have been much weaker at the origin of life.

4.1. Metabolic control and regulation

4.1.1. Terminology

How do we define *control* and *regulation* so that they are not just different words for the same thing? In metabolic control analysis they are clearly different, but in older writing, for example Sauro (1990), they are not clearly distinguished.

From our point of view *control* refers to any effect of a variable or outside parameter, regardless of whether it has any physiological function, whereas *regulation* always implies a physiological function, as discussed in the following description (Hofmeyr and Cornish-Bowden, 1991)[88]:

> Since the discovery of the phenomenon of end-product inhibition in cellular metabolism and the formulation of the first definite ideas on metabolic regulation, a vast amount of research has yielded many intricate ways in which enzyme activity and concentration can be changed: cooperative and allosteric effects, covalent modification cascades, genetic mechanisms of induction and repression, to mention a few. The terms "regulation" and "control" have been applied indiscriminately to all of these phenomena, even to man-made manipulations, so much so that they have almost become devoid of any specific, and therefore useful, meaning. Being terms that are also used, often uncritically, in everyday life, they are admittedly difficult to define qualitatively, and even more so quantitatively, in a specific context such as metabolism, although notable exceptions exist.

[87] It was also implicit in the concept of a *codescript* (Schrödinger, 1944).

[88] When this paper was written not only were most biochemists unaware of metabolic control analysis, but also the leaders in metabolic control analysis paid very little attention to metabolic regulation, even though Kacser and Burns (1973, Appendix C) had included a discussion of feedback inhibition that correctly pointed out that its role is to transfer control of flux *away from* the regulated enzyme.

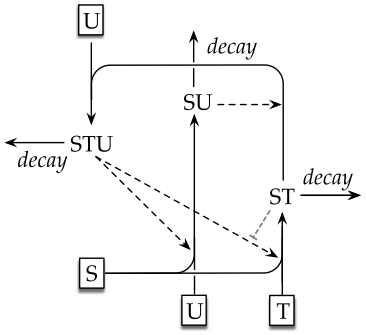


Fig. 37. Addition of inhibitory interactions to the model of Fig. 11. The metabolic product ST is assumed to inhibit the reaction that produces it: S + T → ST.

For example, how much do rates and metabolite concentrations change when an enzyme activity changes? This question comes under the heading *control*: it may have a physiological function, but this is not part of the definition. On the other hand, *regulation* always implies a function, referring, for example, to a mechanism that allows the rate of production of a useful metabolite to correspond to the metabolic need for it. We give examples of where this works and where it fails in Section 4.4: the metabolism of aspartate in *Arabidopsis thaliana* is tightly regulated by the concentrations of the other amino acids derived from it, but the production of glycine in animals is independent of the demand for it, and in large terrestrial animals this gives rise to collagen-related diseases like osteoarthritis.

We regard inclusion of ideas of metabolic regulation as vital in a theory of life, though they are missing from all of the current theories. Leonardo Bich (2018) has expressed the same opinion[89]:

> Living systems employ several mechanisms and behaviours to achieve robustness and maintain themselves under changing internal and external conditions. Regulation stands out from them as a specific form of higher-order control, exerted over the basic regime responsible for the production and maintenance of the organism, and provides the system with the capacity to act on its own constitutive dynamics.

As *control* is a completely general property, all of the processes in all of the theories discussed in Section 2 are subject to control—by the temperature, the external pH, and by all other external parameters, but most control effects have no useful functions: they are just there. For the systems to stop growing before they starve or degenerate to a tarry mess there must be *regulation*.

How easily could regulatory mechanisms be added to the different theories we have discussed? For the theories that do not have specific catalysts (the chemoton and autopoiesis) that seems to us to be impossible, but we would be happy to be shown to be wrong. Autocatalytic sets do have specific catalysts, but as they are supposed to have arisen at random it is not easy to see how all the necessary inhibitory interactions could also have arisen by chance until natural selection took effect. However, simple product inhibition, which occurs in any catalysed reaction, could have some useful effect. In the hypercycle of Fig. 16 the different enzymes E_i are assumed to catalyse metabolic reactions, and no major change would be needed to incorporate feedback inhibition into some of these. Finally, in the case of (*M, R*) systems one could suppose that one of the products, for example ST, inhibits the process that produces it (Fig. 37). Although this is just simple product inhibition, as there is only one step, this can be effective as a regulatory mechanism in very short pathways (Fell and Snell, 1988).

[89] Bich uses *regulation* in a way consistent with the way we define it here, but it is less clear that his *control* agrees with our definition.

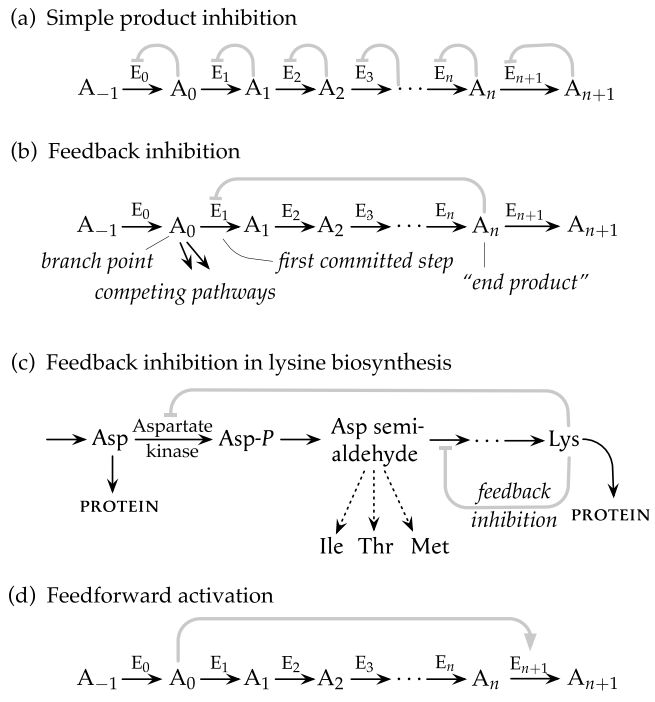


Fig. 38. Regulatory mechanisms. For simplicity all reactions are shown as irreversible, but all are reversible in principle, and some are significantly reversible in practice. (a) Simple *product inhibition* occurs almost universally and is *not* usually a useful regulatory mechanism except in very short pathways. (b) In *feedback inhibition* a metabolite late in a pathway, A_n in this example, inhibits an enzyme early in the pathway, E_1 in this example. The term "end product" is shown in quotation marks because it is not properly the end of metabolism, as indicated by the arrow proceeding from it; it is better regarded as the link metabolite between two pathways. Moreover, the reaction catalysed by E_1 is often called the *first committed step* of the pathway, that is the first step after a branch point, at which A_1 can also participate in other pathways, as indicated by the additional arrows from it. (c) Lysine biosynthesis is a concrete (but less simple) example of feedback inhibition. It is illustrated more fully in Fig. 46. (d) *Feedforward activation* is rare, but does occur, for example (in a more elaborate form) in glycogen synthesis (Cárdenas and Goldbeter, 1996). It allows a metabolite early in a pathway to stimulate removal of the "end product".

4.1.2. Metabolic regulation

Here we are concerned with regulatory effects on enzyme activity, though to avoid confusion we should note that in many papers the authors are concerned with *gene regulation*, that is to say effects on gene expression.

The best known mechanism of metabolic regulation is *feedback inhibition*[90] (Dische, 1940, 1976; Umbarger, 1956; Yates and Pardee, 1956; Stadtman, 1970), recently reviewed by Sauro (2017). It is shown in Fig. 38b and, in the specific case of lysine biosynthesis, in Fig. 38c. Before discussing it we need to dispose of a possible source of confusion. Feedback inhibition is quite different from ordinary product inhibition, shown in Figs. 37 and 38a, which is almost universal, because the products of enzyme-catalysed reactions have structures that resemble those of the substrates, and can therefore compete with them for substrate-binding sites. In short pathways it can play a regulatory role.

$$\mathrm{A} \underset{a}{\overset{V_\mathrm{A},K_\mathrm{mA}}{\rightleftharpoons}} \underset{p}{\overset{V_\mathrm{P},K_\mathrm{mP}}{\mathrm{P}}} \qquad v = \frac{\frac{V_\mathrm{A}a}{K_\mathrm{mA}} - \frac{V_\mathrm{P}p}{K_\mathrm{mP}}}{1 + \frac{a}{K_\mathrm{mA}} + \frac{p}{K_\mathrm{mP}}}$$

Fig. 39. Kinetic equation for a reversible Michaelis–Menten reaction with substrate A and product P, at concentrations a and p. With kinetic parameters V_A and K_mA for the forward reaction A → P, and V_P and K_mP for the reverse reaction A ← P, the equation takes the form shown here.

The simplest equation for a reversible reaction can be written as shown in Fig. 39. Even if the reaction is irreversible for practical purposes (negative term in the numerator negligible), the term in p in the denominator will normally ensure that P inhibits the reaction.[91] In summary, ordinary product inhibition arises from purely structural properties of the substrate and enzyme, without any regard to the metabolic needs of the organism, but it is not usually a useful regulatory mechanism, except in very short pathways, such as the two-step pathway of serine biosynthesis in mammals (Fell and Snell, 1988).

Returning now to feedback inhibition, it is a very common and important regulatory mechanism.[92] It is usually said to occur when the *end product* of a pathway inhibits the enzyme at the *first committed step*. However, both of these terms need some discussion. The metabolite A_n is not in any meaningful sense the end of anything, because metabolites are synthesized to be used. It would be better (but probably now too late) to call it by some name like *link metabolite* and the scheme should indicate this with an arrow out of A_n.[93] Moreover, A_0, the first metabolite in the pathway, is produced by other pathways and has an immediate precursor, A_{-1}, and, more important, A_0 is the substrate of other competing pathways, as illustrated in Fig. 38b.

In other words it occurs at a *branch point*, and, if there are no other branch points between A_1 and A_n then once A_1 is produced the pathway is "committed" to production of A_n, which makes the conversion $A_0 \rightarrow A_1$ the first committed step, the first step that leads nowhere else. In the biosynthesis of lysine (Fig. 38c) there *are*, however, other branch points downstream, so although the conversion of aspartate to aspartyl phosphate is committed to production of amino acids it is not committed to production of lysine in particular, so additional regulation is needed downstream. We shall consider this pathway in more detail in Section 4.4.1, when we discuss regulation according to supply and demand.

We must also briefly consider the mechanistic basis of feedback inhibition, given that the feedback inhibitor may have little structural similarity to the substrates and products of the reaction that it inhibits. Such inhibitors are called *allosteric inhibitors* (Monod et al., 1963),[94] with properties that are specifically evolved (Fig. 40). Another useful property for allosteric inhibition is that it works better if it is *cooperative* (Monod et al., 1965; Koshland et al., 1966; Cornish-Bowden, 2014), which means that the enzyme is more, sometimes much more, sensitive to small changes in inhibitor concentration close to the physiological

[90] It may seem surprising that recognition of feedback inhibition and cooperativity (Cornish-Bowden, 2014) as central to metabolic regulation came so long after the main principles of enzyme kinetics had become established (Henri, 1903; Michaelis and Menten, 1913; Briggs and Haldane, 1925). However, this recognition was possible only after many enzymes had been characterized, and many metabolic pathways elucidated (Cárdenas, 2013). Dische (1940) recognized the importance of his observation from the beginning, but Umbarger (1956) simply referred to "peculiar kinetic behavior" and Gerhart and Pardee (1962), still talked about "complex kinetics" several years later.

[91] The only important exceptions arise when the product spontaneously gains or loses a proton immediately after it is formed. For example, the initial product of the reaction catalysed by aldehyde dehydrogenase is acetic acid, CH_3CO_2H, but this is instantaneously converted in an uncatalysed reaction to acetate, $CH_3CO_2^-$, which is not sufficiently similar to the substrate acetaldehyde, CH_3CHO, to inhibit the enzyme.

[92] At the end of his life Rosen (2000, p. 219) was apparently aware of the importance of feedback inhibition from his reading of the book by Savageau (1976), but he did not connect it with his preferred view that feedforward activation was the fundamental property that allowed metabolic systems to be "anticipatory" (Section 3.1.8), reiterated on p. 249 of the same book.

[93] It is not too late to insist that the arrow should always be drawn in any illustration of feedback regulation. However, we do not know of any general textbook of biochemistry where that is done.

[94] Derived from Greek ἄλλος στερεός strictly "another solid", but "another shape" is clearer, or, clearer still, "another binding site".

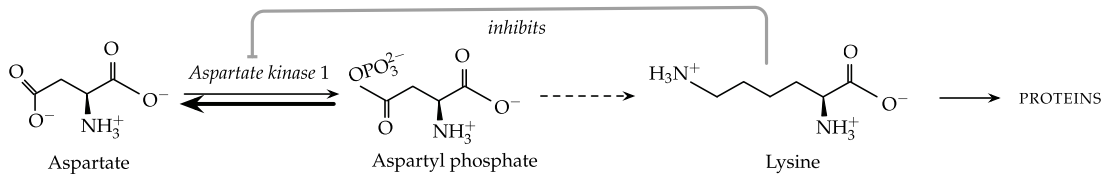


Fig. 40. Allosteric inhibition. Lysine is one of the products of aspartate metabolism in the plant *Arabidopsis thaliana* (and many other organisms). The sidechain is very different in structure from that of the substrate aspartate of aspartate kinase 1 (one of four isoenzymes), and also of its product aspartyl phosphate, but it acts as a feedback inhibitor. The difference in structure makes it difficult to interpret the inhibition as a chance result of binding to the active site. Instead it needs to be interpreted as the result of natural selection of an *allosteric* site of the enzyme that is distinct from the active site where the reaction takes place.

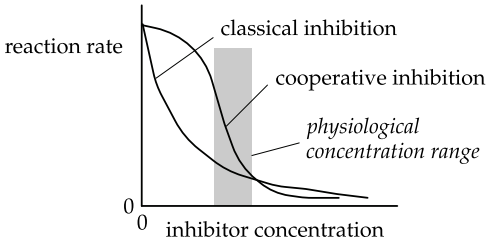


Fig. 41. Cooperative inhibition compared with classical inhibition. In classical inhibition the greatest sensitivity of the rate to the inhibitor concentration occurs at low concentrations. In cooperative inhibition the sensitivity at low concentrations is less, but it is much greater at intermediate concentrations close to the physiological concentration.

concentration than it would be if it followed the simplest equations for enzyme inhibition, typically competitive inhibition of an irreversible reaction, when the greatest sensitivity occurs at zero concentration (Fig. 41). However, this is far too simple to take account of enzyme behaviour *in vivo*: at the very least, reversibility and inhibition by products must be included.

Allosteric inhibition is typically also cooperative. If allostery and cooperativity were just chance properties with no regulatory significance this would be hard to explain, but if both are results of natural selection for effective regulation then it is easy to understand why they occur together.

Mechanisms that can give rise to these properties are described in textbooks of enzyme kinetics (for example, Cornish-Bowden, 2012, pp. 281–325). Here it is sufficient to know that they exist and that they permit efficient regulation of enzyme activity.

4.2. Biology of systems

4.2.1. Homeostasis

The fundamental property of all metabolic systems is that the interior environment of the organism is kept as constant as possible. This concept originated with Claude Bernard (1878, p. 113):

> The *constancy of the interior environment is the condition for a free and independent life*: the mechanism that allows it is the one that ensures the maintenance in the *interior environment* of all the conditions necessary for the life of the components.[95]

It was given the name *homeostasis* by Cannon (1929), who deliberately chose a prefix meaning "similar" (ὅμοιος) rather than one meaning "same" (ὁμοῦ):

> Homeo, the abbreviated form of homoio, is prefixed instead of homo, because the former indicates "like" or "similar" and admits some variation, whereas the latter, meaning the "same", indicates a fixed and rigid constancy.

[95] Italics in the original.

Homeostasis is crucial for studying metabolism, especially in relation to metabolic regulation. Unfortunately, however, the study of the biology of systems is not the same as what is today called "systems biology", as we now discuss.

4.2.2. "Systems biology"

Rosen (1979) cited two important remarks of Robert Hutchins (1931, 1933):

> Science is not the collection of facts or the accumulation of data. A discipline does not become scientific merely because its professors have acquired a great deal of information. Facts do not arrange themselves. Facts do not solve problems.[96]

> The gadgeteers and data collectors, masquerading as scientists, have threatened to become the supreme chieftains of the scholarly world. As the Renaissance could accuse the Middle Ages of being rich in principles and poor in facts, we are now entitled to inquire whether we are not rich in facts and poor in principles.

What Hutchins saw as a threat has become a reality today, at least in relation to the term "systems biology:" it is now just a catchphrase, having lost whatever meaning it once had when it became clear that including it among the keywords would be useful for obtaining finance for research. For practical purposes in biological research it means working with the "big data" that modern instruments can generate.

Mikulecky (2007) has discussed Hutchins's and Nicholas Rashevsky's influence on Rosen, and Thomas (2007) should be consulted for a discussion of Rosen's work in relation to "systems biology".

This is usually regarded as a term that originated in the early 1990s,[97] but the huge increase in its popularity started at the beginning of the 21st century (Fig. 42), appearing as the topic of about 1245 papers in 2018. Taking a recent example at random, Serra et al. (2019) present "a systems biology framework to contextualize the mechanism-of-action of engineered nanomaterials", in an article that does not consider the behaviour of biological systems. Most of the new papers in "systems biology" are concerned with the accumulation of ever-larger mountains of experimental data, not with understanding life. Moreover, it is no coincidence that the period of growth started when abundant data for genome sequences became available, as it has been driven primarily by the desire to analyse vast quantities of data at an ever-increasing rate, and not by a desire to understand systems (Cornish-Bowden, 2006).

[96] Compare this with Darwin's comment, quoted by David Penny (2009): "About thirty years ago there was much talk that geologists ought only to observe and not theorise; and I well remember some one saying that at this rate a man might as well go into a gravel-pit and count the pebbles and describe the colours. How odd it is that anyone should not see that all observation must be for or against some view if it is to be of any service!"

[97] It first appeared in a little-known article by Mihajlo Mesarović (1968), but it is usually thought to be much more recent. As long ago as 1968 Mesarović said that the introduction of systemic thinking into biology had not lived up to its promise. Whatever the origin of the term, the idea itself is much older: Drack et al. (2007) trace it to the ideas of Ludwig von Bertalanffy and Paul Weiss in the 1920s, and even to Immanuel Kant in 1789.

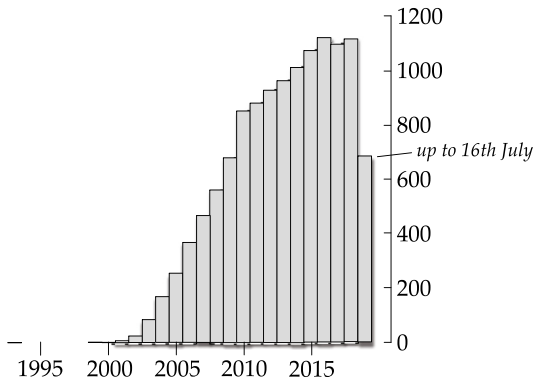


Fig. 42. "Systems biology" in the scientific literature. In 2018 the term "systems biology" appeared 1112 times in the titles or abstracts of papers in the biological literature, as estimated by *PubMed*, growing almost monotonically from one mention each in 1999 and 2000 (and one also in 1993). Unfortunately it is more of a catchphrase than evidence of real interest in the biology of systems.

The important idea that is missing from most applications of "systems biology" is that systems need to be treated as systems. As Kacser (1986) put it:

> One thing is clear; to understand the whole one must study the whole.[98]

The description of systems biology by Sydney Brenner (2010) as "low input, high throughput, no output" biology needs to be put in context to see that it was not quite as dismissive as the bare words suggest:

> No use will be served by regretting the passing of the golden years of molecular genetics when much was accomplished by combining thought with a few well-chosen experiments in simple virus and bacterial systems; nor is it useful to decry the present approach of "low input, high throughput, no output" biology which dominates the pages of our relentlessly competing scientific journals. We should welcome with open arms everything that modern technology has to offer us but we must learn to use it in new ways.

All of the theories that we have examined here satisfy Kacser's requirement, most notably Rosen's (*M, R*) systems. Nonetheless, as we discuss in this section, none of them show even minimal appreciation of the topic for which Kacser is best known, metabolic control and regulation.

Unfortunately much of the current enthusiasm for "systems biology" has led to the adoption of some of the terminology of systemic thinking while leaving its spirit largely ignored: systemic thinking means more than just accumulating huge amounts of data; the accent must be put on the organization more than on the details.

In parallel with the development of theories of the living state there has been a related revolution in the kinetic understanding of metabolism. This followed from the realization by several groups (Kacser and Burns, 1973; Heinrich and Rapoport, 1974; Savageau, 1976) that analysis of multi-enzyme systems needed to go beyond the methods of analysing the kinetics of isolated enzymes.[99]

[98] Compare this with the statement that Drack et al. (2007) attributed to Aristotle: "the whole is of necessity prior to the part".

[99] Kacser and Burns (1973) and Heinrich and Rapoport (1974) derived their ideas independently of each another, apart from taking their inspiration from earlier ideas of Higgins (1963), but they reached rather similar conclusions. The analysis of Savageau (1976) was different, and could be characterized as an engineering approach in which accurate prediction is taken to be more important than adding to understanding: see Cornish-Bowden (1989). The paper of Kacser and Burns (1973) was later rewritten by Kacser et al. (1995) in terms of the standard nomenclature and symbolism agreed by Burns et al. (1985), with some notes added to mention developments after 1973.

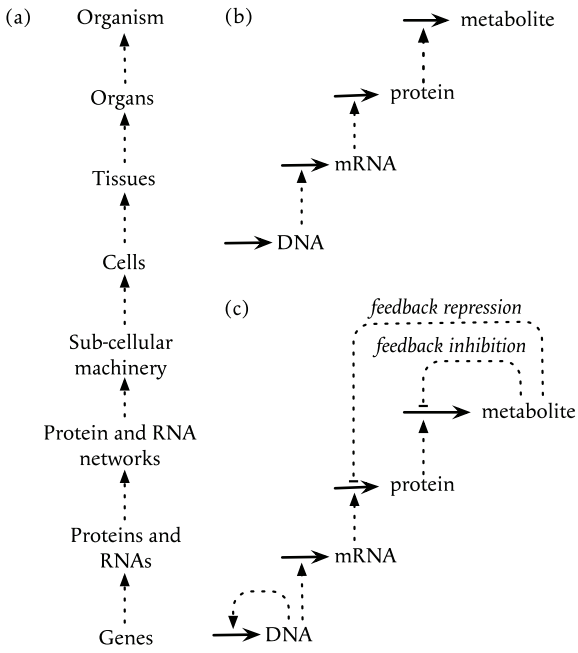


Fig. 43. Direction of causation. (a) The reductionist view assumes an upward flow of causation from genes through several steps to the whole organism. (b) Upward causation in metabolic processes. (c) "Democratic causation" (Westerhoff et al., 1990), in which some elements of downward causation enter into consideration.

In particular, which enzyme, if any, controls the production of any metabolite is a property of the whole system, and must not be confused with the fact that some enzymes are essential for that production: an enzyme may be essential but that does not mean that in normal physiological conditions it controls the pathway. This revolution has been very important, and we shall return to it in Sections 4.3–4.4.

4.2.3. Downward causation

Since its inception at the end of the 19th century biochemical knowledge has been derived from a *reductionist* view of organisms. Long after he took his first steps in endocrinology, Rodbell (1991) wrote as follows:

> I was a biochemist bred to believe in the reductionist philosophy of science, still much in vogue today. Grind, extract, purify, and reconstruct were the key words in my lexicon. Nature, in all its mystery, was at my feet waiting to be dismembered into its constitutive parts and, as with any organic chemistry problem, reassembled as proof of one's unerring biochemical skills.

That is essentially how it was in the 1960s and before, and to some degree still is. The more information you have the better your understanding of the organism, and soon you will arrive at "personalized medicine", in which the treatment of each patient is a function of knowledge of their complete genome. This view of causation has been called *upward causation*, as illustrated in Fig. 43a, which is based on an analysis by Noble (2012), in which he used models of heart function to argue that downward causation is necessary. Nonetheless,

> No one today seriously believes that this diagram represents all causation in biology.[100] Reductive biological discourse, however, privileges this form of causation and regards it as the most important.

[100] This may be too optimistic!

Similar considerations apply to metabolism, in which a causal scheme from DNA to metabolic reactions (Fig. 43b), and "downward" effects such as feedback inhibition and feedback repression are important (Fig. 43c). Westerhoff et al. (1990) described systems in which everything affects everything else as *democratic*. This coincides with our view that there is no strict hierarchy in an organism (Cárdenas et al., 2018), and that it is the absence of a hierarchy in (*M, R*) systems that allows closure to efficient causation.

Reductionism and upward causation should not be dismissed too readily: without a resolutely reductionist spirit during the 20th century it would have been impossible to identify all of the enzymes and metabolic pathways that we now know, and therefore impossible to construct the whole edifice of biochemistry.[101] Nonetheless, the time has come to move beyond the idea that a system can be fully understood in terms of the properties of its components. Instead we need to understand the functions of the parts in terms of the whole (Cornish-Bowden et al., 2004; Cornish-Bowden and Cárdenas, 2005). Everything in a living organism affects everything else and so there is no hierarchy: no up, no down. It ceases to be helpful to draw a firm distinction between upward and downward causation. As we wrote some years ago (Cornish-Bowden and Cárdenas, 2005),

> The reductionist approach remains dominant,... and systems biology is often seen as no more than integration of diverse data into models of systems. This way of thinking needs to be changed if systems biology is to lead to an understanding of life and to provide the benefits that are expected from it. The emphasis ought to be on the needs of the system as a whole for understanding the components, not the converse.

4.3. Metabolic control analysis

4.3.1. Control coefficients

Metabolic control analysis deals with the following type of question: how does a particular output variable, such as the flux through an enzyme-catalysed reaction or the concentration of a metabolite, change when some independent parameter changes, whether an enzyme activity, the concentration of an external effector,[102] or something else? There is no implication here that any of the changes have biological functions: they may do, and some of them explain metabolic regulation, but biological functions are not part of the definitions. The main principles were worked out more than 40 years ago, but they remain little known by biochemists, and almost totally unknown by everyone else, including all the authors of current theories of life.[103] Nonetheless, they are vitally important for various domains, such as biotechnology and drug action. Here we shall just state the basic results, because the detailed theory that justifies them may be found elsewhere (Fell, 1997; Cornish-Bowden, 2012; Sauro, 2018).

The rate through a particular reaction catalysed by an enzyme E_i in a pathway is not conceptually the same as the rate v_i in isolation, and for that reason it is given a different name, the *flux*, and symbol, J. This distinction may seem puzzling, because the flux through the step is the same as the rate of the reaction, $v_i = J$, so what is the difference? The difference is that J is determined by the whole system, not specifically

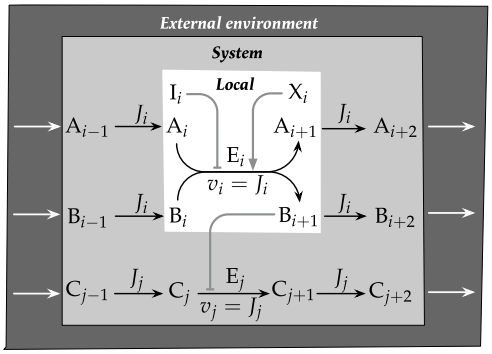


Fig. 44. Degrees of isolation. In principle a metabolic system consists of an entire organism, or at least of an entire cell, but some degree of isolation is needed for analysing it. The *local* level consists of a single enzyme E_i with a rate v_i, determined by the concentrations of its substrates and products and any inhibitors and activators that act on it, all these concentrations being treated as constant when the rate is calculated. However, this local level is contained within the *system* that is being considered, usually with more steps than shown here. Then the substrates of E_i are supplied with a definite flux J_i and the products are removed with the same flux, which is not controlled by E_i. In this system A_{i-1}, B_{i-1}, A_{i+2}, B_{i+2}, C_{j-1}, and C_{j+2} are all treated as constants, as they are supplied and removed by an *external environment* that the system does not control. This classification is related to the concept of a Markov blanket, discussed in Section 2.6.4, and in fact the scheme is a relabelled version of the one in Fig. 35.

by the properties of E_i: these contribute, certainly, but only in concert with the properties of many other enzymes. The rate v_i is, however, the rate of reaction that corresponds to the kinetic equation for E_i at the concentrations of substrates, products and effectors that exist. These concentrations are determined by the whole set of enzymes, not just by E_i, which must adjust them to satisfy the equality $v_i = J$. Fig. 44 illustrates the various degrees of isolation necessary for analysing the system properties.

We do not need a subscript for J in a simple unbranched pathway such as that in Fig. 38 because in such a pathway at steady state all the rates are the same. (However, we did need subscripts in Fig. 44 because J_i and J_j are not the same.) We can now define the *flux control coefficient* with respect to the concentration e_i of the ith enzyme as follows:

$$C_i^J = \frac{\partial \ln J}{\partial \ln e_i} \tag{3}$$

This is a simplified definition because the enzyme concentration is not strictly the quantity that needs to be considered, which is v_i; however, it is convenient for most purposes. A more rigorous definition is one equivalent to the way Heinrich and Rapoport (1974) defined their *control strength*[104]:

$$C_i^J = \frac{\partial \ln J}{\partial \ln u} \Big/ \frac{\partial \ln v_i}{\partial \ln u} = \frac{\partial \ln J}{\partial \ln v_i} \tag{4}$$

in which u is a "perturbing" parameter that affects both the systemic property J and the local property v_i. As the rate of an enzyme-catalysed reaction is normally proportional to the enzyme concentration, at least approximately, especially at a low enzyme concentration,

$$\frac{\partial \ln v_i}{\partial \ln e_i} \simeq 1 \tag{5}$$

it follows that Eqs. (3) and (4) are approximately equivalent. It does *not* imply that regulatory effects are brought about solely by changes in enzyme concentrations.[105] The flux control coefficient answers the

[101] Biochemical knowledge is essential for understanding evolution, though it was largely developed from other characteristics of biological systems, such as comparative anatomy and fossils. Likewise, knowledge of evolution is necessary for understanding why some features of biochemistry are as they are, such as why several coenzyme structures include what appear to be fragments of RNA (Cornish-Bowden et al., 2014b; Cornish-Bowden, 2016, pp. 94–95).

[102] *Effector* is a generic term that embraces both inhibitors and activators.

[103] In defining their "abstract cell model" on the basis of closure to efficient causation (Section 2.2.2) Wolkenhauer and Hofmeyr (2007) mentioned the need for control and regulation. However, they did not relate these needs to metabolic control analysis, or propose mechanisms that can ensure that they are satisfied.

[104] Heinrich and Rapoport's *control strength* is the same as the *sensitivity* of Kacser and Burns (1973), and the *flux control coefficient* in the agreed terminology (Burns et al., 1985).

[105] This may seem to be a serious problem, and critics such as Atkinson (1990) have argued that it undermines the whole basis of metabolic control analysis, as real pathways are not normally regulated over short time

question of how much the flux changes when an enzyme activity changes by a small amount. We can ask the same question about the concentration a_j of a metabolite A_j, and the *concentration control coefficient* is defined in a similar way:

$$C_i^{a_j} = \frac{\partial \ln a_j}{\partial \ln e_i} \tag{6}$$

We have different subscripts i and j because any enzyme can have an effect on any metabolite concentration: in the context of Fig. 38 we could be asking about the effect of the activity of E_n on the concentration of A_2.

4.3.2. Shared flux control

So far this may seem rather pointless, and not clearly linked to theories of life, but the point that *flux control is shared* among all the enzymes becomes clear when we consider the *summation relationships* that express how these coefficients allow us to understand how whole systems behave. The relationship for flux control coefficients is:

$$\sum_{i=1}^{n} C_i^J = 1 \tag{7}$$

The limit n here is the total number of enzymes in the system. In an unbranched pathway all of the flux control coefficients are positive, so the mean flux control coefficient is $1/n$, which must be small if n is large, and even if n is as small as 5 the mean flux control coefficient is only 0.2. It becomes more complicated in a branched system, as negative flux control coefficients are then possible, but they are never normally large enough and numerous enough to seriously overturn the generalization: *the flux control coefficient of an average enzyme is very small,* small enough to be negligible in most circumstances. For a system such as a complete cell, the number of enzymes is very large, and nearly all of them have positive influences on a gross flux such as the rate of growth of the organism, so the effect of changing any one enzyme activity is nearly always far too small to measure.[106] There is a tendency (not an absolute rule) for the flux control coefficients in an unbranched pathway to decrease as one proceeds along the pathway, as Sauro (2018, pp. 95, 104–108) discusses under the name of *front loading,* so the flux control coefficient of E_n in Fig. 38 is likely not to be $1/(n+2)$ but to be *smaller* than $1/(n+2)$. That is why the feedforward activation in Fig. 38d, suggested by Rosen (1985) in his book on anticipatory systems, would have a negligible effect in most biosynthetic processes.

4.3.3. Sensitivity of metabolite concentrations to perturbations

The summation relation for concentration control coefficients is different from Eq. (7), both in detail and in practical effect:

$$\sum_{i=1}^{n} C_i^{a_j} = 0 \tag{8}$$

Now negative values *must* exist, and their absolute sum must be equal to the sum of positive values. They can also be very large, whether positive or negative. In an unbranched pathway control coefficients for metabolites in steps upstream from the enzyme considered are negative, ones for downstream metabolites are positive. This corresponds to the common-sense expectation that increasing the activity of an enzyme will decrease the concentrations of metabolites that it is removing, and increase those of ones that it is producing.

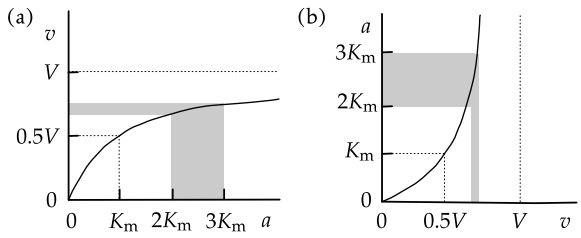


Fig. 45. (a) The usual representation of a Michaelis–Menten dependence of rate v on substrate concentration a, with Michaelis constant K_m and limiting rate V treats a as an independent variable, as it is normally fixed by an experimenter in a kinetic experiment. The rate is then very insensitive to a when $a > K_m$. (b) However, Atkinson (1977, pp. 116–118) pointed out that in many circumstances it is more appropriate to treat v as independent variable, as many enzymes *in vivo* have very little control over the rates of the reactions they catalyse, and must modulate the concentrations of their substrates and products to satisfy the rates they are given. It is then evident that the same region of the curve is one in which a is very sensitive to v. Once pointed out, these characteristics are obvious, and hardly need a graphical illustration, but they were not perceived as obvious before Atkinson drew attention to them.

Thus, whereas Eq. (7) showed that fluxes are in general very insensitive to changes in enzyme activity, Eq. (8) shows that metabolite concentrations can be and usually are very easily perturbed. This effect has been used to show that although many genes are "silent" when fluxes are measured, that is to say that they have no easily observable phenotypes, these become observable when metabolite concentrations are measured (Raamsdonk et al., 2001; Cornish-Bowden and Cárdenas, 2001).

This difference in sensitivity to perturbations between fluxes and metabolite concentrations means that enzymes must juggle the concentrations of their substrates and products to match the required fluxes. This explains something that has been somewhat mysterious for half a century: in some enzyme-catalysed reactions the rate has a low sensitivity to variations in the substrate concentration. This is *negative cooperativity*, first reported for glyceraldehyde 3-phosphate dehydrogenase (Conway and Koshland, 1968) and glutamate dehydrogenase (Dalziel and Engel, 1968), with other examples reported subsequently. But what can be the value of making the rate less sensitive (Cornish-Bowden, 1975)? In some cases it may allow the enzyme activity to vary with conditions in very different ranges of concentration, but that is easily achieved with mixtures of isoenzymes, without requiring special properties for any of them. For example, the half-saturation concentration for glucose of hexokinase D ("glucokinase") is very different from those of the other mammalian hexokinases (Cárdenas, 1995; Cárdenas et al., 1998), so the reaction in the liver can respond to variations of the blood–glucose concentration.

More generally, making the rate less sensitive to the substrate concentration means making the substrate concentration *more* sensitive to the rate, and in many circumstances that may be more important (Cornish-Bowden, 2013a).

Seen in the context of Eqs. (7)–(8) negative cooperativity seems less mysterious, because making the flux less sensitive to metabolite concentrations means inevitably that the concentrations become *more* sensitive to the flux. This increase in sensitivity may be important for a metabolite that acts as an effector, either of its own pathway or another (Cornish-Bowden, 2013a,b), as for example in the inhibition by UTP and CTP of uridine monophosphate kinase in the pathway to pyrimidine of *Escherichia coli* (Reaves et al., 2013). From this point of view it is useful to consider the substrate concentration as a function of the reaction rate, as suggested by Atkinson (1977, pp. 116–118), rather than the more usual inverse. As illustrated in Fig. 45, the concentration

scales by changes in enzyme concentrations or limiting rates. That is a misunderstanding, however, on account of Eqs. (4)–(5). For more information see Cornish-Bowden (2012, pp. 342–344).

[106] Decreasing it to zero will usually have an effect unless the organism has an alternative pathway to by-pass a missing enzyme. However, that can be regarded as an extreme case, and halving an enzyme activity will typically not have much more effect than doubling it. That is the explanation Kacser and Burns (1981) proposed as the basis of genetic *dominance*, whereby most normal alleles in diploid organisms (including humans) are *dominant* over *recessive* mutant alleles, so that heterozygotes do not differ phenotypically from normal homozygotes. A more recent discussion is given by Cornish-Bowden and Nanjundiah (2006). *Heterosis* ("hybrid vigour") is a property that is often considered "mysterious" by researchers unfamiliar with metabolic control analysis, but easily understood in terms of the shapes of curves relating phenotypes to genotypes (Fiévet et al., 2018).

is very sensitive to the rate at rates greater than $0.5V$ (half the limiting rate). For simplicity only one substrate concentration is taken into account in Fig. 45, but in reality all substrates and products for the enzyme concerned are affected in the same sort of way. Similar properties explain why uncompetitive inhibition (decrease in the apparent value of V) differs far more from competitive inhibition *in vivo* than it appears to do *in vitro* (Cornish-Bowden, 1986; Westley and Westley, 1996).

4.3.4. Elasticities

Elasticities are not closely related to the main discussion, but they need to be mentioned because they play an important part in the theory of metabolic control analysis. Their algebraic definitions resemble those of control coefficients, but whereas a control coefficient expresses how a perturbation affects a whole system, an elasticity expresses the effect of a *local* perturbation, such as a change in substrate concentration, on a *local* property, specifically the activity of an enzyme when considered in isolation from the pathway in which it occurs. For example, the local effect of a concentration a on a rate v can be written as an elasticity ε_a^v:

$$\varepsilon_a^v = \frac{\partial \ln v}{\partial \ln a} \tag{9}$$

The similarity to Eq. (3) is obvious.

In a sense, measuring elasticities is what people have been doing since the days of Michaelis and Menten, though the term is only used in the context of metabolic control analysis. It is, indeed, rather obscure, and the term *kinetic order*, used by Savageau (1976) in biochemical systems theory, is much better.

4.4. Two examples of metabolic regulation

4.4.1. Aspartate metabolism

To understand the role of feedback inhibition and other regulatory mechanisms we need to study a more realistic pathway than the abstract one shown in Fig. 38b, for example the one that converts aspartate into lysine, shown in Fig. 46. To understand the regulation we must recognize that the pathway consists of two blocks of reactions, a *supply* block from aspartate to lysine and a *demand* block from lysine to protein (as well as other uses). Even though only a vestigial demand block is shown in Fig. 38c, it is essential to include it if the function of the feedback inhibition is to be understood: analysis of supply and demand is the key to understanding metabolic regulation (Hofmeyr and Cornish-Bowden, 2000).

Lysine inhibits not only the first enzyme, aspartate kinase, but also the third, dihydrodipicolinate synthase. For the moment we shall ignore this second inhibition and ask how inhibition of aspartate kinase produces a redistribution of control among the synthetic steps. If there is no regulation and the flux control coefficients tend to decrease as one proceeds along a pathway (Section 4.3.2) then aspartate kinase should have the largest flux control coefficient and conversion of lysine to protein should have the least. However, that is exactly the opposite of what the system needs: the pathway should produce as much lysine as needed for protein synthesis, and avoid wasting aspartate, which is needed both for protein synthesis and for other purposes. What the feedback inhibition does, therefore, is to increase the share of protein synthesis (demand) for control of the pathway, decreasing as much as possible that of aspartate kinase, i.e. transferring control from the beginning of the pathway, where it is not needed, to the end, where it is.

Although the real pathway, shown in Fig. 47, is considerably more complicated than the version in Fig. 46, the regulatory interactions still make sense in terms of supply and demand. First of all, there is more than one branch point, and so more than one committed step: the aspartate kinase step is committed to amino acid synthesis (as opposed to retaining aspartate for its own incorporation in protein), but there are others as well, one of which explains why lysine inhibits

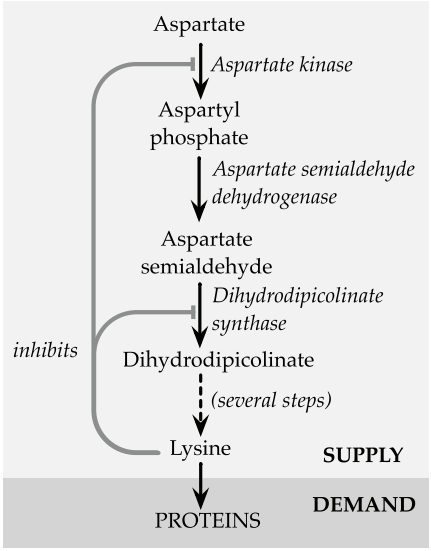


Fig. 46. Biosynthesis of lysine. This is a less abstract representation of feedback inhibition than the one in Fig. 38b. The amino acid lysine is synthesized from aspartate, another amino acid, and acts as a feedback inhibitor not only of the first enzyme, aspartate kinase, but also of the third, dihydrodipicolinate synthase. Lysine is synthesized because there is a *demand* for it, mainly for protein production. The demand step is often omitted from textbook representations of metabolic pathways, but the regulation steps cannot be understood without it.

dihydrodipicolinate synthase as well as aspartate kinase. The feedback inhibition interactions by threonine and isoleucine also prevent excessive production of these amino acids. The potentiation by *S*-adenosylmethionine of the inhibition of aspartate kinase by lysine is another way of regulating the supply of methionine, as is its activation of threonine synthase. The whole set of regulatory interactions is fully consistent with allowing demand and not supply to regulate production of the different amino acids.

The natural question to ask at this point is whether this is just theory, or does it work? This was answered by incorporating all of the available kinetic data into a model that could be simulated by computer (Curien et al., 2009). The result was that the demand for the four amino acids did indeed regulate their synthesis, largely independently and without interference: for example, production of lysine could be varied with almost no effect on the other fluxes.

4.4.2. Glycine production

The sort of feedback seen in Fig. 47 is effective because the demanded products, amino acids in this case, use feedback inhibition to signal a need for increased production. This can fail in pathways that are regulated in other ways, such as the production of glycine in animals. The enzyme glycine hydroxymethyltransferase catalyses its production from serine (Fig. 48), the additional C atom being transferred to tetrahydrofolate, and used for the synthesis of many metabolic products. The stoicheiometry of the reaction means that a glycine molecule can only be made when a tetrahydrofolate-C_1 (5,10-methylenetetrahydrofolate) molecule is made. Excess glycine can be converted irreversibly to tetrahydrofolate-C_1 by the glycine cleavage system, but no pathway for the reverse process exists. Moreover, the reaction is regulated by demand for tetrahydrofolate-C_1, not by demand for glycine. In small or growing animals, as well as aquatic animals that are supported by the water in which they live, this mode of regulation is entirely satisfactory. However, lack of glycine can cause collagen deficiency, manifested as osteoarthritis: this is common in large elderly terrestrial animals, including humans, and is one of the rare degenerative diseases observed in wild animals such as elephants and rhinoceroses (Meléndez-Hevia et al., 2009).

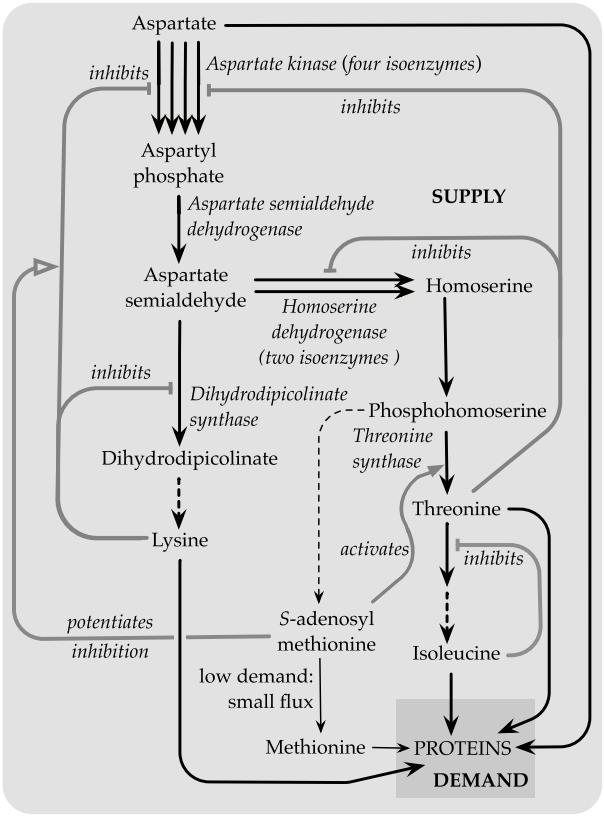


Fig. 47. Metabolism of aspartate in *Arabidopsis thaliana*. Aspartate is itself needed for incorporation into protein, and is the precursor of four other amino acids: lysine, methionine, threonine and isoleucine. The multiple arrows for some steps indicate that these steps are catalysed by *isoenzymes*, multiple proteins catalysing the same reactions. Although less simplified than Fig. 46, this figure also omits various complications, such as co-substrates in most steps, and additional regulatory interactions. The supply block consists of the entire system apart from the part at bottom-right with darker shading. The thinner arrows leading to methionine (and from methionine to proteins) reflect the fact that methionine is one of the least abundant amino acids in proteins, and the flux through these steps is much smaller than that to threonine and isoleucine. *S*-Adenosylmethionine, the precursor of methionine, activates synthesis of threonine and potentiates the inhibition of one of the aspartate kinases by lysine.

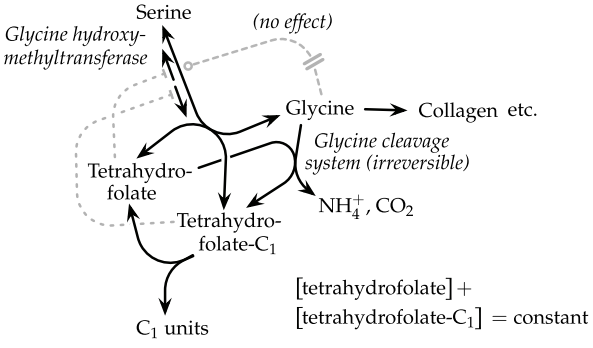


Fig. 48. Biosynthesis of glycine in animals. Glycine and tetrahydrofolate-C_1 are produced in equal amounts in the reaction catalysed by glycine hydroxymethyltransferase: the glycine concentration has no useful effect on the rate, which is determined by the concentrations of tetrahydrofolate and tetrahydrofolate-C_1, as substrates and products of the forward and reverse reactions respectively. The effects are shown as inhibitions, but they are really just ordinary substrate–product effects as given by a two-substrate version of the equation in Fig. 39. The sum of these two concentrations is constant, so if the product concentration in either direction is high the substrate concentration is low, and vice versa. Excess glycine can be converted irreversibly to tetrahydrofolate-C_1 by the glycine cleavage system, but no mechanism is available for converting tetrahydrofolate-C_1 into glycine.

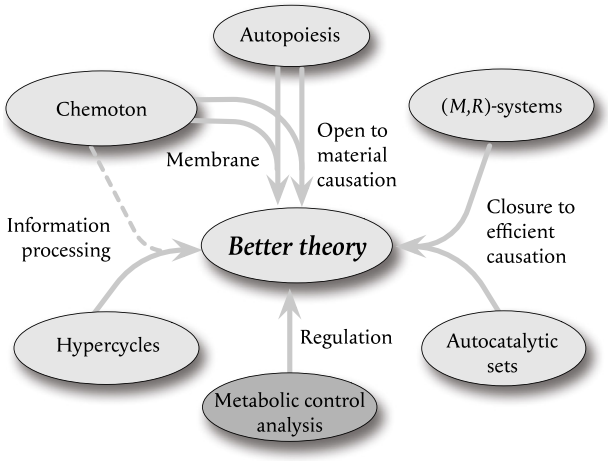


Fig. 49. Towards a better theory of life. This will have to take account of all the current theories: all include some important elements, but omit others or leave them simply implicit. In particular, none provide a mechanism to protect an organism from uncontrolled growth. All lack ideas of metabolic control and regulation. In addition to the theories shown here, Schrödinger (1944) assumed in *What is Life*? a system open to material causation, and proposed a mechanism for information processing.

4.5. Combining all the elements

> Bernard of Chartres used to compare us to dwarfs perched on the shoulders of giants. He pointed out that we see more and farther than our predecessors, not because we have keener vision or greater height, but because we are lifted up and borne aloft on their gigantic stature (translated by MacGarry, 1955, from John of Salisbury, 1159).[107]

Numerous giants contributed to the work discussed in this review. Freeman Dyson, Manfred Eigen, Tibor Gánti, Alfonso Herrera, Henrik Kacser, Stuart Kauffman, Humberto Maturana, Julien Jean Offray de La Mettrie, Stéphane Leduc, Louis Pasteur, Robert Rosen, Erwin Schrödinger, Peter Schuster, d'Arcy Wentworth Thompson, Francisco Varela: all giants in their different ways, but even when they lived through the same period they barely communicated with one another (with the obvious exceptions of those who worked together, Varela with Maturana, Schuster with Eigen) or considered one another's ideas; moreover; none of them attempted a synthesis of the different theories of life. In consequence all of the current theories are seriously incomplete.

The theories appear at first sight to be very different from one another, apart, perhaps, from autopoiesis and the chemoton. However, many of the differences are superficial, derived from the fact that they were developed independently by people who did not communicate with one another. For example, (*M, R*) systems, autocatalytic sets and the hypercycle all incorporate catalytic closure (closure to efficient causation), but that is not obvious at first reading. Likewise autopoiesis and the chemoton explicitly take account of openness to material causation, that is to say, obedience to thermodynamic requirements; probably the others were intended to satisfy this requirement also, but it is less clearly expressed. Autopoiesis and the chemoton also insist that the organism must generate a membrane or other barrier to separate self from not-self (and from the environment): one can argue about whether the first organisms at the origin of life could simply use pre-existing mineral compartments, but there is no doubt that living systems today need to make their own. Incorporating them into autocatalytic sets, (*M, R*) systems, and the hypercycle is certainly possible, but this needs to be explicit.

[107] Isaac Newton made a similar statement in a letter to Robert Hooke.

Whether or not feedback regulation was necessary for the first living systems, there is no doubt that it is essential in living organisms today, as it provides the almost universal mechanism for matching the supply of metabolites to the demand for them. In our view, therefore, mechanisms resulting from natural selection for regulating supply and demand are crucial for a theory of life, though they are lacking from all of the current theories we have discussed.

5. Conclusions

All of the theories we have considered contain some useful features that need to be included in a definitive theory of life (Fig. 49), but all lack some that are important. In particular, none of them incorporate any mechanism of regulation, or any other mechanism to prevent a self-organizing system from growing until it forms a tar (Section 3.1.8). In extreme cases a real living organism may starve to death, or die for some other reason, but, apart from a cancer, which is not a self-organized system, it never forms a tar or otherwise disorganized state.

We have not provided all the answers in this review, but we hope that we have pointed to the direction that future research needs to take in the hope of arriving at a definitive theory of life. There are various courses that future research may take:

1. Each individual researcher may a choose a preferred theory from the current ones and try to extend it. That is essentially what has happened until now, and we do not believe that it is the best way forward.
2. One may try to incorporate all the points in Fig. 49 into a single theory, after first identifying and eliminating any logical inconsistencies. The main points that we see are the following:
    (a) Construction of a membrane needs to be described explicitly, not just left for future development.
    (b) Thermodynamic requirements need to be satisfied explicitly. For a system at the origin of life it may be sufficient to suppose a supply of energy-rich nutrients, but a more long-term system certainly needs to harness gradients across boundaries.
    (c) It is not enough to have a cycle labelled "information cycle": there must be a clear mechanism for collecting, storing and using the information.
    (d) Any living system must be closed to efficient causation: the catalysts (apart from metal ions) must be produced by the organism in such a way that infinite regress is avoided.
    (e) There must be regulation of the metabolism, so that organisms cannot grow indefinitely, and metabolites are produced only as needed.
3. One should identify if there are other essential characteristics not mentioned in Fig. 49 that need to be incorporated. We are not aware of essential characteristics apart from metabolic regulation that are missing from all of the current theories.
4. The really adventurous could start with a completely clean plate and develop a new theory that is not derived from any of the existing ones.

6. Further reading

Despite the length of this review, it has hardly been possible to include all of the relevant work in studies of the nature of life, especially given the reluctance of many authors to cite others. An idea of the vastness of the field can be gained from Fig. 7 of Varela (2000, p. 71), in which he listed various characteristics of extant life:

1. Superorganisms and collective intelligence
    (a) Superorganisms (Wheeler, 1986; Wilson and Sober, 1989)
    (b) Collective intelligence (Deneubourg and Goss, 1989; Lapedes and Farber, 1986)
    (c) Ecology (Grant and Thompson, 1997; Lovelock, 1988)
2. Neuro-cognitive identity
    (a) Neurone webs (Grossberg, 1980; Edelman, 1993)
    (b) Senso-motor closure (Walton et al., 1992)
    (c) Autonomous robots (Brooks, 1991; Steels and Tokoro, 1995)
3. Multicellular identity
    (a) Somatic individuality (Royce and Buss, 1979)
    (b) Morphogenesis (Goodwin, 1978)
    (c) Genetic algorithms (Kauffman, 1969; Holland, 1992)
    (d) Immunity networks (Varela and Coutinho, 1991; Stewart and Varela, 1989)
4. Cellular unity
    (a) Cellular origins (Sagan, 1967)
    (b) Autopoiesis (Maturana and Varela, 1980)
    (c) Chemical autopoiesis (Bachmann et al., 1990)
    (d) Self-reproducing automata (McMullin and Varela, 1997)
5. Pre-cellular epoch
    (a) Chemical algorithms (Fontana and Buss, 1994)
    (b) Dissipative structures (Prigogine et al., 1972)
    (c) Cellular automata (Burks and Farmer, 1984; Langton, 1984)
    (d) Autoreplication (Breaker and Joyce, 1994; Orgel, 1992)

With few exceptions, most of the references cited in these lists have not appeared elsewhere in the text, but they will be useful as sources of additional information. Varela just listed names of authors in his figure, without giving specific references, so we have had to guess which ones he had in mind, probably incorrectly in some cases. In a few cases we were unable to identify plausible candidates, and we have omitted these.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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copy of *Anticipatory Systems*, and John Kineman for helping us to clarify our understanding of formal and final causation. We also thank the organizers of an ELSI[108] workshop in Tokyo in 2016 (Mariscal et al., 2019) that allowed us to discuss the origins of life with many experts. Finally, this review received three detailed expert reports, all of them containing numerous valuable suggestions. We have incorporated most of these, and we are very grateful to the reviewers for taking the time and trouble to read it thoroughly and to comment on it.

Appendix. Biographical notes

Some of the authors whose ideas we have discussed are sufficiently well known not to need much introduction, but most of the others are not. In this Appendix, therefore, we offer some biographical notes.

A.1. Aristotle (384–322 BC)

Aristotle (Ἀριστοτέλης) was a Greek philosopher, born in Stagira, east of Salonica, and died in Euboea. He attended Plato's Academy in Athens, but after Plato's death he was the tutor of the young Alexander the Great. He contributed to many subjects, and is sometimes regarded as the world's first biologist. His classification of *causes* (not a good translation of ἀιτία) formed part of his writings on physics.

A.2. Julien de La Mettrie (1709–1751)

Julien Jean Offray de La Mettrie was born in Saint-Malo. He studied theology and planned to enter the Church, but switched to medicine. His views on the soul, together with his hedonistic attitude to life and the importance of pleasure, created scandals, first in France and then in the more tolerant society of The Netherlands. After his death in 1751 in Prussia, Frederick the Great said in his eulogy, "All those who are not imposed upon by the insults of the theologians mourn in La Mettrie a good man and a wise physician". More information is given by Chisholm (1911).

A.3. Aleksandr Butlerov (1828–1866)

Aleksandr Mikhailovich Butlerov was a Russian chemist who was born and died in the region of Kazan, where his family were landowners. His views on chemical bonding and the way to represent chemical structures, extending the work of Friedrich August Kekulé and including double bonds, are at the basis of modern practices (Leicester, 1959; Rocke, 1981), though he was writing long before the development of quantum mechanics. His reputation in Russia is very high, on the basis of this work on chemical structure, but he is remembered elsewhere mainly for his discovery of the formose reaction, which Juli Peretó (2016) has reviewed.

A.4. Stéphane Leduc (1853–1939)

Stéphane Armand Nicolas Leduc was Professor in the School of Medicine of Nantes, and was one of the first in France to use radiotherapy in the treatment of cancer (Drouin et al., 2014). He devoted his research career to the effect of osmosis on the growth of inorganic crystals, which he believed shed light on the formation of superficially similar structures in the growth of living cells, plants and fungi. His ideas were not well received in his lifetime, but they are enjoying a revival in the work of Barge et al. (2011). Juli Peretó (2016) has reviewed his work, together with that of other contemporary scientists.

[108] Earth-Life Science Institute, Tokyo.

A.5. D'Arcy Thompson (1860–1948)

D'Arcy Wentworth Thompson was Professor of Biology at the University of Dundee for 64 years, from 1884. He held the Chair of Natural History at St Andrews University from 1917. He emphasized the importance of allometry and purely physical considerations in determining the forms of living organisms. He set out his ideas in detail in his book *On Growth and Form*. He was unfortunate in that both editions were published during wartime (1917 and 1945), which doubtless decreased the impact of the book.

A.6. Alfonso Herrera (1868–1943)

Alfonso Luis Herrera López was a Mexican biologist known in particular for his theory of plasmogeny for the origin of life, concerned with the origin of protoplasm. He was the author of several books, including *Nociones de Biología*, and participated in the creation of various important institutions in Mexico, such as the Chapultepec Zoo, precursor of the Institute of Biology of the Universidad Nacional Autónoma de México. Juli Peretó (2016) has reviewed his work, together with that of other contemporary scientists.

A.7. John Burke (1873–1946)

John Benjamin Butler Burke was an English physicist who studied at Trinity College Dublin and Trinity College, Cambridge. He worked under the supervision of J. J. Thomson at the Cavendish Laboratory in Cambridge, and also in Birmingham and Manchester. He was especially concerned with artificial cells and the possibility that life could arise from non-living matter. Juli Peretó (2016) has reviewed his work, together with that of other contemporary scientists.

A.8. Erwin Schrödinger (1887–1961)

Erwin Rudolf Josef Alexander Schrödinger was an Austrian physicist, who was born and died in Vienna. His introduction of wave mechanics led to the Nobel Prize for Physics in 1933. After the *Anschluß*, the forced union of Austria and Germany, he moved to the Dublin Institute of Advanced Studies in Ireland, where he gave the lecture course that led to his book *What is Life?*. He returned to Austria in 1951. Keith Laidler (1993, pp. 336–337) and Walter Gratzer (2002, pp. 191–194) have given short accounts of his life and character, and Walter Moore (1989) has written a full biography.

A.9. Boris Belousov (1893–1976)

Boris Pavlovich Belousov was a Russian chemist, born in Moscow. He studied in Zürich after being forced to leave Russia, having been arrested at the age of 12 for participating in revolutionary activities. He returned to Russia in 1914 but could not enter the army for health reasons. During the Second World War he was a military chemist and worked on remedies for burn injuries, and later on protection against radiation injuries. Afterwards he studied the tricarboxylate cycle, and wrote that the "peculiar behaviour of citric acid in relation to some oxidants lies at the foundation of the periodic reaction". He died in 1976, too soon to know of the posthumous award of the Lenin Prize in 1980. Arthur Winfree (1984) has given some background information on the Belousov–Zhabotinsky reaction.

A.10. Alexander Oparin (1894–1980)

Alexander Ivanovich Oparin was born in Uglich, Russia, north of Moscow. He studied the biochemistry of material processing by plants and enzyme reactions in plant cells, and developed the foundations for industrial biochemistry in the USSR. He originated the theory that life began in a "primordial soup". He was a supporter of Lysenko, not only during the lifetime of Stalin, but afterwards, at least until 1955. Outside Russia he is mainly remembered today for his theoretical work on the origin of life. Juli Peretó (2016) has reviewed his work, together with that of other contemporary scientists.

A.11. Zacharias Dische (1895–1988)

Dische was born in Sambir (now in Ukraine, then in Austria-Hungary) to a merchant family. He studied at the Universities of Lvov (now Lviv) and Vienna. After the *Anschluß* he fled to France, and later to the USA. While a refugee in Marseilles he pursued his main research interest on carbohydrate metabolism, and discovered feedback inhibition, a discovery usually thought to have been made more than a decade later. Information about his life can be found in the Memorial Book Dedicated to the Victims of National Socialism at the University of Vienna 1938: https://tinyurl.com/y29xbcpa

A.12. Henrik Kacser (1918–1995)

Kacser was born in Câmpina (Rumania) of Austro-Hungarian parents. He was educated in Northern Ireland, and spent most of his career in Edinburgh. Trained as a chemist, he regarded himself as a geneticist, but his greatest influence was in biochemistry. He was one of the principal founders of the modern biology of systems, and was the first to argue that the only way to understand whole systems is to study whole systems. He remained active and the undisputed leader of his field until his sudden death in 1995. More information about his life and work can be found in the obituary by David Fell (1996).

A.13. Freeman Dyson (born 1923)

Freeman John Dyson is an American physicist and mathematician. He was born in Berkshire, England, and is known for many different contributions, with wide interests that include the origin of life. He was educated at Cambridge University and Cornell, and is currently professor emeritus at Princeton. He has been accused of being a denier of global warning, but his views (Dyson, 1977) are more subtle than that: he accepts that global warming is occurring, but he rejects the projections of its extent into the future.

A.14. Manfred Eigen (1927–2019)

Eigen was a German biophysicist, best known for his invention of the *temperature-jump* method for studying the kinetics of fast reactions with time constants of the order of microseconds. For this work he was awarded the Nobel Prize in Chemistry in 1967. He was born in Bochum, Germany, and served as a teenager in an anti-aircraft unit during the Second World War. He was captured near Salzburg by American forces on the last day of the war, but he escaped from the camp where he was held, and walked to Göttingen (a distance of hundreds of kilometres). There he obtained his Ph.D., and later became director of the Max Planck Institute for Biophysical Chemistry in Göttingen. The obituary by Georgina Ferry (2019) gives a good account of his life.

A.15. Humberto Maturana (born 1928)

Humberto Maturana Romesín is a Chilean neuroscientist and philosopher. He was born in Santiago, studied first medicine and then biology at the University of Chile, and obtained his doctorate at Harvard. His work spans a broad range, encompassing concepts like cognition, autopoiesis, language, cybernetics and structurally determined systems. Of these, he has been associated in particular with cognition, especially vision. His students have included Francisco Varela, with whom he elaborated the theory of autopoiesis, and Juan-Carlos Letelier.

A.16. Tibor Gánti (1933–2009)

Gánti was a chemical engineer who spent his whole life in Hungary (and published almost all of his work in Hungarian). He taught industrial biochemistry and theoretical biology at Eötvös Loránd University and other universities in Hungary, after working first as head of the yeast laboratory of the Yeast Factory of Budapest. He remained closely in touch with industrial chemistry after his academic functions began, because he continued working in the Factory of Industrial Chemicals of Budapest. His aim in developing the *chemoton* model was to arrive at a minimum definition of life. Eörs Szathmáry (2015) has provided a useful summary of his life and work.

A.17. Robert Rosen (1934–1998)

Rosen was born in New York, and made his career first at the State University of New York in Buffalo and later at Dalhousie University, Nova Scotia. He studied with Nicolas Rashevsky at Chicago, and regarded his work as an extension of Rashevsky's *relational biology*, in which the relations between entities, that is to say their organization, are more fundamental than the entities themselves. His posthumous reminiscences (Rosen, 2006) provide much additional information, as does the hagiographic account by Mikulecky (2001), and papers of Richardson and Louie (2007, Appendix) and Witten (2007).

A.18. Anatol Zhabotinsky (1938–2008)

Anatol Markovich Zhabotinsky was born in Moscow and died in Boston. Although he had intended to study rhythmic behaviour in glucose metabolism, he was assigned to work on the Belousov reaction by his professor, Simon Shnol. He placed this reaction on a firmer experimental basis, but although Belousov approved of his work they were never to meet. He was not permitted to leave the USSR until 1991, but in that year he moved to the USA, and became an Adjunct Professor at Brandeis University. Arthur Winfree (1984) has given some background information on the Belousov–Zhabotinsky reaction.

A.19. Stuart Kauffman (born 1939)

Stuart Alan Kauffman is an American theoretical biologist with a particular interest in complex systems and the origin of life. He was a Marshall Scholar at the University of Oxford, from where he obtained his BA. Afterwards he studied medicine at the University of California Medical Center in San Francisco. He has worked in several different university departments and at the Santa Fe Institute.

A.20. Peter Schuster (born 1941)

Peter Schuster is an Austrian biophysicist. He was born and educated in Vienna, and after post-doctoral work in Göttingen with Manfred Eigen he returned to Vienna, where he has made his career. In addition to the work on the hypercycle that we discuss here, he is very well known for his work on viruses and their replication. He is President of the Austrian Academy of Sciences.

A.21. Francisco Varela (1946–2001)

Francisco Javier Varela García was a Chilean neuroscientist and philosopher. He was born and educated in Santiago, and at the University of Chile he took courses alongside one of the authors of this review. He obtained his doctorate at Harvard, and after his return to Chile, he worked with Humberto Maturana. Together they developed the theory of autopoiesis. He spent much of his career outside Chile, in the USA during the period of the military dictatorship, and in France for the last part of his life. In the later part of his life he became interested in Tibetan Buddhism and participated in dialogues with the Dalai Lama.

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