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On the Crucial Stages in the Origin of Animate Matter

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Abstract. Theories of the origin of life have proposed hypotheses to link inanimate to animate matter. The theory proposed here derived the crucial stages in the origin of animate matter directly from the basic properties of inanimate matter. It asked what were the *general characteristics* of the link, rather than what might have been its *chemical details.* Life and its origin are shown to be one continuous physicochemical process of replication, random variation, and natural selection. Since life exists here and now, animate properties must have been initiated in the past somewhere. According to the theory, life originated from an as yet unknown *elementary autocatalyst* which occurred spontaneously, then replicated autocatalytically. As it multiplied to macroscopic abundance, its replicas gradually exhausted their reactants. Random chemical drift initiated *diversity* among autocatalysts. Diversity led to competition. Competition and depletion of reactants slowed down the rates of net replication of the autocatalysts. Some reached negative rates and became extinct, while those which stayed positive ''survived.'' Thus *chemical natural selection* appeared, the first step in the transition from inanimate to animate matter. It initiated the first animate property, *fitness,* i.e., the capacity to *adapt to the environment* and to *survive.* As the environment was depleted of reactants, it was enriched with *sequels*—namely, with decomposition products and all other products which accompany autocatalysis. The changing environment exerted a selective pressure on autocatalysts to replace dwindling reactants by accumulating sequels. Sequels that were incorporated into the autocatalytic process became *internal components of complex autocatalytic systems. Primitive forms of metabolism* and organization were thus initiated. They evolved further by the same mechanism to ever higher levels of complexity, such as homochirality (handedness) and membranal enclosure. Subsequent evolution by the same mechanism generated cellular metabolism, cell division, information carriers, and a genetic code. Theories of self-organization without natural selection are refuted.

Key words: Origin — Animate matter — Autocatalysis — Natural selection — Sequels — Complexity — Metabolism — Cellular organization — Genetic code

Introduction

Scientific literature abounds with theories of the origin of life (Shapiro 1986). Some say life started with template-replicating polymers (Eigen 1992 and references therein), some say with pyrites (Waechtershaeuser 1992), some say with thioesters (de Duve 1991), some say with clays (Bernal 1967; Cairns-Smith 1982), some say with polypeptides (Oparin 1957; Fox 1988; Dyson 1985; Kauffman 1993), and this list is neither exhaustive nor final. Some say life started in an oceanic thick soup (Oparin 1957), some say in hydrothermal vents (Waechtershaeuser 1992), some say in microscopic confinements (Oparin 1957; Fox 1988; Dyson 1985), and here again the list is neither exhaustive nor final. A thorough critical review of the current work in this field was presented in two excellent articles, one by Joyce (1989) on RNA evolution, and the other by Orgel (1992) on molecular replication. From these one learns how difficult it is (or perhaps impossible) to trace the chemical evolution back from the chemistry of the present cellular metabolism to the chemistry of the prebiotic world. The abundance of specific theories in the study of the origin of life

seems to be due to the general consensus that hypotheses, scenarios, or models are indispensable. They come to fill the vacuum created by the scarcity of more reliable sources of information on prebiotic evolution.

This paper suggests that a *general* theory of the origin of life, based only on established knowledge and commonly accepted premises, is both feasible and desirable (Lifson 1987). The knowledge resides in astrophysics, geology, chemistry, molecular biology, and evolutionary biology. The premises come from quantum mechanics and statistical thermodynamics. The theory should explain the origin of the unique phenomena which sustain life, such as reproduction, metabolism, and their corollaries, independent of the particular composition of animate matter.

Two levels of phenomena should be distinguished with respect to the origin of life. One level contains general, broadly outlined phenomena which *must* have happened or else life could not originate, and which can presumably be deduced from already-established knowledge. A logical and temporal order of the origins of such phenomena should constitute the body of a general theory of the origin of life. For example, *reproduction* is certainly essential for any form of life. Hence, some form of *molecular replication* must have been initiated spontaneously in the prebiotic environment as an elementary, purely physicochemical form of reproduction.

The other level comprises particular phenomena which *might but need not* have happened. Ideas, suggestions, and hypotheses on this level should be compatible with the general theory but not deducible from it, and the theory may be helpful in assessing their credibility. For example, the monomers of template-replicating polymers might have been available either from the start (Eigen 1971, 1992; Orgel 1992) or from early natural selection. Both alternatives are legitimate hypotheses, and the general theory indicates that the latter is more plausible. Hypotheses which are incompatible with a general theory cannot be true, and it should be possible to show where do they go wrong. A critical examination of such hypotheses is presented below.

The Chicken and the Egg

Earth's surface was always teeming with irreversible, stochastic processes, spurred by the high-energy flux of sun radiation as well as by the flux of energy and material from inside the earth and from outer space. The stochastic processes included a random chemical drift of innumerable compounds and reactions, from the most ubiquitous and the most stable to the rarest and the most unstable. A comprehensive knowledge of all such compounds and reactions is not available. Therefore, the search for compounds that could have initiated the origin of life elicited various hypotheses, as noted above. They fall roughly into two classes. One class assumes the primacy of *metabolism and cellular organization.* The other class assumes the primacy of *reproduction and genetic information.* The question, ''Which came first, metabolism or reproduction?'' is metaphorically the question, ''Which came first, the chicken or the egg?''

Many authors (Waechtershaeuser 1992; de Duve 1991; Cairns-Smith 1982; Oparin 1957; Fox 1988; Dyson 1985; Kauffman 1993) favor the chicken-first hypothesis, although otherwise their ideas vary widely. These studies deserve a thorough, separate discussion, where their contributions to the present ''state of the art'' should be recognized and discussed in the light of the theory proposed below. Here, suffice it to point out a common denominator of the chicken-first scenarios. It is the view that metabolism is a complex set of reactions, and random chemical drift is a process that leads to ever-increasing complexity; therefore, random drift could initiate metabolism if it started with the appropriate compounds and reactions and if it lasted long enough.

I challenge this view. While metabolism is certainly complex, complex systems are not necessarily metabolic. *Metabolism is a complex set of reactions which are mutually regulated and coordinated for the survival and replication of the living system. While inanimate complexities result from random drift alone, metabolic complexity requires coordination and regulation. These are specific to life and result from natural selection acting on random drift.* Whenever a hypothesis or a scenario derives metabolism without natural selection, a deliberate selection is performed by the author himself. Mostly, a detailed sequence of steps is chosen by personal convictions. Each step may be an interesting and probable event. However, the convergence of the proposed sequence of steps, each one at its right time and place, to a *predetermined target* is most improbable.

Some supporters of the chicken-first hypothesis claim to have proven theoretically the feasibility of *self-organization and metabolism without natural selection.* Such proofs cannot be true. I studied carefully two such proofs, one by Kauffman (1986, 1993) and the other by Dyson (1985). The findings are presented in some detail in Appendices A and B because their relevance extends, I believe, beyond the present study.

Authors who favor the egg-first hypothesis (Eigen 1971, 1992; Orgel 1992, 1995; Crick 1968) link the origin of animate matter with the origin of information-carrying polymers. Eigen (1971) was the first to propose autocatalysis as the starting point of self-organization of matter and the evolution of biological macromolecules. In his model, autocatalysis took the form of template replication of polymers. One may question whether or not template-replicating polymers were the first autocatalytic molecules which originated spontaneously in inanimate matter. There can, however, be no doubt that some autocatalysis must have been initiated spontane-

ously in one form or another. If template-replicating biopolymers were not generated spontaneously they must have evolved from other autocatalysts by natural selection. Recently, synthetic autocatalysis and in vitro selection have become a center of interest (Eigen 1992; Orgel 1992; Feng et al. 1992; Green and Szostak 1992; Sievers and von Kiedrovsky 1994; Joyce 1994; Li and Nicolaou 1994; Chapman and Szostak 1994; Rebek 1994; Boehler et al. 1995; Wilson and Szostak 1995) because of their resemblance to biological template replication and natural selection. The studies of the egg-first class deserve also, like those of the other class, a thorough discussion. Here suffice it to note that the theory presented below supports the primacy of replication and of natural selection in the earliest stages of the origin of life.

Notwithstanding the above critical comments, the theory which follows rests on ideas taken from both the egg-first and the chicken-first classes. However, it offers a new, comprehensive view of the origin and evolution of reproduction, natural selection, metabolism, and genetic information. In particular, it explains the important role of the mutual interaction between animate matter and its changing environment in the evolution of complexity and metabolism.

Elementary Autocatalysis, Amplification, and Extinction

The theory presented here is based on established knowledge, summarized succinctly in the following observations.

First, life exists on earth, while astrophysical and geological evidence shows that our planet was lifeless during its early days. Furthermore, if life exists (or ever existed) on other planets somewhere in the universe, such planets were also lifeless during their early days. Thus, animate matter must have evolved from inanimate matter.

Second, animate and inanimate matter obey the same general laws of matter, based on quantum mechanics and statistical thermodynamics. These laws suffice, in principle, to explain the physical and chemical processes in animate matter, and therefore should suffice to explain their origin.

Third, Darwin's *The Origin of Species by Means of Natural Selection* offered the only rational explanation of the evolution of animate matter. Quoting Dawkins (1982), ''god and natural selection are, after all, the only two workable theories we have of why we exist.''

Based on these premises, the theory of the origin of animate matter proves that *life and its origin are one continuous process of stochastic physicochemical events directed by natural selection.* For inanimate matter to be subjected to natural selection it has to possess or acquire the appropriate physicochemical properties. These will be introduced in their logical and temporal order. The first such property is molecular replication by autocatalysis, which is the physicochemical analog of biochemical DNA replication and biological reproduction.

Let the term *elementary autocatalysts* denote self-replicating (or mutually replicating) molecules which started autocatalysis spontaneously in a prebiotic environment. Once an autocatalytic molecule originates spontaneously, it can grow exponentially by replication and reach macroscopic abundance if its reactants are abundantly available and if its *rate of net replication* is positive. A positive rate of net replication means that the rate of replication is faster than the rate of decomposition. The prebiotic elementary autocatalysts apparently left no tangible traces, so we don't know how much they resembled polynucleotides. However, the fact that life exists implies that replication did evolve all the way from inanimate elementary autocatalysis to biological reproduction.

Autocatalysis is a unique reaction. This may be appreciated by comparing it with heterocatalysis, using the following notation:

(i) *Italics* for a *single compound,* **boldface** for a **set of compounds.**
(ii) Lowercase for a molecular scale, UPPERCASE for a MOLAR scale.

Consider the *heterocatalytic* reaction scheme

$$\mathbf{R} \xrightarrow{c} p \tag{1}$$

where **R** is a set of macroscopically abundant reactants, catalyzed by a single catalyst molecule c to produce one molecule p each microsecond. To produce one mole P would take 6×10^{23} ms, i.e., about 2×10^{10} years, which is longer than the estimated age of the universe.

Consider next the corresponding *autocatalytic* reaction scheme

$$\mathbf{R} \xrightarrow{a} a \tag{2}$$

where **R** is catalyzed by one elementary autocatalyst molecule a to replicate at the same rate as above, one net replication each microsecond. The reaction would yield two replicas in the next microsecond and would double their number each following microsecond. If autocatalytic doubling were to continue unattenuated, it would produce one mole of A within mere 79 μs ($2^{79} \cong 6 \times 10^{23}$). Even if the autocatalytic reaction were orders of magnitudes slower, say, one replica per day, it would still produce one mole of A within only 79 days.

Thus, a spontaneous synthesis of an autocatalyst initiates an explosion-like phenomenon. However, autocatalytic doubling cannot go on indefinitely. Soon, amplification shifts to different kinetic routes. Some of these routes have been discussed by appropriate models (Eigen 1971; Szathmary 1991). Here we are concerned with general trends rather than with kinetic details. Two such trends determine the course of autocatalysis. *First,*

as long as net replication stays positive, amplification continues. *Second,* autocatalysts necessarily change their environment.

When an autocatalyst, *a,* is amplified to macroscopic abundance, *A,* and its reactants are depleted accordingly, net replication slows down. It eventually reaches a steady state of zero net replication, which prevails if, and as long as, the reactants are supplied steadily. The rate of autocatalysis is then determined by the reactant *r* whose rate of supply is the slowest. The reaction scheme is now

$$\mathbf{R}' + r \xrightarrow{A} A \qquad (3)$$

where $\mathbf{R}'$ represents all reactants except *r*. In the steady state, the rate of supply of *r* and the rates of production and decomposition of *A* are all equal. If the supply is exhausted, *A* decomposes gradually to *total extinction* and leaves behind a chemical environment impoverished in reactants of *A* and enriched with *A*'s decomposition products as well as other by-products.

Thus, autocatalysis possesses two complementary capacities, *tremendous amplification and tremendous reduction.* It amplifies itself as well as its by-products and decomposition products, from microscopic to macroscopic abundance, and it reduces its reactants even to *total extinction.* The fact that these capacities are typical of both inanimate replication and biological reproduction has far-reaching consequences for the origin of animate matter.

Diversity, Fitness, and Natural Selection

An autocatalytic molecule may be modified by reacting with either another autocatalytic molecule or with a somewhat different reactant or by some other chemical process. When such modifications retain autocatalysis, a family of diverse autocatalytic species is formed. Members of the family that share all or some reactants will be named *primitive mutants,* or, in short, *mutants,* because of their functional resemblance to their biological relatives. A new mutant, like the elementary autocatalyst, grows from microscopic up to macroscopic abundance whenever and as long as its net-replication rate is positive.

Mutants whose rates of net replication are not equal cannot maintain together a steady state (see Appendix C). As their reactants are gradually depleted, their rates of net replication are gradually abated. Eventually, some rates become first zero, then negative, while the others are still positive. *Change of sign of the rate of net replication is a singular turning point, irrespective of the kinetic details.* Above this point, when the rate is positive, amplification dominates. Below this point decomposition dominates, leading exponentially to total extinction. Consequently, competition between several mutants leads necessarily to successive extinctions. First to go is the mutant whose net replication is the slowest, then goes the next slowest, and so on. If the number of mutants were limited, the fastest mutant would remain without competitors and would eventually reach a steady state. This is the essence of natural selection among autocatalysts, or *primitive natural selection,* the physicochemical analog of biological natural selection. The fastest mutant may be called *the fittest* and may be said to *survive* by being *selected* against the other, less fit mutants.

Steady states cannot be reached when new mutants occur incessantly and join the process of natural selection. The fittest among the new mutants eventually replace old mutants, and this may continue as long as at least one surviving autocatalyst possesses a non-negative net-replication rate. The other alternative is total extinction, if the last autocatalyst reaches a negative net-replication rate. *Autocatalysis is, therefore, an inherently unstable process.* Since extinction of the unfittest is always final in the long run while the survival of the fittest is not, *natural selection forces autocatalysts to evolve,* namely, to change continuously toward new modes of survival.

Let me conclude this section by alluding to the uniqueness of Darwinian evolution among evolutionary processes in general. Evolution of galaxies and solar systems means the long-time course of a random drift of macroscopic systems toward ever-increasing entropy, or ever-decreasing free energy. Darwinian evolution of autocatalytic systems is no exception in this respect. However, its random drift is directed by natural selection toward all those specific properties that characterize animate matter, namely, fitness, adaptability, organization, and the like. Such properties are neither related directly to thermodynamics nor measurable by its methods. As a consequence, animate organization and inanimate order are totally different and unrelated concepts. Unfortunately, their superficial similarity is a source of much confusion in the context of life and its origin.

Primitive Metabolism

Hitherto, fitness was presented as a property of an autocatalyst relative to other autocatalysts, namely, its ability to survive at the expense of less-fit autocatalysts. In a broader sense, fitness is determined by the relation between the autocatalysts and their environment. A mutant is fitter than other mutants in a given environment if it is better *adapted* to the environment. Therefore, the course of evolution of autocatalysts by natural selection was determined in its broad outlines by the environment to which survivors were being continually adapted. However, the reverse process was equally important. The environment was continually modified by the depletion of the reactants and the accumulation of the autocatalysts as

well as their by-products and decomposition products. Thus, adaptation of autocatalysts to their environment and change of the environment by the autocatalysts were intertwined in a spiral evolutionary process. This process was initiated and mediated through a whole class of compounds, which I shall call *sequels of autocatalysis.*

Sequels of autocatalysis are all those compounds which are derived directly or indirectly from the autocatalysts. Direct sequels are the above-mentioned decomposition products and by-products. Indirect new sequels are produced when already-existing sequels, whether direct or indirect ones, react either with each other, or with other compounds, and/or with high-energy fluxes like sun radiation. Thus, sequels comprise consecutive generations, the first generation being the source of the second, and so on.

Sequels mediate the spiral process of evolution of the autocatalysts and their environment in two complementary ways. On the one hand, all sequels trail more or less the footsteps of their *source autocatalysts.* New sequels rise from microscopic to macroscopic abundance together with their source autocatalysts when net replication is positive. Other sequels fade away following the extinction of their source autocatalysts, when net replication is negative. Thus, autocatalysts change their chemical environment through their sequels. On the other hand, the changing environment exerts a selective pressure on the existing distribution of autocatalysts through the sequels. The selective pressure may materialize in many ways. Here, suffice it to recognize that *adaptation of autocatalysts to their changing environment by incorporating sequels into the autocatalytic process yields a great selective advantage.*

Sequels which participate in the process join their source autocatalysts as *internal components of a complex autocatalytic system.* In addition, compounds which participated in the synthesis of these sequels become *external reactants* of the complex autocatalytic system. The chemical reactions which take place in complex autocatalytic systems are by their very nature coordinated so as to secure the positive net replication of the system, because those which do not do so are eliminated by natural selection. I shall call this kind of complexity ''*primitive metabolism,*'' because it is primitive relative to cellular metabolism but fits the definition suggested above, ''a complex set of reactions which are mutually regulated and coordinated for survival and replication'' of the complex system.

Insomuch as primordial primitive metabolism apparently left no traces, there is room for hypotheses, scenarios, and models concerning the possible roles of sequels. However, these are outside the scope of the present paper, as they may but need not occur. The following simple scenarios are only examples, intended to show how the involvement of sequels in autocatalysis breeds primitive forms of metabolism. Consider an autocatalyst which is heading toward extinction because one of its reactants is continuously depleted. If the autocatalyst or some of its mutants gain a selective edge over their competitors when some of their sequels replace the depleted reactant, they survive together with these sequels. Another example is a complex autocatalytic system which would gain a selective advantage when some of its sequels would promote its net replication by catalyzing or regulating various parts of the complex process.

Furthermore, the process of incorporating sequels as internal components of complex autocatalysis is not limited to single autocatalysts. When sequels of several autocatalysts support each other's replication, they all gain collectively a selective advantage. The result is a metabolic system of higher complexity, comprised of several autocatalysts and their sequels which depend on each other to maintain together a positive net-replication rate. Thus, old and less complex systems may be gradually replaced by new and more complex ones whenever the more complex systems are better adapted to their environment. Note that complexity is not by itself an advantage, but it offers a wider distribution of mutants from which new and fitter mutants might emerge as survivors.

Beyond Primitive Metabolism

The present theory is not fit to trail the evolution of complex autocatalytic systems from primitive to cellular metabolism. The difficulty is that mutations are random microscopic events which cannot be foreseen; therefore evolution can be neither reconstructed backward nor predicted forward. However, some general observations can be made, which offer new insights and raise new questions.

A common paradigm, initiated with classic experiments of Miller (1955), says that monomeric ''building blocks'' of some precursors of biopolymers first accumulated spontaneously and then polymerized to yield functional polymers. This paradigm explains neither how the ''building blocks'' accumulated to sufficiently high concentrations, nor how their synthesis transformed from spontaneous to metabolic synthesis. The present study views the primordial ''building blocks'' as the naturally abundant reactants of the first elementary autocatalysts, whatever their composition might have been. They were generally consumed in the replication process, and were replaced by sequels of autocatalysis which initiated primitive metabolism. The particular case of sequels being identical to the primordial building blocks of their source autocatalysts may not be excluded. However, the more general case of new sequels replacing old reactants may not be excluded either, because it is the major factor in the spiral evolution of complex autocatalysis and its environment, as discussed above. In particular, nucleo-

tides and their template-replicating polymers, which seem too complex and insufficiently stable to accumulate to significant concentrations in a prebiotic environment, could have evolved as metabolites somewhere along the road from primitive to cellular metabolism.

The present study suggests also how autocatalytic systems acquired other characteristic properties of living organisms. One example is homochirality, or handedness, which is so characteristic of cellular metabolism (Bonner 1995). When chiral compounds are synthesized in a nonchiral environment, the amounts of the two enantiomers (the mirror images of each other) are, as a rule, equal. A chiral autocatalyst is a natural exception, provided chirality is retained in the replication. If such a molecule is synthesized either spontaneously or as a chiral mutant of a nonchiral autocatalyst, it may replicate to macroscopic abundance and deplete its reactants, thus reducing the chance of spontaneous synthesis of the other enantiomer (Frank 1953). Homochirality could then spread to many components of metabolism through chiral sequels whenever it offered selective advantages.

Another property which is expected to have originated from sequels during the early evolution of primitive metabolism is its enclosure in membranous vesicles. Primitive vesicles could have been obtained from sequels which could either form membranes directly or catalyze the synthesis of membrane-forming molecules. Such vesicles would carry with them many selective advantages (Dyson 1985) and would gradually evolve toward the highly complex cellular membranes.

Cellular organization, directed by a genetic code which controls cellular metabolism, is extremely complex. The present theory has no answer to the big question of how noncoded metabolism was transformed to coded metabolism and what the links were between the two. It implies, however, that the evolution of the genetic code was intertwined from its very beginning with the evolution of metabolism. For this reason, template replication of polymers which carry coded genetic information should be considered as a high-leveled metabolic phenomenon, while the origin of replication and primitive metabolism are at the roots of the origin of animate matter.

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Appendix A: Kauffman's Self-Organization on the Edge of Chaos

Kauffman (1986, 1993) offered a theory of the ''crystallization of life'' based on a mathematical model in which metabolism is initiated without natural selection. His book (Kauffman 1993) was reviewed by Dover (1993), who described Kauffman as floating in abstract space while losing contact with the ground control of reality. Joyce (1989) characterized the earlier account of Kauffman's theory (1986), together with that of Dyson (1985), as resting on ''a highly over-optimistic estimate of the probability that a random polypeptide can catalyze peptide-bond formation with any significant degree of sequence specificity.'' Here I examine some of the mathematics of Kauffman's model.

All quotations and references to Kauffman (1993) are followed by page number.

Kauffman challenges Neo-Darwinism. (''Since Darwin's theory of evolution, Mendel's discovery of atoms of heredity, and Weismann's theory of the germ plasm, biologists have argued that evolution requires a genome. False, I claim'' [p. 285].) According to Kauffman, 'Self-Organization on the Edge of Chaos' is a universal force in evolution, in addition to, and independent of, Darwinian natural selection. Part II of the book deals with ''The Crystallization of Life''—namely, with its origin. It claims that ''catalytic closure'' of ''reflexively autocatalytic subsets'' (p. 285) leads to ''Crystallization of Connected Metabolism as a Percolation Problem'' (p. 346). Kauffman derives catalytic closure by trying to show that ''a collection of molecules has the property that the last step in the formation of each molecule is catalyzed by some molecule in the system'' (p. 285). There are many problems with the model, but they need not all be discussed because of a major error which renders its conclusions wrong anyhow.

The model discusses a set of polypeptides up to a maximum length M, which contains all linear combinations of two monomer species. The number of different sequences is 2^{M+1} and the total number of bonds between the monomers is $(M-2)\cdot 2^{M+1}$ (pp. 301–302). The polymers may catalyze the ligation and cleavage of the bonds with a constant probability P, and the catalysis is absolutely specific. ''Only one among the $[(M-2)\cdot 2^{M+1}]$ possible reactions is catalyzed by one protoenzyme. . . . I shall suppose that any polymer has a constant probability P of catalyzing any reaction'' (p. 306).

> Kauffman's derivation of catalytic closure includes the following argument: Consider the longest polymers of interest, those of length M. Any specific polymer M^* can be formed in $M-1$ ways by condensation of smaller polymers. The chance **P** that none of the 2^{M+1} polymers in the set catalyzes any of the $M-1$ reactions is just
>
> $$\mathbf{P} = (1-P)^{(M-1)2^{M+1}} \approx \exp(-P\cdot(M-1)\cdot 2^{M+1} \quad (7.6)$$
>
> If we require that **P** be low, say 0.001, then we have stated a condition such that, with a probability 0.999, the formation of M^* will be catalyzed by at least one member of the set (p. 309).

For **P** to be low, the exponent $P\cdot(M-1)\cdot 2^{M+1}$ in equation (7.6) must be high. However, this is impossible due to the dependence of P on M. When M increases, the number of sequences increases exponentially and the number of bonds increases even faster. The probability P depends on both the sequences and the bonds to be catalyzed. It is, therefore, a product of two elementary probabilities. Let P' be the probability that a polymer is a catalyst, without specifying which reaction it catalyzes. Then P' is a small number within the range $0 \leqslant P' < 1$. Next, let P'' be the probability that a catalyst catalyzes a particular reaction. Then, according to the model, P'' is precisely $1/[M-2)\cdot 2^{M+1}]$ because all $(M-2)\cdot 2^{M+1}$ bonds have the same probability to be synthesized, and the catalyst catalyzes only one of them. Consequently, the probability that a particular polymer catalyzes a particular reaction is

$$P = P'\cdot P'' = P'/[(M-2)\cdot 2^{M+1}] < 1/[(M-2)\cdot 2^{M+1}] \quad (A1)$$

Inserting equation (A1) in Kauffman's equation (7.6), we obtain a lower limit for **P**

$$\mathbf{P} \approx \exp[-P'\cdot(M-1)/(M-2)] > \exp[-(M-1)/(M-2)] \quad (A2)$$

which formally approaches $\mathbf{P} > e^{-1}$ with increasing M, but in fact it is very close to 1 because P' is much smaller than 1. Kauffman's error was to increase M at constant P. He presented in Table 7.1 (p. 310) a ''Stringent Criterion for a [Reflexively] Autocatalytic Set Using Only Ligation and Cleavage Reactions.'' To meet this criterion, he chose $\mathbf{P} = e^{-8} < 0.001$, which is far below the lower limit given by (A2). Thus, the derivation of reflexively autocatalytic sets collapses, carrying with it the claim for ''crystallization of connected metabolism as a percolation problem.''

Appendix B: Dyson's Metabolism Without Natural Selection

Dyson (1985) offered a different theory of metabolism without natural selection wherein he addressed fundamental problems in the philosophy of biology. His views were succinctly presented in the closing paragraph (p. 77).

> I have been trying to imagine a framework for the origin of life, guided by a personal philosophy which considers the primal characteristics of life to be homeostasis rather than replication, diversity rather than uniformity, the flexibility of the genome rather than the tyranny of the gene, the error tolerance of the whole rather than the precision of the parts. . . . I hold the creativity of quasi-random complicated structures to be a more important driving force of evolution than the Darwinian competition of replicating monads.

Thus, Dyson stressed points which were perhaps neglected by others, but it seems to me that homeostasis and replication, diversity and uniformity, tolerance and precision are all primal characteristics of life. The present theory agrees with Dyson that the origin of primitive metabolism necessarily preceded the origin of coded information. However, Dyson's derivation of metabolism introduced *unnatural selection.*

Thanks to Dyson's lucid and precise style, it is easy to recognize where and how unnatural selection has been introduced. Dyson based his mathematical model on ten clearly stated assumptions (pp. 44–50). Assumption (3) stated: ''There is no Darwinian selection. Evolution of the population of molecules within a cell proceeds by random drift.'' However, Dyson must have sensed that no metabolism can be reached by unrestricted drift. Therefore he restricted the random drift by assumption (7): ''The active monomers are in active sites where they contribute to the ability of a polymer to act as an enzyme. To act as an enzyme means to catalyze the mutation of other polymers in a selective manner so that the correct species of monomer is chosen preferentially to move into an active site.'' Such a selection or preferential choice is unnatural. An enzyme cannot choose the ''correct'' species and the active site because they are undefined before the effect of the mutant on the cell has been tested. Catalysis is a physicochemical phenomenon, directed by intermolecular forces rather than by subsequent properties of the finished product. In summary, Dyson's emphasis on the role of primitive metabolism, homeostasis, diversity, flexibility, error tolerance, and the like is well taken, but such properties can evolve only through natural selection.

Appendix C: Autocatalysis and Natural Selection

The purpose of this appendix is to show by a simple mathematical model that a single autocatalyst can reach a steady state, while two autocatalysts cannot reach a steady state simultaneously, and are therefore subjected to natural selection.

Consider, first, a single reactant R which is catalyzed by a single autocatalyst A, producing replicas of A, such that one molecule of R is catalyzed by one molecule of A to produce another molecule of A. Let the time-dependent concentrations be denoted by $R(t)$ and $A(t)$ moles per liter, respectively. A replicates at a second-order rate of αRA and decomposes at a first order rate βA, and R is supplied from a source at a constant rate s. The corresponding kinetic equations are

$$\dot{A} = (\alpha R - \beta)A \quad (1)$$

$$\dot{R} = s - \alpha AR \tag{2}$$

In a steady state, both $\dot{R}$ and $\dot{A}$, as well as their higher derivatives, all vanish. The steady-state concentrations R and A are then determined by $\alpha RA = \beta A = s$, namely,

$$R = \beta/\alpha \qquad A = s/\alpha R = s/\beta \tag{3}$$

Consider now two autocatalysts, A_1 and A_2, competing for R. The kinetic equations are now

$$\dot{A}_1 = (\alpha_1 R - \beta_1)A_1 \tag{4}$$

$$\dot{A}_2 = (\alpha_2 R - \beta_2)A_2 \tag{5}$$

$$\dot{R} = s - \alpha_1 A_1 R - \alpha_2 A_2 R \tag{6}$$

In a steady state, $\dot{R}$, $\dot{A}_1$, and $\dot{A}_2$, as well as their higher derivatives, all vanish. However, they cannot vanish simultaneously while the two autocatalysts coexist, namely, as long as $A_1 > 0$ and $A_2 > 0$, unless $R = \beta_1/\alpha_1 = \beta_2/\alpha_2$, which is an exceptional case. In general, the system may reach a steady state only if one of the catalysts, say A_2, vanishes, while the other one, A_1, remains. It then reaches a steady state according to the previous case.

Thus, in the steady state

$$R = \beta_1/\alpha_1 \quad A_1 = s/\alpha_1 R = s/\beta_1 \quad A_2 = 0 \tag{7}$$

In conclusion, two autocatalysts that compete for a common reactant cannot coexist in the steady state. One becomes extinct while the other is selected to survive.