FINAL REPORT

MOLECULAR EVOLUTION IN PROTOBIOLOGICAL SYSTEMS

by

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Introduction

In a proposal to the National Aeronautics and Space Administration, dated October 1961, M.S. Blois and H.H. Pattee of the Biophysics Laboratory, Stanford University, outlined a series of experimental studies in the field of molecular evolution. In particular, these studies contemplated the next level of molecular complexity, beyond the Miller experiment, and included an evaluation of the possibility of the spontaneous origin of catalytic activity.

One of the central questions we asked concerned the possibility of finding any general rules governing the behavior of heterogeneous chemical systems acted upon by a continuous energy source. It was, and remains, our view that the field of molecular evolution is more in need of principles having predictive value in such systems, than in the demonstration that any given molecular species can arise under assumed primeval conditions.

The theoretical and experimental work conducted under this grant has led to a number of results which have been published. These are listed below. Other experimental trials of general interest, which were inconclusive or which led to negative results, are described below very briefly. One doctoral dissertation which resulted from this program accompanies this report (Kenyon, 1964).

In addition to the principal investigators, other individuals who participated in this program were Dr. D.H. Kenyon, Mrs. Lina Taskovich, and Dr. A.N. Tsvetikov.

Results of Experimental Studies

The experimental work conducted under this grant has resulted in the following publications or papers:

(1) "π-electron Photochemistry of DL-phenylalanine in Oxygen Saturated and Oxygen Free Solutions", D.H. Kenyon and M.S. Blois, Photochemistry and Photobiology (in press).

[redacted]

(2) "Random Polymers as a Matrix for Chemical Evolution", M.S. Blois, in The Origin of Prebiological Systems, S. Fox, ed., Academic Press, New York, 1965, p 19.

(3) "The Optical and Photochemical Properties of Melanins", M.S. Blois and D.H. Kenyon, Paper given at the International Congress of Photobiology, Oxford, 1964.

(4) "Photochemistry of DL-Phenylalanine", Dean H. Kenyon Ph.D. Dissertation, Stanford University, B.L. Report No. 124, 1964.

(5) "Experimental Approaches to the Origin of Life Problem", H.H. Pattee, in Advances in Enzymology, Vol. 27, F.F. Nord, ed., Interscience, New York, 1965, p 381 (attached as Appendix I)

(6) "A Note on Chemical and Hereditary Evolution", H.H. Pattee, Paper FC8, 9th Annual Biophysical Society Meeting, San Francisco, Feb. 24-26, 1965 (Abstract). (attached as Appendix II)

In addition to these papers, the expected number of negative results and experimental difficulties ensued. These will be cited here for completeness.

a) Miller type experiments

1) A number of runs were made in the usual Miller type apparatus, starting with mixtures of CH_4, H_2O, and NH_3. After a week's excitation by an arc the solutions were yellowish, opalescent, and in a system having a volume of 4-5 liters, about a half gram of solids were obtained. The UV absorption spectroscopy of this material showed a strong and increasing absorption below 3200 A.

2) A run starting with ethanol, NH_4OH, and H_2O (using iron arc electrodes to provide an Fe source) - the soluble portion showed UV peaks at 2940 and 2100 A which disappeared when the solution was brought to pH 10 with ammonia, and which shifted to slightly longer wavelength at strongly acid pH.

3) A number of runs were made starting with ethanol, NH_4OH, and H_2O, yielding in each instance a yellowish, opalescent solution.

The colloidal material responsible for the opalescence was shown to give the same I.R. spectrum as an authentic sample of silica, and confirms Miller's earlier report. The solution was shown to contain double bonds (by Bayer's reagent), and formic and acetic acids.

4) In order to evaluate the possibilities of dimerization in the gas phase, mixtures of n-pentane and 2-Me pentane were irradiated with 165 kv x-rays to a total dose of 50,000 rads in sealed ampules. Subsequent gas chromatographic analysis showed the presence of both C_{11} and C_{12} chains but no evidence of C_{10} chains.

5) In attempting to learn some of the general properties of organic systems under continuous excitation, aqueous mixtures of formic acid, glycine, and alanine (products of a Miller experiment) were deoxygenated and sealed in ampules and irradiated with Co^{60} gamma rays. After various total doses of radiation, the osmolarity of the solutions were measured with a Fiske osmometer. If there had been a strong tendency of the system to form larger molecular weight compounds the final osmotic pressure would fall; if radiolysis were dominant the osmotic pressure would rise.

The following results were obtained:

X-ray dose	Osmotic pressure	
0	0.327	
2×10^4 rads	0.323	
5×10^4 "	0.332	(0.329)
1×10^5 "	0.323	(0.331)
5×10^5 "	0.321	
1×10^6 "	0.319	
2.2×10^6 "	0.315	
1.5×10^7 "	0.280	(0.277)

The figures in parenthesis refer to ampules which had been opened, and then measured a few hours later to provide for gaseous products (such as H_2 or CO_2) to escape from the solution.

The general fall in osmolarity with radiation dose would be consistent either with the formation of molecules of larger molecular weights, or the production of gaseous products which escaped during the very short intervals between opening the ampules and measuring the osmotic pressure. However, the radiation chemistry of formic acid shows the overall reaction:

$$HCOOH \rightarrow H_2 + CO_2$$

so that for each molecule of formic acid which underwent radiolysis, one molecule of H_2 was formed (which probably would be lost from the solution for solubility reasons) and one molecule of CO_2 was formed, and probably retained in solution. The overall change in number of molecules would be zero under these conditions. The radiation products of glycine and alanine are known and do not include such volatile compounds. We concluded therefore that the fall in osmotic pressure probably implied an increase in the weight average molecular weight of the system under these anaerobic conditions.

The experiment was re-run under similar conditions, except for a three-fold increase in solute concentration, and similar results were obtained. There appears to be an actual rise in osmolarity at a dose of about 10^5 rads and then a decline in osmolarity with doses up to 10^8 rads.

A similar decrease in osmolarity was noted with an aqueous solution of glycine, irradiated with 2537 A , in the presence of O_2. Here the production of gas would not be expected, and the fall in osmolarity is assumed to reflect an increase in the weight average M.W.

6) The next phase of work included the study of spectral absorbance changes produced in organic systems when irradiated with monochromatic ultraviolet light. This work has been described serially in earlier progress reports and more completely in the accompanying publications.

7) Since random polymers of the melanin type are thought to have been produced -- perhaps as the earliest abiogenic macromolecules -- under primeval earth conditions, these systems were examined for catalytic activity. The general approach was to use melanin-like polymers synthesized in the presence of small amounts of trace metals -- ordinarily Fe ion -- and test these with simple substrates for oxidase activity. Tracer techniques and chromotography were employed, and while clear evidence of oxidative activity was found in several systems, the role of contaminating microorganisms could not be convincingly excluded. The use of stringent sterilization methods such as autoclaving inhibited the activity observed, but whether it did so by eliminating microorganisms or by modifying the polymer, is not known. This series of experiments has not yet led to any definite conclusions.

Theories of Molecular Evolution

Dr. Pattee's interest in molecular evolution has centered at the minimum level of complexity at which hereditary control of reactions could have begun; that is, at the stage where some form of molecular memory exists. Memory in this sense means a set of metastable states which differ little in energy but which have significant activation barriers for transitions from one state to another. These conditions are fulfilled by linear copolymers which may be linearly ordered as well as ordered in three dimensions.

A considerable amount of review and study of polymer crystals and tactic polymerization processes has been necessary because of the recent rapid advances in these fields. Both precise conformation control and linear sequence control are the essential operations which must be accomplished in living systems, and it is of great significance that these operations can now be demonstrated in a wide variety of simple nonbiological polymers (1-6).

A review of current experimental approaches to the origin of life including a compilation of abiogenic syntheses was also made and will be

published shortly in Advances in Enzymology, Vol. 27, F.F. Nord, ed., Interscience, New York, 1965. A copy of the proof accompanies this report (Appendix I).

The experimental approach which Dr. Pattee's group is studying is at the copolymer level of molecular organization. From the evidence of the many abiogenic synthesis experiments summarized in Table I, p 382-386, in the above article, it is reasonable to assume the existence of copolyamino acids, copolynucleotides and other polymeric material, at least in dilute solution, in the primitive oceans. The variety of organic material produced by non-specific energy sources on the sterile earth has been shown to be enormous. The next problem, then, is the selection, concentration, or segregation of such a dilute heterogeneous mixture into more ordered aggregations which may possess self-organizing properties. The inorganic minerals of the earth have undergone this segregation process by precipitation, crystallization, sedimentation, and by many other physical or chemical processes, and there is every reason to believe that the organic material in a sterile ocean would also undergo selective processes.

For example, one of the most efficient processes for concentration of surface active material is by surface film formation or foaming (7, 8). The wind and waves near the seashore provide a continuous production and collection mechanism for such surface active material. Copolyamino acids and lipids are of course strongly surface active and would be concentrated by this process. Riley (9) and others have recently demonstrated that the foaming process is an essential step of the ecocycle of zooplankton and many larger marine organisms. Experiments have been started to determine if synthetic copolyamino acids, such as might have been formed abiogenically, can be fractionated and concentrated by foaming.

A second selective process which may have been common on the sterile seashore would occur at the other phase boundary between the solid surfaces of sand and clay minerals on the beach. This is a very complex region involving many cyclic processes such as waves, tides, diurnal UV and visible irradiation, and seasonal changes, as well as the many types

of minerals and states of aggregation of sands and clays, which may strongly affect reactions and separation phenomena (10, 11).

No experiments on simulated sterile primitive seashores have yet been done with the idea of studying the organic polymer chemistry in such systems. Our preliminary studies have been attempts to understand the essential types of selection processes, photochemical, physical and surface activated, which might be studied in a suitable geophysically realistic experiment of this type. While it is apparent that the conditions on a primitive seashore four billion years ago are uncertain, there is no strong evidence for great discontinuities in the general behavior of seashore processes (12). On the other hand, there is good evidence that concentration and segregation of heteropolymers would occur, along with photochemical reactions which would serve as strong selective forces in determining the organic polymeric organizations on the primitive earth. For this reason, we consider simulated sterile seashore experiments of comparable importance to the abiogenic syntheses which generate great chemical variety.

As a complementary approach to experiments with linear copolymers, there is the fundamental question of how such strings of subunits can serve as a hereditary control and gradually accumulate hereditary information by selection processes. Although tactic copolymerization must be treated as a hereditary process, there is no good model of how ordered sequences come to evolve greater and greater complexity in a relatively disordered environment. This problem may be stated as follows: what is the simplest set of monomer units and polymer interactions which will accumulate linear order in copolymers by hereditary selection? This is the general problem of the self-organizing system applied specifically to linear chains. Only a few suggestions have been made (13, 14, 15) along these lines, but the results are suggestive and stimulating. We are presently considering simulation programs of string-processing automata or linear sequence generators which possess the property of accumulating linear order by random search and selection processes. We have attempted to reduce this process to its most elementary logical form and to find the closest chemical representation of this general structure (16).

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EXPERIMENTAL APPROACHES TO THE ORIGIN OF LIFE PROBLEM

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CONTENTS

I. Introduction

No question has aroused the curiosity of man for as long a time and provoked answers from so many disciplines as the primeval source of the persistent, localized, chemical reactions which we now recognize as living organisms. No doubt the question will continue to generate answers from many points of view for a long time to come. As a problem of experimental science it has stimulated only sporadic interest in the past, but for good reasons a general renewal of interest has grown in the last decade. First of all there are now many demonstrations that reasonable, primitive earth-like environments can produce most classes of molecules which are essential for present living organisms. Second, the immanent possibility of landing life-detection instruments on neighboring planets has required some general thinking about a reasonable experimental strategy of extraterrestrial biological exploration. Third, the great advances in understanding the molecular basis of genetic replication and control of protein synthesis has raised deeper questions about the possible origin and evolution of these intricately connected macromolecular activities. Finally, there is a growing appreciation that the evolution of highly ordered systems from a chaos or cosmos poses a worthy fundamental problem in its own right, independent of what the physical or chemical representation of the systems we study may happen to be.

The present point of view of the author is that recent biological and abiological experiments which relate to the origin of life problem have so greatly reduced the gap between what is called "living" and "nonliving" states of matter that new questions must be asked and new experiments designed to help answer them. In some sense during the last decade we have learned to be less puzzled by the complexity of living matter and more puzzled by the complexity of nonliving matter. The general postulates of the biological theory of evolution, which in effect *define* what we mean by "living matter" (*cf.* Crick, 1961), have been reduced to molecular terms. Thus, blind mutation, self-replication, and the storage and expression of genetic information accumulated only by natural selection are described in terms of genetic, messenger, and transfer nucleic acids, protein synthesis, feedback control, the sequence hypothesis, and the central dogma. On the other hand, starting from the simplest molecules, such as water, carbon dioxide, and ammonia, it is possible to produce abiogenically many of the most essential biochemicals of present-day living organisms from common, nonspecific energy sources in a matter of hours (see Table I). Some of these abiogenic polymers easily organize themselves into structured spheres which occasionally cleave or aggregate into more complex forms. (Fox and Yuyama, 1963a, b; 1964). Furthermore, the simplest synthetic organic polymers can be precisely organized at the atomic level both in their primary linear structure (e.g., see Gaylord and Mark, 1959), as well as in their three-dimensional folding (e.g., see Geil, 1963) either autonomously or by the use of simple catalysts. Simplified models such as "perfect" crystals and "random" polymers are no longer of much use for explaining this behavior.

The main body of this chapter will be a brief review and discussion of recent experiments which have possible significance for the origin of life problem. These experiments raise many questions about the organization and behavior of collections of macromolecules which

TABLE I
Abiogenic Syntheses[a]

Substances synthesized	Starting materials[b]	Energy source	References
Formic acid, formaldehyde	CO_2, H_2O, ($FeSO_4$)	Helium ions	Garrison et al. (1951)
Oxalic acid, fumaric acid	Glycine	Heat	Heyns and Pavel (1957)
Formic acid and higher fatty acids, urea	HCN, NH_4OH, H_2O	Heat	Lowe, et al. (1963)
Glycolic acid, lactic acid, formic acid, acetic acid, propionic acid, α-hydroxybutyric acid, succinic acid, urea, methyl urea	CH_4, NH_3, H_2, H_2O	Electric discharge	Miller (1953, 1955, 1959); Miller and Urey (1959)
Glycolic acid, lactic acid, formic acid, glycinamide	Formaldehyde, hydroxylamine	Heat	Oró et al. (1959)
Amino Acids			
α-Alanine, β-alanine, glycine, sarcosine	$CO_2(CO)$, $N_2(NH_3)$, H_2, H_2O	Electric discharge	Abelson (1956)
Aspartic acid, asparagine, arginine, glycine, serine, lysine, proline, histidine, valine	Paraformaldehyde, KNO_3 ($FeCl_3$)	Sunlight	Bahadur (1954, 1959)
Amino acids and amines (unidentified)	CH_4, CO_2, NH_3, H_2, H_2O	X-rays	Dose and Rajewsky (1957)
Aspartic acid, alanine	Malic acid, urea		
Glycine	Glucose urea, α-hydroxyglutaric acid, NH_3	Heat	Fox (1960)
Glutamic acid			
Aspartic acid, alanine	Ammonium fumarate, ammonium malate	Heat	Fox et al. (1955)
Glycine, alanine, sarcosine	CH_4, NH_3, H_2O		
Glycine, alanine, sarcosine, higher amino acids, amines	C_2H_6, NH_3, H_2O	Ultraviolet	Groth (1957)
Aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, allo-isoleucine, isoleucine, leucine, tyrosine, phenylalanine, α-aminobutyric acid	CH_4, NH_3, H_2O	Heat	Harada and Fox (1964)
Alanine, asparagine, glycylglycine	Glycine	Heat	Heyns and Pavel (1957)
Glycine, α-alanine, β-alanine, sarcosine, α-aminobutyric acid	CH_4, CO_2, NH_3, N_2, H_2, H_2S	Ultraviolet	Heyns et al. (1957)

TABLE I (*continued*)

Substances synthesized	Starting materials	Energy source	References
Amino Acids (*continued*)			
Aspartic acid, threonine, serine, glutamic acid, glycine, alanine, isoleucine, leucine, β-alanine, α-β-diaminopropionic acid, α-aminobutyric acid and five others (unidentified)	HCN, NH_4OH, H_2O	Heat	Lowe et al. (1963)
Glycine, alanine, sarcosine, β-alanine, α-aminobutyric acid, *N*-methylalanine, aspartic acid, glutamic acid	CH_4, NH_3, H_2, H_2O	Electric discharge	Miller (1953, 1955, 1959); Miller and Urey (1959)
Glycine, alanine, aspartic acid, asparagine, isoleucine, proline and others (unidentified)	CH_4, C_2H_6, NH_4OH, H_2O	Electric discharge	Oró (1963)
Glycine, alanine, aspartic acid	HCN, NH_4OH	Heat	Oró and Kamat (1961)
Glycine, alanine, β-alanine, serine, aspartic acid, threonine	Formaldehyde, hydroxylamine	Heat	Oró et al. (1959)
Glycine and possibly alanine	$(NH_4)_2CO_3$	X-rays	Paschke et al. (1957)
Glycine, alanine, serine, glutamic acid, valine, isoleucine, phenylalanine, and basic amino acids (unidentified)	Formaldehyde, NH_4Cl NH_4NO_3	Ultraviolet	Pavlovskaya and Pasynskii (1959)
α-Alanine, β-alanine and others (unidentified)	CH_4, CO, NH_3, H_2O (Al_2O_3, aluminosilicates, silicates)	Ultraviolet	Terenin (1959)
Polypeptides			
Polyglycine	Aminoacetonitrile, H_2O (kaolinite)	Heat	Akabori (1959)
Polyglycine with seryl or threonyl side chains	Polyglycine, formaldehyde or acetaldehyde (Kaolinite)	Heat	
Peptide infrared band	CH_4, NH_4Cl, H_2O, FeS	Ultraviolet	Ellengoben (1958)
Copolymers of aspartic acid with each of six other amino acids	Aspartic acid + other amino acids	Heat	Fox (1960)
'Panpolymers' of aspartic acid, glutamic acid, and 16 other amino acids	Aspartic acid, glutamic acid + other amino acids	Heat	Fox and Harada (1960)

TABLE I (*continued*)

Substances synthesized	Starting materials	Energy source	References
Polypeptides (continued)			
Copolymers of glutamic acid or pyroglutamic acid and 6 or 7 other amino acids	Aspartic acid, glutamic acid + other amino acids	Heat	Harada and Fox (1958)
Copolymers of aspartic acid and glutamic acid	Aspartic acid, glutamic acid	Heat	Harada and Fox (1960)
Peptides (uncharacterized)	HCN, NH_4OH, H_2O	Heat	Lowe et al. (1963)
Polyglycine	Glycine, NH_4OH	Heat	Oró and Guidry (1961)
Polyarginine	Arginine (ethyl metaphosphate)	Heat	Schramm et al. (1962)
Poly(alanyl-glycyl-glycine)	Alanyl-glycyl-glycine (ethyl metaphosphate)	Heat	Schramm and Wissman (1958)
Purines, Pyrimidines, Nucleosides, Nucleotides			
Uracil	Malic acid, urea	Heat	Fox and Harada (1961)
Adenine, hypoxanthine	HCN, NH_4OH, H_2O	Heat	Lowe et al. (1963)
Adenine	CH_4, NH_3, H_2O	Electrons (4.5 m.e.v.)	Ponnamperuma et al. (1963)
Adenosine	Adenine, ribose	Ultraviolet	Ponnamperuma et al. (1963)
ATP	Adenine, ribose, ethyl metaphosphate Adenosine, ethyl metaphosphate AMP, ethyl metaphosphate	Ultraviolet	Ponnamperuma et al. (1963)
Adenine	HCN (NH_4OH)	Heat	Oró and Kimball (1962)
Adenosine, deoxyadenosine	Adenine, ribose, deoxyribose (ethyl metaphosphate)	Heat	Schramm et al. (1962)
Polynucleotides			
Polyribonucleotides, e.g., poly-A, poly-U, poly-C, and various copolymers.	Nucleoside 2′-, 3′-, or 5′-monophosphates	Heat	Schramm et al. (1962)
Polydeoxyribonucleotides, e.g. poly-dT.	2′-Deoxynucleoside-5′-monophosphates (ethyl metaphosphate)	Heat	
Poly-C	Cytosine 2′- or 3′-monophosphate (ethyl metaphosphate)	Heat	Schwartz (1964)
Sugars			
Ribose, deoxyribose	Formaldehyde	Ultraviolet	Ponnamperuma and Mariner (1963)
Cellibiose	Glucose, methyl glucose (ethyl metaphosphate)	Heat	Schramm et al. (1962)
Polysaccharides			
Polyglucose	Glucose	Heat	Mora (1958, 1964)
Polyglucose	Glucose (ethyl metaphosphate)	Heat	Schramm et al. (1962)
Polyribose	Ribose	Heat	
Polyfructose	Fructose (ethyl metaphosphate)	Heat	
Other Substances			
Melanic polymers	Phenylalanine	Ultraviolet	Blois (1964); Blois and Kenyon (1964); Kenyon and Blois (1964)
Polymers which yield amino acids and urea upon hydrolysis	HCN, NH_4OH, H_2O	Heat	Lowe, Rees, and Markham (1963)
Porphine-like substances	Pyrrole + an aldehyde	Ultraviolet; x-rays	Szutka (1963, 1964)
High molecular weight hydrocarbon polymer	CH_4, NH_4OH, H_2O, H_2S, yeast ash	Electric discharge	Wilson (1960)

[a] The selection of entries in this table is illustrative rather than exhaustive. Inclusion in this table implies that some attempt has been made to simulate primitive earth or planetary conditions, even though many of these reactions have been known for years, and may have a large number of references in the literature of organic chemistry.

[b] Compounds in parentheses function as catalysts.

must be answered before any *non*biological theory of evolution can explain how simple molecules attain the intricate conditions necessary for biological evolution as we now observe it.

II. Abiogenic Syntheses in Simulated Primeval Environments

A. REVIEW OF EXPERIMENTS

By far the greatest amount of experimental effort on the origin of life problem has been the search for reasonable reactions which produce biologically useful complex molecules from simpler starting materials which are likely to have occurred in abundance on the primitive, sterile earth. Most of these experiments are variations and extensions of experiments conceived first by Calvin (Garrison et al., 1951) and Urey (Miller, 1955). The phrase *chemical evolution* (e.g., see Calvin, 1956; Blum, 1961) is often used to describe this level of reaction in contrast to *biological evolution* by natural selection, although a precise definition of these terms in context is seldom given. Table I presents a brief summary of the data provided by abiogenic synthesis experiments showing the material synthesized, the starting compounds, the source of energy, and the reference. The column of materials synthesized is indeed a remarkable list of biochemicals which fully confirms the ideas first put forth by Oparin (1924) and Haldane (1929) that the primitive environment must have produced the organic materials which are essential for bulding up the first living organisms.

Several important additional points should be borne in mind when considering these data. In the first place, experiments of this type have been performed under laboratory environments which represent possible primitive earth conditions. The best evidence available on these macroscopic conditions is very incomplete and leaves many possibilities open. Furthermore, although improved techniques and more imaginative searches, such as the paleobiochemical experiments of Abelson (1963), will greatly increase our historical knowledge, it is extremely unlikely that direct geochemical evidence can be found to provide the basis for what we might call "crucial" experiments (Rutten, 1962). Nevertheless, the geochemical ground rules provided the essential independent restrictions necessary to give these experiments more than hypothetical significance. Second, even if the precise macroscopic or thermodynamic condition could be specified accurately, the details of the molecular environment may still be of the utmost importance. Although we may not know details with certainty, we are certain that the details were complex. Consider any primitive seashore with periodic waves and tides washing over many forms of sand and clay, and all bathed with ultraviolet and visible light; or consider an active volcanic region with high temperatures and high temperature gradients in complex mineral surfaces cooled by heavy rains which flow into warm stagnant pools or into the sea. Even though we do not know the details, we should recognize that only a few laboratory experiments approach this order of chemical, structural, or sequential complexity (e.g., Fox, 1964) and in this sense many realistic simulated primeval environments remain to be explored. Furthermore, probably only a few per cent of the organic material produced in these experiments has been identified, and there is no reason to believe that the unidentified material is biologically uninteresting or insignificant. Perhaps the most surprising general result of all these abiogenic syntheses is that so many complex organic and biochemical species are produced from such simple starting materials and in such a short time. As Fox (1964) has demonstrated, it is now a reasonable hypothesis that volcanic regions of the primitive earth produced significant quantities of high molecular weight heteropolyamino acids from simple gases in a matter of hours. All these general considerations when combined with the results in Table I reinforce the conclusion that almost any specified class of molecule which we consider essential for life is likely to be produced in a reasonable simulated environment representing some region of the primitive earth.

B. DISCUSSION OF ABIOGENIC SYNTHESIS EXPERIMENTS

What are the conclusions to be drawn from these abiogenic synthesis experiments, and to what type of future experiments do they lead? These experiments were based on the hypothesis that conditions on the primitive earth, whatever they may have been in detail, were at some place favorable for the production of many organic

compounds which make up *life at the present time* (e.g., see Miller and Urey, 1959). The results of these experiments make this hypothesis extremely plausible.

At the same time, these experiments show that an enormous number of other compounds were also produced. Which of these many materials were essential in forming the most primitive hereditary macromolecules which led to present forms of life has not been demonstrated. The most common working hypothesis is that those species of molecules which make up *present living matter* were also the *starting material.* This *biochemical similarity principle* is perhaps the simplest hypothesis, but there is no evidence as yet which excludes alternative possibilities. From the time of the first appearance on earth of such enormously complex chemical heterogeneity, which these abiogenic syntheses lead us to believe existed, there may have been a long period of evolution before the state of biochemical uniformity based on proteins and nucleic acids came into being (e.g., see Pirie, 1959). The fact that an experimenter who has done an abiogenic synthesis can pick out of such complex mixtures so many biochemicals is a valuable starting point for many theories, but it is not in itself sufficient to verify the biochemical similarity principle.

It is important that experiments on abiogenic synthesis continue until it is demonstrated which general sets of conceivable primitive earth conditions produce those chemicals which are considered essential for the different theories of evolving systems; for it may well be that the richness of organic material produced by simulating conditions on the surface of the primeval earth is sufficiently great that within certain limits most classes of biochemical and organic molecules may be found. The only obvious limit is set by the immense number of isomers in heteropolymers which could not all exist simply for lack of space. Furthermore, judging from results of abiogenic experiments using different energy sources and starting materials, the conditions required to produce any class of molecules may not be unique. It is therefore not likely, nor should it be necessary, that a final decision will be made on the basis of abiogenic synthesis experiments alone as to which particular set of primitive earth conditions existed, or which particular molecules were essential for the evolution of present life. The great value of these remarkable experiments is that they have given us a large amount of freedom in formulating reasonable theories for the first evolving molecular systems.

III. Studies of Organized Structure

The complexity and diversity of biological structure is a striking characteristic which has historically often obscured the more fundamental biochemical uniformity at the molecular level. Even if we assume a *structural similarity principle* to guide our observations, the problem of evaluating the significance of "lifelike" structures which arise from abiogenic environments is more difficult than comparing *biochemical similarity,* not only because our knowledge of the formation and development of living structures is very small compared to our chemical knowledge, but also because no quantitative measure of "likeness" has been defined as it has in chemistry. A biologist observing the remarkable structures which are so easily produced with thiocyanates (Herrera, 1942), phospholipids (Nageotte, 1936), or polyamino acids (Fox, Harada and Kendrick, 1959) often finds it difficult to maintain an unbiased attitude. There is often a feeling that the structural complexity and behavioral similarity to living systems is so much greater than we should expect from such simple mixtures that some coincidence or even self-deception is involved, and as a consequence the whole observation is discounted as irrelevant to living systems. At best they are labeled as superficial likenesses, since no metabolism or reproduction is observed. But perhaps this is too strong a reaction. While it is likely that these organized structures contain no genetic information, and may not be precursors of life, on the other hand we actually have far too little detailed molecular knowledge of how genetic information determines the structure in living systems to judge the significance of morphological similarities. It is a lot to expect that we can understand how the conformation and function of an enzyme or a structural protein is affected by a linear sequence of bases in nucleic acids, when we do not even know why the simplest linear carbon chains fold into precise crystals, or why simple metal hydrides can effect the precise sequential positioning of subunits in tactic polymers. Just as there are many biochemical events which can be understood only by studying fundamental nonbiological chemical reactions, so there are probably many biological structures which will be understood only by learning more about molecular structure at a nonbiological level. To be of long-range significance, however, experiments on macromolecular morphology should be done at an elementary level; that is, the systems studied should yield explanations in physical and chemical terms,* not in biological terms. Morphological analogs of living structures certainly should not be discounted as superficial simply because they are not likely to come alive.

* (e.g., Bangham and Horne, 1964)

On the basis of abiogenic synthesis experiments already performed, it is reasonable to go a step further and consider the types of organization and structure which these high molecular weight materials produce. Fox has studied in detail the structure and behavior of microspherical particles formed in aqueous solutions of thermally polymerized amino acids (Fox et al., 1959; Fox and Yuyama, 1963a, b; 1964). These particles are several microns in diameter, often show simple internal structure, and occasionally divide in two. Demonstrations of this type are valuable guides to our concepts of the possible order which we can expect from primitive macromolecules, but their explanation will certainly require a more fundamental understanding of the structure and behavior of simple polymers than we now possess.

Considering the enormously rich possibilities for natural influence of reactions on the primitive earth such as mineral catalysis, photoreactions, natural isolation and mixing processes, concentration and temperature changes, there is no reason to exclude reasonably simple chemical mixtures only because they have not yet been demonstrated to occur in greatly oversimplified, primitive-earth simulation experiments. Following this approach and recognizing the conditional nature of such experiments, Oparin (1964) has introduced proteins, nucleic acids, and other polymers isolated from living matter into structural coacervate systems with the result that certain limited types of protometabolic reactions are observed along with increased structural complexity.

The introduction of extracted biological macromolecules into origin of life experiments must always be considered critically since the introduction of large quantities of evolutionary information is possible. If this happens, the behavior of the system may in some respects become quite lifelike, but it could not answer the basic question of the ultimate source of these evolutionary inventions. On the other hand, the use of non-enzymically synthesized polypeptides and polynucleotides in studying primitive structure and behavior is of great interest and is a logical extension of experiments, if the *biochemical similarity principle* is assumed as a working hypothesis. The only mandatory ground rule in testing this hypothesis is that no molecules be introduced in the experiments which represent large amounts of genetic information accumulated by natural selection. In effect these experiments increase the biochemical complexity of a simulated "primeval broth" and may demonstrate characteristics of structure or function which are in some sense "lifelike" beyond the mere sum of biochemicals which are present. How the likeness is to be defined and measured remains a serious conceptual problem without a clear distinction between what type of structures have or have not the potential for biological evolution. Again, a fundamental understanding of such complexity is beyond our reach without more basic studies of macromolecular aggregations.

IV. The Search for Extraterrestrial Information

A. METEORITES AND PLANETARY LANDINGS

The biochemical and structural similarity hypotheses have also been assumed in the interpretation of organized carbonaceous material in metorites (Claus and Nagy, 1961; Nagy, Meinschien, and Hennessy, 1961). An enormous amount of technical discussion concerning the source and significance of these organized elements has taken place (e.g., see Urey, 1962 and following articles). The basic problem of evaluating these organized structures is deciding the essential differences expected in chemistry and structure between (*1*) extraterrestrial fossils of primitive organisms, (*2*) extraterrestrial prebiota, (*3*) extraterrestrial abiota, (*4*) terrestrial contamination, and (*5*) artifacts of specimen preparation. Whatever differences are assumed, there is the added problem of experimentally resolving these assumed differences on a very small amount of intractable material. Some of the most complex structures in meteorites have been traced to terrestrial contamination of pollen (Fitch and Anders, 1963), but *in situ* microchemical analysis of other organized regions has been interpreted by Nagy et al. (1963) as evidence that some of these organized structures have not originated on earth. In any case, even if some of these structures are of extraterrestrial origin, their significance for the origin of life is at present unknown (e.g., see Morrison, 1962; Fox and Yuyama, 1963b).

With the availability of large rockets and the complex technology of artificial satellite control and communication, there is now the possibility of landing instruments on the moon and nearby planets. Here again, the strategy of exploration is based on the biochemical similarity principle (e.g., Lederberg, 1960a), although gross structural similarity as viewed by televised images from high power tele-

scopes landed on the surface would also be likely to reveal unmistakable characteristics of life if it exists. Other evidence gathered from the many observations of planets have often suggested the possibility of vegetation on Mars (e.g., Sinton, 1959), but as yet this information is so incomplete and conditional that further review is unproductive at this time (see Sagan, 1961).

B. DISCUSSION OF EXTRATERRESTRIAL EXPERIMENTS

In order to better imagine what the next stage of origin of life experiments should seek to discover, it may be useful to consider the possible outcomes of experiments involving planetary landings. One possibility is that earth-like forms of life exist on Mars. By "earth-like" we mean that there are interactions with the exploring instruments which we have predicted on the basis of known biochemical behavior on earth. To find life like our own on a distant planet would undoubtedly be considered the most notable discovery of the century, but, paradoxically, the more biochemical similarity any such extraterrestrial life might possess, the less we may actually learn from it concerning the primeval source of this complexity. For example, if genetic nucleic acids and protein enzymes are identifiable on Mars we might expect life there as we know it; but what else would we be likely to learn? Knowing that life on Mars is not essentially different from life on earth adds very little information to what we have already learned or what we may expect to learn of its origin from studying life and evolution on the earth. We may at least return to our terrestrial experiments knowing that we are missing no great evolutionary innovations.

More generally we must ask what are the minimum essential, remotely observable characteristics which would be acceptable to us as evidence of life? Since we have abiogenically produced protein-like and nucleic acid-like molecules on earth as well as organized structures which resemble living cells in many ways, we could not interpret similar finding on Mars as sufficient evidence that life exists there. As the biochemical list in Table I is gradually extended, abiogenically, there will be a corresponding reduction in the significance of finding such biochemicals on other planets.

Another possibility is that a sufficiently complex type of living system exists so that its outward appearance gives it away, such as tree-like growth or a body with a well-developed form of locomotion. The biochemical nature of such complex structures would be of profound interest, but not essential for the recognition of a live form of matter. However, the basic question of the *evolution* of such highly complex forms, by which we must ultimately define or recognize the living state of matter, would in all likelihood remain for a long time even more obscure than the source of life on earth where we have available an enormous amount of evolutionary information compared to what we may expect to obtain from any other planet. Therefore, there is as yet no very convincing reason why we should expect that a rudimentary knowledge of the existence and nature of life, in one form or another, on other planets should necessarily lead us directly to a nonbiological theory of the *origin* and evolution of this highly organized state of matter from chaotic primeval molecules. There is, of course, a remote possibility that an intelligent form of life on another planet has already solved the problem of its own origin and could in turn explain it to us.

V. The Approach from Molecular Biology

There is always the hope that as we learn to describe in more and more detail the functioning and evolution of living cells there will concurrently emerge some understanding of the origin of evolution of nonliving molecular complexity. In fact, many theories of the earliest form of life are in some sense attempts to simplify and abstract from our knowledge of living systems some characteristic features which, for one reason or another, appear to be the most essential or productive starting point for biological evolution. It is understandable that earlier theories, such as those of Oparin, reflected the biochemical interests of the time in metabolic pathways; later on, the origin of catalytic reactions was often emphasized, whereas today most theories reflect the nucleic acid protein interaction which is now the center of so many productive experiments.

However, instead of providing greater understanding of the source of living complexity, what has actually happened is that our recent detailed understanding of the intricacies of DNA replication and control of protein synthesis through the elaborate mediation of many specific RNA's and enzymes has created a greater mystery; for it

is now even less imaginable how all the necessary conditions for a biochemically similar ancestral threshold system could originate from the primeval broth.

A. CURRENT MOLECULAR BIOLOGICAL THEORIES

Many of the latest discussions of the origin of life have assumed or defined the minimum requirements for life in modern genetic terms involving mutable self-replication of nucleic acid-like macromolecules, and the *assumed* property of such systems of evolving only by the biological process of natural selection. This implies that the central dogma was still valid at this primitive level, i.e., that hereditary information is determined solely by its parental sequence and not by external acquisition.

These assumptions necessarily lead to what we may call *threshold* theories of the origin of life, since biological evolution by natural selection is defined only by assuming *at least* the preexistence of mutable self-replicating units. The origin of the first mutable, self-replicating unit can *not* therefore be explained by the evolutionary process of natural selection from simpler aggregations of molecules which are below this threshold. The term *chemical evolution* has come to mean those chemical processes which lead to increased complexity without assuming natural selection, but as yet there is no theory of chemical evolution, and consequently the term explains nothing. The lowest level of complexity of the threshold which would support persistent evolution by natural selection is usually set at the nucleic acid level (e.g., Muller, 1961). Although Lederberg (1960b) has suggested that nucleic acids are more subtle and specific than we might reasonably expect from chemical evolution alone, or than we might select for our first attempts to synthesize an artificial replicating macromolecule. Lederberg pictures the minimal threshold as follows:

> It must have a rigid periodic structure in which two or more alternative units can be readily substituted. It must allow for the reversible sorption of specific monomers to the units in its own sequence. Adjacent, sorbed monomers must then condense to form the replica polymer, which must be able to desorb from the template.

Crick (1961) and Rich (1962) discuss the necessity for a more elaborate threshold involving both nucleic acid replication and specific coupling with amino acid polymers. This symbiosis of the two types of polymer is, of course, the central theme of present-day molecular biology and it is difficult to imagine a more elementary threshold on which natural selection can operate. A similar point of view is expressed by Horowitz and Miller (1962), Schramm (1962), and Haldane (1964). Rich has emphasized in some detail the rather stringent requirements for the interactions of amino acids and polynucleotides which would be essential for a threshold with evolutionary potential.* In chemically less specific terms Calvin and Calvin (1964) have described the minimum threshold of life as

> (*1*) the ability of such a molecular aggregate to transfer and transform energy in a directed way; and (*2*) its ability to remember how to do this, once having 'learned' it, and to transfer, or communicate, that information to another system like itself which it can construct.

* As Commoner (1962) has pointed out, if the potential for biological evolution is the definition of the threshold of life, then nothing less than a whole live cell has been demonstrated to be alive.

B. DISCUSSION OF BIOLOGICAL APPROACHES TO THE ORIGIN OF LIFE

These descriptions of possible ancestral thresholds for organisms evolving by natural selection are certainly great simplifications of the known organization of living cells, but they are still logically extremely sophisticated and leave an enormous gap beyond what has been demonstrated by chemical evolution. It is sometimes assumed that this gap can be bridged by chance. Thus, Wald (1954) has used the argument that the level of such a threshold makes its attainment highly improbable within the time available in labratory experiments, but nearly inevitable by cosmic time scales. Whether or not such arguments are satisfying depends largely on the epistemological or esthetic standards one demands for scientific explanation. The logical complexity of any biological threshold would make a Maxwell demon appear as an elementary particle by comparison, but such contructs are not acceptable in scientific theories, except insofar as we assume they do not exist. In any case it is difficult to imagine how any theory which depends essentially on auspicious accidents could be experimentally tested (cf. Bridgman, 1954).

Haldane (1961) has pointed out two other difficulties with biological threshold theories. One difficulty involves the problem of the behavior of any "half-life" or prethreshold systems which would presumably contain catalysts arising by chemical evolution which are needed eventually for the threshold nucleic acid–protein system when it "goes critical." Such unorganized catalysts, like those in a dead cell, would be likely to release the energy of any metastable molecules—say, pyrophosphates or sugars—and thereby generally tend to spoil rather than enhance the gains of chemical evolution. The second difficulty is in reconciling the likely minimum biochemical threshold requirements of a specific polypeptide catalyst coupled to a replicating nucleic acid with the probability of attaining these requirements by a stochastic chemical evolution process. Thus a relatively short peptide of only 20 or 25 ordered amino acid residues found by random search might alone be expected to involve some 10^{31} trials, which is probably expecting too much for the space and time available on the earth.

A more general difficulty with any threshold theory is its implicit violation of the Principle of Continuity (e.g., see Weyl, 1949) which in the broadest sense is what any theory of origins should try to preserve. In seeking origins, it is certainly a reasonable strategy to extrapolate backwards from what is now known. In the case of living organisms, following the course of evolution into the past involves many guesses, but whatever hypothesis we choose to consider in our attempt to approach the conditions of the primeval abiogenic reservoir, we must preserve enough organization and function to assure the continuity of the evolutionary pathway that we have retraced. The restriction in this procedure is not the lack of facts about the course of evolution, but the assumptions of the theory of evolution itself. This is a logical restriction, and has nothing to do with the truth of any particular theory. For example, if we define an evolutionary theory which depends on the properties of mutation, replication, and natural selection, then we may not extrapolate backward beyond these properties using this theory. In effect, these properties determine a *conceptual threshold* beyond which we must either invent a new theory of evolution or else relinquish continuity and rely on accidents to organize matter up to this threshold of complexity. It is therefore reasonable to assume that knowledge of biological behavior, including the biological theory of evolution, will be necessary but not sufficient to formulate a theory of its own origin.

VI. Abiological Approaches

A. FUTURE OF ABIOGENIC SYNTHESES

There can be no doubt that abiogenic synthesis experiments have significantly narrowed the gap between inorganic and living matter, and have shown that many essential biochemicals have very primitive origins. Similarly, the demonstrations that some of these molecules when mixed under nonspecific conditions aggregate into formed structures of the size and shape of the simplest living cells certainly suggest that some of the structure of living matter may also be of very primitive origin. The productivity of this approach should stimulate many more experiments which in time will no doubt narrow the gap even further.

We may reasonably ask if we expect this approach to eventually close the gap; that is, do we expect that with the mixing together of more and more complex biochemicals we will continue to learn more about the origin of life? Do we expect to eventually produce a self-replicating macromolecule or aggregate of molecules which will evolve by natural selection?

One criticism of this approach is that while in principle this is possible, or actually happened on the earth, in practice the time and volume of matter necessary to reach the threshold of self-replication effective for biological evolution far exceeds any possibility for an experimental test. However, if the premise is correct then it is reasonable to accelerate the approach toward the replicating threshold by the careful guidance of the experimenter. This has already been done in abiogenic synthesis experiments in which the synthesis of a trace of one chemical in one experiment is followed by its introduction in concentration in subsequent experiments. This is followed by its introduction in concentration in subsequent experiments. This is justified by two arguments: (*1*) that the earth has an immense advantage in size and age over the laboratory, and (*2*) that natural catalysis or concentration is likely to occur in the much greater chemical and structural heterogeneity of the earth than in the oversimplified laboratory conditions. Very likely the size and age of the earth is an advantage only for a theory of prebiological evolution in which random reactions play an essential role. In other words, if, say, over 10^{50} small amino acid copolymers must be randomly synthesized in order to give a reasonable probability of producing

the first polymerase-type catalyst, then the whole surface of the earth working for many millions of years would indeed be necessary to assure success. On the other hand, if we use the hypothesis that some form of prebiological evolution continuously produces increasing molecular order without essential dependence on accidents, then the value of enormous reaction volumes is difficult to imagine. Any mile of seashore (or any volcano) would be about as good as any other, since primitive molecular evolution which we presume went on in such regions would not be significantly influenced by neighboring areas. The value of long time intervals is even more questionable since even for accident-dependent theories the crucial time interval will be the half-life of the fortuitously polymerized catalysts once they appear rather than the time necessary to find them by trial and error. Continuous evolutionary theories, on the other hand, must by their nature depend on a temporal sequence, each element of which has some causal significance in the evolutionary process. In other words, if we are not assuming that accidents are essential for reaching the biological threshold, the size and age of the earth do not present an insurmountable experimental problem for abiogenic synthesis. In future abiogenic synthesis experiments it will be most interesting to more closely simulate the known complexity of the earth such as occurs at the seashore or in volcanic pools, where catalysis, periodic mixing, and natural concentration may all occur.

The question as to whether or not a stage of self-replication can be reached within a reasonable time span is not particularly relevant at this time for evaluating abiogenic synthesis experiments. As a practical matter the problem is really quite the opposite: *abiogenic syntheses have demonstrated more rapid molecular evolution than we can presently explain.* As we have pointed out, experiments have produced almost all essential types of biochemicals plus an even greater amount of unanalyzed organic material, as well as many structures which are beyond our ability to explain in any fundamental way. Therefore, while it is not at all unlikely that abiogenic syntheses will eventually produce a kind of replicating unit, if they have not done so already, this demonstration will be of little significance if we have no theory to explain it. Perhaps the most valuable abiogenic experiments of the future will be those which are designed to verify or disprove specific *nonbiological theories of molecular evolution* rather than to demonstrate the increasing complexity of molecular aggregations supplied with steady energy sources.

B. CONTRIBUTIONS OF POLYMER CHEMISTRY

At present the gap between abiogenesis experiments and molecular biology experiments remains enormous. Some intermediate stage of complexity which could be studied experimentally would be of great value in linking the two approaches as well as in guiding the formulation of theories of molecular evolution. The most obvious possible link is the area of polymer chemistry. Historically, of course, polymer chemistry grew out of studies on biological polymers, and since the commercial availability of synthetic polymers, one of the principal goals of the polymer chemist has been to accomplish an "abiogenic synthesis" which would produce material similar in its properties to macromolecules produced by living systems (e.g., see Flory, 1953; Mark, 1964). Today, however, the gap between polymer chemists and molecular biologists has in many ways grown larger instead of smaller. Much fundamental work is being done on synthetic polypeptides and polynucleotides from the point of view of the molecular biologist both experimental (e.g., see Stahmann, 1962; Khorana, 1960) and theoretical (e.g., see Weissbluth, 1964), and an enormous effort from the chemists' point of view is stimulated by hope of improving the properties of commercial polymers (e.g., see Gaylord and Mark, 1959; Geil, 1963; Ke, 1964). There has been surprisingly little interaction between these groups even though many of the fundamental problems of polymer sequence and conformation are common to both.

Two discoveries have been recognized in the last decade about the behavior of simple linear polymers which are of great potential significance for any theory of molecular evolution. The first discovery is that single linear polymer molecules will spontaneously fold into precise conformations and aggregate with similar molecules to form three-dimensional crystals. The first evidence of this behavior is relatively old (Schweitzer, 1919; Staudinger and Signer, 1929; Sauter, 1932; Storeks, 1938) but only more recently has work on single polymer crystals, beginning with Keller (1957), Fisher (1957), and Till (1957), produced a clear appreciation of the precision in the conformation of individual molecules in the crystal.

The second discovery is that the linear sequential control of the orientation of nonsymmetric monomers in a growing polymer may be precisely accomplished by simple heterogeneous catalysts. Although

the concept of stereoregularity in polymers was clearly stated by Huggins (1944) many years ago, it was the discovery by Ziegler (1952) of catalysts exerting highly specific and detailed steric control of polymer propagation, and the studies by Natta and his coworkers (Natta et al., 1955) on the structures of these polymers that stimulated this new branch of polymer chemistry. Neither this folding of single polymer molecules into crystals nor the catalytic control of sequential orientation in linear polymers is understood, but the observations make it quite clear that both processes are not only possible, but also occur quite generally with a wide range of polymeric material.

Merely to state that polymers will form crystals does not give an adequate picture of polymer morphology, nor is the historical idea of a crystal entirely satisfactory for describing the behavior and structure of such polymers. The essential fact, based on the observation of many types of simple polymers, is that chain folding tends to occur in a *precise* form, probably to within atomic dimension, from either dilute solution or from the melt. The regularity of this chain folding, under fixed conditions, is sufficiently high to produce thin platelet crystals with a hollow pyramid shape. Typically, the thickness of a single platelet is about 100 A. and the platelet may be 1–10 microns across. The polymer chain is faked in a zigzag nearly perpendicular to the large faces of the platelet. Of course, the crystallization conditions will determine the morphology of the crystal habit. The range of structures from single crystals to highly twinned crystals, dendritic growths, and spherulitic textures, depend on primary nucleation rates, growth rate, and rate of generation of deformations, although these relationships are not generally understood (e.g., see Lindenmeyer, 1963). However, the significant point this demonstrates for any theory of molecular evolution is that even at the simplest level of organization in single macromolecules there is the possiblity of precise, three-dimensional positioning of all the monomeric units without breaking the linear chain holding the monomers together. Although this may appear as an obvious or trivial property of chain molecules, nevertheless without this property it would be difficult to imagine molecular storage or transcription of order of any complexity.

Tactic polymerization, in which the positions of each monomer are determined with respect to the linear sequence, is, in effect, one-dimensional crystallization. The order which has been studied so far is extremely simple and may usually be described by Bernoulli statistics, as in isotactic order, or first-order Markov statistics, as in syndiotactic or alternating copolymer order (e.g., see Krigbaum, 1964). But here again, as with the simple polymer crystals, there is clear indication of the property of precise sequential ordering at a very simple macromolecular level.

Most of the work on tactic polymers and polymer crystals has been done from the point of view of the synthetic polymer chemist, and the attitudes and results may appear somewhat far removed from the biological approaches to macromolecular behavior as well as to origin of life studies. This is certainly the case with respect to the type of material which is studied, the descriptive terminology, and the goals of the research. However, with respect to the *elementary* operations involved in the control and propagation of sequential and conformational order in chain macromolecules there is no evidence to suggest that different physical or chemial laws are involved in a plastics factory than in a cell. The significant difference is the order of complexity which one chooses to emphasize in each case.

The description of any experiment must depend to a great degree on what type of order is resolvable by the experimental technique employed. Thus the characterization of a linear heteropolymer as "random" is seldom no more than a statement that the sequence order is unresolved and presumed to obey certain statistics. However, statistical models are useful in formulating theories of propagation, they do not as yet solve the problem of what type of order exists in polymers. Experimentally, all but one of the present tests for tacticity, including x-ray diffraction, melting point determination, mechanical and thermodynamic properties, dipole moments, infrared and nuclear magnetic resonance spectroscopy, etc., depend for their sensitivity to linear order only indirectly through the three-dimensional crystalline structure or conformation, which is conditionally dependent on the linear sequence.* This is also the case for tests of enzyme activity. Furthermore, the absence of detectable crystallinity does not preclude catactivity, as is obvious from our knowledge of proteins. The only test which reveals linear structure directly

even though

(e.g., Krigbaum, 1964)

is chemical sequence determination, which depends for its resolution on the sequential homogeneity of the polymer and highly specific degradation procedures. This has only been accomplished with proteins. The intimate dependence of three-dimensional structure on linear sequence is therefore experimentally difficult to resolve even at the most elementary level, but there can be little doubt that at all levels of organization of polymers the conformation will depend conditionally on the precise linear order of subunits in the chain.

This leads to the question of what determines the linear sequential order of polymers. From the theoretical point of view we may begin with a simple homopolymerization in which order has no significance and the growth kinetics is represented by a propagation rate. Ideally, this rate is constant for a given set of external thermodynamic variables, monomer concentration, catalyst concentration, etc.; but the growing polymer itself represents a changing condition of the system, and its conformation and interactions with catalyst, monomers, and other polymer chains should influence the rate of monomer addition. Such a rate change is observed in the polymerization of amino acids where it has been interpreted as the consequence of a conformation change in the growing chain from an open structure to an α-helix (Lundberg and Doty, 1957; Idelson and Blout, 1957). In stereotactic or copolymers, the possibilities quickly become more complex. Assuming two distinguishable orientations or types of monomer, and their reaction rates dependent on only the last monomer type in the chain, there are four reaction rates to consider, each of which may change with the growing chain conformation. Furthermore, it is quite reasonable to consider monomer interactions with several end units (De Santis et al., 1962; Natta et al., 1962), or to postulate that the polymer itself has more than one state with different sets of probabilities of addition for each state (Coleman and Fox, 1962). Pattee (1964) has suggested that a higher degree of order could reasonably be expected if monomer addition rates were influenced by two or more polymer subunits widely separated from each other in the linear chain, but brought together by the precise three-dimensional folding, as for example in a helix or by zigzag folding. Such interactions would be favored by heterogeneous systems or in poor solvents (Ham, 1959), but experimentally such order would be difficult to resolve since the sequential complexity could easily match that of proteins, even assuming very simple rules of sequence propagation (Pattee, 1961). However, the work of Fox and Harada (1960) shows clearly that thermally polymerized amino acids are not random with respect to terminal residues, and the fact that they exhibit catalytic activity which is thermally inactivable in aqueous solution suggests some degree of preferred conformation (Fox et al., 1962). This might suggest some form of sequential order, but as yet no sequence analysis has been done.

These experiments on chain macromolecules are difficult to interpret, and there is no fundamental understanding of either chain crystallization or tactic polymerization. But certainly the elementary processes or precise conditional control of conformation and sequence in the simplest heteropolymers are basically similar to the conformation and sequence control of the more intricate type which has evolved in nucleic acid and protein interactions. In the last section we shall discuss some possible theories of how this intricate control could have evolved.

VII. Theories of Macromolecular Evolution

The experimental evidence now clearly substantiates the basic hypothesis of Oparin and Haldane that the primitive earth provided the essential starting materials for living systems. Consequently we may expect that the origin of life problem will shift away from the evolution of the building blocks and the elementary operations of joining them together, to the more difficult problem of the *evolution of control* in complex organizations. This problem is more difficult because the idea of "control" is not defined in the same sense as we can define biochemicals. Nevertheless, as biologists have so often emphasized, the essential characteristics of life cannot be separated from the total *process* of cellular activity, and as Weiss (1962) has put it, the elements of a complex process are not elementary particles or biochemicals, but elementary *processes*. A live cell and a dead collection of the identical biochemicals in the same structural organization differ essentially in the amount of intermolecular control that exists in each unit. From this point of view, the question of the orgin of life becomes the problem of understanding elementary molecular control processes, and of formulating a theory of the evolution of molecular control.

What no one can say at this stage is how much effort should be aimed at gathering data in the hope that it will stimulate a good theory,

and how much effort should be directed at formulating a theory which will generate some good experiments. Historically, a mixed strategy has often proven the most productive, and since the main body of this review discusses experimental approaches to the problem, a few ideas on experimentally testable theories of evolution may be of some value.

A. ORDER FROM DISORDER

Perhaps the simplest physical example of ordering is the process of crystallization. Ideally we may picture a large collection of subunits confined within some kind of box. Initially these subunits move with a distribution of energy high enough to keep them in disarray. If the energy is now reduced by removing it from the box, the subunits will begin to appear ordered, by which we mean that we recognize some simple rule which relates one subunit to another. This type of order is explained physically by a combination of more general laws which include the inherent structure and forces of the subunits on each other, the minimum energy principle, statistical thermodynamics, or the general properties of three-dimensional Euclidean space, depending on the point of view. But since every subunit in the box must also be "ordered" by these same general physical principles, we may find it more instructive to consider carefully why we choose to "recognize" the particular order which we call a crystal. This is the point of view which Burgers (1963) has adopted in his discussion of the emergence of patterns of order in simple examples from classical physics, and which Ashby (1962) presents in analyzing the "self-organizing" system in general. It is difficult to escape the conclusion that the concept of "order" is not so much a property of the system as of the frame of mind of the observer. Furthermore, the frame of mind of an observer, especially one trained as a scientist, is often sensitive to some form of simplicity. We cannot pursue the idea of simplicity in an experimental sense, although its relationship to scientific generalization and physical laws is certainly fundamental (e.g., see Frank, 1957). However, there is discernible here some kind of paradox when we see that in our search for a theory of the evolution of complexity we tend to focus our attention on those aspects of a physical situation which are the *simplest*. There is some reason, then, to consider the possibility that complexity as we find it in living systems is the end result of a kind of divergent phenomenon which, although obeying physical laws, leaves us conceptually impotent with respect to the formulation of general theories. This pessimistic idea, once stated by Condon (1959), is a slightly better working hypothesis than any "accident" theory, since there is at least the possibility of significant experimental observations and formulation of partial theories up to the point where our conceptual mechanisms of abstraction fail. Elsasser (1963) has argued that the inhomogeneity or "individuality" of biological organisms requires a separate type of law which is not logically reducible to physical laws; but this approach is even farther from experimental test at this stage.

B. ORDER IN AUTOMATA

One of the most promising experimental approaches to the evolution of complexity is by the construction of devices of complexity in which each element and operation is thoroughly understood beforehand. These automata may be designed or programmed to simulate the behavior of other complex systems which are not understood in detail, and with which they may be compared by some objective test (Turing, 1956). Such deterministic machines have the advantage that any abstract subjective "order" which an observer may find interesting can in principle be reduced to a set of objective "states" of the machine. Furthermore, the behavior of such complex automata does not depend in any essential way on the physical building blocks from which they are assembled. The logical "control" aspects of behavior are then more clearly separated from any particular physical representation, which in itself may be entirely too complex to efficiently describe in physical terms.

Von Neumann (1956) has formulated the control aspects of *self-reproduction* in terms of automata theory, based on the idea of the Turing machine (Turing, 1936), which may be thought of as the simplest type of automaton which is not limited by its elementary logical operations, although it may compute very slowly in comparison to more complicated automata. The conclusions of this analysis are discouraging from the point of view of a primitive theory of evolution; for although von Neumann designs a self-reproducing machine, his careful description also shows how logically complex the threshold of any complete self-replicating organization must be. Furthermore, he suggests that "complication" in some sense is degenerative below a certain level, and thereby places the concept of evolution up to such a level outside the scope of his theory. On the other hand, there is ample experimental evidence, such as shown in Table I, that simple systems do form more complicated organization in a fairly persistent way, but we are inclined to expect that this degree of order can be

explained by chemical statistics and the properties of the starting molecules, and that no additional theory of evolution is necessary up to this stage. The gap in theories of evolution is therefore between the levels of molecular complexity on the outskirts of statistical distributions representing chemical reaction probabilities and the threshold of persistent self-replicating units with the potential for evolution by natural selection.

C. ORDER ACQUIRED BY LEARNING

An obvious case of the evolution of complexity which occurs without self-replication is the process of learning. This may at first be considered a poor example on at least two counts: the first is the association of learning with highly evolved biological systems, and the second is that we do not understand the process of learning any better than evolution itself. In fact, several authors have used the analogy of biological evolution by trial and selection to explain the process of learning (e.g., Pringle, 1951; Bremermann, 1958; Campbell, 1960). However, an analogy may be used both ways. Furthermore, the recent contributions to the theory of learning in automata have reduced many aspects of learning to practical levels of simulation on general purpose computers (e.g., see Fiegenbaum and Feldman, 1963).

We have the same advantage in using the concept of learning at the macromolecular level as we do in using the concept of biological evolution, that is, we may attempt to reduce these ideas to their most primitive operations. We have reviewed the various hypothetical thresholds of molecular complexity which might support biological evolution in Section V-A, and pointed out the large gap that still exsists between these thresholds and what we have observed in abiological experiments, What is the corresponding primitive threshold for a learning process?

Both learning and biological evolution certainly require the acquisition and storage of order or information, which is another way of saying that learning and evolution produce increasing complexity of control. The simplest information-storage structure which we can imagine is a linear chain with ordered, distinguishable subunits; and, as we have seen, this condition is fulfilled in the simplest abiogenic heteropolymers. The essential difference between the processes of learning and biological evolution at this elementary level is in the method of acquisition of order in such a linear chain. First of all, biological evolution requires chance alteration in sequence, whereas learning more often implies a predictable or causal change in order. Second, biological evolution requires self-replication of linear sequence without regard to the order being replicated, whereas learning only implies storage or propagation of order. Finally, biological evolution can be said to increase its order only by the natural selection process which does not involve any direct interaction of the environment with the replicated sequential order; whereas in the learning process, especially in the sense of "training," there is selection by direct feedback interaction of the environment with the newly acquired order. Therefore, in spite of our lack of detailed understanding of either learning or the behavior of simple polymers, we may more reasonably compare the elementary sequence and conformation control processes which are observed in polymers to the elements of learning rather than to a process of biological evolution. The question now becomes: can we formulate a theory which explains why a collection of growing linear sequences in the form of interacting heteropolymers should learn to control its molecular processes so that self-replication and natural selection becomes an effective method of evolution?

At this point we are at the fringe of our knowledge of both macromolecular behavior and theories of self-organizing systems. To proceed further will require first an experimental determination of the basic functional operations which are accomplished by tactic polymer sequence and conformation control. From what is already known of conditionality and specificity of polymer interactions, it is not unlikely that growing collections of heteropolymers behave like molecular automata (Pattee, 1961; Stahl and Goheen, 1963) but until these interactions are better understood no detailed theory is likely to be formulated.

On the other hand, general theories of learning in automata may be the most productive approach in deciding what type of experimental information about polymer interaction is most significant for molecular evolution. No general theory of self-organization exists at the present time, although many aspects of the problem have been analyzed (e.g., see Shannon and McCarthy, 1956; Yovits and Cameron, 1960; von Foerster and Zopf, 1962; Yovits et al., 1962). Ashby (1962) has postulated that reproduction in some sense is a dynamical state which will be reached in time by any "sufficiently large system" if it does not first reach equilibrium. We might also postulate that replication is favored by "the principle of the most unstable solution"

by which Burgers (1963) accounts for the choice of particular patterns of order in certain hydrodynamic convection problems. There is also the analogy with learning in higher organisms with well-developed nervous systems, in which the most primitive learning is by rote, direct training, or simple, immediate interaction with the environment. Gradually, as the brain develops more conditional relationships, the learning process becomes increasingly similar to random search and selection (e.g., Hadamard, 1945; Campbell, 1960). This same strategy of learning has also proven effective for the programming of computers to simulate learning (e.g., Newell, 1962; Minsky, 1963). We might therefore consider this strategy as a valuable source of order at the molecular level (Pattee, 1964), beginning with learning order by direct feedback with the environment, and proceeding *continuously* through stages of increasing delay and hereditary isolation from the environment to the ultimate random search and natural selection process which is now observed in highly evolved organisms. At the present time any tests of such a theory must be simulated by computers, since no techniques of polymer characterization are likely to resolve such sophisticated behavior. However, experimental studies of the basic elements of control of polymer sequence and conformation are essential before any such computer-simulated process could be designed to represent actual macromolecular interactions.

VIII. Conclusion

The Oparin-Haldane theory of the origin of the organic molecules necessary for living organism by abiogenic synthesis on the primeval earth has been largely confirmed by many experiments. We now face the problem of the evolution of the interaction and control of macromolecular sequence and conformation. Only recently have experiments shown that precise sequence control and conformation control is a general possibility in a wide variety of simple polymers, but much more experimental work must be done before these properties can be understood. Similarly, studies of the theory and behavior of automata suggest that many types of self-organization are possible with extremely simple, fundamental operations, not unreasonably different in a logical sense from the basic operations in collections of growing macromolecules. As we learn more of macromolecular control and theories of organization, we may not only expect a more fundamental understanding of biological behavior, but we may also fill in the gap between our present conceptions of chemical evolution and the process of biological evolution.

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A NOTE ON CHEMICAL AND HEREDITARY EVOLUTION. H. H. Pattee, Biophysics Laboratory, Stanford University, Stanford, California.

When mixtures of simple molecules supplied with an energy source react to form increasingly complex structures and interactions, there is generally no corresponding increase in hereditary information or homeostatic control. Growth of chemical complexity in itself does not account for accumulation and propagation of hereditary control. Since hereditary control in living systems is stored and transmitted by linear sequences of copolymers, a theory of the origin of living systems should consider (a) the most elementary set of operations in linear copolymer systems which delay and propagate hereditary order, (b) the types of feedback interactions which may selectively accumulate hereditary order, and (c) measures of fitness at this primitive level, which will determine the sequence of early evolutionary steps. A set of simple tactic copolymerization and polymer crystallization processes is proposed as one reasonable chemical system capable of hereditary propagation. Direct and delayed feedback interactions within and between copolymers and with the environment are considered as selective forces insofar as they affect the hereditary information. Two alternative hypotheses are considered: The first significant hereditary process in the origin of life was (I) template replication of single linear copolymers followed by blind trials and natural selection of coupled homeostatic control systems; or (II) homeostatic control of hereditary copolymer propagation causing increasing isolation of the hereditary sequences from environmental control, gradually leading to self-replication of total systems. Possible approaches for experimentally testing these alternatives are suggested. (Work supported by NASA Grant NsG 218-62).