Die Naturwissenschaften 64. Jahrgang Heft 11 November 1977

The Hypercycle

A Principle of Natural Self-Organization

Part A: Emergence of the Hypercycle

Manfred Eigen
Max-Planck-Institut für biophysikalische Chemie, D-3400 Göttingen

Peter Schuster
Institut für theoretische Chemie und Strahlenchemie der Universität, A-1090 Wien

This paper is the first part of a trilogy, which comprises a detailed study of a special type of functional organization and demonstrates its relevance with respect to the origin and evolution of life.
Self-replicative macromolecules, such as RNA or DNA in a suitable environment exhibit a behavior, which we may call Darwinian and which can be formally represented by the concept of the quasi-species. A quasi-species is defined as a given distribution of macromolecular species with closely interrelated sequences, dominated by one or several (degenerate) master copies. External constraints enforce the selection of the best adapted distribution, commonly referred to as the wild-type. Most important for Darwinian behavior are the criteria for internal stability of the quasi-species. If these criteria are violated, the information stored in the nucleotide sequence of the master copy will disintegrate irreversibly leading to an error catastrophy. As a consequence, selection and evolution of RNA or DNA molecules is limited with respect to the amount of information that can be stored in a single replicative unit. An analysis of experimental data regarding RNA and DNA replication at various levels of organization reveals, that a sufficient amount of information for the build up of a translation machinery can be gained only via integration of several different replicative units (or reproductive cycles) through *functional* linkages. A stable functional integration then will raise the system to a new level of organization and thereby enlarge its information capacity considerably. The hypercycle appears to be such a form of organization.

Preview on Part B: The Abstract Hypercycle

The mathematical analysis of dynamical systems using methods of differential topology, yields the result that there is only one type of mechanisms which fulfills the following requirements:
The information stored in each single replicative unit (or reproductive cycle) must be maintained, i.e., the respective master copies must compete favorably with their error distributions. Despite their competitive behavior these units must establish a cooperation which includes all functionally integrated species. On the other hand, the cycle as a whole must continue to compete strongly with any other single entity or linked ensemble which does not contribute to its integrated function.
These requirements are crucial for a selection of the best adapted functionally linked ensemble and its evolutive optimization. Only hypercyclic organizations are able to fulfil these requirements. Noncyclic linkages among the autonomous reproduction cycles, such as chains or branched, tree-like networks are devoid of such properties.
The mathematical methods used for proving these assertions are fixed-point, Lyapunov- and trajectorial analysis in higher-dimensional phase spaces, spanned by the concentration coordinates of the cooperating partners. The self-organizing properties of hypercycles are elucidated, using analytical as well as numerical techniques.

Preview on Part C: The Realistic Hypercycle

A realistic model of a hypercycle relevant with respect to the origin of the genetic code and the translation machinery is presented. It includes the following features referring to natural systems:
1) The hypercycle has a sufficiently simple structure to admit an origination with finite probability under prebiotic conditions.
2) It permits a continuous emergence from closely interrelated (t-RNA-like) precursors, originally being membres of a stable RNA quasi-species and having been amplified to a level of higher abundance.
3) The organizational structure and the properties of single functional units of this hypercycle are still reflected in the present genetic code in the translation apparatus of the prokaryotic cell, as well as in certain bacterial viruses.

I. The Paradigm of Unity and Diversity in Evolution

Why do millions of species, plants and animals, exist, while there is only one basic molecular machinery of the cell: one universal genetic code and unique chiralities of the macromolecules?
The geneticists of our day would not hesitate to give an immediate answere to the first part of this question. Diversity of species is the outcome of the tremendous branching process of evolution with its myriads of single steps of reproduction and mutation. It in-

volves selection among competitors feeding on common sources, but also allows for isolation, or the escape into niches, or even for mutual tolerance and symbiosis in the presence of sufficiently mild selection constraints. Darwin's principle of natural selection represents a principle of guidance, providing the differential evaluation of a gene population with respect to an optimal adaptation to its environment. In a strict sense it is effective only under appropriate boundary conditions which may or may not be fulfilled in nature. In the work of the great schools of population genetics of Fisher, Haldane, and Wright the principle of natural selection was given an exact formulation demonstrating its capabilities and restrictions. As such, the principle is based on the prerequisites of living organisms, especially on their reproductive mechanisms. These involve a number of factors, which account for both genetic homogeneity and heterogeneity, and which have been established before the detailed molecular mechanisms of inheritance became known (Table 1).

Table 1. Factors of natural selection (according to S. Wright [1])

Factors of genetic homogeneity	Factors of genetic heterogeneity
Gene duplication	Gene mutation
Gene aggregation	Random division of aggregate
Mitosis	Chromosome aberration
Conjugation	Reduction (meiosis)
Linkage	Crossing over
Restriction of population size	Hybridization
Environmental pressure(s)	Individual adaptability
Crossbreeding among subgroups	Subdivision of group
Individual adaptability	Local environment of subgroups

Realizing this heterogeneity of the animate world there is, in fact, a problem to understand its homogeneity at the subcellular level. Many biologists simply sum up all the precellular evolutionary events and refer to it as 'the origin of life'. Indeed, if this had been one gigantic act of creation and if it – as a unique and singular event, beyond all statistical expectations of physics – had happened only once, we could satisfy ourselves with such an explanation. Any further attempt to understand the 'how' would be futile. Chance cannot be reduced to anything but chance.

Our knowledge about the molecular fine structure of even the simplest existing cells, however, does not lend any support to such an explanation. The regularities in the build up of this very complex structure leave no doubt, that the first living cell must itself have been the product of a protracted process of evolution which had to involve many single, but not necessarily singular, steps. In particular, the genetic code looks like the product of such a multiple step evolutionary process [2], which probably started with the unique assignment of only a few of the most abundant primordial amino acids [3]. Although the code does not show an entirely logical structure with respect to all the final assignments, it is anything but random and one cannot escape the impression that there was an optimization principle at work. One may call it a principle of least change, because the structure of the code is such that consequences of single point mutations are reduced to minimum changes at the amino acid level. Redundant codons, i.e., triplets coding for the same amino acids, appear in neighbored positions, while amino acids exhibiting similar kinds of interaction differ usually in only one of the three, preferentially the initial or the terminal position of the codon. Such an optimization, in order to become effective during the evolutionary process, requires by trial and error the testing of many alternatives including quite a number of degenerated assignments. Hence, precellular evolution should be characterized by a similar degree of branching as we find at the species level, provided that it was guided by a similar Darwinian mechanism of natural selection.

However, we do not encounter any alternative of the genetic code, not even in its fine structure. It is quite unsatisfactory to assume that it was always accidentally the optimal assignment which occurred just once and at the right moment, not admitting any of the alternatives which, undoubtedly, would have led to a branching of the code into different fine structures. On the other hand, it is just as unsatisfactory to invoke that the historical route of precellular evolution was uniquely fixed by deterministic physical events.

The results of our studies suggest, that the Darwinian evolution of species was preceded by an analogous stepwise process of molecular evolution leading to a unique cell machinery which uses a universal code. This code became finally established, not because it was the only alternative, but rather due to a peculiar 'once-forever'-selection mechanism, which could start from any random assignment. Once-forever selection is a consequence of hypercyclic organization [4]. A detailed analysis of macromolecular reproduction mechanism suggests that catalytic hypercycles are a minimums requirement for a macromolecular organization that is capable to accumulate, preserve, and process genetic information.

II. What Is a Hypercycle?

Consider a sequence of reactions in which, at each step, the products, with or without the help of addi-

Naturwissenschaften 64, 541–565 (1977)

tional reactants, undergo further transformation. If, in such a sequence, any product formed is identical with a reactant of a preceding step, the system resembles a *reaction cycle* and the cycle as a whole a catalyst. In the simplest case, the catalyst is represented by a single molecule, e.g., an enzyme, which turns a substrate into a product:

$$S \xrightarrow{E} P$$

The mechanism behind this formal scheme requires at least a three-membered cycle (Fig. 1). More involved reaction cycles, both fulfilling fundamental catalytic functions are presented in Figures 2 and 3. The Bethe-Weizsäcker cycle [5] (Fig. 2) contributes essentially to the high rate of energy production in massive stars. It, so to speak, keeps the sun shining and, hence, is one of the most important *external* prerequisites of life on earth. Of no less importance, although concerned with the *internal* mechanism of life, appears to be the Krebs- or citric acid cycle [6], shown in Figure 3. This cyclic reaction mediates and regulates the carbohydrate and fatty acid metabolism in the living cell, and has also fundamental functions in anabolic (or biosynthetic) processes. In both schemes, energy-rich matter is converted into energy-deficient products under conservation, i.e., cyclic restoration of the essential material intermediates. Historically, both cycles, though they are little related in their causes, were proposed at about the same time (1937/38).

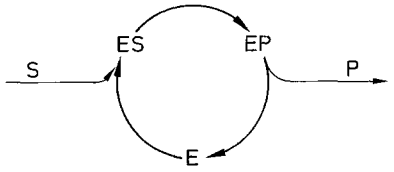

Fig. 1. *The common catalytic mechanism of an enzyme* according to Michaelis and Menten involves (at least) three intermediates: the free enzyme (E), the enzyme-substrate (ES) and the enzyme-product complex (EP). The scheme demonstrates the equivalence of catalytic action of the enzyme and cyclic restoration of the intermediates in the turnover of the substrate (S) to the product (P). Yet, it provides only a formal representation of the true mechanism which may involve a stepwise activation of the substrate as well as induced conformation changes of the enzyme.

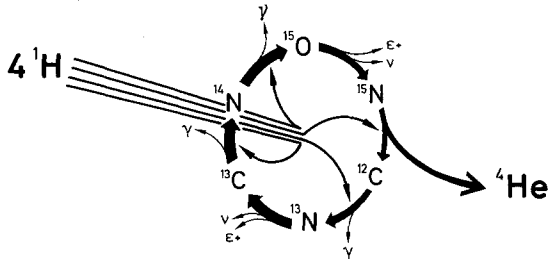

Fig. 2. *The carbon cycle,* proposed by Bethe and v. Weizsäcker, is responsible – at least in part – for the energy production of massive stars. The constituents: ^{12}C, ^{13}N, ^{13}C, ^{14}N, ^{15}O, and ^{15}N are steadily reconstituted by the cyclic reaction. The cyclic scheme as a whole represents a catalyst which converts four ^{1}H atoms to one ^{4}He atom, with the release of energy in the form of γ-quanta, positrons (ε^+) and neutrinos (ν).

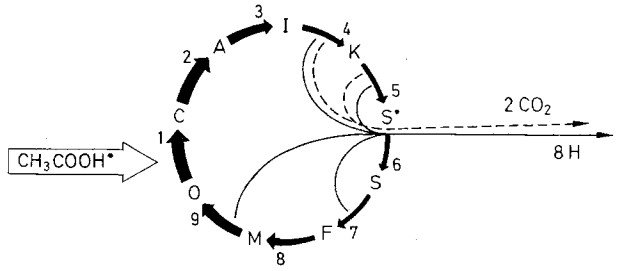

Fig. 3. *The tricarboxylic or citric acid cycle* is the common catalytic tool for biological oxidation of fuel molecules. The complete scheme was formulated by Krebs; fundamental contributions were also made by Szent-Györgyi, Martius, and Knoop. The major constituents of the cycle are: citrate (C), cis-aconitate (A), isocitrate (I), α-ketoglutarate (K), succinyl-CoA (S*), succinate (S), fumarate (F), l-malate (M) and oxaloacetate (O): The acetate enters in activated form as acetyl-CoA (step 1) and reacts with oxaloacetate and H_2O to form citrate (C) and CoA ($+H^+$). All transformations involve enzymes as well as co-factors such as CoA (steps 1, 5, 6), Fe^{2+} (steps 2, 3), NAD^+ (steps 4, 5, 9), TPP, lipoic acid (step 5) and FAD (step 7). The additional reactants: H_2O (steps 1, 3, 8), P, and GDP (step 6) and the reaction products: H_2O (step 2), H^+ (steps 1, 9), and GTP (step 6) are not explicitly mentioned. The net reaction consists of the complete oxidation of the two acetyl carbons to CO_2 (and H_2O). It generates twelve high-energy phosphate bonds, one formed in the cycle (GTP, step 6) and 11 from the oxidation of NADH and $FADH_2$ [3 pairs of electrons are transferred to NAD^+ (steps 4, 5, 9) and one pair to FAD (step 7)].

N.B.: The cycle as a whole acts as a *catalyst* due to the cyclic restoration of the substrate intermediates, yet it does *not* resemble a *catalytic cycle* as depicted in Figure 4. Though every step in this cycle is catalyzed by an enzyme, none of the enzymes is formed via the cycle

CoA = coenzyme A, NAD = nicotine amide adenine dinucleotide, GTP = guanosine triphosphate, FAD = flavine adenine dinucleotide, TPP = thiamine pyrophosphate, GDP = guanosine diphosphate, P = phosphate

Unidirectional cyclic restoration of the intermediates, of course, presumes a system far from equilibrium and is always associated with an expenditure of energy, part of which is dissipated in the environment. On the other hand, equilibration occurring in a closed system will cause each individual step to be in detailed balance. Catalytic action in such a closed system will be microscopically reversible, i.e., it will be equally effective in both directions of flow.

Let us now, by a straight forward iteration procedure, build up hierarchies of reaction cycles and specify

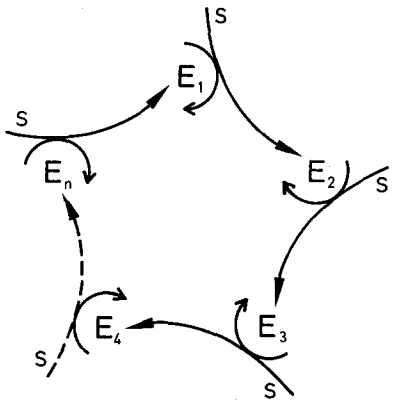

Fig. 4. *The catalytic cycle* represents a higher level of organization in the hierarchy of catalytic schemes. The constituents of the cycle $E_1 \rightarrow E_n$ are themselves catalysts which are formed from some energy-rich substrates (S), whereby each intermediate E_i is a catalyst for the formation of E_{i+1}. The catalytic cycle seen as an entity is equivalent to an autocatalyst, which instructs its own reproduction. To be a catalytic cycle it is sufficient, that only one of the intermediates formed is a catalyst for one of the subsequent reaction steps.

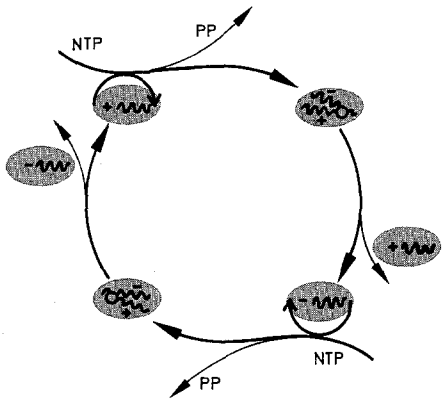

Fig. 5. *A catalytic cycle of biological importance* is provided by the replication mechanism of single-stranded RNA. It involves the plus and minus strand as template intermediates for their mutual reproduction. Template function is equivalent to discriminative catalysis. Nucleoside triphosphates (NTP) provide the energy-rich building material and pyrophosphate (PP) appears as a waste in the turnover. Complementary instruction, the mechanism of which will be discussed in connection with Figure 11, represents inherent autocatalytic, i.e., self-reproductive function

their particular properties. In the next step this means we consider a reaction cycle in which at least one, but possibly all of the intermediates themselves are catalysts. Notice that those intermediates, being catalysts, now remain individually unchanged during reaction. Each of them is formed from a flux of energy-rich building material using the catalytic halp of its preceding intermediate (Fig. 4). Such a system, comprising a larger number of intermediates, would have to be of a quite complex composition and, therefore, is hard to encounter in nature. The best known example is the four-member cycle associated with the template-directed replication of an RNA molecule (Fig. 5). *In vitro* studies of this kind of mechanism have been performed using a suitable reaction medium, buffered with the four nucleoside-triphosphates, as the energy-rich building material and a phage replicase present as a constant environmental factor [7, 8], (A more detailed description will be given by B.-O. Küppers [9]). Each of the two strands acts as a template instructing the synthesis of its complementary copy in analogy to a photographic reproduction process.

The simplest representative of this category of reaction systems is a single autocatalyst, or – in case of a whole class of information carrying entities I_i – the self-replicative unit. The process can be formally written as:

$$X \xrightarrow{I} I$$

Reactions of this type will be considered frequently in this paper, we characterize them by the symbol

Ⓘ

Double-stranded DNA, in contrast to single-stranded RNA, is such a truely *self*-reproductive form, i.e., both strands are copied concomitantly by the polymerase [10] (cf. Fig. 6). The formal scheme applies to the prokaryotic cell, where inheritance is essentially limited to the individual cell line.

Both plain catalytic and autocatalytic systems share, at buffered substrate concentration, a rate term which is first order in the catalyst concentration. The growth curve, however, will clearly differentiate the two sys-

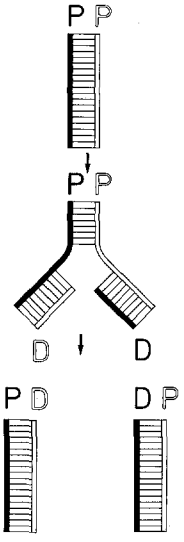

Fig. 6. *A true self-reproductive process* exemplified by a one-member catalytic cycle can be found with DNA replication. The mechanism which is quite involved (cf. Fig. 12), guarantees that each daughter strand (D) is associated with one of the parental strands (P)

tems. Under the stated conditions, the product of the plain cytalytic process will grow linearly with time, while the autocatalytic system will show exponential growth.

In strict terminology, an autocatalytic system may already be called hypercyclic, in that it represents a cyclic arrangement of catalysts which themselves are cycles of reactions. We shall, however, restrict the use of this term to those ensembles which are hypercyclic with respect to the *catalytic function*. They are actually hypercycles of second or higher degree, since they refer to reactions which are at least of second order with respect to catalyst concentrations.

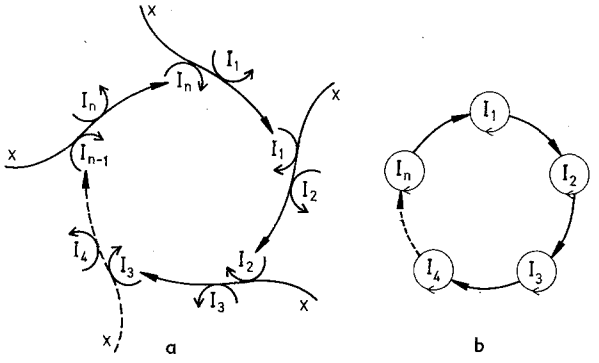

Fig. 7. *A catalytic hypercycle* consists of self-instructive units I_i with two-fold catalytic functions. As autocatalysts or – more generally – as catalytic cycles the intermediates I_i are able to instruct their own reproduction and, in addition, provide catalytic support for the reproduction of the subsequent intermediate (using the energy-rich building material X). The simplified graph (b) indicates the cyclic hierarchy

A catalytic hypercycle is a system which connects autocatalytic or self-replicative units through a cyclic linkage. Such a system is depicted in Figure 7. The intermediates I_1 to I_n, as self-replicative units, are themselves catalytic cycles, for instance, combinations of plus- and minus-strands of RNA molecules as shown in Figure 5. However, the replication process, as such, has to be directly or indirectly furthered, via additional specific couplings between the different replicative units. More realistically, such couplings may be effected by proteins being the translation products of the preceding RNA cycles (Fig. 8). These proteins may act as specific replicases or derepressors, or as specific protection factors against degradation. The couplings among the self-replicative cycles have to form a superimposed cycle, only then the total system resembles a hypercycle. Compared with the systems shown in Figures 4 and 5, the hypercycle is self-reproductive to a higher degree.

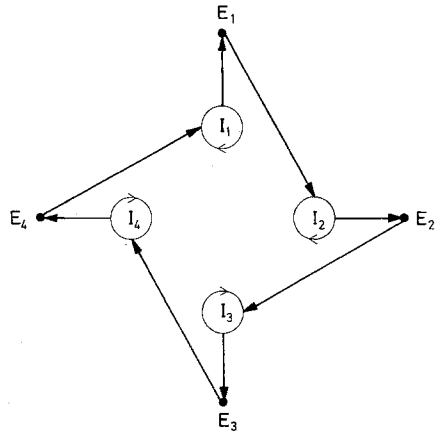

Fig. 8. *A realistic model of a hypercycle of second degree*, in which the information carriers I_i exhibit two kinds of instruction, one for their own reproduction and the other for the translation into a second type of intermediates E_i with optimal functional properties. Each enzyme E_i provides catalytic help for the reproduction of the subsequent information carrier I_{i+1}. It may as well comprise further catalytic abilities, relevant for the translation process, metabolism, etc. In such a case hypercyclic coupling is of a higher than second degree

The simplest representative in this category is, again, the (quasi)one-step system, i.e., the reinforced autocatalyst. We encounter such a system with RNA-phage infection (Fig. 9). If the phage RNA (+strand) is injected into a bacterial cell, its genotypic information is translated using the machinery of the host cell.

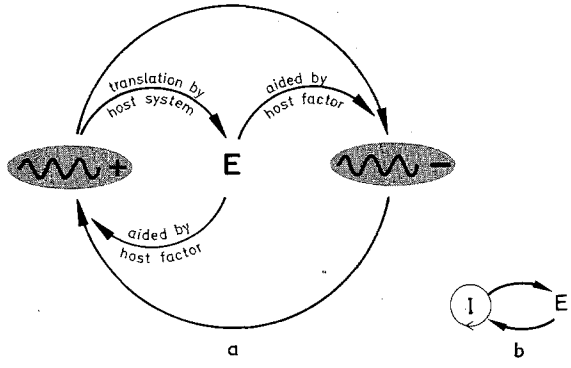

Fig. 9. *RNA-phage infection* of a bacterial cell involves a simple hypercyclic process. Using the translation machinery of the host cell, the infectious plus strand first instructs the synthesis of a protein subunit (E) which associates itself with other host proteins to form a phage-specific RNA-replicase. This replicase complex exclusively recognizes the phenotypic features of the phage-RNA, which are exhibited by both the plus and the minus strand due to a symmetry in special regions of the RNA chain. The result is a burst of phage-RNA production which – owing to the hypercyclic nature – follows a hyperbolic growth law (cf. part B, Fig. 17) until one of the intermediates becomes saturated or the metabolic supply of the host cell is exhausted. Graph (b) exemplifies that it is sufficient if one of the intermediates possesses autocatalytic or self-instructive function presuming that the other partners feed back on it via a closed cyclic link

One of the translation products then associates itself with certain host factors to form an active enzyme complex which specifically replicates the plus and minus strand of the phage RNA, both acting as templates in their mutual reproduction [11]. The replicase complex, however, does not multiply – to any considerable extent – the messenger RNA of the host cell. A result of infection is the onset of a *hyperbolic* growth of phage particles, which eventually becomes limited due to the finite resources of the host cell.

Another natural hypercycle may appear in Mendelian populations during the initial phase of speciation, as long as population numbers are low. The reproduction of genes requires the interaction between both alleles (M and F), i.e., the homologous regions in the male and female chromosome, which then appear in the offsprings in a rearranged combination. The fact that Mendelian population genetics [12] usually does not reflect the hypercyclic non-linearity in the rate equations (which leads to hyperbolic rather than exponential growth), is due to a saturation occurring at relatively low population numbers, where the birth rate (usually) becomes proportional to the population number of females only.

As is seen from the comparative schematic illustration in Figure 10, hypercycles represent a new level of organization. This fact is manifested in their unique properties. Non-coupled self-replicative units guarantee the conservation of a limited amount of information which can be passed on from generation to generation. This proves to be one of the necessary prerequisites of Darwinian behavior, i.e., of selection and evolution [13]. In a similar way, catalytic hypercycles are also selective, but, in addition, they have integrating properties, which allow for cooperation among otherwise competitive units. Yet, they compete even more violently than Darwinian species with any replicative entity not being part of their own. Furthermore, they have the ability of establishing global forms of organization as a consequence of their once-forever-selection behavior, which does not permit a coexistence with other hypercyclic systems, unless these are stabilized by higher-order linkages.

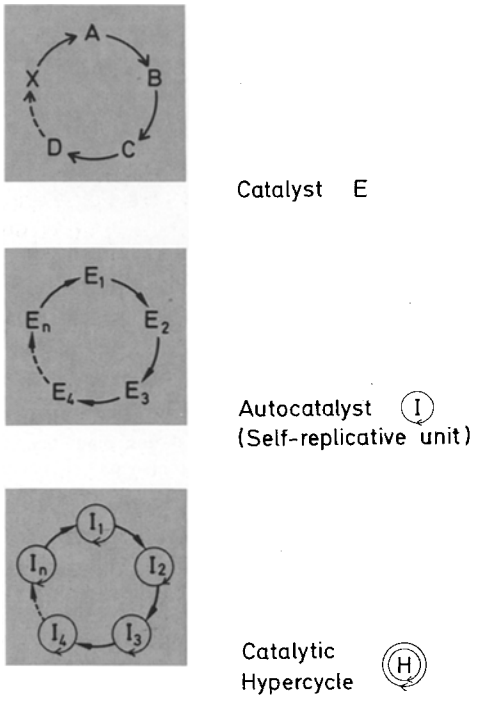

Fig. 10. *The hierarchy of cyclic reaction networks* is evident from this comparative representation (→ chemical transformation, ⇢ catalytic action)

The simplest type of coupling within the hypercycle is represented by straight-forward promotion or derepression introducing second-order formation terms into the rate equations. Higher-order coupling terms may occur as well and, thus, define the degree p of the hypercyclic organization.

Individual hypercycles may also be linked together to build up hierarchies. However, this demands intercyclic coupling terms which depend critically on the degree of organization. Hypercycles H_1 and H_2, having the degrees p_1 and p_2 of internal organization, require intercyclic coupling terms of a degree $p_1 + p_2$, in order to establish a stable coexistence.

It is the object of this paper to present a detailed theoretical treatment of the category of reaction networks we have christened *hypercycles* [4] and to discuss their importance in biological self-organization, especially with respect to the origin of translation, which may be considered the most decisive step of precellular evolution.

III. Darwinian Systems

III. 1. The Principle of Natural Selection

In physics we know of principles which cannot be reduced to any more fundamental laws. As axioms, they are abstracted from experience, their predictions being consistent with any consequences that can be subjected to experimental test. Typical examples are the first and second law of thermodynamics.

Darwin's principle of natural selection does not fall into the category of first principles. As was shown in population genetics [14], natural selection is a consequence of obvious, basic properties of populations of living organisms subjected to defined external constraints. The principle then makes precise assertions about the meaning of the term fittest in relation to

environmental conditions, other than just uncovering the mere tautology of 'survival of the survivor.' Applied to natural populations with their variable and usually unknown boundary conditions, the principle still provides the clue for the fact of evolution and the phylogenetic interrelations among species. This was the main objective of Charles R. Darwin [15] and his contemporaty Alfred R. Wallace [16], namely, to provide a more satisfactory foundation of the tenet of descendence.

Actually, most of the work in population genetics nowadays is concerned with more practical problems regarding the spread of genetic information among Mendelian populations, leaving aside such academic questions as whether 'being alive' really is a *necessary* prerequisite of selective and evolutive behavior. The fact that obvious attributes of living organisms, such as metabolism, self-reproduction, and finite life span, as well as mutability, suffice to explain selective and evolutive behavior under appropriate constraints, has led many geneticists of our day to believe that these properties are *unique* to the phenomenon of life and cannot be found in the inanimate world [14]. Test-tube experiments [7], which clearly resemble the effects of natural selection and evolution *in vitro*, were interpreted as post-biological findings rather than as a demonstration of a typical and specific behavior of matter. Here one should state that even laser modes exhibit the phenomenon of natural selection and an analysis of their amplification mechanism reveals more than formal analogies. Yet, nobody would call a laser mode 'alive' by any standard of definition.
Wsuch questions may not have much appeal to those who are concerned with the properties of actual living organisms, they become of utmost importance in connection with the question of the *origin of life*. Here we must, indeed, ask for the *necessary* prerequisites in order to find those molecular systems which are eligible for an evolutionary self-organization. The underlying complexity we encounter at the level of macromolecular organization requires this process to be guided by similar principles of selection and evolution as those which apply to the animated worled.
Recent work (loc. cit.), both theoretical and experimental, has been concerned with these questions. In the following we shall give a brief account of some previous results concerning Darwinian systems.

III. 2. Necessary Prerequisites of Darwinian Systems

What is the molecular basis of selection and evolution? Obviously, such a behavior is not a global attribute of any arbitrary form of matter but rather is the consequence of peculiar properties which have to be specified.

The essential requirement for a system to be self-selective is that it has to stabilize certain structures at the expense of others. The criteria for such a stabilization are of a dynamic nature, because it is the distribution of competitors present at any instant that decides which species is to be selected. In other words, there is no static stability of any structure, once selected; it may become unstable as soon as other more 'favourable' structures appear, or in a new environment. The criteria for evaluation must involve some feedback property, which ensures the indentity of value and dynamic stability. An advantageous mutant, once produced as a consequence of some fluctuation, must be able to amplify itself in the presence of a large excess of less advantageous competitors. Therefore, advantage must be indentical with at least some of those dynamic properties which are responsible for amplification. Only in this way can the system selectively organize itself in absence of an 'external selector'. The feedback property required is represented by inherent autocatalysis, i.e., self-reproductive behavior.
In a general analysis using game models [18], we have specified those properties of matter which are necessary to yield Darwinian behavior at the molecular level. They can be listed as follows:

1) *Metabolism.* Both formation and degradation of the molecular species have to be independent of each other and spontaneous, i.e., driven by positive affinities. This cannot be achieved in any equilibrated system, in which both processes are mutually related by microscopic reversibility yielding a stable distribution for all competitors once present in the system. Complexity, i.e., the huge multiplicity of alternative structures in combination with time and space limitations simply doesn't allow for such an equilibration, but rather requires a steady degradation and formation of new structures. Selection can become effective only for intermediate states which are formed from energy-rich precursors and which are degraded to some energy-deficient waste. The ability of the system to utilize the free energy and the matter required for this purpose is called metabolism. The necessity of maintaining the system far enough from equilibrium by a steady compensation of entropy production has been first clearly recognized by Erwin Schrödinger [19].

2) *Self-reproduction.* The competing molecular structures must have the inherent ability of instructing their own synthesis. Such an inherent autocatalytic function can be shown to be necessary for any mechanism of selection involving the destabilization of a

population in the presence of a single copy of a newly occurring advantageous mutant. Furthermore, self-copying is indispensable for the conservation of information thus far accumulated in the system. Steady degradation, a necessary prerequisite with respect to condition 1) and 3), would otherwise lead to a complete destruction of the information.

3) *Mutability*. The fidelity of any self-reproductive process at finite temperature is limited due to thermal noise. This is especially effective if copying is fast, more precisely, if it requires for each elementary step an energy of interaction not too far above the level of thermal energy. Hence mutability is always physically associated with self-reproducibility, but it is also (logically) *required* for evolution. Errors of copying provide the main source of new information. As will be seen, there is a threshold-relationship for the rate of mutation, at which evolution is fastest, but which must not be surpassed unless all the information thus far accumulated in the evolutionary process is to be lost.

Only those macromolecular systems which fulfil these three prerequisites are eligible as information carriers in a virtually unlimited evolutionary process. The properties mentioned have to be inherited by all members of the corresponding macromolecular class, i.e., by all possible alternatives or mutants of a given structure and, furthermore, they have to be effective within a wide range of concentration, i.e., from one single copy up to a macroscopically detectable abundance. The 'prerequisite of realizibility' excludes systems of a complicated composition and structure, in which the features mentioned would result from a particular coincidence of molecular interactions rather than from a general principle of physical interaction. As an example, consider the nucleic acids as compared with proteins. Reproduction in nucleic acids is a general property based on the physical forces associated with the unique complementarities among the four bases. Proteins, on the other hand, have a much larger functional capacity, including instructive and reproductive properties. Each individual function, however, is the consequence of a very specific folding of the polypeptide chain and cannot be attributed to the class of proteins in general. It might even be lost completely by a single mutation.

Systems of matter, in order to be eligible for selective self-organization, have to inherit physical properties which allow for metabolism, i.e., the turnover of energy-rich reactants to energy-deficient products, and for ('noisy') self-reproduction. These prerequisites are indispensible. Under suitable external conditions they also prove to be sufficient for selective and evolutive behavior.

III. 3. Dynamics of Selection

The simplest system in accordance with the quoted *necessary* prerequisites can be described by a system of differential equations of the following form [4] ($\dot{x}=\mathrm{d}x/\mathrm{d}t$; $t=$time):

$$\dot{x}_i=(A_iQ_i-D_i)x_i+\sum_{k\neq i} w_{ik}x_k+\Phi_i \qquad (1)$$

where i is a running index, attributed to all distinguishable self-reproductive molecular units, and hence characterizing their particular (genetic) information. By x_i we denote the respective population variable (or concentration). The physical meaning of the other parameters will become obvious from a discussion of this equation. The set of equations first of all involves those self-reproductive units i, which are present in the sample under consideration and which may be numbered 1 to N. It may be extended to include all possible mutants, part of which appear during the course of evolution.

In these equations describing an open system, *metabolism* is reflected by *spontaneous* formation ($A_iQ_ix_i$) and decomposition (D_ix_i) of the molecular species. 'Spontaneous' means that both reactions proceed with a positive affinity and hence are not mutually reversible. The term A_i always contains some stoichiometric function $f_i(m_1, m_2 \ldots m_\lambda)$ of the concentrations of energy-rich building material (λ classes) required for the synthesis of the molecular species i, the precise form of which depends on the particular mechanism of reaction. This energy-rich building material has to be steadily provided by an influx of matter as have the reaction products to be removed by a corresponding outflux (Φ_i). For a spontaneous decomposition, the D_i-term is linearly related to x_i reflecting a common first-order-rate law. In more complex systems both A_i and D_i may include further concentration functions if the corresponding reactions are enzyme-catalyzed or if further couplings among the reactions are present.

Self-reproduction, the second prerequisite, manifests itself in the x_i-dependence of the formation rate term. A straightforward linear dependence represents only the simplest form of inherent autocatalysis. Other more complicated, yet still linear mechanisms, such as complementary instruction or cyclic catalysis, can be treated in an analogous way as will be shown Nonlinear autocatalysis, on the other hand, is the main object of this paper.

Mutability is reflected by the quality factor Q_i, which may assume any value between zero and one. This factor denotes the fraction of reproductions that take place at a given template i and result in an *exact* copy of i. There is, of course, a complementary term related to imprecise reproduction of the template i; $A_i(1-Q_i)x_i$. It means the production of a large variety of 'error copies' which in most cases are quite closely related to the species i. The production of error copies of i will then show up with corresponding terms in the rate equations of each of its 'relatives' k. Correspondingly, the copy i will also receive contributions from those relatives due to errors in their replication. These are taken into account by the sum term; $\sum_{i\neq k} w_{ik}x_k$. The individual mutation rate parameter w_{ki}, will usually be small compared with the reproduction rate parameter A_iQ_i—the smaller the more distant the 'relative' k. If all species present and their possible mutants are taken into account by the indices i and k (running from 1 to N), the following conservation relation for the error copies holds:

$$\sum_i A_i(1-Q_i)x_i=\sum_i\sum_{k\neq i} w_{ik}x_k \qquad (2)$$

The individual flow or transport term Φ_i, finally, describes any supply or removal of species i other than by chemical reaction. It is required due to the metabolic turnover (cf. above). In most

Naturwissenschaften 64, 541–565 (1977)

cases each species contributes to the total flow Φ_t in proportion to its presence:

$$\Phi_i = \Phi_t \frac{x_i}{\sum_k x_k} \tag{3}$$

In evolution experiments the overall flow can be adjusted in order to provide reproducible global conditions, such as constant overall population densities:

$$\sum_k x_k = \text{const} \equiv c_n \tag{4}$$

In this case, the flow Φ_t has to be steadily regulated in order to compensate for the excess overall production, i.e.

$$\Phi_t = \sum_k A_k x_k - \sum_k D_k x_k \equiv \sum_k E_k x_k \tag{5}$$

where we call $E_i \equiv A_i - D_i$ the 'excess productivity' of the template i. Notice that the error production does not show up explicitly in this sum as a consequence of the conservation relation (2).

If, in addition, the individual fluxes of the energy-rich building material are also regulated, in order to provide for each of the λ classes a constantly buffered level $(m_1 \ldots m_\lambda)$, the stoichiometric functions $f_i(m_1 \ldots m_\lambda)$ appearing in the rate parameters A_i are constant and as such do not have to be specified explicitly. We shall refer to this constraint, in which, via flux control, both the non-organized, as well as the total organized material is regulated to a constant level, as 'constant overall organization'. It is usually maintained in evolution experiments, e.g., in a flow reactor [9], or – on average – in a serial transfer experiment [7]. An alternative straightforward constraint would be that of 'constant fluxes'. In this case, the concentration levels are variable, adjusting to the turnover at given in- and outfluxes. Both constraints will cause the system to approach a steady state with sharp selection behavior. The quantitative results may show differences for both constraints, but the qualitative behavior turns out to be very similar [4]. It is, therefore, sufficient to consider here just one of both limiting cases. The constraints to be met in nature may vary with time and, hence, will usually not correspond to either of the simple extremes – just as little as weather conditions usually resemble simple thermodynamic constraints (e.g., constant pressure, temperature, etc.). However, the essential *principles* of natural selection can only be studied under controlled and reproducible experimental conditions.

For the constraint of constant overall organization the rate equations (1) in combination with the auxiliary conditions (2) to (5) reduce to:

$$\dot{x}_i = (W_{ii} - \bar{E}(t))\, x_i + \sum_{k \neq i} w_{ik} x_k \tag{6}$$

where

$$W_{ii} = A_i Q_i - D_i \tag{7}$$

may be called the (intrinsic) selective value and

$$\bar{E}(t) = \sum_k E_k x_k \Big/ \sum_k x_k \tag{8}$$

the average excess productivity, which is a function of time. Only when the population variables $x_k(t)$ become stationary, will $\bar{E}(t)$ reach a constant steady-state value which is metastable since it depends on the population of the spectrum of mutants. For constant (i.e., time-invariant) values of W_{ii} and w_{ik} the non-linear system of differential equations (6) can be solved in a closed form. Approximate solutions of the selection problem have been reported in earlier papers. In recent years, an exact solution has been worked out by C.J. Thompson and J.L. McBride [20] and independently by B.L. Jones, R.H. Enns, and S.S. Rangnekar [21, 22]. The explicit expressions obtained from the exact solutions by second-order perturbation theory are in agreement with the formerly [4] reported approximations*. The following discussion is based on the exact solutions given by B.L. Jones et al. [21] which offers an elegant quantitative representation of the selection problem.

III. 4. The Concept "Quasi-Species"

The single species is not an independent entity because of the presence of couplings. Conservation of the total population number forces all species into mutual competition, while mutations still allow for some cooperation, especially among closely related species (i.e., species i and k with non-vanishing w_{ik} and w_{ki} terms).

Let us, therefore, reorganize our system in the following way. Instead of subdividing the total population into N species we define a new set of N quasi-species, for which the population variables y_i are linear combinations of the original population variables x_i, whereby, of course, the total sums are conserved:

$$\sum_{k=1}^{N} x_k = \sum_{k=1}^{N} y_k \tag{9}$$

How to carry out this new subdivision is suggested by the structure of the differential equations (6). It corresponds actually to an affine transformation of the coordinate system, well known from the theory of linear differential equations. One obtains a new set of equations for the transformed population variables y_i which reads:

$$\dot{y}_i = (\lambda_i - \bar{E}(t))\, y_i \tag{10}$$

An application of this procedure to the non-linear equations (6) is possible because the term causing the non-linearity, $\bar{E}(t)$ according to Eq. (8), remains

* Jones et al. [21] pointed out that a neglection of the backflow term $\sum_{k \neq i} w_{ik} x_k$ in [4] is not valid for the approach to the steady state because the term $W_{mm} - \bar{E}(t)$ becomes very small. They referred to Eq. II-49 in [4], where any error rate was deliberately neglected (i.e. $Q = 1$) for the purpose of demonstrating the nature of solutions typical for selection. They overlooked, however, our explicit statement (p. 482 in [4]) that such an assumption may apply approximately only to a dominant species with a well established selective advantage, while cell mutants owe their existence solely to the presence of the w_{ik} terms. The approximations obtained previously (Eq. II-33a; II-43; II-59; II-69; II-72 in [4]; cf. also [22]) indeed agree quantitatively with those following from the exact solution by application of perturbation theory (Eq. (21) and (22) in [21] and Eq. (13), (18) and (19) in this paper).

On the other hand, we should like to state that we appreciate very much the availability of the exact solutions, as obtained by Thompson and McBride [20] as well as by Jones et al. [21] which aid tremendously the presentation of a consistent picture of the quasi-species.

invariant in the transformation and can be expressed now as the average of the λ_i's.

$$\bar{E}(t)=\sum_k \lambda_k y_k / \sum_k y_k \tag{11}$$

The λ_i's are the eigen-values of the linear dynamic system. They – as well as the eigen-vectors which correlate the x_i's whith the y_i's – can be obtained from the matrix, consisting of the coefficients W_{ii} and w_{ik}.

The solutions of the system (10) are physically obvious. Any quasi-species (characterized by an eigen-value λ_i and a population variable y_i), whose λ_i-value is below the threshold represented by the average, $\bar{E}(t)$, will die out. (Its rate is negative!) Correspondingly, each quasi-species with a λ_i above the threshold will grow. The threshold $\bar{E}(t)$, then, is a function of time, and – due to equation (11) – will increase, the more the system favours quasi-species associated with large eigen-values. This will continue until a steady state is reached:

$$\bar{E}(t)\rightarrow\lambda_{\max} \tag{12}$$

i.e., the mean productivity will increase until it equals the maximum eigen-value. By then all quasi-species but one, namely the one associated with the maximum eigen-value – will have died out. Their population variables have become zero.

Darwinian selection and evolution can, thus, be characterized by an extremum principle. It defines a category of behavior of self-replicative entities under stated selection constraints.

Such a process, for instance, can be seen in analogy to equilibration, which represents a fundamental type of behavior of systems of matter under the constraint of isolation and which is characterized by a general extremum principle. The extremum principle (12) is related to the stability criteria of I. Prigogine and P. Glansdorff [23]. As an optimization principle it holds also for certain classes of non-linear dynamical systems [21]. Furthermore, the validity of the solutions of the quasi-linear system (10) is not restricted to the neighborhood of the steady state.

What is the physical meaning of a quasi-species?

In biology, a species is a class of individuals characterized by a certain phenotypic behavior. On the genotypic level, the individuals of a given species may differ somewhat, but, nevertheless, all species are represented by DNA-chains of a very uniform structure. What distinguishes them individually is the very sequence of their nucleotides. In dealing now with such molecules, being the replicative units, we just use these differences of their sequences in order to define the (molecular) species. The differences are, of course, expressed also by different phenotypic properties, such as replication rates, life times, error rate, etc.

The single (molecular) species, however, is not the true target of selection. Eq. (10) tells us that it is rather the quasi-species, i.e., an organized combination of species with a defined probability distribution which emerges via selection. As such it is selected against all other distributions. Under selection strains the populations numbers of all but one quasi-species really will disappear. The quasi-species is closely correlated to what is called the "wild-type" of a population.

The wild-type is often assumed to be the standard-genotype representing the optimally adapted phenotype within the mutant distribution. The fact that it is possible to determine a unique sequence for the genome of a phage supports this view of a dominant representation of the standard copy. Closer inspection of the wild-type distribution of phage Q_β (in the laboratory of Ch. Weissmann) [24], however, clearly demonstrated that only a small fraction of the sequences actually is exactly identical with that assigned to the wild-type, while the majority represents a distribution of single and multiple error copies whose *average* only resembles the wild-type sequence. In other words, the standard copies might be present to an extent of (sometimes much) less than a few percent of the total population. However, although the predominant part of the population consists of non-standard types, each individual mutant in this distribution is present to a very small extent (as compared with the standard copy). The total distribution, within the limits of detection, then exhibits an *average* sequence, which is exactly identical with the standard and, hence, defines the wild-type. The quasi-species, introduced above in precise terms, represents such an organized distribution, characterized by one (or more) *average* sequences. Typical examples of distributions (related to the RNA-phage Q_β) are given in Table 2. One unique (average) sequence is present only if the copy which *exactly* resembles the standard is clearly the dominant one, i.e., if it has the highest selective value within the distribution. Mutants, whose W_{ii} are very close to the maximum values, will on average be present in correspondingly high abundance (cf. Table 2). They will cause the wild-type sequence to be somewhat blurred at certain positions. If two closely related mutants actually have (almost) identical selective values, they may both appear in the quasi-species with (almost) equal statistical weights. How closely the W_{ii}-values have to resemble each

Table 2.
The abundance of the standard sequence in the wild-type distribution is determined by its quality function Q_m and its superiority σ_m. At given number of nucleotides ν_m the quality function can be calculated from the average digit quality $\bar{q}_m$ of the nucleotides referring to a particular enzymic read-off mechanism. Both $\bar{q}_m$ and σ_m also determine the maximum number of nucleotides ν_{max}, which a standard sequence must never exceed, otherwise the quasi-species distribution becomes unstable. The data refer to RNA sequences consisting of 4500 nucleotides (phage Q_β).

The values in the dark fields (a) show the relative abundance of the standard sequence within the wild-type distribution (in percent) according to Eq. (18) and (25). Negative values mean that the distribution is unstable. The light fields show the threshold numbers ν_{max} for given $\bar{q}_m$ and σ_m values. The data clearly demonstrate the sensitivity of ν_{max} towards the parameter $\bar{q}_m$. For a well-adapted species, $1-\bar{q}_m$ should be slightly larger than $1/\nu_m$ (e.g., $=1-\bar{q}_m=0.0005$ for $\nu_m=4500$ nucleotides requiring σ_m values $\geqq 10$).

correct copies / ν_{max}	$\bar{q}_m$ average digit copying quality: 0.9980	0.9990	0.9995	0.9998
superiority σ_m = 2	unstable (<0) / 346	unstable (<0) / 693	unstable (<0) / 1386	unstable (<0) / 3466
20	unstable (<0) / 1498	unstable (<0) / 2996	5.8 % / 5991	37.6 % / 14979
200	unstable (<0) / 2649	0.6 % / 5298	10 % / 10597	40 % / 26492

a

In part b a more realistic example of a quasi-species distribution comprising $1\cdot 10^9$ individuals is presented. A sequence of 4500 nucleotides would have 13500 one-error mutants, supposed that for each correct nucleotide (A, U, G, or C) there are three incorrect alternatives. Experiments with RNA-replicases, however, show that purine → purine and pyrimidine → pyrimidine substitutions are by far more frequent than any cross-type substitutions: purine ↔ pyrimidine. Hence—in order to be more realistic—we have assumed only one incorrect alternative for any position. Accordingly the multiplicity of any k-error copy is just $\binom{\nu}{k}$. The total of 4500 different one-error mutants have been subdivided with respect to their degenerate (average) selective values into five classes. There is one mutant in class M_{1a} which resembles the standard quite closely. Its selective value (W_{kk}) differs by only one percent. Class M_{1b} contains four degenerate mutants possessing selective values within 10% of that of the standard while 495 mutants of class M_{1c} show W_{kk} values 30% of W_{mm}. A bulk of 2000 mutants is by one order of magnitude lower in their W_{kk} values and an equal amount of mutants is not viable at all, i.e., they do not reproduce with any speed comparable to that of the standard. Furthermore all the copies with more than two errors have been assigned W_{kk} values $\ll W_{mm}$. Although this may not be an realistic assumption, it is of no serious consequence with respect to the population numbers of *individual* sequences which are extremely small because of the large multiplicity of different error copies. Despite this fact, the sum of all multiple error copies represents the largest group in this example, followed by the total of one-error copies. On the other hand, the standard is by far the most abundant individual in the quasi-species distribution.

mutant class	degeneracy of class	assumed relative selective value W_{kk}/W_{mm}	population number of individual mutant (× degeneracy)
error-free	1	1	8.9×10^7 (×1)
one-error			
M_{1a}	1	0.99	4×10^6 (×1)
M_{1b}	4	0.9	5×10^5 (×4)
M_{1c}	495	0.3	6.3×10^4 (×495)
M_{1d}	2000	0.1	4.9×10^4 (×2000)
M_{1e}	2000	~0	4.3×10^4 (×2000)
$\sum M_1$	4500		2.2×10^8 (×1)
multiple-error			
M_2	$\sim10^7$	~0	<30 ($\times10^7$)
$\sum M_{k>1}$	$\sim2^{4500}$	~0	6.88×10^8 (×1)

b

An alternative calculation has been made in which the relative selective value of the one-error mutant 1a has been raised from 0.99 to 0.9995. While the gros of the distribution changes only slightly, the population number of the particular mutant 1a rises to the value found for the standard type (i.e., both result to 8.4×10^7). This example shows the limitation of the approximations behind Eqs. (18) and (19) requiring $w_{km}\ll W_{kk}-W_{mm}$. A more rigorous numerical evaluation yields a population number of mutant 1a amounting to only about 60% of that of the standard. Even for small differences of selective values the standard clearly remains the dominant species. Only if a one-error mutant resembles the standard within limits of $W_{mm}-W_{kk}\ll 1/\nu_m$ it may be considered to be a degenerate and hence undistinguishable individual.

other for both mutants to become selectively indistinguishable, depends on their 'degree of affinity.' For distant relatives, the correspondence has to be much more precise than for one-error copies. A special class of 'reversible neutral' mutants, hence, can be quantitatively defined. There is, of course, a second wider class of neutral mutants which belong to different quasi-species being degenerate in their eigenvalues λ_i. Most of these neutral mutants die out after appearance but a minor part may spread through the population and coexist with, or displace, the formerly established quasi-species. This diffusional spread of neutral mutants can be understood only on the basis of stochastic theory (cf. below).

III. 5. Realistic Approximations

An explicit representation of the eigen-value of the selected quasi-species can be obtained with the help of perturbation theory. The result of second-order perturbation theory resembles the previously reported expression for W_{max} ([4], Eq. II-33a):

$$\lambda_{max} \approx W_{mm} + \sum_{k \neq m} \frac{w_{mk} w_{km}}{W_{mm} - W_{kk}} \tag{13}$$

Here the index m refers to that (molecular) species which is distinguished by the largest selective value. The approximation holds only if no other W_{kk} approaches this value too closely and the dominant copy m can be considered as representative of the wild-type. Table 2 shows, how effective the approximation indeed is for any system of realistic importance. The larger the information content the smaller the individual w-values. The approximation thereby reveals a very important fact; selection (under strain) is extremely sharp with respect to distant relatives (the smaller the w_{mk}- and w_{km}-values the closer may W_{kk} resemble W_{mm} without being of any restriction to m). However, selection is smooth with respect to very close relatives. These are always present in the distribution if their selective value W_{kk} is much smaller than W_{mm} (or even zero). If the sum term in Eq. (13) can be numerically neglected (cf. values in Table 2), the extremum principle (Eq. (12)) can be expressed as:

$$W_{mm} > \bar{E}_{k \neq m} \tag{14}$$

or

$$Q_m > \sigma_m^{-1} \tag{15}$$

where

$$\bar{E}_{k \neq m} = \sum_{k \neq m} E_k x_k / \sum_{k \neq m} x_k \tag{16}$$

represents the average productivity of all competitors of the selected wild-type m and

$$\sigma_m = \frac{A_m}{D_m + \bar{E}_{k \neq m}} \tag{17}$$

is a superiority parameter of the dominant species.
With the same approximation the relative stationary population numbers can be calculated, yielding for the dominant copy

$$\bar{x}_m / \sum_{k=1}^{N} \bar{x}_k = \frac{W_{mm} - \bar{E}_{k \neq m}}{E_m - \bar{E}_{k \neq m}} = \frac{Q_m - \sigma_m^{-1}}{1 - \sigma_m^{-1}} \tag{18}$$

and for the one-error copy

$$\bar{x}_{1k} / \bar{x}_m = \frac{w_{km}}{W_{mm} - W_{kk}} \tag{19}$$

which is valid, as long as $w_{km} \ll W_{mm} - W_{kk}$ (cf. Table 2).
Higher approximations could be obtained using λ_{max} in which-ever form it is expressed. Eqs. (16)–(18) would change accordingly.

On the basis of these approximations we can quantitatively characterize Darwinian behavior of macromolecular systems. Eq. (13) shows to which extent the dynamics of selection is determined by the individual properties of the dominant (standard) species (m), while Eqs. (18) and (19) indicate the relative weights of representation of the standard species and its mutants (cf. also Table 2). It is surprising how small a fraction of the standard species is actually present within the wild-type distribution, despite the fact that its physical parameters almost entirely determine the dynamic behavior of the distribution. This provides a great adaptive and evolutive flexibility of the quasi-species and enables it to react quickly to environmental changes.

The approximations break down only in the case of the presence of two or more dominant species (cf. Table 2) which are (almost exactly) neutral mutants. However, those reversible neutral mutants can be combined to a selectively indistinguishable subclass of species and as such will determine the dynamic behavior like a single dominant species, according to the relations given above. It is interesting to note the fact that within the quasi-species there is no selection against reversible neutral mutants ('reversible' being defined as sufficiently large w_{ik} and w_{ki} to guarantee a reproducible representation). These reversible neutral mutants being part of the quasi-species have to be distinguished from unrelated neutral mutants, (w_{ik} and w_{ki} too small to provide a reproducible representation according to Eq. (18)). Those unrelated neutral mutants can still coexist, to a minor extent, as a consequence of random fluctuations [25]. The stochastic treatment shows that competition among those neutral species is a random drift phenomenon leading to extinction with an indeterminate 'survival of the survivor' as well as to a certain upgrowth and spread of new mutants, a phenomenon to which geneticists [26] refer as non-Darwinian behavior (i.e., survival without selective advantage). It should be realized that this stochastic behavior of unrelated neutral mutants, although it was not anticipated by Darwin and his followers, is not in contradiction with those properties which lead to the deterministic Darwinian behavior. The selection of reversible neutral mutants is even in accordance with the Darwinian principle, if this is interpreted in the correct way as a derivable physical principle, where it then applies to the concept of quasi-species.

III. 6. Generalizations

In the quantitative representation of Darwinian system by Eqs. (2) and (6) we have made a number of special assumptions in connection with the structural prerequisites and external constraints. In this section we want to find out how far we can generalize those assumptions without losing the characteristic features of Darwinian behavior.

1) *Rate Terms.* The linear rate terms for both autocatalytic formation and first-order decomposition may be substituted by more general expressions. Most common for enzymic mechanisms is the Michaelis-Menten form:

$$\text{rate} \sim \frac{x_i}{1 + a_i x_i} \quad \text{or} \quad \frac{x_i}{1 + \sum_k a_k x_k}$$

which replaces the simple x_i-dependence. It has been shown [4] that for autocatalytic mechanisms of this kind, selection remains effective for low population numbers and, hence, in the range which is critical for selection. Saturation will not prevent the upgrowth of advantageous mutants, but may allow for some coexistence due to a change from exponential to linear growth in the unconstrained mechanism.
In general, if the reaction order is defined by a term x_i^k, Darwinian behavior may be found for exponents:

$$0 < k \leqq 1$$

For $k = 0$ coexistence would result in a growth-limited system. Under the constraint of constant fluxes, such a situation may occur for self-reproductive species if their formation rate is limited by the constant rate of supply of energy-rich building material. Species which feed on different (mutually independent) sources will not compete with each other. The independent sources then provide

'niches' for coexistence. The multiple varieties of different species often owe their existence to such or similar devices, the consequence of which is in complete accord with the scope of Darwin's theory.
The behavior for exponents $k>1$ is analyzed in more detail in part B of this paper. It leads to extremely sharp selection with some consequences which were not compatible with Darwin's view, especially regarding descendence.

2) *Autocatalysis.* According to our discussion in section II, straightforward self-reproduction is only the simplest example among a whole class of linear autocatalytic mechanisms. Self-reproduction may generally be effected via a cyclic catalytic process. Single-stranded RNA-phages, for instance, reproduce via mutual instruction through the two complementary strands. The rate equations for such a two-membered catalytic cycle [4] yield two eigen-values for the dominant species:

$$\lambda_{1,2}=-\frac{D_{+}+D_{-}}{2}\pm\sqrt{A_{+}A_{-}Q_{+}Q_{-}+\tfrac{1}{4}(D_{+}-D_{-})^{2}} \tag{20}$$

These expressions are based on similar approximations as Eq. (13) neglecting the mutation terms. One of these eigen-values, if it is positive (i.e., $A_{+}A_{-}Q_{+}Q_{-}>D_{+}D_{-}$), replaces the W_{mm} referring to the *self*-replicative unit. Here, the kinetic parameters of both strands contribute with equal weight (geometric mean) to the selective value. They both have to be optimized in order to yield optimal performance. Wherever phenotypic properties of the RNA strands are of importance, equivalence can be most suitably reached by structural symmetry (cf. t-RNA, midi-variant of Q_{β}-RNA [27]). The second always negative eigen-value refers to an 'equilibration' between plus- and minus-strands, the concentrations of which then assume a fixed ratio. Whenever this equilibration is reached, plus- and minus-strand will act like one replicative ann competitive *unit.*
The general treatment of catalytic cycles has been shown [4, 20–22], to resemble the results for simple self-instructive systems. A n-membered cycle again is characterized by one positive eigen-value which corresponds to the selective value of the single self-reproductive unit. The catalytic properties of all members contribute to this eigen-value, in the simplest case, in the form of the geometric mean of their AQ-values, hence, requiring finite AQ-values for all members of the cycle. Furthermore, a n-membered cycle is characterized by $(n-1)$ negative eigen-values, which are representative of the internal equilibration of the concentration ratios of all members of the cycle.

3) *Mutations.* The main source of mutations, especially in the early stages of evolution, is mis-copying, i.e., the inclusion of a nucleotide with a non-complementary base during the process of replication. The experiments with Q_{β}-phage show that error rates for replacing a given purine or pyrimidine by its homologue differ considerably from those for exchanging a purine by one of the pyrimidines or *vice versa* [24]. In the formal treatment, using the parameters Q and w, no distinction has to be made regarding the kind of mutation, such as point mutation or frame shifts resulting from deletion or insertion. Those distinctions, of course, are important if the functional properties of the mutants are to be studied. The formal representation is also invariant to the causes of mutation, such as misreading in replication, chemically induced changes, or radiation damage. It may be necessary to correlate some of the mutations more closely with the decomposition term (cf. below) which, however, has no influence on the formal structure of the equations.

4) *Decomposition.* Mutations as a consequence of external influences (e.g. radiation) should be attributed to the decomposition term. In general, decomposition of the individual i may lead to species k belonging to a different class comprising only fragments of i. Again, those processes do not change the formal structure of the differential equations, if the corresponding conservation relations are appropriately taken into account.

5) *External Constraints.* The explicit form of the solutions of the selection equations depends on the constraints to be imposed. We have discussed in detail the case of constant overall organization. Similar, though quantitatively different, results are obtained for the constraint of constant fluxes [4, 28, 29]. The externally regulated parameters may, of course, involve temporal (e.g., any type of periodical) variations. This may lead to mechanistic advantages, but does not alter the essential prerequisites and consequences of Darwinian behavior. The extremum principle (12) then assumes the general form:

$$\lim_{\tau\to\infty}\frac{1}{\tau}\int_{0}^{\tau}\bar{E}(t)\,\mathrm{d}t=\lambda_{m} \tag{21}$$

A further generalization of the extremum principle has been achieved by Jones et al. [21].
Selection of a quasi-species relative to its competitors may also be considered in a growing system. It will be shown that a simple normalization procedure is applicable, which allows a generalized treatment of non-steady-state systems.

6) *Stochastic Treatment.* A final generalization is of a more principal nature. Deterministic rate equations describe generally the average behavior of ensembles consisting of a large number of individuals. The elementary processes, however, can be represented only by reaction probabilities. Game models which have been developed together with R. Winkler-Oswatitsch [18] demonstrate clearly three basic types of behavior which can be treated by stochastic theory:
a) Internal self-control of fluctuations as found around stable steady states and, in particular, at thermodynamic equilibrium,
b) self-amplification of fluctuations characterizing an instability, and
c) indifference towards fluctuations yielding random drift behavior.
In the first case, fluctuations are of importance only for small population numbers and simply mean an uncertainty of any momentary microstate. For the macrostates, which are accessible to experimental tests, they yield expectation values within specifiable average fluctuation limits.
In the second case deterministic behavior is restricted to the response to a given fluctuation. In other words, this 'if-then' determinacy will predict what happens if a certain fluctuation occurs and the accuracy of the prediction will increase with the extent of the fluctuation. The occurrence of the fluctuation itself, however, is uncertain and this microscopic uncertainty is mapped macroscopically via the finally deterministic amplification process. This case is of particular relevance for Darwinian systems. Most mutations represent fluctuations of the first type, i.e., they are not of any selective advantage and do not endanger the stability of the wild-type. After occurrence, they cease deterministically as in the case of equilibrium. However, there are also mutations which bring along a selective advantage, and they have the tendency to amplify themselves, hence, causing an instability. Whether or not they are successful in reaching dominance depends on the magnitude of their selective advantage and the extent of the fluctuation. A single copy still has a fairly high chance of dying out before it is reproduced, especially if its W-value is only slightly larger than $\bar{E}(t)$. The stochastic theory shows that for small advantages, i.e. $(W_{m+1}-W_{m})\ll W_{m}$, the fluctuation has to reach a certain extent, i.e., a number of copies which corresponds to the magnitude of $W_{m}/(W_{m+1}-W_{m})$, before the probability of growing up becomes larger than $1-e^{-1}$. This is equivalent to saying that only mutants

identified by distinct advantages will *deterministically* influence the evolutionary behavior. Nearly neutral mutants behave stochastically almost like truly neutral mutants, and, hence, refer to the third category of games which resembles random drift behavior. Neutral mutants of high frequency, of course, are part of a given quasi-species and as such are somewhat stabilized due to a finite mutation rate. They thereby utilize a fluctuation response which was characteristic of the first category. Since such a coupling is rather weak, there may be quite large fluctuations in the relative representation of neutral relatives. Unrelated (i.e., very rare) neutral mutants, on the other hand, may be considered as different quasi-species whose eigen-values have the same magnitude as that of the wild-type. Most of those neutral quasi-species will have to die out, but if they happen to grow up they may become persistent and even replace the former wild-type ('survival of the survivor'). This kind of behavior can be deduced only from stochastic theory. Corresponding calculations have been carried out for the spread of genes in Mendelian populations, especially by M. Kimura [25] and his school.

In conclusion, evolution is a deterministic process with respect to its progressive character. There will always be favorable competition of the wild-type with less advantageous mutants, coexistence of neutral or nearly neutral, closely related mutants, and upgrowth of new clearly advantageous quasi-species. However, evolution is indeterminate with respect to the temporal sequence of the appearance of mutants, as well as with respect to genetic drift caused by unrelated neutral mutants. Only a small fraction of these neutral quasi-species actually can grow up. Evolution via rare neutral mutations may, therefore, be more important in the later than in the earlier stages of evolution, where many advantageous alterations are still possible and, hence, occur with relatively high frequency.

III. 7. Information Content of the Quasi-species

In our approach to molecular evolution we have not yet dealt explicitly with the concept of genetic information. We have defined the (molecular) species as the replicative unit with a distinct information content, represented by a particular arrangement of molecular symbols. We have also taken into account the similarity relations among different such symbol arrangements, which led to the concept of the quasi-species. In deriving the criteria of selection and evolution, it proved sufficient, just as in population genetics, to notice the individual differences in genotypic information and correlate them with their characteristic dynamic properties as expressed by the selective value W_{ii}.

On the other hand, questions such as, "How much information can be accumulated in a given quasi-species?" or, "Where is the limit of reproducibility and, hence, of the evolutionary power of a quasi-species?" would remain unanswered in such a treatment. We, therefore, now introduce more specifically the concept of information.

In the theory of communication, the information content of a message consisting of ν_k symbols can be expressed as

$$I_k = \nu_k i \tag{22}$$

where i is the average information content of a single symbol. According to Shannon i can be correlated with the probability distribution of the symbols [30, 31]:

$$i = -K \sum_j p_j \ln p_j \tag{23}$$

with

$$0 < p_j < 1 \quad \text{and} \quad \sum_j p_j = 1 \tag{24}$$

The constant K usually is taken to be $1/\ln 2$ in order to yield the unit 'bits/symbol'. The alphabet generally includes classes of symbols (e.g., $\lambda = 4$ for nucleic acids). The practical use of Eq. (23) is limited to those cases where the *a-priori* probabilities of all symbols are known and where the number of symbols in the message is large enough that the averages apply. In order to account for all cooperative effects or redundancies influencing the probability distribution, it might be necessary to know the probabilities of all λ^ν possible alternatives of symbol combinations.

In our case we are actually not so much interested in any statistical a-priori probability of a symbol, but rather in the probability that a given symbol is correctly reproduced by the genetic mechanism however sophisticated the machinery may be at the various levels of organization. This probability refers to the dynamical process of information transfer and, therefore, must be determined experimentally from kinetic data (wherever it is possible; cf. below). Let us call these probabilities for correct symbol reproduction q_j. A message consisting of ν_i (molecular) symbols then will be reproduced correctly with the probability or quality factor:

$$Q_i = \prod_{j=1}^{\nu_i} q_{ij} \equiv \bar{q}_i^{\nu_i} \tag{25}$$

Even if the symbols consist of only λ classes, the quality factor for each symbol of the message might be dependent on its particular environment so that the determination of the geometric mean $\bar{q}_i$ may require the consideration of various cooperative effects, specifically associated with the message 'i'. Nevertheless, this average $\bar{q}_i$ for any given message i can be determined and it turns out that for a particular enzymic reproduction machinery those averages apply quite universally, whenever the message is sufficiently long. Moreover, the individual q-values in general are so close to one that the geometric mean can be replaced by the arithmetic mean, i.e.,

$$\left(\prod_{j=1}^{\nu_i} q_{ij}\right)^{1/\nu_i} \approx \frac{\sum_{j=1}^{i} q_{ij}}{\nu_i} \quad \text{if } (1-q_{ij}) \ll 1. \tag{26}$$

Eq. (25) then comprises the information-theoretical aspect of reproduction where $\bar{q}$, however, refers to a dynamic rather than to a static probability. The numerical values of $\bar{q}$ may take into account all mechanistic features of symbol reproduction including any static redundancy which reduce the error rate of the copying process. Nature actually has invented ingenious copying devices ranging from complementary base recognition to sophisticated enzymic checking- and proof-reading mechanisms.

Genetic reproduction is a continuously self-repeating process, and as such differs from a simple transfer

of a message through a noisy channel. For each single transfer it requires more than just recovery of the meaning of the message, which, given some redundancy, would always allow a fraction of the symbols to be reproduced incorrectly. It is also necessary to prevent any further accumulation of mistakes in successive reproduction rounds. In other words, a fraction of precisely correct wild-type copies must be able to compete favorably with the total of their error copies. Only in this way can the wild-type be maintained in a stable distribution. Otherwise the information (now in a semantic sense, i.e., the copy with optimum W_{ii}-value) would slowly seep away until it finally is entirely lost.

The condition which guarantees the stable conservation of information is the selection criterion Eq. (14) or Eq. (15). This criterion can be expressed in a general form as:

$$Q_m > Q_{\min} = \sigma_m^{-1} \tag{27}$$

and as such applies to any reproduction mechanism, even if σ_m cannot be expressed in as simple a form as valid for the linear mechanism (cf. Eq. (17)). If we combine the selection criterion, as derived from dynamics theory, with the information aspect, as expressed by Eq. (25), we obtain the important threshold relationship for the maximum information content of a quasi-species:

$$\nu_{\max} = \frac{\ln \sigma_m}{1-\bar{q}_m} \tag{28}$$

The number of molecular symbols of a self-reproducible unit is restricted, the limit being inversely proportional to the average error rate per symbol: $1-\bar{q}_m$

There is another way of formulating this important relationship. The expectation value of an error in a sequence of ν_m symbols; $\varepsilon_m = \nu_m(1-\bar{q}_m)$ must always remain below a sharply defined threshold:

$$e^{\varepsilon_m} < \sigma_m \tag{29}$$

otherwise, the information accumulated in the evolutionary process is lost due to an error catastrophe.

Table 2 contains examples which demonstrate the relevance of Eq. (28). The threshold is not sensitively dependent on the magnitude of the superiority function, but σ_m must be larger than one, i.e. $\ln \sigma_m > 0$, in order to guarantee finite values for $\nu_{\max}$. In practice (cf. below), $\ln \sigma_m$ is usually between one and ten. Relation (28) allows a quantitative estimate of the evolutionary potential, which any particular reproduction mechanisms can provide. It states, for instance, that an error rate of 1% (or a symbol-copying accuracy of 99%) is just sufficient to collect and maintain reproducibly an information content not larger than a few hundred symbols (depending on the value of $\ln \sigma_m$) or that the maintainance of the information content of the genome as large as that of *E. coli* requires an error rate not exceeding one in 10^6 to 10^7 nucleotides. It is a relation which lends itself to experimental testing, and we shall report corresponding measurements below. Eq. (28) also gives quantitative account of what has been called in population genetics the 'genetic load,' the importance of which has been stressed long ago.

The results of this section can be summarized as follows: Any mechanism of selective accumulation of information involves an upper limit for the amount of digits to be assembled in a particular order. If this limit is surpassed, the order, i.e., the equivalent of information, will fade away during successive reproductions. Stability of information is equivalent to internal stability of the quasi-species. It is as much based on competition as is selection of the quasi-species. However, two quasi-species can be brought to coexistence, while violation of the threshold relation will result in a total loss of genetic information. Hence internal stability of the quasi-species distribution, more than its 'struggle for existence' is the characteristic attribute of Darwinian behavior.

IV. Error Threshold and Evolution

IV. 1. Computer Test of the Error Catastrophe

The physical content of the threshold relation (Eq. 28) may be exemplified with a little computer game (cf. Table 3). The goal is to create a meaningful message from a more or less random sequence of letters. For this purpose the computer is initially given a set of N random sequences and programmed:

a) to remove each sequence from its memory after a defined (average) lifetime,

b) to reproduce each sequence which is present in the storage with a characteristic rate, and

c) to introduce random errors in the reproduced copies, again with a chosen average rate of substitutions per symbol.

The average rates for a) and b) are matched in such a way that a steady representation of N copies of sentences is maintained in the store, each sentence having a finite lifetime. Hence any information gained during the game can be preserved only via faithful reproduction of the sequences present. The gain of information, on the other hand, must result from a selective evaluation of the various mutant sequences

Table 3. Self-correction of sentences is the result of the evolution game, exemplified in this table.
The target sentence reads:

TAKE ADVANTAGE OF MISTAKE

It has been chosen because it provides 'selectively advantageous' information with respect to the mechanism of evolution. Its special form permits a cyclic closure, whenever functional links among the single words are introduced (as will be done in part B). Using a code, in which each letter (and word spacing) is represented by a quintet of binary symbols, the information content amounts to $v_m = 125$ bits, allowing for about 4×10^{37} alternatives. This number excludes appearance of information by mere chance. The sequences of letters shown in this tables for given generations have been sampled as being representative for the total population of sequences in the computer store.

INITIAL SEQUENCE: BAK GEVLNT GUPIF LESTKKM

DIGIT QUALITY $\bar{q}_m$: 0.995

SELECTIVE ADVANTAGE PER BIT: 10

1. GENERATION:	RAK GEVNNT GUPQF KESTKKM
5. GENERATION:	NAK AEZ,NS GEPOF MESTMKU
10. GENERATION:	VAKF ADV!NT.GE OF MISD!KE
16. GENERATION:	TAKE ADVANTAGE OF MISTAKE
	(GOAL REACHED)

The first example demonstrates that evolution is very efficient near the critical value $1-\bar{q} \approx 1/v_m$, which with $v_m = 125$ amounts to $\bar{q}_m = 0.992$. Starting with a random sequence of letters the target sentence is usually reached within $20(\pm 6)$ generations for any value of $\bar{q}$ between 0.995 and 0.990. This efficiency near the threshold is even more evident if we compare the evolutionary progress at a given generation for various values of $\bar{q}_m$:

INITIAL SEQUENCE: BAK GEVLNT GUPIF LESTKKM
SELECTIVE ADVANTAGE PER BIT : 10

$\bar{q}_m$	BEST SEQUENCE AFTER 11 GENERATIONS	NUMBER OF MISTAKES
0.999	LAKD AEV,NTAGU AF KISTQKM	9
0.995	TAKE ADV!NT GE OF MISTAKE	2
0.990	TAKEBADV!NTAGE OF MISXAKE	3
0.985	VATA ADBKMDI DHOD ?CSYBKE	18

Analogous behavior is found for the disintegration of information for $v_m > v_{max}$. At an error rate $(1-\bar{q}_m) = 1.5\%$, a selective advantage per bit of 2.5 corresponds to a σ_m value of about 5. Under these conditions the information is not stable any more. For small selective values (as in this example), however, disintegration (or accumulation) of information is a comparatively slow process.

INITIAL SENTENCE: TAKE ADVANTAGE OF MISTAKE

DIGIT QUALITY $\bar{q}_m$: 0.985

SELECTIVE ADVANTAGE PER BIT: 2.5

NUMBER OF GENERATION	BEST SENTENCE	NUMBER OF MISTAKES
1	TAKE ADVANTAGE OF MISTAKE	0
5	TAKF !DVALTAGE OF MISTAKE	3
10	TALF ADVALTACE OF MISTAKI	5
20	DAKE ADUAVEAGE OF MJUTAKE	6
40	TAKE ADVONTQCU OF MFST!ME	7
71	TAKEB ?VALTAGI LV MIST!KE	8

71	GENERATION FOR $\bar{q}_m = 0.97$	
	?AMEBADTIMOACFHQEBA!STBMF	18

Comparison of the last two rows (referring to generation 71) shows that disintegration is much faster at an error rate of 3% ($\bar{q}_m = 0.97$).
For the other example (selective advantage per bit = 10) the threshold is not yet passed at $\bar{q}_m = 0.985$; so that information is stable, as seen below, where the process starts with the correct sentence.

INITIAL SENTENCE: TAKE ADVANTAGE OF MISTAKE

DIGIT QUALITY $\bar{q}_m$: 0.985

SELECTIVE ADVANTAGE PER BIT: 10

71 GENERATION OUTPRINT OF 8 REPRESENTATIVE SENTENCES	NUMBER OF MISTAKES
TAKE ADVANTAGE OF MISTAKE	0
TAKE ADVANTAGIPOF MISTAKE	2
TAKE ADVANTAGE OF MISTAKE	0
TBKE !DVANTAGE OF MISTAKE	2
SAKE ADVANTAGE OF MGSTAME	3
TAOE ADVANVAGE OF MISTAKE	2
TAKE ADVAVTAGE OF MISTAKE	1
TAKE .DVANTAGE OF MISTAKE	1

according to their meaning, or better, according to their more or less close relationship to any meaning. This evaluation is to be effected by intrinsic meaning-dependent properties of the sequences, which influence their rates of reproduction and (or) removal as expressed by the selective value.

In natural selection, the target of evolution is always the genotype which represents the phenotype with the optimum selective value. Evaluation then is effected via the phenotypic, i.e., physical and chemical properties of the particular individual which determine the reproduction rate and quality, as well as the lifetime of the genotype in relation to the average of its competitors present in the population. Likewise, in our psychic memory we can associate with any sequence of letters a reproductive value which is related to its meaning. In our game we could, thus, evaluate any sequence of letters according to its relation to meaning, using our imagination. The computer, of course, can achieve such a goal only via a particular program, in which the evolving sentences are compared with one or several possible target sentences.

Let us then assume, that each sequence is reproduced with a rate which depends on the number of symbols that coincide in their position with those of a (meaningful) target sentence. Using binary coding we may define, that with each bit closer to the target, we enhance the reproduction rate by a certain factor; with each bit more distant we slow it down correspondingly.

The information about possible target sentences which thereby has to be supplied to the computer in advance, however, is not used for any other purpose than to provide the computer with a value scheme. The evaluation procedure could easily be made more sophisticated, i.e., more closely resembling our mental evaluation of 'meaning,' up to a level that the particular target finally reached is not at all predetermined. However, these mechanistic details of simulation are not of so much importance for exemplifying the physical meaning of the threshold relation, after we can well define the intrinsic evaluation procedure of molecular evolution (an example is provided by a game model demonstrating the evolution of t-RNA molecules [18]). The results of the computer experiment, according to Table 3, can be summarized as follows:

At high symbol-reproduction quality, e.g., for a sentence of 100 bits with average error rates per symbol

$$1-\bar{q}_m \ll 10^{-2}$$

the evolutionary progress is very slow, even if large σ_m-values, i.e., large selective advantages are involved. Maximum progress is achieved if we choose average error rates $(1-\bar{q}_m)$ which are of the same order of magnitude as $1/\nu_m$ (e.g., for 100 bits: $\bar{q} \approx 0.99$).

For sufficiently large σ_m-values (>3) the target sentence is then obtained within a number of generations which corresponds to the order of magnitude of the evolutionary distance between the target and the initial (more or less random) sequence (e.g., 100 generations). However, as soon as the threshold for $1-\bar{q}_m = \ln\sigma_m/\nu_m$ is surpassed, no more information can be gained, regardless of how large a selective advantage per bit is chosen. If one starts out with a nearly correct sentence, the information disintegrates to a random mixture of letters, rather than to evolve to an error-free copy. The threshold is very sharp but the rate of disintegration varies near the threshold. There is only a weak dependence of the threshold value on the magnitude of σ_m, unless this parameter gets very close to unity. The superiority σ_m is calculated from the relative selective advantages, and, hence, some knowledge about the error distribution (relative to the respective optimal copy) is required. This distribution of course depends on the magnitudes of the selective advantages. The computer experiment closely resembles the expected error distribution, which near the critical value $1-\bar{q}_m \approx 1/\nu_m$ (with $\ln\sigma_m \approx 1$) yields an almost equal representation of the optimum copy, all one-error copies (relative to optimum), and the sum of all multiple-error copies (in which distribution the two-error copies again are dominantly represented, with strongly decreasing tendency for copies with more errors). For smaller selective advantages (e.g., $W_{mm}-W_{kk}<3$) this representation shifts in favor of the error copies and in disfavor of the (relative) optimum, which for $\ln\sigma_m=1$ is already present with less than 10% of the total.

IV. 2. Experimental Studies with RNA-Phages

As trivial as this game may appear—after one has rationalized its results—as relevant has it turned out in nature in determining the information gained at the various levels of precellular and cellular self-organization. An experiment resembling almost exactly the above game has been carried out with phage Q_β by Ch. Weissmann and his coworkers [32, 33].

An error copy of the phage genome has been produced by site-directed mutagenesis. The procedure consists of *in vitro* synthesis of the minus strand of the phage RNA containing at the position 39 from the 5′-end the mutagenic base analog N^4-hydroxy CMP, instead of the original nucleotide UMP. Using this strand as template with the polymerizing enzyme Q_β-replicase, an infectious plus strand could be obtained in which at position 40 from the 3′-end this

position corresponds to position 39 from the 5′-end in the minus strand and is located in an extra-cistronic region – an A-residue is substituted by G. *E. coli* spheroplasts then were infected with this mutant plus-strand yielding complete mutant phage particles, which could be recovered from single plaques. Serial transfer experiments *in vivo* (infection of *E. coli* with complete phage particles) as well as *in vitro* (rate studies with isolated RNA strands using Q_β-replicase) allowed for a determination of reproduction rate parameters for both the wild-type and the mutant-40 including their distributions of satellites. Combined fingerprint and sequence analysis, applied to successive generations, indicated changes in the mutant population due to the formation of revertants. Studies with different initial distributions of wild-type and mutant revealed the fact that natural selection involves the competition between one dominant individual and a distribution of mutants. The quantitative evaluation shows that the value depends on the particular selective advantage as well as on distribution parameters of the mutant population. The wild-type as compared with the particular mutant shows a selective advantage

$$W_{\text{wild-type}} - W_{\text{mutant}} \approx 2 \text{ to } 4$$

while the rate of substitution was estimated to be

$$1 - q \approx 3 \times 10^{4}.$$

The q-value is based on the rate of revertant formation and hence applies to the particular (complementary) substitutions

$$G \rightarrow A \quad \text{or} \quad C \rightarrow U, \text{ respectively.}$$

According to Eq. (20) the quality factors of both the plus and the minus strand contribute equivalently to the fidelity of reproduction. G → A and C → U substitutions are, therefore, equivalent. They may not differ too much from A → G and U → C replacements, the main cause being the similarity of wobbling for GU and UG interactions.

Since the replicating enzyme requires the template to unfold in order to bind to the active site, the q-values should not further depend on the secondary or tertiary structure of the template region. *In vitro* studies with a midi-variant of Q_β-RNA [27] yield rates for C → U substitutions which are consistent with the values reported above. Purine → pyrimidine and pyrimidine → purine substitutions seem to occur much less frequently and, hence, do not contribute materially to the magnitude of $\bar{q}$.

A determination of σ_m is more difficult, since it depends on the magnitude of $\bar{E}_{k \neq m}$. First of all, it is noteworthy that modification of an extracistronic region – which does not influence any protein encoded by the phage RNA – has such a considerable effect upon the replication rate. S. Spiegelmann was the first in stressing the importance of *phenotypic* properties of the phage RNA molecule with respect to the mechanism of replication and selection. The σ_m value reported above refers to a particular mutant and its satellites. Other mutants might influence the tertiary structure of Q_β-RNA in a different way and, hence, exhibit different replication rates. Moreover, mutations in intracistronic regions may be lethal and, therefore do not contribute to $\bar{E}_{k \neq m}$ at all. If we consider the measured value as being representative for the larger part of mutations we obtain for the maximum information content, then, a value only slightly larger than the actual size of the Q_β genome, which comprises about 4500 nucleotides. One might be somewhat suspicious with such a close agreement and we have mentioned our reservations. However, they refer mainly to the value of σ_m which enters only as a logarithmic term. Larger σ_m value would still yield acceptable limits for $\nu_{\max}$. Thus the value obtained may finally be not too far beyond reality.

There is another set of experiments, carried out by Ch. Weissmann and his coworkers [24], which indicates the presence of a relatively small fraction of standard phage in the wild-type distribution. These data suggest that $\sigma_m^{-1} \approx Q_m$ and that the actual number of nucleotides is indeed very close to the threshold value $\nu_{\max}$ (cf. Eq. 18).

The midi-variant, used in the evolution experiments of G. Mills and S. Spiegelmann et al. [27] consists of only 218 nucleotides and, hence, is not as well adapted to environmental changes as Q_β-RNA. It is, of course, optimally adapted to the special environment of the 'standard reaction mixture,' used in the test-tube experiments (which does not require the RNA particles to be infectious). However, its response to changes in the environment, e.g., to the addition of the replication inhibitor ethidium bromide, is fairly slow. The mutant obtained after twenty transfers, each allowing for a hundredthousand-fold amplification differs in only three positions from the wild-type of the midi-variant and shows a relative small selective advantage in the new environment. The reason for the slow response is that 218 nucleotides with an average single digit quality of 0.9995 yield Q values close to one and lead to wild-type sequences, that are very faithfully replicated, carrying along only a small fraction ($\lesssim 10\%$) of mutants in their error distribution.

The remarkable result of these studies in the light of theory is not the fact that the threshold relation as an inequality is fulfilled. Since its derivation is based on quite general logical inferences, any major disagreement would have indicated serious mis-

conceptions in our understanding of Darwinian systems. There is not the slightest reason for any such discrepancy, since we know quite well the molecular mechanism of self-replication in such a 'lucid' system as phage Q_β. The truly surprising result is that the actual value of ν not only remains below the threshold ν_{max}, but, in fact, resembles it so closely. The number of nucleotides could easily have been restricted by factors other than symbol quality $\bar{q}$, thereby yielding ν values far below the threshold allowing for a reproducible accumulation of information.

Hence, we are forced to conclude that these RNA phages during their evolution, indeed, tried to accumulate as much information as was possible, utilizing also large extracistronic, but phenotypically active regions in their genome. This fact does not exclude that under other (e.g., artificial) conditions much smaller RNA molecules, such as the mentioned midi-variant, could win the competition, or that under different natural circumstances also much smaller viable phages exist.

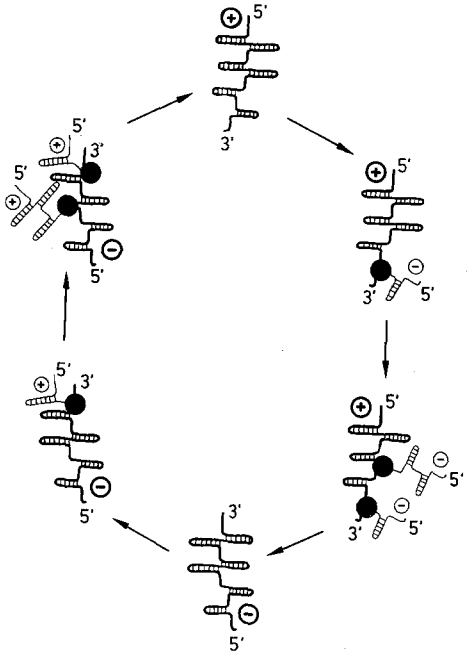

Fig. 11. *The replication cycle encountered in RNA phages* leads to single-stranded units of RNA with highly specific secondary and tertiary structures utilized as phenotypic targets [11, 34]. The formation of complete or partial duplices between plus and minus strands (the so-called Hofschneider or Franklin pairs, resp.) is prevented by an immediate internal folding of the newly synthesized strand. Using a midi-variant of the phage Q_β, S. Spiegelmann and coworkers [35] were able to demonstrate the existence of inhibitory effects due to duplex formation. Single-strand replication is based on an efficient interaction of the replicase with both the plus and the minus strand, requiring a certain symmetry in those regions of the tertiary structure which are phenotypically important.

Even more important is to realize that in nature no (single-stranded) RNA phage exists which comprises more than about 10000 nucleotides in its genome. This suggests that the enzymic mechanism of RNA replication, especially with respect to the discrimination of the bases A and G or U and C has reached its optimum and could not be improved further. It is not possible for any single-stranded RNA to maintain reproducibly more information than is equivalent to the order of magnitude of 1000 to 10000 nucleotides (the precise number depending on the value of σ_m).

Larger molecules could exist, of course, according to chemical criteria, but they would be of no evolutionary value. Moreover, the requirements for selective conservation of information must be fulfilled by both the plus and the minus strand, as prescribed by Eq. (20), although only one of the strands needs to carry the genetic information to be translated by the host mechanism. These conclusions apply only to RNA molecules which in their replication phase act as *single*-stranded templates and for which the mechanism depicted in Figure 11 has been proposed [11].

IV. 3. DNA-Replication

With double-stranded molecules, especially with DNA, we encounter a quite different situation. They generally reduplicate in the form of double-stranded units, i.e., they may be considered to be truly *self*-reproductive, at least in a phenomenological sense (even if the instruction conveyed is based again on the complementarity of the nucleotides).

Let us briefly summarize what is known [10] about reproduction of such double-stranded DNA molecules (cf. Fig. 12):

a) Replication is a semi-conservative process. Each of the two strands of the DNA duplex is copied during the reproduction phase, leading to two (essentially identical) duplices, each of which contains one parental strand.

b) Replication starts at a defined growing point and may proceed in both directions of the strand. The unwinding of the double strand is aided by so-called unwinding proteins, some of which have been isolated and identified. They enhance the rate of unwinding as much as thousand-fold, yielding a relatively fast movement of the replication fork. At the same time it is necessary to relieve the torque caused by the unwinding in some part of the molecule. It is suggested, that a chain break and repair mechanism, effected by endonucleases and ligases, may permit intermediate rotation of chain sections around a phosphodiester bond.

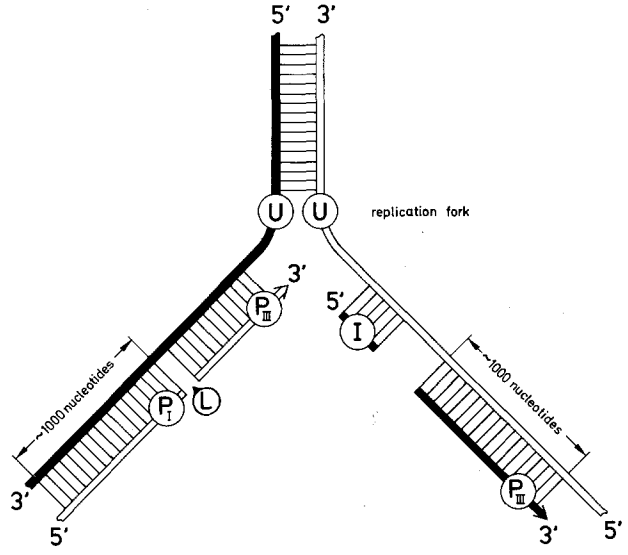

Fig. 12. *The semi-conservative replication of double-stranded DNA* is a highly sophisticated process including many steps of reaction and control of which the most important ones are indicated here [10]. Both daughter strands are polymerized in the $5' \rightarrow 3'$ direction. The unwinding of the parental double helix is effected concomitantly by an unwinding protein (U). The synthesis of new fragments is initiated by RNA primers formed with the help of an enzymic system (I) and later hydrolyzed away presumably by the nuclease function of polymerase-I (P_I). Chain elongation up to fragments with 1000 to 2000 nucleotides is believed to be effected mainly by the polymerase-III complex (P_{III}). Those nascent so-called Okazaki fragments have to be linked together by a ligase (L). Mispaired nucleotides at the 3'-end of the fragments (and only those) are excised by the $3' \rightarrow 5'$-exonuclease function, most likely of the polymerase-I complex (P_I), whose activity is predominantly associated with gap filling and repair. Other features, such as repair by $5' \rightarrow 3'$-exonuclease action, through which whole fragments of DNA can be removed, are not included in this scheme since they may not be as important for the synthesis of new strands.

c) Replication of *both* strands proceeds by inclusion of nucleotide residues in the 5'-3' direction. The DNA-polymerases can include the monomers only in this unique vectorial way, which cannot take place concomitantly at both strands. Electron microscopy with a resolution of about 100 Å has revealed the existence of single-stranded regions at only one side of the growing fork, suggesting that the other strand is completed only after a larger stretch, sufficient for a 5'-3' progression, has been created.

d) Replication occurs in short, discontinuous pulses. In prokaryotes, the fragments produced in a single pulse are about 1000 to 2000 nucleotides long. They are initiated by the formation of primers, for which very short segments of RNA serve. The fragments of copied DNA occurring along both strands behind the replication fork are later sealed together by ligases.

e) The various functions required in DNA replication have been identified by isolation of the particular enzymes and by demonstration of their activity. In particular, several polymerase complexes (I, II and III) have been characterized, which comprise polymerizing as well as several chain-decomposing functions. Of special interest here is the $3' \rightarrow 5'$ exonuclease activity of polymerase I. It allows for a preferential excision of a non-base-paired nucleotide at the 3'-terminus of the growing chain. Since chain growth occurs only in the $5' \rightarrow 3'$ direction this exonuclease function allows for a proofreading of the newly synthetized chain fragments. Its optimal activity is about 2% of that of polymerizing functions. The $3' \rightarrow 5'$ exonuclease is to be distinguished from a $5' \rightarrow 3'$ exonuclease, which also is part of the DNA-polymerase I complex and probably involved in excision repair. It acts only at the 5'-terminus and cleaves the di-ester bond at a base-paired region, possibly up to 10 residues apart from the 5'-end. It, hence, can remove oligonucleotides, while the proofreading $3' \rightarrow 5'$ enzyme only removes single non-base-paired nucleotides at the end of the growing chain.

We may conclude now an important difference between RNA and DNA replication, which is expressed in the average symbol quality factors for both mechanisms. In RNA replication the accuracy of information transfer has to be established in the continuous polymerization. However the RNA-replicase solves the problem, it achieves apparently a limiting value of $\bar{q}$ between 0.9990 and 0.9999. Approximately the same fidelity should be reached by any continuous DNA-polymerizing mechanism.

Mutant bacteriophage DNA-polymerases devoid of $3' \rightarrow 5'$-exonuclease activity have been tested *in vitro* and shown to incorporate errors with the relatively high frequency of about one for every thousand nucleotides. Similar results have been reported for purified *avian myoblastosis virus* DNA-polymerase. However, also lower error rates have been found. For instance, studies with small eukaryotic DNA-polymerases, lacking proofreading exonuclease activity, yielded values one order of magnitude lower than in the cases mentioned above (i.e., one for every five- to tenthousand nucleotides) [36–39].

The observed DNA fragment of 1000 to 2000 nucleotides, appearing during DNA polymerization (in prokaryotic cells) may also directly refer to the limited fidelity of the polymerase function. Apparently, the polymerase cannot easily extend a mispaired terminus generated by itself ([10], p. 88), although this has been observed in the absence of exonucleases. On the other hand, 3'-5'-exonuclease if present will recognize the mismatch and excise the wrong nucleotide. There is

no reason to assume that the optimal resolving power at this step is much different from that in polymerization. Hence, proofreading may reduce the error rate (optimally) by another three orders of magnitude.

Correction of errors, on the other hand, cannot be postponed to any later stage, i.e., after both chains have been completed. Although repair systems using $5' \rightarrow 3'$-nuclease activities do exist, they cannot deter-

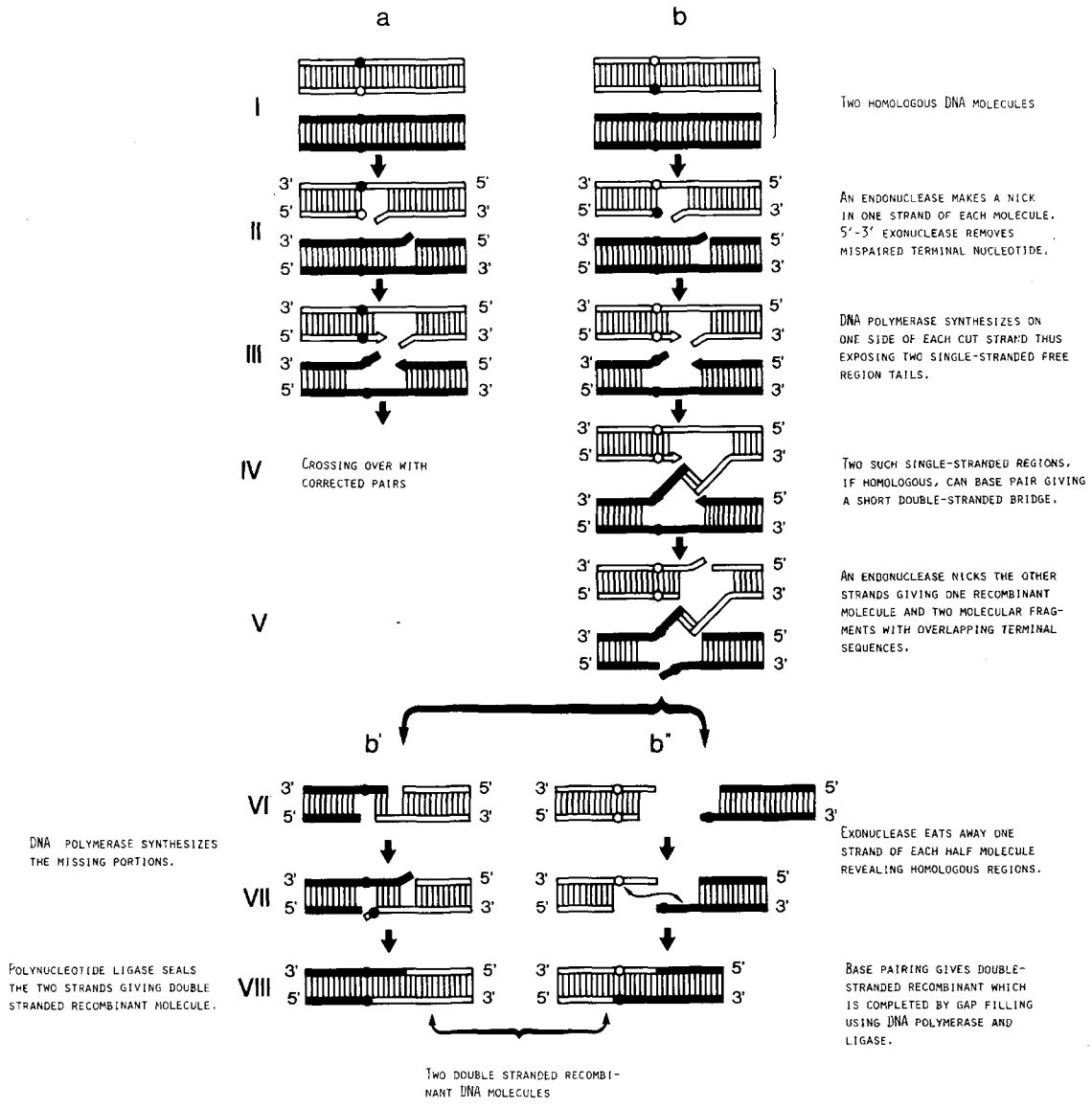

Fig. 13. *Genetic recombination allows for error detection* in completed double strands of DNA. This model was originally proposed to explain the mechanism of crossing over. It can be applied to error correction as well. The symbol ● designates a genetically correct, the symbol ○ an erroneous nucleotide. Accordingly ● always resembles the correct complementary, ○ the mismatched (non-complementary) and ○ the complementary, but erroneous nucleotide pair, regardless of which of the four nucleotides is involved. Assume that strand nicking is triggered by a mismatched pair (stage II). Then $3' \rightarrow 5'$-exonuclease action will correct the error: ○ → ● in 50% of the cases (stage III), while in the other 50% the wrong nucleotide will complement itself: ○ → ○. The incorrect (though complementary) pair, however, is not fixed as in a simple repair mechanism. Recombination with the correct copy (stage VI, VII) will restore the original situation (stage I and VIII) in which only one of four homologous positions is occupied by an incorrect nucleotide. Hence iteration of the procedure can lead to a steady reduction, rather than to a 50%-irreversible fixation of errors. This scheme points out that crossing over is associated with error checking in completed strands. The scheme (copied from [45]) can be understood on the basis of known functions of DNA-polymerase, which does not exclude the existence of other hypothetical and, even more efficient mechanisms. A complete understanding of the fidelity problem, which has to include a consideration of vegetative multiplication processes, requires a more detailed knowledge of the mechanism than is available hitherto

mine which of the two strands contains the mismatched member of the incorrect pair (cf. [36]). More detailed mechanisms of kinetic proofreading have been proposed [40] and experimentally tested [41, 42] (for a review cf. [43]).

We may therefore conclude: The optimum average symbol quality for DNA replication reaches values of 0.999999 or somewhat higher, thus allowing for an accumulation of information of up to an equivalent of one to ten million nucleotides (depending on the magnitude of σ_m). It is gratifying to notice that this number coincides with known sizes of genomes in prokaryotic cells (e.g. *E. coli:* 4×10^6 base pairs). Again there is no need at all for any individual to reach this limit. Other restrictions, such as packing requirements in the case of DNA phages, etc., may limit the actual size of a genome. As for RNA phages, any intermediate size below the threshold may, thus, be observed.

There is an upper limit for the genetic information content of a prokaryotic cell. Just as any extension beyond the single-strand information capacity of 10^4 nucleotides requires a new mechanism involving double-stranded templates and proofreading enzymes, the new limit of about 10^7 nucleotides set by the prokaryotic DNA-reproduction mechanism could not have been exceeded until another mechanism for further reduction of errors was available. Such a mechanism, namely genetic recombination was invented by nature at the prokaryotic level. However, it took about two to three billion (10^9) years before it reached perfection in order to give rise to another extension of the genetic information content of single individuals.

The process of genetic recombination utilized by all eukaryotic cells requires two alleles to be identified at their homologous positions. Since the error rate for the enzymic DNA reproduction is below 10^{-6} per nucleotide, uncorrected mistakes are very rare and cannot be present in more than one of the four equivalent sites of the two alleles. Hence, there is a further opportunity to identify and correct those errors in the recombinants, even if they occur in formerly completed duplices. A possible scheme is depicted in Figure 13. However, the mechanism of recombination is neither yet known in sufficient detail, nor is it clear how many steps finally are responsible for the further reduction of the error rate. The fact is that such a reduction *has* been achieved, as is revealed by an analysis of evolutionary trees, and that it is an important prerequisite for the expansion of genetic information capacity up to the level of man.

IV. 4. The First Replicative Units

For a discussion of the origin of biological information we have to start at the other end of the evolutionary scale and analyze those mechanisms which led to the first reproducible genetic structures. The physical properties inherent to the nucleotides effect a discrimination of complementary from non-complementary nucleotides with a quality factor $\bar{q}$ not exceeding a value of 0.90 to 0.99. The more detailed analysis based on rate and equilibrium studies of cooperative interactions among oligonucleotides has been presented elsewhere [4, 44]. In order to achieve a discrimination between complementary and non-complementary base pairs according to the known differences in free energies, the abundant presence of catalytically active, but otherwise uncommitted proteins as environmental factors might have helped. However, uncommitted protein precursors in some cases will favor the complementary, in other cases the non-complementary, interaction. Any preference of one over the other can only be limited to the difference of free energies of the various kinds of pair interaction. Any specific enhancement of the complementary pair interaction would require a convergent evolution of those particular enzymes which favor this kind of interaction. In order to achieve this goal they must themselves become part of the self-reproducing system which in turn requires the evolution of a translation mechanism.

The first self-reproductive nucleic acid structures with stable information content – given optimal $\bar{q}$ values of 0.90 to 0.99 – were t-RNA-like molecules. For any reproducible translation system, however, an information content larger by at least one order of magnitude would be required. As we know from the analysis of RNA-phage replication, such a requirement can be matched only by optimally adapted replicases, which could not have evolved without a perfect translation mechanism. The phages, we encounter today, are late products of evolution whose existence is based on the availability of such a mechanism, without which nature could not afford to accumulate as much information in one single nucleic acid molecule. Hence, there was a barrier for molecular evolution of nucleic acids at the level of t-RNA-like structures similar to those barriers we find at later stages of evolution, requiring some new kind of mechanism for enlarging the information capacity.

The t-RNA's or their precursors, then, seem to be the 'oldest' replicative units which started to accumulate information and were selected as a quasi-species, i.e., as variants of the same basic structure.

The first requirement was stability towards hydrolysis. It has been shown by a game model, similar to the one described in IV. 1., that the presently known secondary (and tertiary) structure of t-RNA (cf. Figs. 14a and b) is a direct evolutionary consequence of this requirement. The symmetry of this structure, furthermore, reflects the optimization of its replicative mechanism which, according to Eq. (20), supposes equivalent behavior of the plus and the minus strand

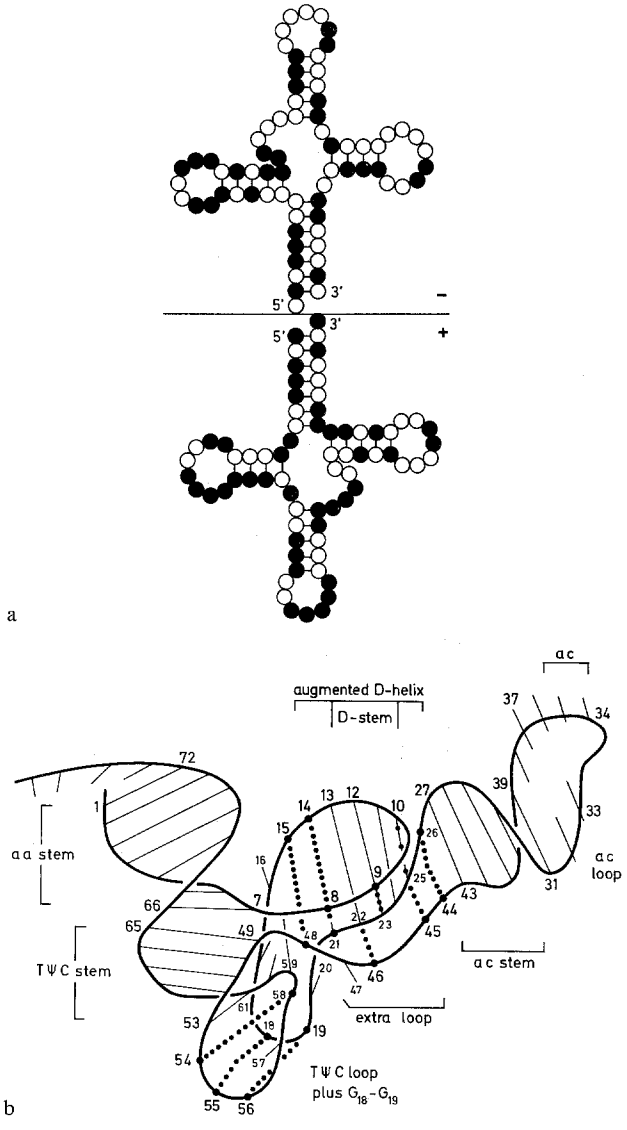

Fig. 14. *Symmetry of functional RNA molecules,* as exemplified with t-RNA^{phe}, aids single-strand replication by specifically adapted enzymes. The plus and minus strands of the symmetrical structure are distinguished by common phenotypic features. Although t-RNA's in present organisms are genotypically encoded, their symmetry might still reflect the ancestrial mechanism of single-stranded RNA reproduction, for which plus and minus strand are equally important. The symmetry is most obvious in the secondary structure (a), but shows up accordingly also in the tertiary structure (b) (reproduced from [46])

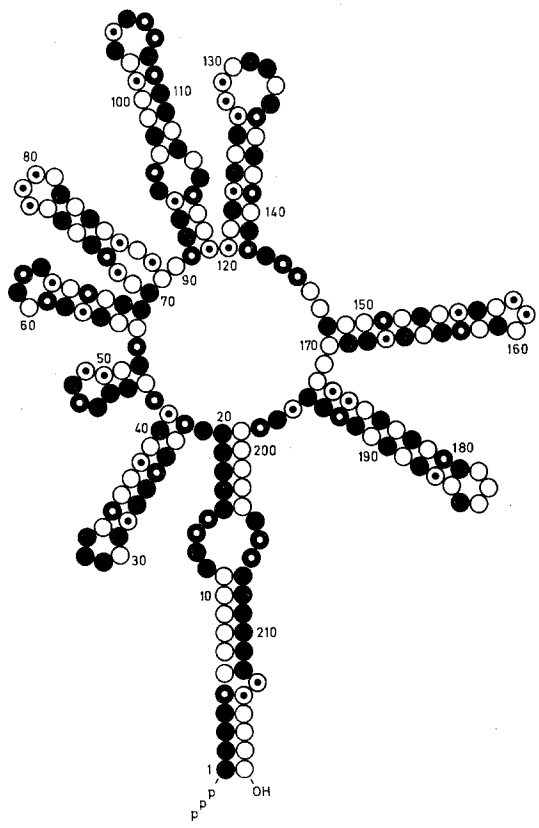

Fig. 15. *'Flower' model of Spiegelmann's midi-variant of Q_β-RNA (plus strand).* Symmetry requirements are less important where the information is mapped in genotypes which are reproduced via standardized polymerization mechanisms. The midi-variant of phage Q_β is selected *solely* for its phenotypic information, in that it exhibits an optimal target structure for recognition by the enzyme Q_β-replicase. This property must be inherited by both the plus and the minus strand. The symmetry of the structure becomes most obvious in the 'flower' model, although this arrangement probably does not represent the natural structure of the active molecule. According to the mechanistic conditions of single-strand replication shown in Figure 11, a model admitting immediate chain folding during synthesis [27] should be advantageous

in order to yield optimal performance. This symmetry can also be found at the level of RNA phages, especially for variants which are selected for being phenotypically most efficient with respect to *in vitro* replication, but otherwise not carrying genetic information (Fig. 15).

IV. 5. The Need for Hypercycles

It is the object of this paper to show, first that the breakthrough in molecular evolution must have been brought about by an integration of several self-reproducing units to a cooperative system and, second that a mechanism capable of such an integration can be provided only by the class of hypercycles. This conclu-

sion again can be drawn from logical inferences, based on the following arguments:

The information content of the first reproductive units was limited to $\nu_{max} \lesssim 100$ nucleotides. Several of those units representing similar functions but different specificities were required to build up a translation system. Such a system might have emerged from one quasi-species, but the equivalent partners had to evolve simultaneously. This is neither possible by linking them up into a larger self-reproductive unit (because of the error threshold), nor could it result from compartmentation, because of the strong competition among the equivalent self-reproductive units within the compartment. Such a process rather requires functional linkages among all self-reproductive units, to be distinguished by the following qualities:

a) The linkage must still permit competition of each self-reproductive unit with its error copies, otherwise these units cannot maintain their information.

b) The linkage must 'switch off' competition among those self-reproductive units which should be integrated to a new functional system and allow for their cooperation.

c) The integrated functional system then must be able to compete favorably with any other less efficient system or unit.

These three requirements can be fulfilled only by a cyclic linkage among self-reproductive units or, in other words, the functional linkage among autonomous self-reproductive units itself has to be of a self-enhancing cyclic nature, otherwise their total information content cannot be maintained reproducibly. Hypercyclic organization, thus, appears to be a necessary prerequisite for the nucleation of integrated self-reproductive systems of larger information content, as were required for the origin of translation. This statement is the conclusion of what is to be shown in the subsequent parts by a more detailed analysis of linked systems.

If we are asked, "What is particular to hypercycles?", our answer is, "They are the analogue of Darwinian systems at the next higher level of organization." Darwinian behavior was recognized to be the basis of generation of information. Its prerequisite is integration of self-reproductive symbols into self-reproductive units which are able to stabilize themselves against the accumulation of errors. The same requirement holds for the integration of self-reproductive and selectively stable units into the next higher form of organization, in order to yield again selectively stable behavior. Only the cyclic linkage—as an equivalent of autocatalytic reinforcement at this level (cf. II.)—is able to achieve this goal.

Table 4. The essential stages of information storage in Darwinian systems

Digit error rate $1-\bar{q}_m$	Superiority σ_m	Maximum digit content ν_{max}	Molecular mechanism and example in biology
5×10^{-2}	2	14	enzyme-free RNA replication[a]
	20	60	
	200	106	t-RNA precursor, $\nu=80$
5×10^{-4}	2	1386	single-stranded RNA replication via specific replicases
	20	5991	
	200	10597	phage Q_β, $\nu=4500$
1×10^{-6}	2	0.7×10^6	DNA replication via polymerases including proofreading by exonuclease
	20	3.0×10^6	
	200	5.3×10^6	*E. coli*, $\nu=4\times10^6$
1×10^{-9}	2	0.7×10^9	DNA replication and recombination in eukaryotic cells
	20	3.0×10^9	
	200	5.3×10^9	vertebrates (man), $\nu=3\times10^9$

[a] Uncatalyzed replication of RNA never has been observed to any satisfactory extent; however, catalysis at surfaces or via not specifically adapted proteinoids (as proposed by S.W. Fox) may involve error rates corresponding to the values quoted.

The results of section IV are summed up in Table 4, showing the essential stages of information storage in Darwinian systems, which could be facilitated by various storage mechanisms of reproduction.

This table will be useful for a discussion of a model of continuous evolution from single molecules to integrated cellular systems, as presented in part C.

1. Wright, S.: Genetics *16*, 97 (1931)
2. Woese, C.R.: The Genetic Code. New York: Harper and Row 1967
3. Crick, F.C.R., et al.: Origins of Life *7*, 389 (1976)
4. Eigen, M.: Naturwissenschaften *58*, 465 (1971)
5. Bethe, H., in: Les Prix Nobel en 1967, p. 135. Stockholm 1969
6. Krebs, H., in: Nobel Lectures, Physiology or Medicine 1942–1962, p. 395. Amsterdam: Elsevier 1964
7. Spiegelmann, S.: Quart. Rev. Biophys. *4*, 213 (1971); Haruna, I., Spiegelmann, S.: Proc. Nat. Acad. Sci. USA *54*, 579 (1975); Mills, D.R., Peterson, R.L., Spiegelmann, S.: ibid. *58*, 217 (1967)
8. Sumper, M., Luce, R.: ibid. *72*, 1750 (1975)
9. Küppers, B.-O.: Naturwissenschaften (to be published)
10. Kornberg, A.: DNA Synthesis. San Francisco: W.H. Freeman 1974
11. RNA-Phages (Zinder, N.D., ed.). Cold Spring Harbor Monograph Series, Cold Spring Harbor Laboratory 1975
12. Fisher, R.A.: Proc. Roy. Soc. B *141*, 510 (1953); Haldane, J.B.S.: Proc. Camb. Phil. Soc. *23*, 838 (1927); Wright, S.: Bull. Am. Math. Soc. *48*, 233 (1942)
13. Eigen, M.: Ber. Bunsenges. physik. Chem. *80*, 1059 (1976)
14. Dobzhansky, Th.: Genetics of the Evolutionary Process. New York: Columbia Univ. Press 1970
15. Darwin, Ch.: Of the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle

for Life. Paleontological Society 1854. The Origin of Species, Chapter 4, London 1872; Everyman's Library, London: Dent and Sons 1967
16. Darwin, Ch., Wallace, A.R.: On the Tendency of the Species to Form Varieties and on the Perpetuation of the Species by Natural Means of Selection. J. Linn. Soc. (Zoology) *3,* 45 (1858)
17. Eigen, M., Winkler-Oswatitsch, R.: Ludus Vitalis, Mannheimer Forum 73/74, Studienreihe Boehringer, Mannheim 1973
18. Eigen, M., Winkler-Oswatitsch, R.: Das Spiel. München: Piper 1975
19. Schrödinger, E.: What is Life? Cambridge Univ. Press 1944
20. Thompson, C.J., McBride, J.L.: Math. Biosci. *21,* 127 (1974)
21. Jones, B.L., Enns, R.H., Rangnekar, S.S.: Bull. Math. Biol. *38,* 15 (1976); Jones, B.L.: ibid. *38,* XX (1976)
22. Küppers, B.-O.: Dissertation, Göttingen 1975
23. Glansdorff, P., Prigogine, I.: Thermodynamic Theory of Structure, Stability and Fluctuations. New York: Wiley-Interscience 1971
24. Sabo, D., et al.: to be published
25. Kimura, M., Ohta, T.: Theoretical Aspects of Population Genetics. Princeton, New Jersey: Princeton Univ. Press 1971
26. King, J.L., Jukes, T.H.: Science *164,* 788 (1969)
27. Kramer, F.R., et al.: J. Mol. Biol. *89,* 719 (1974)
28. Hoffmann, G.: Lecture at Meeting of the Senkenbergische Naturforscher Gesellschaft, April 1974
29. Tyson, J.J., in: Some Mathematical Questions in Biology (ed. Levin, S.A.). Providence, Rhode Island: AMS Press 1974
30. Shannon, C.E., Weaver, W.: The Mathematical Theory of Communication. Urbana: Univ. of Illinois Press 1949
31. Brillouin, L.: Science and Information Theory. New York: Academic Press 1963
32. Domingo, E., Flavell, R.A., Weissmann, Ch.: Gene *1,* 3 (1976)
33. Batschelet, E., Domingo, E., Weissmann, Ch.: ibid. *1,* 27 (1976)
34. Weissmann, Ch., Feix, G., Slor, H.: Cold Spring Harbor Symp. Quant. Biol. *33,* 83 (1968)
35. Spiegelmann, S.: Lecture at the Symposium: Dynamics and Regulation of Evolving Systems, Schloß Elmau, May 1977
36. Hall, E.W., Lehmann, I.R.: J. Mol. Biol. *36,* 321 (1968)
37. Battula, N., Loeb, L.A.: J. Biol. Chem. *250,* 4405 (1975)
38. Chang, L.M.S.: ibid. *248,* 6983 (1973)
39. Loeb, L.A., in: The Enzymes, Vol. X, p. 173 (ed. P.D. Boyer). New York-London: Academic Press 1974
40. Hopfield, J.J.: Proc. Nat. Acad. Sci. USA *71,* 4135 (1974)
41. Englund, P.T.: J. Biol. Chem. *246,* 5684 (1971)
42. Bessman, M.J., et al.: J. Mol. Biol. *88,* 409 (1974)
43. Jovin, T.M.: Ann. Rev. Biochem. *45,* 889 (1976)
44. Pörschke, D., in: Chemical Relaxation in Molecular Biology, p. 191 (Pecht, I., Rigler, R., eds.). Heidelberg: Springer 1977
45. Watson, J.D.: The Molecular Biology of the Gene. New York: Benjamin 1970
46. Ladner, J.E., et al.: Proc. Nat. Acad. Sci. USA *72,* 4414 (1975)

Received July 8, 1977