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Selforganization of Matter and the Evolution of Biological Macromolecules

MANFRED EIGEN*

Max-Planck-Institut für Biophysikalische Chemie,
Karl-Friedrich-Bonhoeffer-Institut, Göttingen-Nikolausberg

I. Introduction

I.1. „Cause and Effect"

The question about the origin of life often appears as a question about "cause and effect". Physical theories of macroscopic processes usually involve answers to such questions, even if a statistical interpretation is given to the relation between "cause" and "effect". It is mainly due to the nature of this question that many scientists believe that our present physics does not offer any obvious explanation for the existence of life, which even in its simplest forms always appears to be associated with complex macroscopic (i.e. multimolecular) systems, such as the living cell.

As a consequence of the exciting discoveries of "molecular biology", a common version of the above question is: *Which came first, the protein or the nucleic acid?*—a modern variant of the old "chicken-and-the-egg" problem. The term "first" is usually meant to define a causal rather than a temporal relationship, and the words "protein" and "nucleic acid" may be substituted by "function" and "information". The question in this form, when applied to the interplay of nucleic acids and proteins as presently encountered in the living cell, leads ad absurdum, because "function"

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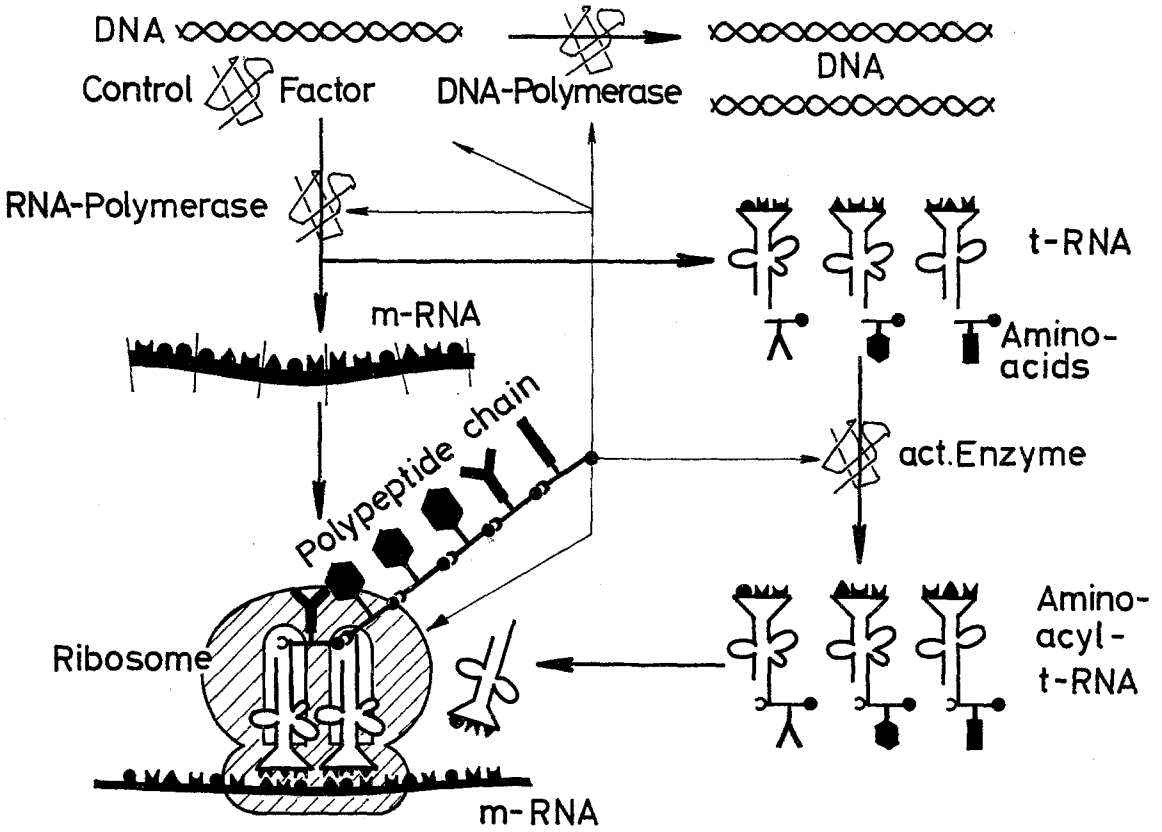

Fig. 1. The selfreproducing biosynthesis cycle of the cell

Table 1. *Nucleic acids and proteins are intimately linked together in their reproduction cycle*

Their important functional links are:

1. DNA and DNA Polymerase

Deoxyribonucleic acid (DNA), the stable source of information, is copied with the help of the enzyme DNA polymerase. The Kornberg enzyme, which catalyzes DNA polymerization was thought to be the functional unit; but there are indications[3] that a different protein complex of higher molecular weight, probably bound to membrane structures, represents the actual replication machine of the cell, whereas the Kornberg enzyme has repair functions.

Literature. DNA: J. D. Watson and F. H. C. Crick [1]. DNA polymerase: A Kornberg [2], P. de Lucia, J. Cairns [3].

2. m-RNA and RNA Polymerase

Information containing the instruction for protein synthesis is "transcribed" from DNA to a single stranded, more easily "readable" form, the messenger-RNA, again with the help of an enzyme. The RNA polymerase (e.g. from *E. coli*) is well characterized: M. W. $\sim 5 \cdot 10^5$; it has several subunits ($\beta' \beta \alpha_2 \omega \sigma$) containing specific factors for control.

Literature. "Transcription of Genetic Mutagenicity" (Cold Spring Harbor Symposium, cf. Ref. [4]).

3. t-RNA's and Amino Acyl Synthetases (Activating Enzymes)

The recognition of the different amino acids by their adaptors, the transfer-RNA molecules, requires a "second code" provided by the recognition sites of the amino acyl synthetases. The *t*-RNA's are molecules of relatively low M.W. (about 70 to 80 nucleotides, cf. Fig. 2) whose compositions (base sequences) in some cases are known. The amino acid is linked to its specific *t*-RNA adaptor in an energy coupled reaction with the help of its specific activating enzyme, the amino acyl synthetase. Several laboratories are concerned with the study of this enzyme which represents the important link between nucleic acid and protein code [6].

Literature. *t*-RNA: R. W. Holley [5a], H. G. Zachau [5b], G. Khorana [5c]. — Activating enzymes: P. Berg [6].

4. Ribosomal RNA and Proteins

Protein synthesis occurs at the ribosome, which is a complex consisting of RNA and protein subunits with a total molecular weight of about $2.7 \cdot 10^6$. It can be split easily into two fragments which sediment in the ultracentrifuge as 50 S and 30 S particles, respectively. The smaller fragment contains the binding site for the messenger, the larger one the catalytic site for peptide bond formation. Both subunits participate in the amino acyl *t*-RNA binding in response to the *m*-RNA. It has recently been possible to take apart the fragments into their single protein and RNA-subunits, to characterize and also to reassemble them.

Literature. M. Nomura [7], R. A. Garret and H. G. Wittmann [8].

5. Operon, Operator, Promotor and Repressor

Transcription is a highly regulated process, involving induction and repression. The regulation occurs by protein subunits (e.g. σ-factor) which cooperate with, or are part of RNA polymerase. A well studied case of gene control is the repression and derepression of the lac operon. The repressor is a protein of M.W. 150000 (4 identical subunits) which interacts with a specific repressor site of the DNA molecule (containing about 10 to 20 base pairs). Derepression occurs via complexation of the repressor with a (low molecular weight) inducer. The reaction mechanisms have been studied in detail.

Literature. Lac operon: F. Jacob and J. Monod [9]. — Repressor: W. Gilbert and B. Müller-Hill [10]. — Kinetics: A. D. Riggs, S. Bourgeois and M. Cohn [11].

The literature quoted refers to some key publications. For a more detailed study of problems of molecular biology reference is made to J. D. Watson: "The Molecular Biology of the Gene" [12] and to "Molekularbiologie" [13] (in German).

cannot occur in an organized manner unless "information" is present and this "information" only acquires its meaning *via* the "function" for which it is coding.

Such a system may be compared to a closed loop. Although it is evident that the line from which the loop is formed must have originated somewhere, the starting point will have lost all its importance as soon as the circle is closed. The present interplay of nucleic acids and proteins corresponds to a complex hierarchy of "closed loops" (cf. Fig. 1 and Table 1). What is required in order to solve such a problem of interplay between cause and effect is a *theory of selforganization* which can be applied to molecular systems, or more precisely, to special molecular systems under special environmental conditions. We may envisage that such a process of molecular selforganization includes many random events which do not have any instructed functional significance. What really matters is how certain such random effects are able to feed back to their origin and thus become themselves the cause of some amplified action. Under certain external conditions such a multiple interplay between cause and effect may build up to a macroscopic functional organization, which includes selfreproduction, selection and evolution to a level of sophistication where the system can escape the prerequisites of its origin and change the environment to its own advantage.

I.2. Prerequisites of Selforganization

I.2.1. Evolution Must Start from Random Events

At the "beginning"—whatever the precise meaning of this may be—there must have been *molecular chaos*, without any functional organization among the immense variety of chemical species. Thus, the self-organization of matter we associate with the "origin of life" must have *started* from random events[1]. This statement, however, does not imply that any—even primitive—organisms as we know them today can assemble in a random fashion.

Some years ago, E. Wigner [14] wrote an article: *"On the Probability of the Existence of a Self-Replicating Unit"*, in which he implicitly made such an assumption. In essence, his reasoning was as follows: Assuming that a "living state" as well as the states of its nutrients and metabolic products are completely given in the quantum mechanical sense and therefore can be described by state vectors in Hilbert space, the reproduction process—i.e. the interaction of the organism with the nutrient resulting in the reduplication of the organism—can be expressed by a transformation involving a unitary *"collision matrix"* S. If S is assumed to be a random matrix, then it turns out that the number of equations representing the transformation is in very large excess of the number of the unknowns, i.e. the components of the vectors—the excess being as large as N^2R compared to $(N+R+NR)$ for any large number of N and R. Actually for any realistic case, N and R are such large numbers that—as Wigner correctly concludes—"it would be a miracle" if the transformation equations were satisfied by the unknowns (a statement which still holds if many alternative states represent the living organism). However, the whole argument rests on the assumption that S *is* essentially a random matrix and the interaction is therefore not "instructed", so that any given state is almost infinitely unlikely as compared to the large number of possible states. Thus, the result can lead only to the conclusion that any sophisticated state of matter which we now call "living" cannot come about by random assembly. The presence of instruction at the molecular level, which implies that the transformation matrix S must have a very specific form, may require an adaptation of statistical mechanics to special prerequisites of selective and evolutionary processes, but does not necessarily indicate "that the present laws and concepts of quantum mechanics will have to undergo modifications before they can be applied to the problem of life" (E. Wigner [14]).[2]

I.2.2. Instruction Requires Information

I believe it was N. Wiener who once proposed that information be regarded as a new variable in physics. We do have at our disposal a fairly advanced "information theory" which originated from the pioneering work of J. von Neumann, N. Wiener, C. Shannon and others (cf. Refs. [15, 16]). Can we use this theory to solve our problem of selfinstruction?

Information theory as we understand it today is more a *communication theory*. It deals with problems of processing information rather than of "generating" information. It requires information to be present *"ab initio"* in a well-defined form. Then the theory can tell us how to code a message and how to utilize redundancy; it can also tell us how to match a message with the capacities of processing machinery in order to transmit it through noisy channels, to filter out the noise and to retrieve the message with the help of code checking devices; but it always requires "somebody"—usually man—to define beforehand what to call "information" and what to call "nonsense".

This is expressed already in the definition of *information*. If—in the simplest case—we want to select a situation with Z_1 out of Z_0 original outcomes of equal a priori probability, then in order to reduce the Z_0 to Z_1 possibilities, we need the information content

$$I_1 = K \ln (Z_0/Z_1). \tag{I-1}$$

If Z_1 represents one defined outcome, i.e. $Z_1 = 1$, then the required information content is

$$I = K \ln Z_0. \tag{I-2}$$

This is a definition. It has been chosen to convert joint probabilities—which are multiplicative combinations of the probabilities of the single independent events—into quantities which are additive. Thus, if Z_0 represents all possibilities of combining λ types of digits to sequences of ν, we have with $Z_0 = \lambda^\nu$:

$$I_{\lambda\nu} = K\nu \ln \lambda. \tag{I-3}$$

1 The term "random", of course, refers to the non-existence of functional organization and not to the absence of physical (i.e. atomic, molecular or even supramolecular) structures.

2 *Note Added in Proofs.* It is not my intention to discuss here certain difficulties of the application of quantum mechanics to "macroscopic processes". These difficulties occur as well with other well known physical processes, which are not associated with the phenomenon of "life". This point was brought to my attention by H. Primas Zürich.

The constant K is chosen to fit a binary code, i.e. $K = 1/\ln 2$. Hence, if $\lambda = 2$, I is expressed by the total number ν of binary digits ("bits") which represent the message.

The information content of a sequence of n nucleotides ($\lambda = 4$) thus involves $2n$ bits. The translation from a nucleotide to an amino acid code ($\lambda = 20$) requires at least triplets as coding units. This also allows for some redundancy as well as for start and termination signals. The table of the genetic code (Table 2) which we shall have to consult in more detail in part VI, is completely known today, mainly thanks to the work of G. Khorana, H. Matthaei, M. Nirenberg, S. Ochoa and their coworkers (cf. Refs. [17–19]).

Table 3 demonstrates quite dramatically the *enormous information capacity of biological macromolecules.* The probability of finding reproducibly, under any reasonable circumstances (i.e. in volumes of reasonable dimensions), any *given* sequence from a random distribution approaches practically zero for a relatively short chain length. Such sequences cannot yet contain any appreciable amount of information.

Table 2. *The genetic code*

first position	*second position* U	C	A	G	*third position*
U	phe	ser	tyr	cys	U
	phe	ser	tyr	cys	C
	leu	ser	term	term	A
	leu	ser	term	trp	G
C	leu	pro	his	arg	U
	leu	pro	his	arg	C
	leu	pro	gln	arg	A
	leu	pro	gln	arg	G
A	ile	thr	asn	ser	U
	ile	thr	asn	ser	C
	ile	thr	lys	arg	A
	met[a]	thr	lys	arg	G
G	val	ala	asp	gly	U
	val	ala	asp	gly	C
	val	ala	glu	gly	A
	val[a]	ala	glu	gly	G

Each amino acid denoted by its initial letters—is coded by a triplet of the four bases U, C, A, G. At first glance one sees the high redundancy within each segment indicating the minor importance of the third letter of the triplet. (For details of third letter substitution cf. Crick's "wobble-hypothesis" Ref. [20].) One also sees a certain similarity of amino acids in the vertical direction, indicating the high significance of the second letter. Triplets containing only U and A show a larger variety of functional amino acids (phe, leu, tyr, ile, asn, lys + 1 signal) than triplets containing only G and C (pro, arg, ala, gly). All these facts may contain certain information about the origin of the code and the various theories must be checked against them. Although the code seems to be universal, the assignments (especially the "signals") refer to *E. coli.*

The amino acids are:

ala	= alanine	met	= methionine[a] (formyl methionine)
arg	= arginine		
asn	= asparagine	phe	= phenylalanine
asp	= aspartic acid	pro	= proline
cys	= cysteine	ser	= serine
glu	= glutamic acid	thr	= threonine
gln	= glutamine	trp	= tryptophane
gly	= glycine	tyr	= tyrosine
his	= histidine	val	= valine[a]
ile	= isoleucine	term	= end chain
leu	= leucine	met, val	= start chain[a]
lys	= lysine		

[a] The triplets AUG and GUG mean "start chain" or *f*-met if they occur at the beginning of a cistron; they mean met or val respectively within the cistron.

Table 3. *ν digits of basis λ have $N_{\lambda\nu} = \lambda^\nu$ possible sequences*

Examples	λ	ν	$N_{\lambda\nu}$
Small subunits of natural proteins (e.g. M.W. 1200)	20	100	10^{130}
Polypeptides, resulting from AU code only (cf. Table 2)	6	100	10^{78}
DNA chain, coding for 33 amino acids	4	99	10^{60}
AU copolymer coding for 33 amino acids of the AU codon class	2	99	10^{30}
Oligopeptide containing any 12 out of the 20 natural amino acids	20	12	$4 \cdot 10^{15}$
Oligopeptide containing 20 amino acids of the AU codon class	6	20	$4 \cdot 10^{15}$

For comparison: Number of protein molecules of M.W. 10^4

a) assuming closest packing

Universe	10^{103}
1 m thick layer on surface of earth	$2 \cdot 10^{40}$
1 m^3	$6 \cdot 10^{25}$

b) contained in a 10^{-3} M solution (corresponding to a "soup" of appreciable viscosity)

All oceans	10^{42}
pond (100 × 100 m 10 m deep)	$6 \cdot 10^{28}$
puddle (1 liter)	$6 \cdot 10^{20}$

Note that most natural proteins and nucleic acids have much higher molecular weights than the quoted examples. Note further, that the age of earth is "only" about 10^{17} seconds, so that assuming even a fast turnover of protein molecules one never could scan through all possible sequences (the life and assembly times for each molecule are certainly longer than 1 sec). Only orders of magnitude are quoted. The "universe" has been assumed to be a sphere with a diameter of 10 billion light years; closest packing was interpreted as space filling with a density of 1 g/cm^3.

On the other hand, the total information content of a highly sophisticated living entity, stored in the DNA chains of the chromosomes may exceed 10^{10} bits, representing one choice out of about $10^{3\,\text{billion}}$ possible. There have been attempts to correlate such numbers with the information contained in the structures and functions the DNA is coding for. As will be seen later, this is not possible if one disregards the environment and history of the corresponding entity. The information resulting from evolution is a "valued" information and the number of bits will not tell us too much about its functional significance.

The *definition of information* given requires some modification if we have a set of digits with different a priori probabilities p_1, $p_2 \ldots p_\lambda$. This is certainly the case for amino acids in a polypeptide chain, as it is true also for letters in the different languages. Here the average information per digit is, according to C. Shannon [21] (the symbol is chosen in analogy to Boltzmann's H-function):

$$h = -K \sum_{i=1}^{\lambda} p_i \ln p_i \quad \text{with} \quad \sum_{i=1}^{\lambda} p_i = 1. \qquad \text{(I-4)}$$

It is seen that any constraint (e.g. different redundancies) lowers the average information content per letter as compared to equal a priori probabilities. This con-

straint in the English language, for instance, reduces the average information content from 4.76 to 4.03 bits per letter. (Other constraints, such as preferred successions of letters or words will further modify this number.)

The analogy of Shannon's concept to Boltzmann's statistical interpretation of entropy (cf. Eqs. (I-2) and (I-4)) is obvious and was always recognized as of more than a formal nature (cf. Ref. [22]). It was particularly stressed by E. Schrödinger in his remarkable booklet: "What is Life?" [23]. If entropy characterizes the amount of "unknowledge", then any decrease of "unknowledge" is equivalent to an increase of "knowledge" or "information". This complementarity between information and entropy shows clearly the limited application of classical information theory to problems of evolution. Whereever information has a defined meaning, e.g. as in language by agreement, or as in biology (after evolution has brought about the fixation of a code), there are numerous applications of this theory. However, it is of little help as long as information has not yet reached its "full meaning", or as long as there are still many choices for generating new information. Here we need a new variable, a "value" parameter, which characterizes the level of evolution. A complementary description of order and disorder—as somewhat overemphasized by E. Schrödinger in his booklet—is not sufficient. This inadequacy of present information theory in treating biological problems was clearly pointed out by L. Brillouin [16] in his excellent monograph, "Science and Information Theory".

We see that to a certain extent "information theory" is complementary to classical statistical mechanics, at least with respect to the concept of entropy and information describing the degree of unknowledge and knowledge. For a theory of evolution, this concept is not sufficient. We need further specification of what we call knowledge or unknowledge. We need "valuation" in order to characterize the degree of selforganization of a functional order and to define a gradient for evolution.

I.2.3. Information Originates or Gains Value by Selection

This statement implies one of the essential principles of biology: Darwin's principle of natural selection. Darwin himself considered it a characteristic property of the living:

> *"This preservation of favourable individual differences and variations, and the destruction of those which are injurious, I have called Natural Selection, or the Survival of the Fittest"* [24].

He actually did not make any commitment about its physical origin: *"It is no valid objection that science as yet throws no light on the far higher problem of the essence or origin of life"* [25].

In population genetics, especially in the fundamental work of the great schools of R. A. Fisher [26], J. B. S. Haldane [27] and S. Wright [28], Darwin's principle was given a mathematical formulation. The difficulty of giving the *"value concept"*—which is behind this principle—a physically objective foundation has led to a reinterpretation, which will be considered in more detail at the end of this paper (cf. VIII.5.). If we want to close the gap between physics and biology, we have to find out what "selection" means in precise molecular terms which can ultimately be described by quantum-mechanical theory. We have to derive Darwin's principle from known properties of matter.

In order to associate the term selection more closely with molecular properties and to illustrate its meaning, let us play some games with proteins and nucleic acids.

We shall use dice, to represent randomness:

icosahedral dice for proteins,
tetrahedral for nucleic acids.

Each face of a die then will represent one of the twenty natural amino acids or one of the four nucleotides respectively. The aim of the game will be to reach a specified sequence of ν—say 100—digits by throwing the die for all digits in cyclic iteration and moving the corresponding digit into position.

Without any additional selection rule this game will turn out to be rather dull. Except for relatively small numbers of ν, it would be almost endless. We have already seen that a protein molecule with 100 amino acid residues has about 10^{130} different choices of sequences and we would have to throw the die a corresponding number of times in order to arrive at a specified one. It is just another example of Wigner's conclusion, showing that not even one single protein molecule with specified structure (and function) could come about by random assembly.

A quite simple *modification of the rules* would allow us to finish the game within a relatively short time. We introduce "selection" by attributing to each correctly occupied position a "selective advantage". In the extreme, this would mean that correctly occupied positions are not subject to further throws. Since (for proteins) N/20 positions in any random sequence are correctly occupied by chance, we see that now on average $20\,(N-N/20)=19\,N$ (e.g. for $N=100:1900$) throws will be sufficient to approach the specified sequence. Fluctuations are large enough to give each player an equal chance of winning. It is still quite a dull game—all the excitement would have to arise from the prize—but we see clearly the effect of extreme selection on a trial and error process. To approach the correct sequence takes only about 20 times as many trials as the fully instructed assembly.

Of course, nature plays much more sophisticated games. "Selective advantage" is usually not only a property of the one final amino acid or nucleotide residue, nor is it independent of the development in other positions. These couplings make the game intellectually more interesting, but they will require more "moves" and demand certain strategies. A—still fairly simple—example of such a "strategic" game, this time with nucleic acids, is represented in Table 4. It can show us why, in the assembly of t-RNA, *nature preferred "clover leaves" for the secondary structure.* For nucleic acids "advantage" is usually somehow related to the presence or absence of complementary base pairs. In the turnover of nucleic acids complementarity has a large effect on both the synthesis and decomposition rate (cf. Section IV). For proteins it is much more difficult to relate catalysis and control function—a property of the spatial, i.e. tertiary or quarternary structure—to primary sequence.

Table 4. *The t-RNA Game or How to Make Clover Leaves*

Given	For each player a random sequence of N digits of 4 classes, denoted by the letters A, U, G, C and a tetrahedral die, each face of which represents one of the 4 letters.
Aim	By throwing the die in turn and substituting (a position in the sequence) with the obtained digit, each player should try to approach a double-stranded structure with as many as possible AU or GC pairs.
Rules	The game is over whenever a player announces a "complete" structure. The winner is the one who then has the largest number of points, where each GC counts twice as many points as each AU pair. The constraint is that pairs must not be formed unless there is a succession of at least 2 GC, or 1 GC and 2 AU, or 4 AU pairs (cooperativity rule). Otherwise one is free to form any kind of structure, e.g. hairpins, paper clips, clover leaves etc., provided that one obeys the "cooperativity rule" for any started sequence. For each loop in the structure one has to leave at least 5 positions unpaired (cf. also *t*-RNA model in Fig. 2). The players throw in turn. Each player can throw for any position he wants, but must announce beforehand for which. Another possible constraint is to require "end to end" matching, i.e. the two terminal digits must be opposite each other.

Note on procedure. One may be surprised to find out that the winner invariably ends up with a clover leaf structure, similar to the known secondary structures of *t*-RNA molecules (which may involve further spatial folding).
The "trick" of the game is to find initially that structure which has the maximum number of potential base pairs (including noncooperative pairs). The probability of finding in a fixed structure with n possible pairs, k and only k, can be calculated according to the binomial distribution formula

$$P_k = \left(\frac{1}{4}\right)^k \left(\frac{3}{4}\right)^{(n-k)} \frac{n!}{k!\,(n-k)!}.$$

Among any fixed structures, *the hairpin* (with only one loop) *has the largest number of bases which are allowed to pair.* However, the *clover leaf is more flexible* in that one can try many more combinations than for the hairpin. The reason is that one can shift single leaves independently of others and thus get many more combinations to start with. This will turn out to be decisive. Too many leaves, on the other hand, cannot form because of the cooperativity rule. For 80 nucleotides the optimum is around 3 to 4 leaves (+1 stalk), for longer chains it will shift to higher values.
Nature has apparently played such a game long ago. It should be noted that the cooperativity rule mentioned above corresponds to the stability constants we have found for the different base combinations of oligonucleotides (cf. Section IV), i.e. about twice as large a free energy of interaction for the GC pair as for the AU pair.
The details of this game were worked out by Ruthild Winkler [47]. Quantitative estimates about the most favorable secondary structures of polynucleotide chains were previously reported by J. R. Fresco, B. M. Alberts and P. Doty [48].

Our knowledge obtained from experiments correlating structure and function is still limited to the very few enzymes studied in detail so far (cf. Ref. [29]).
It is the main object of this paper to introduce the concept of "selection" into molecular dynamics and to correlate it with known molecular parameters. I have stressed the relatively trivial examples of games in order to show that selection rules can be based on chemical properties. What is still to be seen is how the system will utilize such structural advantages and how the mechanism of valuation will result from the dynamic behavior of the system.

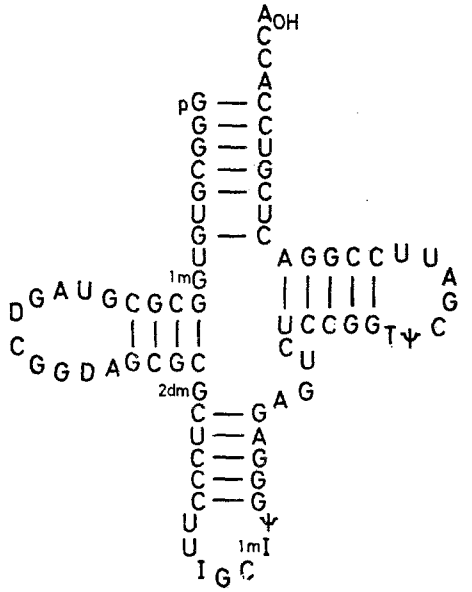

Fig. 2. "Cloverleaf" model of *t*-RNA. Sequence of alanine-specific *t*-RNA from yeast (cf. [5a]). The unusual bases are: ψ = pseudouridine, I = inosine, D = dihydrouridine, T = ribothymidine; 1 mG = 1-methylguanosine, 2 dmG = N(2)-dimethylguanosine, 1 mI = 1-methylinosine. The anticodon is: 5′ IGC 3′(*t*-RNA). The codon (GCC) is read 3′ CCG 5′ (*m*-RNA).

I.2.4. Selection Occurs with Special Substances under Special Conditions

What Properties of Matter are Required to Start Self-organization?

Logically, we should distinguish several phases of evolution, which temporally are not completely separated:

1. a prebiotic "chemical" phase,
2. the phase of selforganization to replicating "individuals",
3. the evolution of individual species.

For a long time biologists were mainly concerned with the third phase, which itself involves many noticeable stages: from *differentiation* and the development of *sex*, through the development of *nerve cells*, of *autonomous control* and modes of *communication*, leading finally to *selfconsciousness* and *logical reflexion*, which are unique properties of the human mind. I do not wish to give the reader the impression that the changes among these stages are less dramatic or less incisive than changes among the three phases mentioned before. However, in this article I am concerned with the *second phase*, the transition from the "non-living" to the "living". As F. H. C. Crick [30] points out in his charmingly written essay "Of Molecules and Men", it is "*notoriously difficult to define the word living*" because the transition is anything but sharp. Thus, if we are concerned with this "step", we should start at systems which clearly are "non-living" (such as minerals) and end up with those which at least can be seen to develop into what we definitely call "living": bacterial cells, plants, animals.
Selforganization is based on certain chemical prerequisites—as well as on special environmental conditions. It is not "just plainly" a property of matter. The prebiotic phase is chemistry and as such

is described "in principle" by quantum mechanical theory. However, it has to be shown, of course, that *conditions on the early earth were such as to favor the formation of the required material.* Another problem is that in absence of any functional order a much larger variety will be formed than is actually required. The complexity of what chemistry can account for is tremendous, a glance through the "Beilstein" may serve to illustrate this. Anything which *can* form under appropriate conditions *will* do so; the first phase of evolution is a divergent one and only by functional organization can it be turned into convergent reproduction and "valued" evolution.

It would be beyond the scope of this paper to go into any detailed discussion of the problems of prebiotic "chemical" evolution. The quite extensive literature is excellently reviewed in several monographs and articles, e.g. by A. I. Oparin [31], one of the early pioneers in this field, M. Calvin [32], S. Fox [33] *et al.*, C. Ponnamperuma [34] and others. Two recent papers by F. H. C. Crick and L. Orgel [35] deserve particular mention since they critically review—in the light of experimental results—the conditions for the occurrence of the second phase, with which we are mainly concerned in this paper. All authors agree on the conclusion that the essential building stones of biological macromolecules—amino acids, energy-rich nucleoside phosphates such as ATP and its base homologues, as well as many other "biochemical" compounds—could form, where required, and polymerize under prebiotic conditions, e.g. in a reducing atmosphere involving the use of various energy sources. In saying this, I do not want to create the impression, that all problems concerning this phase of evolution are solved. Many questions remain, e.g. how ordered polymerization of 3′–5′ polynucleotides occurred (whereas G. Schramm's [36] and L. Orgel's [37] experiments showed preference for other links, e.g. 2′–5′), or how activated amino acids can be polymerized to long polypeptide chains rather than to short oligomers, apparently a problem of appropriate catalytic condition (e.g. surface catalysis), as recent experiments by A. Katchalsky [38] and his group show. Another problem is the abundance of the various precursors required for a synthesis of biologically active material. All these questions may still keep a generation of chemists busy, but they represent typical "chemical" problems.

All we need at the moment is to assume that substances like energy-rich phosphates, activated amino acids etc. were present and could be condensed into macromolecular substances exhibiting simple catalytic functions, e.g. by cooperative action of certain acid and base groups located in the side chains of amino acids, or by enhancing simple electron donating and accepting functions of cofactors such as metal ions in different valence states, or—as in the case of nucleic acids—as simple templates.

Catalytic function in combination with various feedback mechanisms causing certain selfenhancing growth properties of the system will be shown to be one of the decisive prerequisites for selforganization (cf. Part II). The catalyst—according to its classical definition—enhances the rate of equilibration without shifting the equilibrium accordingly. It is true that most catalysts also interfere with the equilibria of their substrates because the free energies of their interactions with the substrates are not negligible. However, if there is any true catalytic effect present, it will show up in both the forward and reverse reactions in exactly the same way. As a consequence, "autocatalytic growth" cannot occur in completely or nearly equilibrated systems. This introduces our second question:

Under what Environmental Conditions can Selforganization Occur?

One fundamental answer was given by E. Schrödinger (loc. cit.) who wrote: "Living matter evades the decay to equilibrium". Equilibrium (in an isolated system) is a state of maximum entropy. If we keep the system away from equilibrium, we have to compensate steadily for the production of entropy, which means we have to "feed" the system with free energy or energy-rich matter. This energy is used by the machinery to "drive" certain reactions which keep the system from "fading away" into the inert or "dead" state of equilibrium. This statement is obviously correct and Schrödinger deserves the credit for having expressed it so clearly. However, he also realized that this statement is only of limited help in explaining "how" order is maintained via (other) order and "how" it came about from disorder. The cause of the difficulty is that it is not sufficient just to divide the world of living into order and disorder.

Let us enlarge a little further on the thermodynamic aspect; we shall need it anyway for the theory of selection, in order to start from solid ground. The thermodynamic theory of irreversible processes was developed by J. Meixner [39], I. Prigogine [40], S. de Groot [41] and others. It was based on Onsager's [42] reciprocity relations, expressing microscopic reversibility, and was consequently applied only to systems near equilibrium. More recently, P. Glansdorff and I. Prigogine [43] have extended it to include systems near a steady state. The important quantity we have to consider is not so much entropy itself but its temporal derivative: dS/dt. It includes two contributions: fluxes from and to the exterior, i.e. d_eS/dt, and internal production of entropy, i.e. d_iS/dt which we denote by σ. For macroscopic systems (to which Gibbs' formula applies) σ can be expressed as a sum of terms, each of which is a product of a flux J_i and a generalized force X_i (for inhomogeneous systems σ has to be integrated over the whole volume, but we shall disregard them here for reasons of simplicity). The essence of the theory for systems near equilibrium (i.e. where linear relations between fluxes and forces hold) is then expressed by the relation

$$\sigma = \sum_k J_k X_k \geqq 0 \qquad \text{(I-5)}$$

i.e. entropy will always increase with time for any system close to equilibrium. At equilibrium it will reach a maximum, thus σ will be zero.

Table 5 represents the application to systems of chemical reactions where each reaction is characterized by an "extent of reaction" ξ_i or its temporal derivative (i.e. reaction rate V_i = non vectorial flux) and an "affinity" A_i (non vectorial force A_i/T). The example shows that close to equilibrium σ always can be expressed by a quadratic (positive definite) form either in "extents of reaction" or affinities. The linear relationship between fluxes (reaction rates) and forces (affinities)

Table 5. *Definitions and relations of chemical thermodynamics*

Affinity of reaction i:

$$A_i = -\sum_k \nu_{ik}\mu_k, \tag{1}$$

$$= RT\left[\ln K_i - \ln \prod_k a_k^{\nu_{ik}}\right]. \tag{2}$$

Stoichiometric coefficients:

ν_{ik} $\begin{cases} >0 \text{ for reaction products} \\ <0 \text{ for reactants.} \end{cases}$

Example: $4\,O_2 + Hb \rightleftharpoons Hb(O_2)_4$:

$\nu_{O_2} = -4$; $\nu_{Hb} = -1$; $\nu_{Hb(O_2)_4} = +1$;

Chemical potential of substance k:

$$\mu_k = \mu_k^0 + RT \ln a_k \tag{3}$$

μ_k^0 = standard chemical potential; a_k = activity (to be replaced by concentration c_k for ideal solutions).

Equilibrium constant:

$$K_i = \prod_k \bar{a}_k^{\nu_{ik}} \tag{4}$$

$\bar{a}_k$ refers to equilibrium state, where $A_i \equiv 0$;

$$-RT \ln K_i = \sum_k \nu_{ik}\mu_k^0 \quad \text{(cf. Eq. (2))}. \tag{5}$$

Extent of reaction ξ_i according to:

$$d\xi_i = dn_k/\nu_{ik} \quad (n_k = \text{mole number of component } k). \tag{6}$$

At const T and P:

$$dG = \sum_i A_i d\xi_i \tag{7}$$

(G = Gibbs' free energy; ξ_i is conjugate to A_i).

Reaction Rate:

$$V_i = d\xi_i/dt = \sum_k \varepsilon_{ik} \cdot A_k/T \tag{8}$$

(ε_{ik} = phenomenological coefficients).

Onsager's Relations:

$$\varepsilon_{ik} = \varepsilon_{ki}. \tag{9}$$

Linearization:

$$V_i = \frac{1}{T}\sum_k \varepsilon_{ik} \sum_l (\partial A_k/\partial \xi_l)_{T,P}\,\delta\xi_l. \tag{10}$$

The matrix (ε_{ik}) and the tensor $(\partial A_k/\partial \xi_l)_{T,P}$ can be written in diagonal form. The transformed rate equations assume the form:

$$V_i' = d\xi_i'/dt = \lambda_i \delta\xi_i', \tag{11}$$

where $\delta\xi_i'$ = normal mode;

$\lambda_i = -1/\tau_i$ = eigenvalue; τ_i = relaxation time;

the solution of the rate equation is

$$\delta\xi_i' = \delta\xi_{i0}' e^{-t/\tau_i}. \tag{12}$$

Entropy production:

$$\sigma = \frac{1}{T^2}\sum_{ik} \varepsilon_{ik} A_i A_k \geqq 0. \tag{13}$$

Table 6. *Example of a (simple) autocatalytic reaction:* $X + Y \rightleftharpoons 2X$, i.e. Y is transformed to X with the catalytic help of X.[a]

1. Far from Equilibrium

Reaction Rate:

$$V = \vec{k}[X][Y]; \tag{1}$$

At constant $[Y]$: $\delta V \sim \delta[X]$.

Affinity:

$$A = RT[\ln K - \ln([X]/[Y])]; \quad \delta A \sim -\delta[X]. \tag{2}$$

Excess Entropy Production:

$$T\delta_x\sigma \to \delta V \delta A \leqq 0. \tag{3}$$

In absence of other processes which provide a stabilization, such a system cannot reach a stable state for constant supplement of Y.

2. Close to Equilibrium (Fluctuation $\delta[X]$):

Reaction Rate:

$$V = \vec{k}[X][Y] - \overleftarrow{k}[X]^2 = \{\vec{k}[Y] - \overleftarrow{k}[X]\}[X] \tag{4}$$

since $\vec{k}Y \approx \overleftarrow{k}X$, {}-term small, at equilibrium equal to zero.

$$\delta V \approx -\overleftarrow{k}[X]\delta[X] \tag{5}$$

(neglecting second-order terms).

Affinity: as above

Excess Entropy Production:

$$T\delta_x\sigma \geqq 0 \quad \text{(stable equilibrium)}. \tag{6}$$

[a] The symbol X denotes here a chemical compound and is to be distinguished from the generalized forces X_i. The subscript x of d or δ refers to the change of forces.

will hold for any reaction system close to equilibrium, irrespective of the reaction orders or of the presence of any couplings among the different reactions. The solutions of the system of linear differential equations are exponential functions with real and negative arguments; in other words, any deviation of a concentration from its equilibrium value will decay exponentially with time:

$$(c_i - \bar{c}_i) = \sum_k a_{ik}\, e^{-t/\tau_k}. \tag{I-6}$$

Equilibration is a "relaxation" process characterized by a spectrum of time constants τ_k. Many such relaxation spectra of quite complicated reaction systems have been studied during the last two decades (cf. Ref. [44]). The fact that no periodic solutions (complex exponentials) or instabilities (exponentials with positive argument) occur is a consequence of Onsager's relations (which imply symmetric matrices for the rate coefficients) and of the sign of the rate coefficients (which in the characteristic equation lead to a polynomial with only positive coefficients) [45]. The different roots are the negative, reciprocal relaxation times.

Analogous to Eq. (I-5) is a stability criterion which can be derived from Einstein's classical formula for fluctuations around an equilibrium state (cf. Ref. [43]). Any fluctuation around a stable equilibrium will always result in an entropy change smaller than zero:

$$\delta_i S \leqq 0. \tag{I-7}$$

For a fluctuation $\delta\xi_k$ around a stable chemical equilibrium we have to require:

$$\delta_i S = \frac{1}{T}\sum A_k \delta\xi_k \leqq 0 \tag{I-8}$$

or after expansion and diagonalisation ($A_k = 0$ at equilibrium)

$$\sum_k (\partial A_k'/\partial \xi_k')\,(\delta\xi_k')^2 \leqq 0, \tag{I-9}$$

since $(\partial A_k'/\partial \xi_k') < 0$.

According to the type of solutions common for all systems near true equilibrium, selection and evolution cannot occur in equilibrated or nearly equilibrated systems, even if the right types of substances are present.

Autocatalysis will not result in growth for systems near equilibrium, since catalytic enhancement influences both the forward and reverse reactions in the same way.

Restrictions of this kind are not present in systems at a steady state. Both equilibria and steady states are characterized by a zero net change of concentrations, but they differ with respect to symmetry relations. Onsager's reciprocity relations do not apply to steady states. As a consequence, *oscillations* around a steady state can occur.

I. Prigogine and P. Glansdorff [43] have derived a relation analogous to Eq. (I-5) which holds for the neighborhood of steady states:

$$d_x \sigma = \sum_k J_k \, dX_k \leqq 0 \qquad \text{(I-10)}$$

in words: the change of entropy production due to a change of forces X_k (e.g. the change of σ at const. fluxes), will always be smaller than zero near to, and zero at, the steady state, or, entropy production is at a minimum with respect to a variation of forces for steady state systems. (Note that the relation does not apply to $d_J \sigma$ or $d\sigma = d_x \sigma + d_J \sigma$.) Again there is a stability criterion analogous to Eq. (I-7). It states that any fluctuation around a *stable* steady state will show up in the "excess entropy production" as a positive term

$$\delta_x \sigma = \sum_k \delta J_k \, \delta X_k \geqq 0 \qquad \text{(I-11)}$$

which due to relation (I-10)—as for the entropy production around equilibrium—contains only second order contributions, e.g. for a chemical system:

$$\delta_x \sigma = \frac{1}{T} \sum_k \delta V_k \, \delta A_k \geqq 0. \qquad \text{(I-12)}$$

Or: a steady state is *unstable* whenever a negative fluctuation $\delta_x \sigma$ occurs. As shown in Table 6, autocatalytic reaction systems ("autocatalytic" will later be interpreted in the widest sense) are the candidates for such instabilities. Here the change in rate due to a fluctuation in concentration and the corresponding change in affinity have opposite signs (note that this is true only far from equilibrium, where rates of "reverse" reactions can be neglected). It will be seen that such instabilities are a prerequisite for selective growth and evolution.

Prigogine and his coworkers have shown that a combination of autocatalytic reaction behavior with transport processes may lead to peculiar spatial distributions of the reaction partners, which he called "dissipative structures", i.e. structures resulting from a dissipation of energy rather than from conservative molecular forces.

Prigogine [46] believes that these structures were of importance for the formation of functional order in the evolution of life. They certainly are, whenever conditions for their appearance are favorable. So they may have been of some influence in early morphogenesis, but I do not think that macroscopic spatial structures are the key to understanding the first steps of biological selforganization. These steps may even have occurred in a structureless "soup", certainly involving functional macromolecular structures such as nucleic acids and proteins. The type of organization we need at the beginning is not so much organization in physical (i.e. geometrical) space. We need functional order among a tremendously complex variety of chemical compounds—possibly in a homogeneous phase. We need organization in a different "space", which one may call "information space". This order will also be based on the principle of Prigogine and Glansdorff, to which I assign great significance, but its utilization will require new parameters and may lead us beyond the realm of present thermodynamic theory.

II. Phenomenological Theory of Selection

II.1. The Concept "Information"

Orderliness in a complex reaction system which involves a large variety of different chemical compounds requires the formation of a selfreproducing "functional code". The word "functional code" specifies two properties: one executive and one legislative. The executive property requires machinery which can control all reactions going on in the system and may be represented by an ensemble of interacting and selfregulating catalysts, preferably made of uniform material, but involving practically unrestricted functional capacity. Regardless of whether the primary structure of this executive machinery also provides the instruction for its reproduction, or whether this has to be translated from a different legislative source, selforganization and further evolution of correlated and reproducible functional behavior must start at the level of a selfreproducing molecular code.

We shall now consider the *code carrier*. The fact that we know of the existence of such code carriers, i.e. nucleic acids and proteins, will aid us in arriving at a useful concept.

Let us define a set of N_ν information carriers

$$i_{\nu k} \quad (k = 1, 2 \ldots N_\nu) \qquad \text{(II-1)}$$

which are characterized by sequences of ν digits of basis λ (e.g. $\lambda = 4$ for nucleic acids or $\lambda = 20$ for proteins). For simplicity we shall often refer to such a uniform class of information carriers, i.e. sequences of uniform length containing a specified number ν of digits.

In classical information theory the "information content" of a specified sequence $i_{\nu i}$ is expressed by the number of bits:

$$I_\nu = \nu \ln \lambda / \ln 2 \quad [\text{bits}] \qquad \text{(II-2)}$$

N_ν then represents a "structural capacity" of the class ν, i.e. the total number of sequences of length ν and basis λ which could be formed

$$N_\nu = \lambda^\nu = 2^{I_\nu} \qquad \text{(II-3)}$$

If we allow for any length with an upper limit of ν digits we obtain

$$\sum_\nu N_\nu = \frac{\lambda^\nu - 1}{1 - \lambda^{-1}} \qquad \text{(II-4)}$$

different sequences. This number may be of importance if we consider systems of independent competitors (including any length 1 to ν), in which each

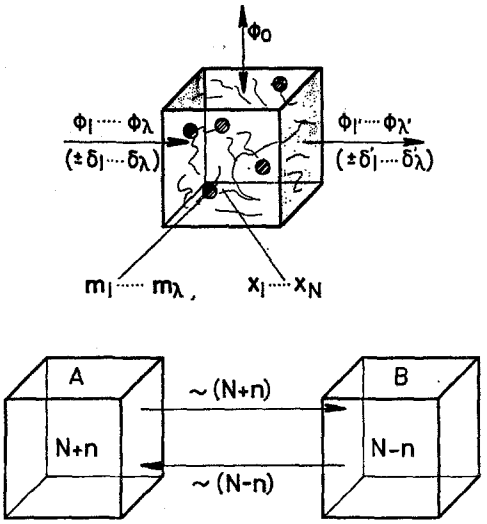

Fig. 3. The "information box". The upper box is assumed to have semipermeable walls through which energy-rich and energy-deficient monomeric digits can flow in and out. Inside the box polymeric sequences—representing "information"—are assembled and decomposed. Both template-instructed assembly and decomposition may be enzyme-catalyzed processes. Steady state can be maintained via a control of fluxes ($\phi_1 \ldots \phi_\lambda$; $\phi_{1'} \ldots \phi_{\lambda'}$) or of concentrations ($m_1 \ldots m_\lambda$; $x_1 \ldots x_N$ regulated by dilution flux ϕ_0). For comparison, equilibrium is represented by two boxes A and B, among which the system is distributed: $N_A = N_B$, involving fluctuations $\pm n$ (cf. Ehrenfest's urn model, as discussed in Part III.2).

sequence represents a certain message (of different "selective value", cf. below).

The system of information carriers may assemble within a "box" of finite volume having permeable walls through which energy-rich and energy-deficient monomeric digits can flow in and out (cf. Fig. 3). Within the box each information carrier may be present in $x_{\nu i}$ copies (per unit volume), the total population of each class being:

$$n_\nu = \sum_{k=1}^{N_\nu} x_{\nu k}. \qquad \text{(II-5)}$$

For most of the N_ν possible carriers $i_{\nu k}$ the concentration $x_{\nu k}$ will be zero. The concentrations of unorganized energy-rich monomeric digits $1, \ldots, \lambda$ in the box are $m_1 \ldots m_\lambda$, their fluxes into the box: $\phi_1 \ldots \phi_\lambda$.

The total number of digits—organized or unorganized—amounts to:

$$M_0 = \sum_{k=1}^{\lambda} m_k + \sum_\nu \nu n_\nu. \qquad \text{(II-6)}$$

The "degree of organization" D_0 within the box, i.e. the ratio of organized to the sum of organized and unorganized digits is

$$D_0 = \frac{\sum_\nu \nu n_\nu}{M_0}. \qquad \text{(II-7)}$$

It will turn out that, for nearly all practical cases, the total population of a given class in the volume V will always be extremely small compared to the information capacity of that class N_ν:

$$n_\nu \cdot V \ll N_\nu \qquad \text{(II-8)}$$

(e.g. for $\nu = 100$, $\lambda = 20$ as small as 10^{20} or less as compared to 10^{130}; cf. Table 3). An important consequence is that in a random distribution in the absence of selfinstruction, the expectation value for any given sequence is practically zero. Furthermore, for those sequences which are formed by chance, the probability of finding another copy of the same sequence *by chance* again is practically zero. This property of "unsaturated" information capacity is of great importance for the optimization procedure of evolution.

Our further task is to assign certain dynamic properties to the information carriers and to develop a theory of selection. This theory should involve the derivation of a parameter which expresses "selective advantage" in molecular terms.

For the treatment of coupled systems, we may conceive the idea of an "information space" defined by the set of population variables $x_{\nu i}$ and the functional relations among them. For quasi-linear systems—true linear systems cannot "select"—this may lead to a "normal mode" treatment similar to that of linear relaxation phenomena.

II.2. Phenomenological Equations

Given a class of information carriers $i_{\nu i}$, each of which is present in $x_{\nu i}$ copies per unit volume:

$$0 \leqq x_{\nu i} < n_\nu \quad (n_\nu \cdot V \ll N_\nu) \qquad \text{(II-9)}$$

selection in the Darwinian sense must involve dynamical properties of the system, represented by the rates of assembly and decomposition of the information carriers. Consequently, we have to start from the rate equations for generation and turnover of the macromolecular species which are the representatives of the evolutionary behavior.

For systems far from equilibrium we cannot expect a simple linear relationship between fluxes and forces. Thus, there is no advantage in starting with the formalism of phenomenological equations used in the linear range of thermodynamic theory of irreversible processes (i.e. simple relations between rates and affinities). Since quite a specific reaction behavior was shown to be required for selection, it may be of advantage to use the terminology of chemical rate theory which specifies more explicitly the class and order of the reactions involved.

Let us make three assumptions which will turn out to be necessary prerequisites for selection:

1. The system must be open and far from internal equilibrium. In order to prevent the system from decaying to equilibrium, we have steadily to feed in free energy, e.g. in the form of energy-rich monomers (such as ATP and its base analogues or activated amino acids). Decomposition, on the other hand, will lead to energy-deficient products. Thus, both reactions, formation and decomposition of the information carriers, are driven by positive affinities; there is no relationship of "microscopic reversibility" for the two processes such as would be present for formation and decomposition close to equilibrium.

2. The formation rate must exceed the decomposition rate and be—at least—of the same order in $x_{\nu i}$. Since decomposition is usually (at least) first order

in $x_{\nu i}$[1], the formation must be of an "autocatalytic" nature. If the formation rate were of lower order in $x_{\nu i}$ than the decay rate, the system would not possess the intrinsic growth property which is required for selection against less efficient competitors. In such a case all $i_{\nu i}$ would grow only to a constant level, where their formation rate is "matched" by the decomposition rate. Such a system would carry along all the useless information of previous mutations, which would finally block any further evolution.

3. Due to the condition $n_\nu \cdot V \ll N_\nu$, non-instructed formation of any individual information carrier is completely negligible.

It will be shown in Sections IV to VI that autocatalytic behavior involves many different classes of reactions of which only certain types will qualify for evolutionary behavior. One very important specification with respect to macromolecular information carriers will be the distinction of "selfinstructive" from "general autocatalytic" behavior. A process may be autocatalytic in that the product of a reaction feeds back on its own formation—possibly via some catalytic reaction cycle involving several intermediates (cf. Section V). The formation rate of a given $i_{\nu i}$ may not then be proportional to its concentration $x_{\nu i}$ but rather to the concentration $x_{\nu j \neq i}$ of some information carrier, while the ratio $x_{\nu j}/x_{\nu i}$ eventually reaches a constant value. Here autocatalysis is a special property of a particular ensemble. A "selfinstructive" information carrier is required to have general "template" properties. This means that *any* sequence of digits will instruct *its own* reproduction. If an error occurs in the reproduction, the *error copy* will be further reproduced. This kind of behavior is typical for nucleic acids whereas "general autocatalytic" behavior can also be found with proteins.

The phenomenological rate equation—for each information carrier present—can now be written in the general form ($\dot{x} \equiv dx/dt$):[2]

$$\dot{x}_i = (\mathcal{F}_i - \mathcal{R}_i)\, x_i + \sum_{l \neq i} \varphi_{il}\, x_l \qquad \text{(II-10)}$$

The first and second terms in this rate equation refer to the *selfinstructed* formation of the information carrier $i_{\nu i}$ and its removal (e.g. by decomposition, dilution etc.). The third term includes all further (non-spontaneous) production terms resulting from imprecise copying of other sequences which closely resemble $i_{\nu i}$. $\mathcal{F}_i$ and $\mathcal{R}_i$ are general rate parameters which may include several individual terms. The particular form of Eq. (II-10) is chosen in order to express the requirement of "inherent" autocatalysis, but it does not necessarily imply first-order reaction behavior. The rate parameter $\mathcal{F}_i$ certainly is a function of the concentrations of monomeric digits ($m_1 \ldots m_\lambda$), and both $\mathcal{F}_i$ and $\mathcal{R}_i$ may depend further on x_i or the population variables x_k of other species.

We may specify $\mathcal{F}_i$ and $\mathcal{R}_i$ further in purely phenomenological terms (possibly involving a sum of individual reaction terms)[3]

$$\mathcal{F}_i = k_0 \mathcal{A}_i \mathcal{Q}_i; \qquad \mathcal{R}_i = k_0 \mathcal{D}_i + \varphi_{0i} \qquad \text{(II-11)}$$

k_0 represents a *general* first-order rate constant with the dimension $\sec^{-1}$. It defines a suitable constant reference (or threshold) value for all competitive formation processes. The remaining individual parameters $\mathcal{A}_i$, $\mathcal{Q}_i$ and $\mathcal{D}_i$ then do not contain the dimension time.

The product term $\mathcal{A}_i \mathcal{Q}_i$ characterizes the *individual* magnitude and form of the different formation rate parameters $\mathcal{F}_i$. $\mathcal{A}_i$ is called an "amplification factor"; $k_0 \mathcal{A}_i$ is actually a rate constant which describes how fast synthesis is directed by the template i_i. Thus $k_0 \mathcal{A}_i$ counts *all* formation processes (per unit time) which occur via instruction by the template i_i, regardless of whether they lead to precise copies of i_i or to mutants. With $\mathcal{Q}_i$ we introduce then a "quality factor" which tells us which fraction $\mathcal{Q}_i$ of these processes leads to the *precise* copy of i_i. The fraction $(1 - \mathcal{Q}_i)$ of all "copying" processes directed by i_i describes the formation of mutants i_j which still partly resemble the master copy i_i, but which involve individual errors occurring with a certain probability distribution. These mutants usually are characterized by rate parameters $\mathcal{F}_j \leqq \mathcal{F}_i$, but occasionally an advantageous copy ($\mathcal{F}_j > \mathcal{F}_i$) may occur.

The definition of $\mathcal{A}_i$ and $\mathcal{Q}_i$ is purely phenomenological. One may count the number of copying processes (per unit itime) instructed by a given template as well as determine—by sequence or other analysis—the number of correctly formed species i_i; thus both parameters have a defined physical meaning. This is also expressed if we consider the total production rate

$$\sum_{k=1}^{N} \mathcal{F}_k x_k + \sum_{k=1}^{N} \sum_{l} \varphi_{kl} x_l = k_0 \sum_{k=1}^{N} \mathcal{A}_k x_k \qquad \text{(II-12)}$$

where the right-hand term no longer contains the quality parameters $\mathcal{Q}_i$, since the total production involves both the fractions $\mathcal{Q}_i$ of correct copying as well as the fractions $(1 - \mathcal{Q}_i)$ of error (or mutant) production. Actually, the last term in Eq. (II-10) accounts for the fact that the occurrence of any error must show up in the production term of a closely related copy. This term may be very small or even negligible for a specified selected species, but it may be of importance for the reproducible formation of certain "error satellites" of a selected master copy. By conservation requirements, we obtain—in the absence of any spontaneous, noninstructed synthesis—for the total sum of error production:

$$k_0 \sum_{k=1}^{N} \mathcal{A}_k (1 - \mathcal{Q}_k)\, x_k = \sum_{k=1}^{N} \sum_{l} \varphi_{kl} x_l. \qquad \text{(II-13)}$$

The products $\mathcal{A}_i \mathcal{Q}_i$ also contain stoichiometric functions f_i ($m_1 \ldots m_\lambda$) describing the dependence of formation rate on the concentration of monomeric (energy-rich) digits. Their precise form depends on the particular mechanism of polymerization (cf. Part IV), especially in the rate-limiting step. If the concentrations $m_1 \ldots m_\lambda$ are buffered—a condition which will be chosen for most evolutionary experiments (cf. Part VII)—f_i can be included in $\mathcal{A}_i \mathcal{Q}_i$ as a constant factor. Otherwise we have to specify

$$\mathcal{A}_i \mathcal{Q}_i = f_i (m_1 \ldots m_\lambda)\, \mathcal{A}_i' \mathcal{Q}_i' \qquad \text{(II-14)}$$

(and possibly also corresponding averages for the different error copies). $\mathcal{A}_i' \mathcal{Q}_i'$ does not contain any more concentration terms of $m_1 \ldots m_\lambda$, but may still involve concentration terms of x_i or any x_k. ($f_i (m_1 \ldots m_\lambda)$ can be normalized, e.g. to initial or final conditions.)

With the relation (II-11) for $\mathcal{R}_i$ we distinguish an individual decomposition term $\mathcal{D}_i$ (again related to the general rate constant k_0) from a dilution term φ_{0i}, which in the rate equation also appears to be related to x_i ("proportional" dilution). If this proportionality is straightforward, we may leave out the index i and φ_0 is related to the total "dilution"

1 In enzyme-catalyzed decomposition processes, the reaction rate may become independent of substrate concentration if the enzyme is saturated; however, such cases will not at all invalidate the above statement. The substrate always passes a "non-zero order range" during its growth, and competition under saturation conditions still involves the population variables of the individual species. (Example in Part VII.)

2 In the following we leave out the index ν unless we want to specify the corresponding class.

3 The particular symbols were chosen in order to emphasize the general form of the rate and quality parameters, which may be complicate functions of concentrations and involve several terms.

flow ϕ_0[1] by

$$\varphi_0 = \frac{\phi_0}{\sum_{k=1}^{N} x_k}. \qquad \text{(II-15)}$$

The overall removal rate then can be expressed as

$$\sum_{k=1}^{N} \mathscr{R}_k x_k = k_0 \sum_{k=1}^{N} \mathscr{D}_k x_k + \phi_0. \qquad \text{(II-16)}$$

As mentioned already, the $\mathscr{D}_i$-parameters may be functions of the concentrations x_i or x_k: but here we cannot distinguish any "quality" parameter, since decomposition of any individual species leads to useless products ("garbage")—unless we introduce more sophisticated repair mechanisms.
The occurrence of mutants caused by effects other than error copying can be formally included in the three parameters $\mathscr{A}_i$, $\mathscr{Q}_i$ and $\mathscr{D}_i$.

Eq. (II-10) now assumes the form:

$$\dot{x}_i = k_0 [\mathscr{A}_i \mathscr{Q}_i - \mathscr{D}_i] x_i + \sum_{l \neq i} \varphi_{il} x_l - \varphi_{0i} x_i. \qquad \text{(II-17)}$$

This phenomenological rate equation describes generally any reaction system which is classified by the following properties:

a) Metabolism, as represented by the two overall rate terms $k_0 \sum \mathscr{D}_k x_k$ and $k_0 \sum \mathscr{A}_k x_k$ which describe the turnover of energy-rich into energy-deficient material.

b) Selfreproduction, as indicated by the form of the rate equation. Both the formation and decomposition terms are supposed to be proportional to x_i, and $\mathscr{F}_i > \mathscr{D}_i$ for $0 < x_i < n$, regardless of any further concentration dependence of $\mathscr{F}_i$ and $\mathscr{D}_i$.

c) Mutability, as expressed by a quality factor $\mathscr{Q}_i < 1$.

It turns out that the first part of Eq. (II-17) is determinant for selection behavior and that $\mathscr{A}_i$, $\mathscr{Q}_i$ and $\mathscr{D}_i$ are the decisive phenomenological parameters. Even for complex "living" entities, selection is determined by these parameters, which may involve complicated concentration terms due to "internal" couplings and depend on many environmental variables.
However, in this form Eq. (II-17) does not yet describe a selection process. It defines some segregation, due to the threshold property

$$\mathscr{A}_i \mathscr{Q}_i \gtrless \mathscr{D}_i. \qquad \text{(II-18)}$$

Those species which are above the threshold ($\mathscr{A}_i \mathscr{Q}_i > \mathscr{D}_i$) will grow, those which are below ($\mathscr{A}_i \mathscr{Q}_i < \mathscr{D}_i$) will die out.

If we disregard the second part of Eq. (II-17), the solutions of Eq. (II-17) could be generally written as

$$x_i(t) = x_i^0 \quad \exp \left\{ k_0 \int_0^t (\mathscr{A}_i \mathscr{Q}_i - \mathscr{D}_i)\, dt \right\} \qquad \text{(II-19)}$$

which for constant $\mathscr{A}_i$, $\mathscr{Q}_i$ and $\mathscr{D}_i$ represents a real exponential with either positive or negative argument. If any of these reactions were to come close to equilibrium, the solution would approach an exponential form with a negative argument, as shown in Part I.2.4. It results from an expansion of the reversible (generally nonlinear) rate terms, yielding as variable the deviation of the "extent of reaction" from its equilibrium value (cf. Table 5).

1 φ_0 may also have a negative sign corresponding to "concentration" of the system. Usually, however, the term φ_0 will be used to compensate for growth.

Nonlinear systems may show much sharper "segregation" behavior. For instance, a differential equation of the type

$$\dot{x}_i = (\mathscr{A}_i \mathscr{Q}_i - \mathscr{D}_i) x_i \quad \text{with} \quad (\mathscr{A}_i \mathscr{Q}_i - \mathscr{D}_i) = a_i + b_i x_i \qquad \text{(II-20)}$$

yields singularities at finite t if b_i and $(a_i + b_i x_i^0)$ are larger than zero (allowing even for negative values of a_i). With $x_i^0 = x_i(t=0)$ we have

$$x_i(t) = x_i^0 \frac{a_i e^{a_i t}}{a_i + b_i x_i^0 (1 - e^{a_i t})} \qquad \text{(II-21)}$$

i.e. $x_i(t)$ approaches infinite at

$$t = a_i^{-1} \ln \left(1 + \frac{a_i}{b_i x_i^0} \right). \qquad \text{(II-22)}$$

If a_i is negative, its absolute amount has to be smaller than $b_i x_i^0$, otherwise the solution decays. If $a_i \ll b_i x_i^0$, the solution reduces to a simple hyperbola

$$x_i(t) = \frac{x_i^0}{1 - b_i x_i^0 t} \qquad \text{(II-23)}$$

with a singularity at $t = 1/(b_i x_i^0)$.

These and similar solutions provide quite a sharp selection behavior (cf. Part VI) and turn out to be of great importance for the initiation of evolution.

I have intentionally called the behavior described so far "segregative" rather than "selective", because it leads only to a separation of the system into two parts, discriminated by the threshold property ($\mathscr{A}_i \mathscr{Q}_i \gtrless \mathscr{D}_i$). If we want to interpret "selection" by some extremum principle, we need not only growth properties of the single components but also some "external" *selection strains* in order to force the system into real competition for survival.

II.3. Selection Strains

We could think of many external constraints and internal couplings (cf. the treatment of "struggle" problems by V. Volterra [49]) which would make the reaction system more competitive. However, there are two straightforward procedures which will provide a general basis for a theory of selection. Both procedures can be correlated with Prigogine and Glansdorff's treatment [43] of reaction processes in the neighborhood of a steady state.
We force the system to maintain a steady state. In the thermodynamic theory (cf. Section I) one may consider such systems either at constant reaction forces or at constant reaction flows.
Likewise, if we refer to the information box introduced in II.1., we may keep constant either the overall organization (and thus some overall affinity, cf. Table 5) or the overall flow of digits (as determined by the in- and outflux of monomeric digits as well as by the overall reaction flows within the box).
More specifically, the first condition means that the total number of both the organized and the unorganized digits as well as the "degree of organization" in the box have to be kept constant. Physically, this can be facilitated by buffering the concentrations $m_1 \ldots m_\lambda$ of the energy-rich monomers and controlling (via ϕ_0) the total flux in such a way that the total number of information carriers is kept constant.
The constraints are then for constant overall organization:

$$m_1 \ldots m_\lambda = \text{const} \qquad \text{(II-24)}$$

which means also that $f_i(m_1 \dots m_\lambda) = \text{const}$ and can be included in $\mathscr{A}_i\,\mathscr{Q}_i$

$$\sum_{k=1}^{N} x_k = \text{const} = n \qquad \text{(II-25)}$$

(or if we consider different classes ν: $\sum_\nu \nu n_\nu = \text{const}$).

The dilution flux ϕ_0 has to be adjusted so as to compensate for the overall excess production:

$$\phi_0 = k_0 \sum_{k=1}^{N} [\mathscr{A}_k - \mathscr{D}_k]\, x_k. \qquad \text{(II-26)}$$

The alternative selection condition allows the content of the box to vary, but both the influx of monomeric energy-rich material ($\phi_1 \dots \phi_\lambda$) as well as the reaction flow, i.e. the total assembly and turnover rate of information carriers (including also the outflux of energy deficient decomposition products), are invariant:

$$\phi_1 \dots \phi_\lambda = \text{const} \qquad \text{(II-27)}$$

or if the monomers result from the same source:

$$\phi_M = \sum_{k=1}^{\lambda} \phi_k = \text{const},$$

and

$$k_0 \sum_{k=1}^{N} \mathscr{A}_k\, x_k = k_0 \sum_{k=1}^{N} \mathscr{D}_k\, x_k = \phi_M = \text{const}. \qquad \text{(II-28)}$$

Experimental implementation and theoretical treatment of the latter case are more difficult. Some processes in nature may come close to these conditions. For instance, over a certain period energy may be supplied at a constant rate (e.g. by constant influx of sun energy) so that the level of the energy-rich material may adjust so as to yield a constant rate of production. (Increase in rate parameters is compensated by a decreased concentration level of monomeric digits.) Similarly, the information carriers may grow to a level where their decomposition is adjusted to their formation rate. Exact maintenance of this condition, however, requires sophisticated control. For evolution experiments it is easier to maintain the conditions of constant overall organization (cf. S. Spiegelman's serial transfer experiments with phage $Q\beta$ as described in Part VII). One may build "evolution machines" which automatically control and maintain the specified conditions, and one could also imagine other constraints involving various combinations of the two mentioned conditions.

Note: *Important as these specifications of defined conditions are for an understanding of the principles of evolution and for a quantitative evaluation of experimental data, it is not in the least necessary that any real evolutionary process in nature should have taken place under these special conditions—just as no steam engine ever had to work under the exactly specified thermodynamic equilibrium conditions of the Carnot cycle.*

Let us now return to the phenomenological equations and rewrite them with due consideration of the two different selection constraints.

a) Constant Overall Organization

Let us call the terms

$E_i = \mathscr{A}_i - \mathscr{D}_i$ the (excess) productivity, (II-29)

$\bar{E} = \dfrac{\sum_{k=1}^{N} E_k x_k}{\sum_{k=1}^{N} x_k}$ the "mean productivity", (II-30)

$W_i^0 = \mathscr{A}_i\,\mathscr{Q}_i - \mathscr{D}_i$ the "selective value". (II-31)

All these quantities refer to the constraint of constant overall organization.

By substituting φ_0 according to Eq. (II-15) and (II-26), we may write Eq. (II-17) in the form

$$\dot{x}_i = k_0 [W_i^0 - \bar{E}]\, x_i + \sum_{l \neq i} \varphi_{il}\, x_l. \qquad \text{(II-32)}$$

The term $\left(\sum_{l \neq i} \varphi_{il}\, x_l\right)$—corresponding to a "repair of errors" or a "back flow of information"— will usually turn out to be negligible for any selected master copy (i_m). Such a "master copy", however, will always carry along a "comet tail" of error copies, whose stationary presence is mainly due to the formation term $\varphi_{im}\, x_m$ (the index m referring to the selected master copy). Eq. (II-32) is inherently nonlinear— even for constant values of $\mathscr{A}_i$, $\mathscr{Q}_i$ and $\mathscr{D}_i$—due to the fact that each population variable x_i occurs in the mean productivity $\bar{E}$. Thus, all equations are coupled via this term, which provides a sliding and selfadjusting threshold value reflecting the self-organization of the system. Only those information carriers will grow whose "selective values" W_i^0 are above the threshold $\bar{E}$. As a consequence of their growth they shift the threshold $\bar{E}$ steadily to higher values until an optimum of $\bar{E}$ is reached which matches the maximum selective value of all species present:

$$\bar{E} \to W_m^0 \qquad \text{(II-33)}$$

or, more generally (e.g. in the case of oscillating systems), for any oscillation period Δt

$$\int_t^{t+\Delta t} \bar{E}\, dt \to \int_t^{t+\Delta t} W_m^0\, dt \quad \text{or} \quad \langle \bar{E} \rangle \to \frac{1}{\Delta t} \int_t^{t+\Delta t} W_m^0\, dt. \qquad \text{(II-34)}$$

Depending on the type of reaction system (i.e. the couplings among various components) W_m^0 will belong to a single information carrier (or a degenerate class) if the parameters $\mathscr{A}_m$, $\mathscr{Q}_m$ and $\mathscr{D}_m$ are constants, or it may refer to a catalytic cycle and then be a function of the concentrations of all members of the cycle, or it may even include a whole hierarchy of reaction cycles, expressed by concentration terms of higher order. For nonlinear systems, the final value of W_m^0 for any species may depend on the initial conditions of concentrations as for instance indicated by Eq. (II-21). It is important to note that the index m in W_m^0 refers to the species with "maximum" selective value relative to all competitors present in the final phase. Furthermore, the relations (II-33) or (II-34) are only approximations for the case of negligible "error repair" terms. Otherwise one may substitute W_m^0 by

$$W_m^0 + \frac{1}{k_0} \sum_{l \neq m} \varphi_{ml} \frac{\bar{x}_l}{\bar{x}_m},$$

where the second term represents an average "back flow" of information from mutants (which also may be modified by specific repair function terms).
The next higher approximation of (II-33) for small repair term contributions could then be written as:

$$\bar{E} \to W_m^0 + \frac{1}{k_0^2} \sum_{l \neq m} \frac{\varphi_{ml}\, \varphi_{lm}}{W_m^0 - W_l^0} \,. \qquad \text{(II-33a)}$$

This approximation is valid as long as the second term is small compared to any $(W_m^0 - W_{l \neq m}^0)$, which always can be fulfilled if $\mathcal{Q}_m$ approaches closely one.
We have thus characterized selection by some extremum principle, in a certain analogy to thermodynamic equilibrium. There, however, we have a "true" maximum of entropy or minimum of free energy, whereas here we are dealing only with "optima", i.e. maxima relative to alternative compositions in the presence of certain constraints. We may as well call the state characterized by the criterion (II-33) or (II-34) "selection equilibrium", but we should be aware that we are dealing here with a metastable state of equilibrium. It stabilizes the information for the reproduction of what we may call the "fittest" among a population, but only as long as no "selective advantage"—characterized by $W_{m+1}^0 > W_m^0$—occurs among the fluctuating error distribution (cf. $\mathcal{Q} < 1$ and $nV \ll N$). As soon as such a new copy (or ensemble) i_{m+1} appears (cf. the stochastic treatment in Part III), the former equilibrium will collapse and a new (metastable) state of equilibrium will be reached. It is characterized by a different $\bar{E}$, the whole change of which corresponds to an optimization procedure. If W_m^0 is independent of any x_i, the change of $\bar{E}$ (at constant environmental conditions) corresponds to a monotonic increase

$$W_m^0 < W_{m+1}^0 < \cdots < W_{\text{opt}}^0. \qquad \text{(II-35)}$$

The final state is an "optimum state", i.e. a maximum under constraints, given in the form of inequalities. The system then can reach only certain states among the total set of N possibilities; it is bound to a certain path by which the system is required to "climb".
If internal couplings are present—expressed by concentration dependences of the W_i^0-parameters—the whole optimization process is more complex. The maximum of a selective value among a population has no "absolute" meaning since it refers now to a given distribution of concentrations x_i. In such a system any change in the distribution of the x_i represents a "change of environment". Optimization here refers to a "differential" process; it may well be accompanied by a general decrease of (possibly all) selective values (e.g. as a consequence of "pollution" caused by one of the selected information carriers). It may also consist of the utilization of a larger information content (in order to cope with the changed environment). In general, the optimization procedure of evolution does not need to be a simple *monotonic* variation of selective values. Whatever the final state is, here $\bar{E}$ refers to the maximum value of W^0 of all competitors present in the final phase. Those species which belong to a cooperative system will reach the same value of W_m^0 and the equality relation can be used to calculate the "equilibrium" distribution of selected species in analogy to a "law of mass action".
It is important to notice that the distinction of "selection" (occurring among a given set of populated states at fixed environmental conditions) from "evolution" (as a further optimization procedure with respect to changing population and environment) is an abstraction. If we consider the whole process of evolution as a game, this abstraction serves to use the selection mechanism as an executive tool for evaluating the state of the game according to certain rules and thus to replace the player. This abstraction is approximately verified only for systems with $nV \ll N$ and $\mathcal{Q}$ close to 1. It also requires experimental conditions which allow selection to occur within times which are short as compared to the time of evolutionary change.

b) Constant Overall Fluxes of Organized and Unorganized Digits

The system of phenomenological equations has a somewhat more complicated form than that referring to the first type of constraints. Let us therefore consider a simplified case which, however, still possesses all the essential features: All information carriers (including error copies) are assumed to have an approximately uniform overall composition and their formation rate to be described by the same (average) stoichiometric function $f(m_1 \ldots m_\lambda)$. Furthermore, a uniform and constant influx ϕ_M of the energy-rich monomers (in constant proportion) is assumed. The constraint introduced with Eq. (II-27) and (II-28) then leads to:

$$f(m_1 \ldots m_\lambda) = \frac{\phi_M}{k_0 \sum_k^N \mathscr{A}_k' x_k} \qquad \text{(II-36)}$$

and the rate equations can be written in analogy to Eq. (II-32) (neglecting "back flow" terms)

$$\dot{x}_i = \frac{k_0 \mathscr{D}_i}{\bar{P}+1} [W_i^F - \bar{P}] x_i. \qquad \text{(II-37)}$$

Here $\bar{P}$ is again mean of a "productivity", however, with the definition

$$\bar{P} = \frac{\bar{\mathscr{A}'}}{\bar{\mathscr{D}}} - 1, \qquad \text{(II-38)}$$

with

$$\bar{\mathscr{A}'} = \frac{\sum_{k=1}^N \mathscr{A}_k' x_k}{\sum_{k=1}^N x_k} \quad \text{and } \bar{\mathscr{D}} \text{ accordingly.}$$

Both, productivity and selective value

$$P_i = \frac{\mathscr{A}_i'}{\mathscr{D}_i} - 1; \quad W_i^F = \frac{\mathscr{A}_i' \mathscr{Q}_i}{\mathscr{D}_i} - 1 \qquad \text{(II-39)}$$

refers here to the constraints of constant flows.
If $f(m_1 \ldots m_\lambda)$ does not represent a generally valid stoichiometry function, one may still formally obtain Eq. (II-36) using suitable averages besides the individual stoichiometric terms.

The case of constant flows is of special interest with respect to an application of the principle of Prigogine and Glansdorff, which was discussed at the end of Section I. This principle refers to changes of forces at constant fluxes. The system due to the sliding threshold again selects for a maximum selective value among the present population. Here, it can be shown that each mutation leading to a further increase of the "selective value" corresponds to a negative

fluctuation of entropy production, indicating instability of the existing steady state. Evolution at constant flows corresponds to a sequence of such instabilities, in which the dominant species i_m die out in favor of new species i_{m+1} according to a finite (positive) selective advantage $(W^F_{m+1} - W^F_m)$.

II.4. Selection Equilibrium

We have called the state of maximum productivity of a given population "selection equilibrium". This "equilibrium" involves fluctuations of the error distribution and is metastable with respect to the occurrence of species with selective advantages. Nevertheless, as in chemical thermodynamics, we can derive "equilibrium constants" from the conditions (II-33) or (II-34) or their analogues for the constraint of constant flows, respectively.

a) Constant Overall Organization

We write the mean productivity $\bar{E}$ as

$$\bar{E} = \frac{E_m x_m + \sum_{k \neq m} E_k x_k}{\sum x_k} = \bar{E}_{k \neq m} + \frac{x_m}{n}(E_m - \bar{E}_{k \neq m}) \qquad \text{(II-40)}$$

by defining a mean value of the residual productivity

$$\bar{E}_{k \neq m} = \frac{\sum_{k \neq m} E_k x_k}{\sum_{k \neq m} x_k} \qquad \text{(II-41)}$$

using

$$\sum_{k=1}^{N} x_k = \text{const} = n \quad \text{and} \quad \sum_{k \neq m} x_k = n - x_m. \qquad \text{(II-42)}$$

The equilibrium condition (II-33) then yields for the "equilibrial fraction" of the selected species in first approximation ($\mathcal{Q} \lesssim 1$, neglecting "back flow" terms, cf. II.6.c)

$$\frac{\bar{x}_m}{n} = \frac{W^0_m - \bar{E}_{k \neq m}}{E_m - \bar{E}_{k \neq m}}. \qquad \text{(II-43)}$$

The selection criterion can be written

$$W^0_m > \bar{E}_{k \neq m}$$

giving a physical definition to the Darwinian term "fittest".

We notice that while the survival ratio $\bar{x}_m/n$ is not directly proportional to $\mathcal{Q}_m$, the stationary error fraction $(1 - \bar{x}_m/n)$ is proportional to $(1 - \mathcal{Q}_m)$, i.e.

$$1 - \frac{\bar{x}_m}{n} = \frac{\mathcal{A}_m}{E_m - \bar{E}_{k \neq m}}(1 - \mathcal{Q}_m). \qquad \text{(II-44)}$$

If $\mathcal{Q}_m$ were equal to one, W^0_m would equal E_m and $\bar{x}_m$ would approach n. This would be the extreme of a selection process, but without any usefulness for further evolution. The "value" such a system has acquired is restricted to a choice from a relatively limited (random) variety of maximally n species. We see also that the term "value" has no meaning unless we specify "for what". Value for selection under special circumstances already differs from a more general value for optimal evolution. What we would need for the latter would be a quality factor $\mathcal{Q}_m$ just large enough to ensure survival

$$\mathcal{Q}_m > \mathcal{Q}_{\min} = \frac{\bar{\mathcal{A}}_{k \neq m} + \mathcal{D}_m - \bar{\mathcal{D}}_{k \neq m}}{\mathcal{A}_m} \qquad \text{(II-45)}$$

but otherwise as small as possible to provide as many as possible mutants from which further "progress" could arise. The system has to fulfil the very important selection condition (II-45) in order to preserve the "information" thus far gained. Then the effect of a low $\mathcal{Q}_m$ ($> \mathcal{Q}_{\min}$) is twofold: first, it produces a larger *variety* of mutants among which the system can select and thus allows a higher ultimate optimum value of W_m, second, it speeds up the rate of evolution.

Selection equilibrium in coupled reaction systems involves the survival of whole ensembles of information carriers. Here we have to solve systems of algebraic equations. The "selective values" as well as the "productivities" may become quite involved expressions containing all the $\mathcal{A}$, $\mathcal{Q}$ and $\mathcal{D}$-parameters of the coupled system. Examples will be discussed in Sections V and VI. For linear systems we can transform the variables and represent the whole ensembles by "normal modes" which behave analogously to the concentrations of single selfinstructing species. Also, for certain nonlinear systems equilibrium relations can be calculated explicitly. It is obvious that for those systems the term "equilibrium" has much in common with what we usually call chemical equilibrium, since it correlates the concentrations of several and sometimes even many components. However, the difference is that in a true equilibrium the partners interconvert, whereas in selection equilibrium the partners are assembled from one reservoir and decompose into another reservoir without interconverting; but they do it in a correlated way which ensures fixed proportions as long as fixed environmental conditions are maintained.

b) Constant Information Flow

In analogy to Eq. (II-43) we may calculate the equilibrium ratio of a selected species using the definitions introduced in II.3.b) and obtain [again as an approximation for $\mathcal{Q} \lesssim 1$]

$$\frac{\bar{x}_m}{\phi_M / k_0 \mathcal{D}_m} \equiv \frac{\bar{x}_m}{\bar{n}} = \frac{W^F_m - \bar{P}_{k \neq m}}{P_m - \bar{P}_{k \neq m}}. \qquad \text{(II-46)}$$

Here $\bar{x}_m$ is not normalized to $\sum_{k=1}^{N} x_k$, which is not invariant, as in the case of constant overall organization, but rather to the analogous (at a given $\mathcal{D}_m$) constant quantity

$$\frac{\phi_M}{k_0 \mathcal{D}_m} = \frac{\sum_{k=1}^{N} \mathcal{D}_k x_k}{\mathcal{D}_m} \equiv \bar{n}. \qquad \text{(II-47)}$$

The flux ϕ_M here is the conserved quantity and $\phi_M / k_0 \mathcal{D}_m$ is the analogue of an overall concentration. Again the selection criterion is given by $W^F_m > \bar{P}_{k \neq m}$ in analogy to selection equilibrium at constant organization.

Selective advantages can be introduced via any of the three parameters $\mathcal{A}$, $\mathcal{Q}$ and $\mathcal{D}$ which determine the selective value. Accordingly, three limiting cases may be distinguished (cf. Table 7), which include repression, derepression, specific promotion, digestion blocked by specific protection etc.

Table 7. *Selection criteria (limiting cases with respect to $\mathcal{A}_m$, $\mathcal{Q}_m$ and $\mathcal{D}_m$)*

1. $\mathcal{D}_m = \mathcal{D}_{k\neq m}$, i.e. $\phi_M/\mathcal{R}_m = \sum_k x_k = n$.

Constant forces and constant fluxes

$$\frac{\bar{x}_m}{n} = \frac{\mathcal{A}_m\mathcal{Q}_m - \bar{\mathcal{A}}_{k\neq m}}{\mathcal{A}_m - \bar{\mathcal{A}}_{k\neq m}};\ \text{selection, if } \mathcal{A}_m\mathcal{Q}_m > \bar{\mathcal{A}}_{k\neq m}.$$

2. $\mathcal{A}_m = \bar{\mathcal{A}}_{k\neq m}$.

a) Constant forces

$$\frac{\bar{x}_m}{n} = \frac{(\bar{\mathcal{D}}_{k\neq m} - \mathcal{D}_m) - (1-\mathcal{Q}_m)\bar{\mathcal{A}}_{k\neq m}}{\bar{\mathcal{D}}_{k\neq m} - \mathcal{D}_m};$$

selection, if $\bar{\mathcal{D}}_{k\neq m} > \mathcal{D}_m + \bar{\mathcal{A}}_{k\neq m}(1-\mathcal{Q}_m)$.

b) Constant fluxes

$$\frac{\bar{x}_m}{n} = \frac{\mathcal{Q}_m\bar{\mathcal{D}}_{k\neq m} - \mathcal{D}_m}{\bar{\mathcal{D}}_{k\neq m} - \mathcal{D}_m};\ \text{selection, if } \mathcal{Q}_m\bar{\mathcal{D}}_{k\neq m} > \mathcal{D}_m.$$

3. $\mathcal{A}_m\mathcal{Q}_m = \bar{\mathcal{A}}_{k\neq m}$.

a) Constant forces

$$\frac{\bar{x}_m}{n} = \frac{\bar{\mathcal{D}}_{k\neq m} - \mathcal{D}_m}{(\mathcal{A}_m - \bar{\mathcal{A}}_{k\neq m}) + (\bar{\mathcal{D}}_{k\neq m} - \mathcal{D}_m)};$$

selection, if $\bar{\mathcal{D}}_{k\neq m} > \mathcal{D}_m$.

b) Constant fluxes

$$\frac{\bar{x}_m}{n} = \mathcal{Q}_m\frac{\bar{\mathcal{D}}_{k\neq m} - \mathcal{D}_m}{\bar{\mathcal{D}}_{k\neq m} - \mathcal{Q}_m\mathcal{D}_m};\ \text{selection, if } \bar{\mathcal{D}}_{k\neq m} > \mathcal{D}_m.$$

II.5. Quality Factor and Error Distribution

It is quite obvious that "selective value" as a dynamical property depends on rate parameters such as $\mathcal{A}$ and $\mathcal{D}$. Less evident is the role of the quality factor $\mathcal{Q}$, which describes the exactness of reproduction. For simple models $\mathcal{Q}$ can be explicitly related to molecular parameters, such as the preciseness of single-digit recognition q_i, which may be measured in terms of free energies (or activation parameters) for the interaction of matching (complementary) and mismatching (non-complementary) pairs of single digits. Usually such a recognition involves cooperative interactions, for which a specification of the nearest (and possibly next-nearest) neighbor pair is required. This will enlarge appreciably the number of possible combinations. An example concerning the enzyme-free recognition of the nucleobases A, U, G and C ($\lambda = 4$) is discussed in Part IV.

If only two stabilities have to be distinguished, i.e. one for all (degenerate) complementary and another for all (degenerate) non-complementary pairs, the relations between the phenomenological parameter $\mathcal{Q}$ and a molecular (single-digit) recognition parameter q are quite straightforward. The corresponding relations for such an example are compiled in Table 8. They can be generalized for models involving several non-degenerate classes of digits (e.g. nucleotides). The significance of $\mathcal{Q}$ being a quality factor is clearly demonstrated by the graphic representation of the error distributions in Fig. 4. The smaller the expectation value for errors, the sharper the corresponding $\mathcal{Q}$ curve, i.e. the higher the "quality" factor. The important evolution criterion, Eq. (II-45), which correlates the "spread" of rate parameters with a minimum quality factor $\mathcal{Q}_{\min}$, defines a maximum information content ($\nu_{\max}$) which can be reproducibly preserved for a given recognition parameter q (cf. Eq. (10) in Table 8):

$$\nu_{\max} = \frac{|\ln \mathcal{Q}_{\min}|}{1-q}.$$

As a consequence, the elementary recognition mechanism (i.e. q) *must improve with the increase of the information content I_ν of a species during evolution.* At higher levels of molecular evolution quite sophisticated control mechanisms are required to improve the accuracy of single-digit recognition (e.g. enzymic code checking) and to allow for a sufficient spread of the $\mathcal{A}$ and $\mathcal{D}$ parameters (cf. Parts IV to VI).

Table 8. *The quality factor $\mathcal{Q}$ for a "two-state" model*

Probability for exact reproduction of a *single digit:* q.

Probability that one of the $(\lambda-1)$ fold *degenerate errors* occurs: $(1-q)$.

Expectation value of errors in sequence of ν digits:

$$\varepsilon = \nu(1-q). \tag{1}$$

Degeneracies:

1	arrangement with	0 defects
$\nu(\lambda-1)$	arrangements with	1 defect
$\binom{\nu}{k}(\lambda-1)^k$	arrangements with	k defects,

(2)

$$\text{sum: } \sum_{k=0}^{\nu}\binom{\nu}{k}(\lambda-1)^k = \lambda^\nu \quad \text{possible sequences (cf. Table 4).} \tag{3}$$

Probability for occurrence of *error-free* copy:

$$\mathcal{Q} = Q_{\nu 0} = q^\nu \to e^{-\varepsilon}. \tag{4}$$

Probability distribution for the occurrence of sequences with *k and only k defects:*

$$Q_{\nu k} = q^{(\nu-k)}(1-q)^k\binom{\nu}{k}\ \text{(binomial distribution)} \tag{5}$$

for $k(1-q) \ll 1$ approximated by *Poisson distribution:*

$$Q_{\nu k} = \frac{\varepsilon^k \cdot e^{-\varepsilon}}{k!}. \tag{6}$$

Sum of error copies:

$$\sum_{k=1}^{\nu} q^{(\nu-k)}(1-q)^k\binom{\nu}{k} = 1-q^\nu = 1-Q_{\nu 0}. \tag{7}$$

Probability for occurrence of *one specific mutant* containing *k errors* in *defined positions:*

$$P_{\nu k} = \frac{Q_{\nu k}}{\binom{\nu}{k}(\lambda-1)^k} = \frac{q^{(\nu-k)}(1-q)^k}{(\lambda-1)^k} \to \left[\frac{q^{-1}-1}{\lambda-1}\right]^k e^{-\varepsilon}. \tag{8}$$

The production rate of a given mutant (e.g. with a selective advantage) is proportional to $P_{\nu k}$.

The probability distributions (5), (6) and (8) are shown in Fig. 4.

A minimum value of $Q_{\nu 0}$ for stable selection has been defined by Eq. (II-45). For a given recognition factor q, the criterion for stable selection can be expressed as

$$\nu < \frac{|\ln \mathcal{Q}_{\min}|}{|\ln q|} \tag{9}$$

or for $(1-q) \ll 1$

$$\nu < \frac{|\ln \mathcal{Q}_{\min}|}{1-q} \tag{10}$$

defining a limiting value of information content $I_{\max}$ which can be correctly reproduced and maintained.

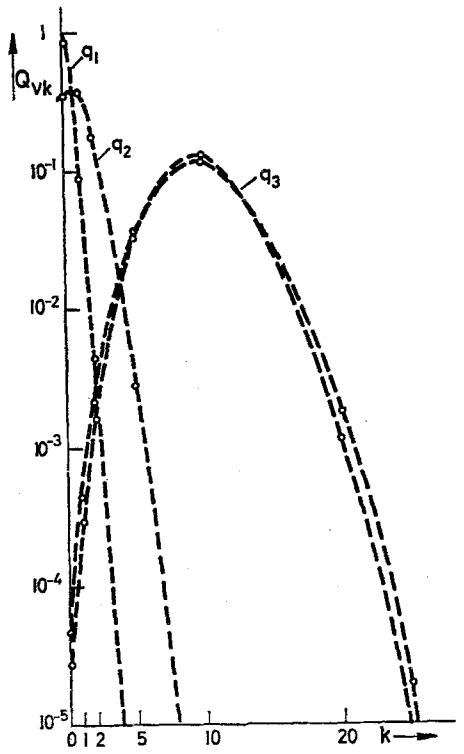

Fig. 4. Probability distributions for the occurrence of error copies: Q_{vk} according to Eq. (5) in Table 8

The parameters are:

$$\begin{array}{llll} \nu = 100 & q_1 = 0.999 & \varepsilon_1 = 0.1 \\ \lambda = 4 & q_2 = 0.990 & \varepsilon_2 = 1 \\ & q_3 = 0.900 & \varepsilon_3 = 10. \end{array}$$

The two curves for q_3 represent a comparison of the binominal and the Poisson distribution Eqs. (5) and (6). For q_1 and q_2 these curves coincide (within the accuracy of the plot) for small k-values. However, large deviations occur for $k \to \nu$. The maxima of the curves occur at $k = \varepsilon$. If plotted on a linear scale the distributions are extremely sharp. P_{vk} according to Eq. (8) is not represented graphically, because it drops too sharply with increasing k. Examples for q_2:

k	P_{vk}
0	$3.7 \cdot 10^{-1}$
1	$1.2 \cdot 10^{-3}$
2	$4.2 \cdot 10^{-6}$

It was the aim of this discussion to demonstrate the importance of the quality factor $\mathcal{Q}_i$, not only for selection among a given population, but also for the rate and ultimate optimum state of further evolution. Among a population characterized by comparable rate parameters the system seeks high $\mathcal{Q}$ values, and this seems to be disadvantageous for the rate of further evolution. On the other hand, higher $\mathcal{Q}$ values allow the formation of species with a higher information content, which ultimately will turn out to be of advantage for further evolution. We see that the process of optimization may sometimes involve contradictory requirements. For instance, specific substrate recognition by the enzyme requires high stability constants for the enzyme-substrate complex, yet too high stability constants limit the rate of turnover. This problem of optimal evolution resulting from a generalization of the value concept will be taken up in Section VIII. We see here already that "value" always requires specification of the property which is valued, the more so, the higher the level of evolution.

The essential result of these considerations is that $I_{\max}$—the maximum information content $\nu_{\max} \frac{\ln \lambda}{\ln 2}$ to be maintained reproducibly—has to be adapted to the accuracy of elementary digit recognition. The quantity, as defined by the variation of the $\mathscr{A}$ and $\mathscr{D}$ parameters, enters only as a logarithmic term, and hence it will be of restrictive influence only for small variations of $\mathscr{A}$ and $\mathscr{D}$ (i.e. $\mathscr{A}_i \approx \bar{\mathscr{A}}_{k \neq i}$ *and* $\mathscr{D}_i \approx \bar{\mathscr{D}}_{k \neq i}$).

II.6. Kinetics of Selection

The phenomenological equations for both constraints always represent systems of nonlinear differential equations. Explicit solutions, of course, depend on the special form of the equations as determined by the particular reaction mechanism. Several mechanisms of selforganizing systems involving proteins and nucleic acids will be discussed in Parts IV to VI. Here we shall deal only with some prototypes of solutions for constant parameters W_i in order to characterize the process of selection. Let us consider three cases of increasing complexity:

a) Constant Overall Organization; $\bar{E}_{k \neq i}$ Variable; $\mathcal{Q}_i \approx 1$, i.e. $W_i^0 \approx E_i$

An exact solution of the system of differential Eqs. (II-32) can be given, as long as any flow of information into and out of mutant copies—as represented by the terms $\sum_{l \neq i} \varphi_{il} x_l$—is completely negligible ($\mathcal{Q}_i = 1$). The system of equations then has the simple form:

$$\dot{x}_i = k_0 [W_i^0 - \bar{E}]\, x_i \qquad \text{(II-48)}$$

and the solution reads $[x_i^0 = x_i(t=0)]$:

$$x_i(t) = \frac{x_i^0\, n \exp(k_0 W_i^0 t)}{\sum_{k=1}^{N} \frac{x_k^0 E_k}{W_k^0} \exp(k_0 W_k^0 t)}. \qquad \text{(II-49)}$$

where any W_k^0 could as well be replaced by E_k.

This solution can be arrived at by starting from the implicit form, obtained by integration of (II-48):

$$x_i(t) = x_i^0 \frac{\exp(k_0 W_i^0 t)}{\exp\left\{k_0 \int_0^t \bar{E}(\tau)\, d\tau\right\}}. \qquad \text{(II-50)}$$

The integral term drops out for any ratio (x_k/x_i) which can be inserted into $\bar{E}$, if this is written as

$$\bar{E} = \frac{x_i}{n} \sum_{k=1}^{N} \frac{x_k}{x_i} E_k. \qquad \text{(II-51)}$$

The rate equation (II-48)

$$\dot{x}_i = k_0 W_i^0 x_i - k_0 \frac{\sum_{k=1}^{N} x_k^0 E_k \exp[k_0 (W_k^0 - W_i^0)\, t]}{n x_i^0} x_i^2 \qquad \text{(II-52)}$$

then represents a special form of Bernoulli's differential equation

$$\dot{x} + g(t)\, x + f(t)\, x^2 = 0 \qquad \text{(II-53)}$$

with the well-known solution [50]

$$\frac{1}{x(t)} = e(t)\int \frac{f(t)}{e(t)}\,dt; \qquad e(t) = \exp\left[\int g(t)\,dt\right]. \quad \text{(II-54)}$$

Inserting

$$g(t) = \text{const} = -k_0 W_i^0 \quad \text{(II-55)}$$

$$f(t) = \frac{k_0}{n x_i^0}\sum_{k=1}^{N} x_k^0 E_k \exp\left[k_0 (W_k^0 - W_i^0) t\right] \quad \text{(II-56)}$$

yields the solution Eq. (II-49).

This solution describes explicitly a selection procedure. At $t=0$ each x_i is given by its initial value x_i^0. For $t\to\infty$ the sum of exponentials can be represented by the largest term, which belongs to the species with the highest "selective value": W_m^0. This species will be selected. It approaches the stationary value

$$\bar{x}_m = \frac{W_m^0}{E_m}\, n \quad \text{(II-57)}$$

which is equal to n as long as $Q_m = 1$ (i.e. $W_m^0 = E_m$). All other species must ultimately decay according to:

$$x_i(t) = n\,\frac{x_i^0}{x_m^0}\,\frac{W_m^0}{E_m}\exp\left[(W_i^0 - W_m^0)\, k_0\, t\right]. \quad \text{(II-58)}$$

Before x_m has grown to its "dominant" level, some of the $x_i(t)$ might initially increase and pass through a maximum before they decay. Fig. 5 shows the example of four competing species.

This treatment may still provide a useful solution for the mastercopy, if Q_m is not exactly equal but close to one. When the selected "master copy" has grown to a dominant level, it will compete mainly with mutants resulting from incorrect reproduction. The preceding treatment does not account for the behavior of these mutants, for which according to Eq. (II-13) additional rate terms (especially $\varphi_{im}\, x_m$) have to be taken into consideration. As a consequence, their concentrations will not decay to zero. For the selected master copy Eqs. (II-49) and (II-57) provide a good approximation as long as $\bar{E}_{k\neq m} \ll W_m^0$. Here we do not specify any error copy, but realize that a certain amount of errors is present. (If $W_m^0 = E_m$, i.e. $Q_m = 1$, the system could not evolve further.) Before we consider in more detail the interactions among master copy and mutants, we may treat a special case where a straightforward solution for $\mathcal{Q}_i \lesssim 1$ can be given.

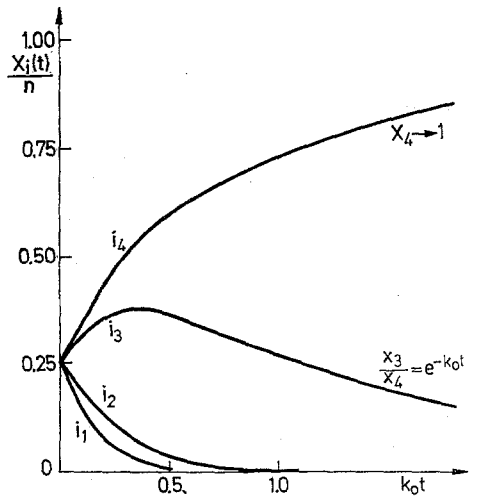

Fig. 5. Selection among four competing species, according to Eq. (II-49). $W_1^0 = 1$; $W_2^0 = 4$; $W_3^0 = 9$; $W_4^0 = 10$

b) Constant Overall Organization or Constant Flows; $\bar{E}_{k\neq m}$ or $\bar{P}_{k\neq m}$ Constant

This case describes either the competition between two species m and k or the competition with a whole degenerate class of species $k \neq m$ with constant $\bar{E}_{k\neq m}$ or $P_{k\neq m}$. We see also that this case represents a good approximation for selection among species which are not degenerate in $E_{k\neq m}$ or $P_{k\neq m}$ but show a fairly constant distribution around average values $\bar{E}_{k\neq m}$ and $\bar{P}_{k\neq m}$, whereas the selected species has a distinctive reproduction rate with $W_m^0 > \bar{E}_{m\neq k}$ or $W_m^F > \bar{P}_{k\neq m}$.

In the case of constant overall organization, the solution for the selected species reads:

$$x_m(t) = x_m^0\,\frac{\exp\left[(W_m^0 - \bar{E}_{k\neq m})\, k_0\, t\right]}{1 + \frac{x_m^0}{\bar{x}_m}\left\{\exp\left[(W_m^0 - \bar{E}_{k\neq m})\, k_0\, t\right] - 1\right\}} \quad \text{(II-59)}$$

where

$$\bar{x}_m = n\,\frac{W_m^0 - \bar{E}_{k\neq m}}{E_m - \bar{E}_{k\neq m}} \quad \text{(cf. Eq. (II-43))}$$

is the "equilibrium" value of x_m as introduced in II.4., which will be reached for $t\to\infty$. Again, all "independent" species with $W_{k\neq m}^0 < W_m^0$ will ultimately decay to zero, whereas the sum of all mutants (which are degenerate according to the assumption $\bar{E}_{k\neq m} =$ const) will reach the stationary level expressed by Eq. (II-44).

There is still one limitation with respect to $\mathcal{Q}_m$: it must not be too small, so that any recurrence of the species i_m from mutants (by reversal of the error in subsequent reproductions) is negligible. The tolerance limit for $\mathcal{Q}_m$ here depends on the information content, i.e. on the number ν of the digits involved. The same restriction also holds for the "equilibrium" value according to Eq. (II-43). Otherwise, solution (II-59) is a good approximation for the final phase of selection within a population, where virtually only competition among mutants (and master copy) is involved. Here the mean value $\bar{E}_{k\neq m}$ can indeed be approximated by a constant so that this solution reproduces the correct "equilibrium" value of x_m (and also holds for the neighborhood of selection "equilibrium"). The first case, i.e. Eq. (II-49), on the other hand, does not lead to the correct equilibrium value except for $\bar{E}_{k\neq m} \ll W_m^0$; but it is a good approximation for the initial phase of selection, where the number of mutants is small compared to the number of unrelated copies. Solutions similar to (II-59), but with $\mathcal{Q} = 1$, have been repeatedly discussed in literature on population genetics [51]. A well-known example is the Ross equation for the spread of malaria [52]. A. J. Lotka also has given general function theoretical criteria for the stability of the solutions of such equations [53].

The problem of "two-species" competition can also be solved explicitly under the constraint of *constant fluxes*, at least with the assumption of uniform stoichiometry $f(m_1 \ldots m_\lambda)$ for all species, as made in connection with Eq. (II-36) and (II-46). In the rate equation (II-37) the terms $\bar{P}_{k\neq m}$, W_m^F and $n\,\mathcal{A} = n\,\bar{\mathcal{D}} = \phi_M$ are constants (assuming simple first-order formation and decomposition rates), whereas the terms $n\,\mathcal{A}'$ and thus

$(1+\bar{P}) \equiv \mathscr{A}'/\bar{\mathscr{D}}$ are linear functions of x_m. The individual rate equations, having the form

$$\dot{x}_m = \frac{A + B x_m}{C + D x_m} x_m \qquad \text{(II-60)}$$

with

$$A = \phi_M \mathscr{D}_m (W_m^F - \bar{P}_{k\neq m}); \quad C = \frac{\Phi_M}{k_0}(1+\bar{P}_{k\neq m})$$

$$B = -k_0 \mathscr{D}_m^2 (P_m - \bar{P}_{k\neq m}); \quad D = -B/k_0 \mathscr{D}_m$$

can be integrated and yield the solutions

$$\frac{[x_m(t)]^{\alpha_m}}{\bar{x}_m - x_m(t)} = \frac{[x_m^0]^{\alpha_m}}{\bar{x}_m - x_m^0} \exp\{k_0 \mathscr{D}_m [1-\alpha_m] t\}$$

or alternatively

$$\frac{[x_m(t)]^{\alpha_m}}{[x_m(t)]^{\alpha_m} + [\bar{x}_m - x_m(t)]} = \frac{[x_m^0]^{\alpha_m} \exp\{k_0 \mathscr{D}_m [1-\alpha_m] t\}}{[\bar{x}_m - x_m^0] + [x_m^0]^{\alpha_m} \exp\{k_0 \mathscr{D}_m [1-\alpha_m] t\}} \qquad \text{(II-61)}$$

with

$$\alpha_m = \frac{1+\bar{P}_{k\neq m}}{1+W_m^F} \quad \text{or} \quad (1-\alpha_m) = \frac{W_m^F - \bar{P}_{k\neq m}}{1+W_m^F}. \qquad \text{(II-62)}$$

This solution behaves quite similarly to Eq. (II-59). For $t \to 0$ the "selected" species grows according to

$$x_m(t) = x_m^0 \exp\left\{\frac{k_0 \mathscr{D}_m}{1+\bar{P}_{k\neq m}} (W_m^F - \bar{P}_{k\neq m}) t\right\} \qquad \text{(II-63)}$$

(approximation for $x_m(t) \ll \bar{x}_m$), whereas for $t \to \infty$, $x_m(t)$ approaches the "equilibrium value"

$$\bar{x}_m = \bar{n} \frac{W_m^F - \bar{P}_{k\neq m}}{P_m - \bar{P}_{k\neq m}} \quad \text{(cf. Eq. (II-46))}.$$

c) Constant Overall Organization, $\bar{E}_{k\neq m}$ Variable, Approximate Consideration of Error Production: $\mathscr{Q} < 1$ (but not $\ll 1$)

During the growth of a selected species, reproducibly occurring mutants of the master copy i_m will contribute increasingly to the total production. These mutants can be divided into classes according to the number of defective positions (as compared to the master copy), i.e. $\sum_k i_{1k}$, $\sum_k i_{2k}$ etc. For sequences of ν digits the number of copies in each class is given by the binomial coefficients and for λ types of digits each defect at a given position is $(\lambda-1)$-fold degenerate; thus we have $\binom{\nu}{l}(\lambda-1)^l$ different copies in a class of l defects. The frequency of production of the different individual copies i_{lk} will decrease with increasing l—e.g. for the simplest model assuming uniform q-factors for single digit recognition (cf. Table 8) proportional to

$$\frac{q^{(\nu-l)}(1-q)^l}{(\lambda-1)^l}.$$

Correspondingly, each class of defects will also contribute to a restoration of the master copy, again decreasingly with increasing number of defects.

In order to get some quantitative idea of this influence of mutants on selection, let us consider a simple approximation: the influence of single-digit defects. This approximation holds only as long as the expectation value of errors $\nu(1-q)$ is still appreciably smaller than one, i.e. $0 \ll \mathscr{Q} < 1$.

Then we have to distinguish three classes of rate equations ($W \equiv W^0$ always refers to constant organization).

a) For one master copy i_m

$$\dot{x}_m = k_0 (W_m - \bar{E}) x_m + k_0 \sum_{k=1}^{\nu(\lambda-1)} \frac{E_{1k} - W_{1k}}{\beta_{1k}} x_{1k}. \qquad \text{(II-64a)}$$

b) For $\nu(\lambda-1)$ mutants i_{1i} having one single digit defect

$$\dot{x}_{1i} = k_0 (W_{1i} - \bar{E}) x_{1i} + k_0 \frac{E_m - W_m}{\beta_{mi}} x_m. \qquad \text{(II-64b)}$$

c) For $(n - x_m - \sum_k x_{1k})$ independent competitors i_{ji}

$$\dot{x}_{ji} = k_0 (W_{ji} - \bar{E}) x_{ji}. \qquad \text{(II-64c)}$$

The third category of independent competitors can be considered also as mutants of the master copy having $j \geqq 2$ defects. For sufficiently long sequences, this number of possible competitors is so large that the probability of finding any given copy by chance is practically zero. Their production as mutants of the master copy is assumed to be negligily small. This includes those mutants of the master copy which have defects in only two positions. The approximation then requires (as long as independent competitors contribute essentially to the solution) that

$$\sum_k x_{2k} \ll \sum_{j>2} \sum_k x_{jk}. \qquad \text{(II-65)}$$

The factors β in Eq. (II-64a and b) generally correlate the production of the particular mutant with the total defect production, expressed by

$$\mathscr{A}_i (1 - \mathscr{Q}_{i0}) = (E_i - W_i).$$

For a uniform q with $\mathscr{Q}_{i0} = q^n$, and for uniform rates of error production, β_{mi}— for instance—reduces to

$$\beta_{mi} = \nu(\lambda-1) \qquad \text{(II-66)}$$

since the fraction $1/\{\nu(\lambda-1)\}$ of all errors produced from the master copy will correspond to the production of any particular single defect copy.

In order to get a self-consistent approximation we have to neglect any mutant formation other than production of single defects in the master copy and reproduction of the master copy from single defect copies. This requires to assume $W_{ji} \approx E_{ji}$, $(E_{1k} - W_{1k}) = \mathscr{A}_{1k}(1-q)$ and $\beta_{1k} = 1$. The latter assumption, i.e. to replace $\mathscr{Q}$ by q for any of the single defect copies seems at first glance somewhat unrealistic, since it allows only for one kind of mistake, namely the one which leads to restauration of the master copy. However, the neglection of other errors in the reproduction of single defect copies (which would lead to copies with two defects) is consistent with this approximation and affects only small correction terms. For any of the single copies i_{1k} the difference of E_{1k} and W_{1k} is certainly negligibly small; however the sum of all these terms occuring in Eq. (II-64a) must be taken into consideration for this approximation.

For the master copy we obtain the following time dependence of concentration $(x_m^0 = x_m(t=0))$

(Eq. (II-67) see below)

with

$$A_k = -\frac{E_m - W_m}{\beta_{mk}(W_{1k} - W_m')}$$

$$B_k = \frac{W_m'}{W_{1k}} E_{1k} \left(x_{1k}^0 + x_m^0 \frac{E_m - W_m}{\beta_{mk}(W_{1k} - W_m')}\right) \qquad \text{(II-68)}$$

$$C_{jk} = \frac{W_m'}{W_{jk}} E_{jk} x_{jk}^0.$$

W_m' can be expressed as

$$W_m' \approx W_m + \frac{E_m - W_m}{W_m - \bar{E}_{1k}} \left\langle \frac{E_{1k} - W_{1k}}{\beta_{1k}} \right\rangle \qquad \text{(II-69)}$$

$$\frac{x_m(t)}{n} = \frac{W_m' x_m^0}{x_m^0 \left[E_m + \sum_{k=1}^{\nu(\lambda-1)} A_k E_{1k}\right] + \sum_{k=1}^{\nu(\lambda-1)} B_k \exp\{k_0 (W_{1k} - W_m') t\} + \sum_{i,k} C_{jk} \exp\{k_0 (W_{jk} - W_m') t\}} \qquad \text{(II-67)}$$

where the symbol $\langle\ \rangle$ represents the "equilibrium average" taken over all $\nu(\lambda-1)$ single defect copies. It is seen that W'_m reduces to W_m for $\mathcal{Q}_m \to 1$. For $t \to \infty$ the solution approaches the equilibrium ratio (cf. Eq. (II-43))

$$\frac{\bar{x}_m}{n} = \frac{W'_m - \bar{E}_{1k}}{E_m - \bar{E}_{1k}} \qquad \text{(II-70)}$$

where any residual term is negligibly small within the limits of the present approximation, which requires the master copy to be distinguished by a sufficiently large selective advantage: $W'_m > W_{1k}$.
How small the difference between W'_m and W_m is, can be seen rom the model of uniform digit recognition which yields

$$W'_m - W_m \approx \frac{\nu(1-q)^2}{\lambda-1} \frac{\mathscr{A}_m \bar{\mathscr{A}}_{1k}}{W_m - \bar{E}_{1k}} \qquad \text{(II-71)}$$

with $(1-q) \ll 1/\nu$. (Note that the averages $\bar{\mathscr{A}}_{1k}$ and $\bar{E}_{1k}$ in this model replace the previous averages $\bar{\mathscr{A}}_{k+m}$ and $\bar{E}_{k+m}$.)
The solutions for any of the single-defect copies i_{1i} as well as for the independent competitors i_{ji} are related to the solution for the master copy by

$$\frac{x_{1i}(t)}{x_m(t)} = \left\{\frac{x^0_{1i}}{x^0_m} + \frac{E_m - W_m}{\beta_{mi}(W_{1i} - W'_m)}\right\} \cdot \exp\left[k_0 (W_{1i} - W'_m)t\right] - \frac{E_m - W_m}{\beta_{mi}(W_{1i} - W'_m)} \qquad \text{(II-72)}$$

$$\frac{x_{ji}(t)}{x_m(t)} = \frac{x^0_{ji}}{x^0_m} \exp\{k_0 (W_{ji} - W'_m)t\}. \qquad \text{(II-73)}$$

In order to obtain such explicit solutions for the single species, it was necessary to make the assumption that "equilibrium" among master copy and mutants is attained before the competitors are outgrown, so that in the (small) correction term in Eq. (II-64a) the ratio

$$\sum_{k=1}^{\nu(\lambda-1)} x_{1k}/x_m$$

can be replaced by the (constant) equilibrium ratio. The rate equation for the master copy then assumes the simple form

$$\dot{x}_m = k_0 [W'_m - \bar{E}]\, x_m \qquad \text{(II-74)}$$

where W'_m differs from W_m only by a term which is small as long as $\sum_k x_{1k}$ remains small compared with x_m (i.e. $(1-\mathcal{Q}_m) \ll 1$). The further procedure is analogous to case a). We can easily solve for x_{1i}/x_m with

$$\frac{d}{dt}\left(\frac{x_{1i}}{x_m}\right) = \frac{\dot{x}_{1i}\, x_m - \dot{x}_m x_{1i}}{x_m^2}$$

inserting from Eq. (II-64) and accordingly for x_{ji}/x_m. The ratios are used to express $\bar{E}$ in the form of (II-51) which after insertion into (II-74) leads to a Bernoulli-type differential equation for x_m. The integration can be carried out in analogy to case a).

So far our discussion has been restricted to the theoretical behavior of selfselecting reaction systems rather than to any realistic application. For those, satisfactory solutions can always be obtained with the help of a computer. The above considerations show us to what extent we can use simpler approximations. In fact, there will be only a few reaction systems to which the simple linear form of primary rate equations is applicable. In general we shall have to consider various interactions, first between "informational" and "functional" molecules, but then also between individual members of these classes. This may lead to whole *reaction cycles or networks* including nonlinear rate equations—e.g. of the Michaelis-Menten type or even more complex—for each individual reaction partner. These more realistic systems will be treated in detail and correlated with experimental results in Parts IV to VI. It will be seen that the theoretical behavior of selection—as described in this section—is clearly reproduced by the more complex systems, although the explicit solutions may show important qualitative differences, such as periodicities of various forms as well as singularities providing very sharp selection. We shall also see that these qualitative differences will turn out to be most important in drawing conclusions about the origin of a selforganizing "living" system.

III. Stochastic Approach to Selection

III.1. Limitations of a Deterministic Theory of Selection

We have so far treated selection as a deterministic process. The phenomenological equations clearly specify which copy among a given population is to be selected. Whenever a mutant with selective advantage ($W_i > \bar{E}$) occurs, it will inevitably outgrow the former distribution.
There are two important limitations to such a deterministic description of selection:

1. The elementary process leading to a specific mutant is inherently non-deterministic. The autocatalytic amplification leads to a macroscopic mapping of "uncertain" microscopic events[1].

2. The growth process itself is subject to statistical fluctuations. Since growth starts from single copies, such fluctuations have to be taken into account. They may modify appreciably the results of the deterministic theory, which only holds for the average of large numbers of the species involved.

There is an additional difficulty arising from the fact that certain steady states—in contrast to true equilibria—are metastable. They cannot stabilize themselves and therefore require regulation if they are to be maintained over long periods of time. It is due to all these facts that we have to reexamine the problem of selection from the point of view of probabilistic theory. It will be seen that important modifications of the (deterministic) phenomenological theory will result from a stochastic treatment[2].

III.2. Fluctuations Around Equilibrium States

In order to characterize the difference between fluctuations around a steady state and a stable equilibrium, we start this discussion with a reconsideration of a classical example of equilibrium fluctuation, i.e. Ehrenfest's urn model (cf. also Fig. 3).
Given two urns and a (large) number—say $2N$—of spheres which are arbitrarily distributed among the two urns. The spheres are numbered from 1 to $2N$.

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1 P. Jordan [54]—according to my knowledge—was the first to draw attention to the "amplification" of elementary events which are subject to quantum-mechanical uncertainty.

2 "Stochastic" theory is the extension of the theory of probability to dynamical problems. From στοχαζομαι → aim, hit or guess. An excellent review is given in "Encyclopedia of Physics" III/2 by A. Ramakrishnan [55].

The "game" is to choose a number randomly—e.g. by some suitable mechanism such as lots, dice or any other fancy lottery machine—and then transfer the corresponding sphere from one urn to the other. If this procedure is repeated often enough, the result will be, independent of the initial distribution, an equipartition of the $2N$ spheres among the two urns.

The model was conceived by P. and T. Ehrenfest [56] and more recently treated stochastically by D. ter Haar and C. D. Green [57], M. Kac [58], M. J. Klein [59] and others. K. W. F. Kohlrausch and E. Schrödinger [60] tested the model experimentally. According to the stochastic treatment (e.g. by M. Kac) the model is described by the following features:

1. The equilibrium, although subject to fluctuations, is represented by a stable distribution. On average, each urn will contain N spheres.

2. There will be fluctuations around the equilibrium state denoted by a number n; i.e. one urn will contain $N+n$, the other $N-n$ spheres, where n can assume all values from $-N$ to $+N$. In analogy to Boltzmann's theorem, we can then describe the model by a distribution function

$$H=(N+n)\ln(N+n)+(N-n)\ln(N-n) \quad \text{(III-1)}$$

or for $n \ll N$:

$$H=\frac{2n^2}{N}+\text{const} \quad \text{(III-2)}$$

showing that fluctuations occur symmetrically with respect to $n=0$.

3. The probability of finding $(N+n)$ spheres in one and $(N-n)$ spheres in the other urn is

$$P_0(n)=2^{-2N}\frac{(2N)!}{(N+n)!\,(N-n)!}\rightarrow\frac{e^{-\left(\frac{n}{\sqrt{N}}\right)^2}}{\sqrt{\pi N}}. \quad \text{(III-3)}$$

This probability is stationary with respect to the stochastic equations, i.e. independent of time, while n always fluctuates. The probability distribution is symmetrical with respect to $n=0$ (Gaussian), the half-width being proportional to $\sqrt{N}$. It is extremely unlikely that fluctuations as large as $n=N$ occur. We have

$$\frac{P_0(n=N)}{P_0(n=0)}=2^{-2N}\sqrt{\pi N}. \quad \text{(III-4)}$$

4. The same relation holds for $\tau(n=0)/\tau(n=\pm N)$, the ratio of the "recurrence times" (i.e. the average times for reappearance of identical macro-states). This time shows a minimum for $n=0$.

The important conclusions are as follows:

The equilibrium is a "stable" state. *The fluctuations are selfregulating*; the larger the deviation in one direction, the larger the probability for its reversal, i.e. the restoration of equilibrium. The average fluctuations are proportional to $\sqrt{N}$, thus they are unimportant for large N. The ratio of the recurrence times, as given by Eq. (III-4), shows how rare large fluctuations really are if N is a large number. The model was of historical importance in clarifying the nature of an irreversible process as compared to a fluctuation [61].

III.3. Fluctuations in the Steady State

In the preceding example, the two urns represent two equivalent states between which the $2N$ equivalent spheres assume a stable equilibrium. Let us now change our model: instead of two urns we take only one, but instead of one lot per sphere we provide two, a white and a black. Whenever we draw a white lot we add another sphere to the urn, whereas drawing of a black lot requires the removal of a sphere from the urn. Actually, if we consider the spheres to be equivalent, we no longer need the numbering. However, if we are interested in the evolution of single species, we may maintain the numbered spheres, whereby certain numbers become duplicated whenever the corresponding white lot is drawn. We must also ensure that every sphere in the urn is represented by two lots (a black and a white), so that the removal or addition of a sphere always requires the removal or addition of both corresponding lots.

This model represents a typical steady state problem, where the probabilities of formation and decomposition are equal and both are proportional to the number of particles present. A deterministic equation would again indicate a time-independent distribution. However, this distribution is metastable due to the fact that fluctuations in the rate of addition and removal are independent of each other and therefore not selfregulating. The example is closely related to the chemical rate problem which we shall discuss below: therefore let us rephrase it in more realistic terms.

We may recall our "information box" introduced in § II.1. The walls of this box are semi-permeable, and monomeric digits in both the energy-rich and the energy-deficient state can pass through them whereas all macromolecular information carriers as well as any replication machinery (enzymes etc.) are kept inside the box. The conditions inside the box are such that macromolecular synthesis is favored; however, only template-instructed synthesis, i.e. replication, can occur. *Let us consider three problems* which will throw more light on the stochastic aspect of selection and which will be treated quantitatively in the next paragraph.

1. Given a large number N of different sequences (for simplicity, of uniform length), only one copy of each sequence being present; formation of new sequences can only occur by a template-directed process and the reduplication is precise, i.e. no mistakes are made ($\mathcal{Q}_k=1$). All formation and decomposition rate factors $\mathcal{F}_k$ and $\mathcal{R}_k$ are the same, i.e. $W_i=E_i=W_k=E_k=0$ $(i,k=1,2\ldots N)$. (The identity $W_i=E_i$ is due to $\mathcal{Q}_i=1$.) According to the deterministic theory, the system would be at a steady state and nothing should happen. This obviously cannot be true for any of the specified single copies. It may be approximately true for the total set, at least during a certain time interval, if we do not distinguish the different copies (which all are degenerate with respect to their rate properties). Our question is, what is the real fate of both the total content and the single specified class of information carriers? (Note that we start with N different copies—each of which may represent a different "message".)

2. We suppose the same conditions as in the first problem, but now allow for errors in the reproduction, i.e. $\mathcal{Q}_k < 1$. However, we still require for all copies $W_i = W_k$ $(i, k = 1, 2 \ldots N)$. Now, due to errors in the replication process, new sequences may be generated. We ask again for the time dependence of both the total and individual information content.

3. Starting from the conditions of example 2, we finally allow for different rate parameters of formation and decomposition, i.e. W_i, $\overline{W}_k$ $(i, k = 1, 2 \ldots N)$. Without flux control, this system could only initially be at a steady state, where the average total formation rate equals the average total decomposition rate. However, one may maintain a steady state by controlling the influx of monomeric digits and (or) of solvent according to the constraints specified in Part II. Here we are especially interested in the evolutionary behavior of individual species, especially if they appear as single mutant copies with selective advantages $W_i > \bar{E}$.

Before starting any mathematical treatment we may try to rationalize what behavior is to be expected. *In the first problem,* the system obviously is "closed" with respect to any addition of new information (which could have been introduced only via errors of reproduction). On the other hand, information is lost whenever a single nonredundant information carrier decomposes before being reduplicated. This will steadily occur, thus the amount of individual nonredundant information will steadily decrease. If the total number of information carriers is very large, such "negative" fluctuations may be compensated for by "positive" fluctuations (i.e. multiplication) of other copies. Thus the individual information content will first narrow down to a few (or even one) highly redundant sequences, before *the total population finally dies out.* This fate of the total population is inevitable since fluctuations of formation and decomposition occur independently of each other. Total extinction may even occur within relatively short times, as is shown by the graph in Fig. 6 (cf. the discussion in the legend of Fig. 6). "Relatively short" is meant in comparison to corresponding recurrence times of equilibrial fluctuations, as discussed in III.2.

As long as we do not distinguish the single information carriers, we would expect exactly the same fate for the total population in the second example.

The rates for all species are the same and overall formation is exactly compensated by overall decomposition. However, for the single information carrier (i.e. the individual information content) the evolutionary behavior differs considerably from that in the first case. If the amplification factors $\mathcal{A}_k$ equal exactly the decomposition factors $\mathcal{D}_k$, but the quality

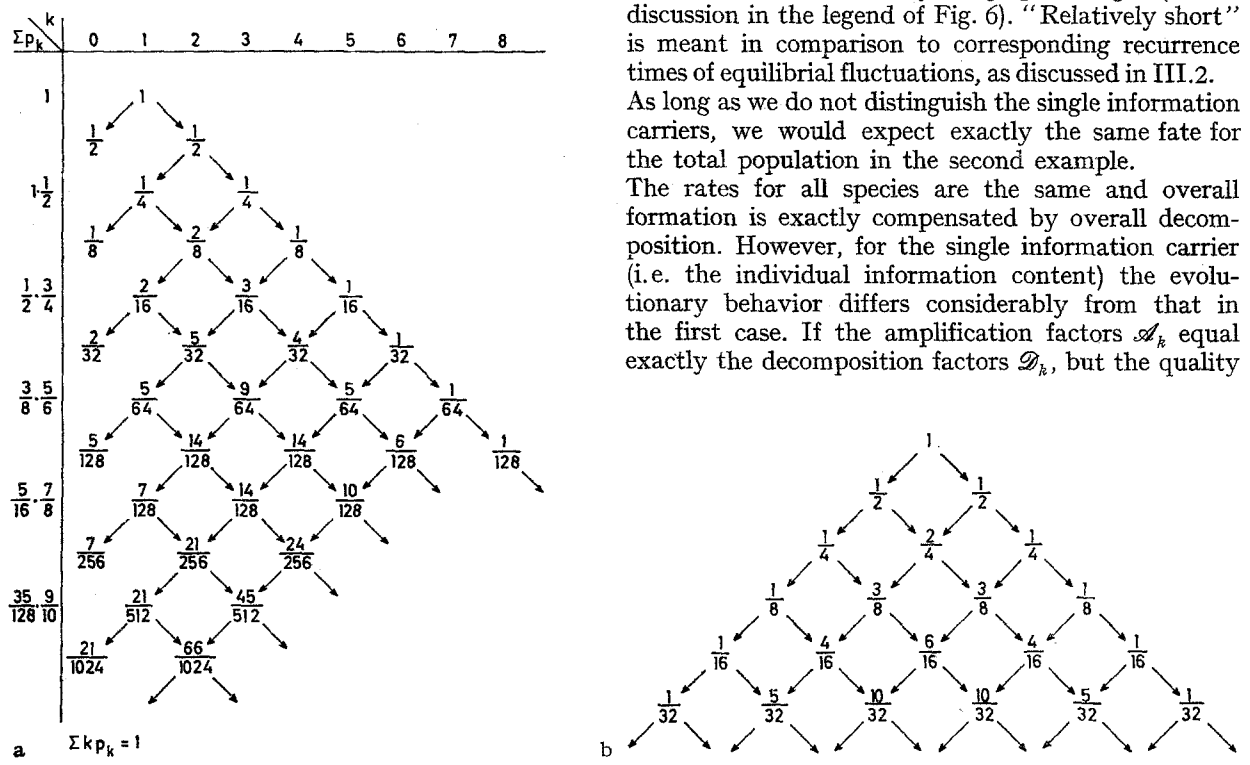


Fig. 6. Graph representation of the probabilities in the steady state model. The horizontal numbers k indicate the redundancy of each copy. Note that this graph merely represents probabilities rather than the temporal evolution. Each step represents the change of population by one, and there is an equal chance for addition or removal. The time intervals for transition shrink with increasing k, i.e. are inversely proportional to k. This representation was chosen in order to demonstrate the dissymetry of the random walk problem brought about by the "extinction" of states at $k = 0$.
If this dissymmetry were absent, i.e. if each subsequent state followed from the preceding one with a probability of $\frac{1}{2}$ (allowing also for negative numbers k), the "Pascal triangle" would result in which the numbers are the binomial coefficients divided by the sum of the coefficients of the corresponding row. As can be seen, the condition of extinction changes the probabilities in a dissymmetrical way so that only the right borderlines in both triangles are identical.
If we want to make a prediction about the probability of extinction, we start at any number k and consider the random walk in the corresponding triangle headed by the starting point. Since the transition probabilities for each direction are equal, it will take on the average k^2 steps before the state of extinction (i.e. $k = 0$) is reached. Since the shrinkage of the time interval is inversely proportional to k, we could predict that a time between k and k^2 times the elementary time $1/\mathcal{F}_i$ on the average would be required for extinction. The time should be larger than, but closer to $k/\mathcal{F}_i$. At $t = k^2/\mathcal{F}_i$ extinction should be almost certain. The shortest time in which the zero state (starting from k) could be reached in the model would be given by

$$1/\mathcal{F}_i \left(\sum_{i=1}^{k} 1/i \right) \to \frac{1}{\mathcal{F}_i} (C + \ln k), \quad \text{for large } k,$$

C being Euler's constant:

$$C = -\int_0^\infty e^{-t} \ln t \, dt \approx 0.5772.$$

factors $\mathcal{Q}_k$ are smaller than one, then every species must die out, since for each species: $W_k = \mathcal{A}_k \mathcal{Q}_k - \mathcal{D}_k < 0$. This system compensates by steadily *producing new information through errors* in the copying process. It "drifts irregularly through the information space" until, as in the first example, its total population is wiped out by a "*fluctuation catastrophy*".

Only in the *third example* can we expect *stable and reproducible behavior*. Here the system would select the species of maximal W_k (supposing there is any species for which $W_k > 0$). As long as this species exists in only a few copies, it still may be in danger of decaying by fluctuations. However, the more it grows, the more stable will it become, until it finally dominates the total population according to the deterministic equations. An interesting question arises: Is there any "point of no return" in the random walk as represented by the graph in Fig. 6, if the probabilities are in favor of growth? Every pilot knows such a point on the runway; when he has passed it, he must take off. Similarly, we may look for such a "critical" point on the concentration axis, which—once it is reached by a mutant—leaves no possibility of "return".

Quantitative answers to all these questions can only come from a quantitative theoretical treatment.

III.4. Stochastic Models as Markov Chains

A stochastic examination of various evolutionary models is under way at present. In the meantime we may give the principal answers to most of the questions raised above by using the results obtained previously for some simple linear models.

A stochastic treatment of the stationary linear "birth and death process" was recently given by A. F. Bartholomay [62] and similar problems have been treated by other authors, e.g. the simple autocatalytic (forward) reaction as early as 1940 by M. Delbrück [63]. A review of the literature on applications of stochastic theory to chemical rate processes can be found elsewhere (cf. D. A. McQuarrie [64]).

The following discussion is based on Bartholomay's elegant treatment [62] in which he used Doob's Q-Matrix method [65]. Both the elements of Doob's method and Bartholomay's procedure are summarized in Tables 9 and 10.

The problem to which the deterministic Eq. (II-10), with $\varphi_{il} = 0$, $\mathcal{F}_i$ and $\mathcal{R}_i = \text{const}$, applies is represented by a stationary Markov chain:

$$\{x_t,\ 0 \leqq t < \infty\}. \qquad \text{(III-5)}$$

Time (t) is a continuous parameter; x_t, a random population variable, refers to a discrete sequence of denumerably many states $S_0, S_1 \ldots$. The change from one state (S_i) to another (S_j) is described by a transition probability $p_{ij}(t)$. It is the probability of a system starting from a state S_i at $t=0$ reaching state S_j at time t. A knowledge of $p_{ij}(t)$ allows the determination of expectation values as well as mean variances for the population of any state at time t, starting from defined initial conditions for $t=0$. The transition probabilities applying to the linear birth and death process, which are calculated in Table 10, form the basis of our further discussion.

Table 9. *Doob's Q-Matrix method for stochastic processes according to A. F. Bartholomay* [62]

I. Requirements

Definition of transition probability function $p_{ij}(t)$ for passage from state S_i to S_j $(i, j = 0, 1, 2, \ldots)$ as a conditional probability

$$p\{x_{t_0+t} = S_j \mid x_{t_0} = S_i\} \qquad (1)$$

that the random variable x_t will have the value S_j, if it had the value S_i t units previously. The process $\{x_t,\ 0 \leqq t < \infty\}$ qualifies as a stationary Markov chain if the transition probabilities fulfil the following conditions:

$$p_{ij}(t) \geqq 0 \qquad (i, j = 0, 1, 2, \ldots), \qquad (2)$$

$$\sum_j p_{ij}(t) = 1 \qquad (i = 0, 1, 2, \ldots), \qquad (3)$$

$$\sum_j p_{ij}(s)\, p_{jk}(t) = p_{ik}(s+t) \qquad (i, k = 0, 1, 2, \ldots), \qquad (4)$$

$$\lim_{t \to 0} p_{ij}(t) = \delta_{ij} = \begin{cases} 1 \text{ for } i = j \\ 0 \text{ for } i \neq j. \end{cases} \qquad (5)$$

II. Definition of Matrix $\boldsymbol{Q} = (q_{ij})$

$$q_{ii} = \lim_{t \to 0} \frac{p_{ii}(t) - 1}{t} \equiv \frac{dp_{ii}}{dt} \quad (t = 0), \qquad (6)$$

$$q_{ij} = \lim_{t \to 0} \frac{p_{ij}(t)}{t} \equiv \frac{dp_{ij}}{dt} \quad (t = 0). \qquad (7)$$

Requirements I and definitions II are used to construct the following system of differential equations ($\dot{p} = dp/dt$).

III. a) Forward System

$$\dot{p}_{ik}(t) = q_{kk} p_{ik}(t) + \sum_{j \neq k} q_{jk} p_{ij}(t). \qquad (8)$$

b) Backward System

$$\dot{p}_{ik}(t) = q_{ii} p_{ik}(t) + \sum_{j \neq i} q_{ij} p_{jk}(t). \qquad (9)$$

The forward system describes what happens *in the last time interval* $(t \to 0)$ *prior to transition*, whereas the backward system asks what happens *in the first time interval after transition*. This is expressed in the sums where the final state is varied for the forward system, whereas it is the initial state which is varied for the backward system.

IV. General Solution

For a finite number of states and given initial conditions, e.g. condition (5), a unique solution for both systems may be given in matrix form, as was shown by Doob [65]:

$$\boldsymbol{P}(t) = e^{t\boldsymbol{Q}}. \qquad (10)$$

$\boldsymbol{P} = (p_{ij})$ and $\boldsymbol{Q} = (q_{ij})$ being matrices, where $e^{t\boldsymbol{Q}}$ is obtained from the element-by-element sum of the exponential series expansion:

$$1 + t\boldsymbol{Q} + t^2\boldsymbol{Q}^2/2! + \cdots. \qquad (11)$$

In determining the $p_{ij}(t)$ and the corresponding q_{ij}, certain properties of the deterministic equations are given a probabilistic interpretation (cf. Table 10).

III.5. Quantitative Discussion of Three Prototypes of Selection

Case 1. Let us go back to the first of the three steady state problems introduced in § III.3 and ask what is the probability of survival or extinction of a system specified by the parameters

$$\mathcal{F}_k \equiv \mathcal{F} = \mathcal{R}_k \equiv \mathcal{R} \qquad \text{(for any species } k \text{ present)}.$$

Starting with a total population of N species at $t=0$, the probability of extinction (for $\mathcal{F} = \mathcal{R}$) follows from Eq. (19) in Table 10:

$$p_{N0}(t) = \left[\frac{\mathcal{F}t}{1 + \mathcal{F}t}\right]^N \qquad \text{(III-6)}$$

$$\to e^{-N/\mathcal{F}t} \quad \text{for } \mathcal{F}t \gg 1. \qquad \text{(III-7)}$$

Table 10. *Linear birth and death processes as Markov chains according to A. F. Bartholomay [62]*

Given the deterministic equation

$$\dot{x} = (\mathscr{F} - \mathscr{R})\, x \tag{1}$$

and its solution

$$x(t) = x(t=0)\, e^{(\mathscr{F}-\mathscr{R})t} \tag{2}$$

where $\mathscr{F}$ and $\mathscr{R}$ are constants.

For a small time interval between t_1 and $t_1 + \Delta t_1$, the net change Δx_1 in the population is

$$\frac{\Delta x_1}{x_1} = \mathscr{F}\Delta t_1 - \mathscr{R}\Delta t_1 + O(\Delta t_1), \tag{3}$$

where $O(\Delta t_1)$ includes all infinitesimals of higher order. Δt_1 is chosen small enough to ensure that only the probability for *one* single formation or decomposition event in the time interval is finite and given by

$$\mathscr{F} x_1 \Delta t_1 + O(\Delta t_1) \quad \text{or} \quad \mathscr{R} x_1 \Delta t_1 + O(\Delta t_1). \tag{4}$$

Then the population can only change by plus or minus one individual species, thus only transitions $S_i \to S_{i-1}$ or $S_i \to S_{i+1}$ $(i = 1, 2, \ldots)$ are permitted and these transitions can only occur via one elementary event (multiple birth and death events which compensate to a net change of ± 1 are excluded). Furthermore, the transition $S_0 \to S_1$ has zero probability, on the understanding that the system "dies out" when it reaches the state S_0. From expansion of $p_{ij}(t)$, i.e.

$$p_{ij}(\Delta t_1) = p_{ij}(O) + \dot{p}_{ij}(O)\Delta t_1 + \ddot{p}_{ij}(O)\frac{(\Delta t_1)^2}{2} + \cdots$$

one can construct the $\boldsymbol{Q}$-matrix according to Eqs. (6) and (7) in Table 9, and in comparison with the expressions (4) in this table one obtains

$$p_{i,i-1}(\Delta t_1) = q_{i,i-1}\Delta t_1 + O(\Delta t_1); \tag{5}$$

$$p_{i,i+1}(\Delta t_1) = q_{i,i+1}\Delta t_1 + O(\Delta t_1); \tag{6}$$

$$q_{i,i-1} = i\mathscr{R}; \quad q_{i,i+1} = i\mathscr{F}; \quad q_{ii} = -i(\mathscr{F} + \mathscr{R}) \tag{7}$$

$(i = 0, 1, 2, \ldots$ represents the state of the population).

The "forward" and "backward" equations specified in Table 9 can then be constructed

$$\dot{p}_{ik}(t) = -k(\mathscr{F} + \mathscr{R})\, p_{ik}(t) + (k-1)\,\mathscr{F} p_{i,k-1}(t) + (k+1)\,\mathscr{R} p_{i,k+1}(t), \tag{8}$$

$$\dot{p}_{ik}(t) = -i(\mathscr{F} + \mathscr{R})\, p_{ik}(t) + i\mathscr{F} p_{i+1,k}(t) + i\mathscr{R} p_{i-1,k}(t). \tag{9}$$

These equations are solved by the method of "probability generating functions", i.e. with the definition of a function

$$\phi_i(s,t) = \sum s^k p_{ik}(t). \tag{10}$$

From $\partial\phi/\partial s$ and $\partial\phi/\partial t$ one obtains

$$(\partial\phi_i/\partial t) - (s-1)(\mathscr{F}s - \mathscr{R})(\partial\phi_i/\partial s) = 0, \tag{11}$$

with the auxiliary equation

$$\frac{dt}{-1} = \frac{ds}{(s-1)(\mathscr{F}s - \mathscr{R})} \tag{12}$$

integration of which for the two cases $\mathscr{F} \neq \mathscr{R}$ and $\mathscr{F} = \mathscr{R}$ leads to the general solution of the partial differential equation for the probability generating functions:

1. $\mathscr{F} \neq \mathscr{R}$:

$$\phi_i(s,t) = \left[\frac{(\mathscr{R}e^{(\mathscr{F}-\mathscr{R})t} - \mathscr{R}) - (\mathscr{R}e^{(\mathscr{F}-\mathscr{R})t} - \mathscr{F})\,s}{(\mathscr{F}e^{(\mathscr{F}-\mathscr{R})t} - \mathscr{R}) - (\mathscr{F}e^{(\mathscr{F}-\mathscr{R})t} - \mathscr{F})\,s}\right]^i. \tag{13}$$

Expansion in powers of s leads to the values for the coefficients of s^k which by comparison with (10) leads to the probabilities

$$p_{ik}(t) = \sum_{n=0}^{k \text{ or } i} (-1)^n \binom{i}{n}\binom{i+k-n-1}{k-n} \mathscr{F}^{(k-n)}\mathscr{R}^{(i-n)} \cdot [e^{(\mathscr{F}-\mathscr{R})t} - 1]^{i+k-2n} \cdot [\mathscr{F}e^{(\mathscr{F}-\mathscr{R})t} - \mathscr{R}]^{-i-k+n}[\mathscr{R}e^{(\mathscr{F}-\mathscr{R})t} - \mathscr{F}]^n. \tag{14}$$

The upper summation limit is k if $0 < k < i$ and i if $k \geqq i$. We have

$$\lim_{t\to 0} p_{ik}(t) = \begin{cases} 1 & (k = i) \\ 0 & (k \neq i). \end{cases} \tag{15}$$

The expectation value of i is given by

$$\varepsilon_i(t) = \left(\frac{\partial\phi_i}{\partial s}\bigg|_{s=1}\right) = i \cdot e^{(\mathscr{F}-\mathscr{R})t} \tag{16}$$

i.e. the result of the deterministic theory,

and the mean variance

$$\sigma_i^2(t) = \left(\frac{\partial^2\phi_i}{\partial s^2}\bigg|_{s=1}\right) + \varepsilon_i(t) - \varepsilon_i^2(t) = i\frac{\mathscr{F}+\mathscr{R}}{\mathscr{F}-\mathscr{R}} e^{(\mathscr{F}-\mathscr{R})t}[e^{(\mathscr{F}-\mathscr{R})t} - 1]. \tag{17}$$

2. $\mathscr{F} = \mathscr{R}$: Analogously, we obtain

$$\phi_i(s,t) = \left[\frac{\mathscr{F}t - (\mathscr{F}t-1)\,s}{(1+\mathscr{F}t) - \mathscr{F}ts}\right]^i, \tag{18}$$

$$p_{ik}(t) = \sum_{n=0}^{k \text{ or } i} (-1)^n \binom{i}{n}\binom{i+k-n-1}{k-n} \cdot (\mathscr{F}t)^{i+k-2n}(\mathscr{F}t-1)^n(\mathscr{F}t+1)^{-i-k+n}, \tag{19}$$

$$\varepsilon_i(t) = \left(\frac{\partial\phi_i}{\partial s}\bigg|_{s=1}\right) = i; \tag{20}$$

$$\left(\frac{\partial^2\phi_i}{\partial s^2}\bigg|_{s=1}\right) = i(2\mathscr{F}t + i - 1). \tag{21}$$

This probability approaches one for $\mathscr{F}t \gg N$. At $\mathscr{F}t = N$ we have already

$$p_{N0}(t = N/\mathscr{F}) = 1/e \tag{III-8}$$

i.e. a probability of more than 1/3 for the extinction of the population. At $\mathscr{F}t = N^2$ with

$$p_{N0}(t = N^2/\mathscr{F}) = 1 - 1/N \tag{III-9}$$

extinction is almost certain.

The expectation value of the population remains equal to N, independent of t, (cf. Eq. (20) in Table 10). However, the mean variance increases with time. At $t = N/\mathscr{F}$ it becomes

$$\sigma_N^2(t = N/\mathscr{F}) = 2N^2. \tag{III-10}$$

Thus we find, as suggested in the discussion of Fig. 6, *that a stationary population consisting at $t = 0$ of N species will live on average for a time between $N/\mathscr{F}$ and $N^2/\mathscr{F}$. Extinction is almost certain for $t = N^2/\mathscr{F}$.*

We now ask about the fate of the N different individual species, only one copy of each of which was assumed to be present at the beginning.

According to Eq. (19) in Table 10, we have for the probability that a single species is multiplied k-fold:

$$p_{1k}(t) = \frac{1}{(\mathscr{F}t)^2}\frac{1}{(1+1/\mathscr{F}t)^{k+1}} = \frac{1}{\mathscr{F}t(1+\mathscr{F}t)}\left[\frac{\mathscr{F}t}{1+\mathscr{F}t}\right]^k \quad (\text{for } k \geqq 1) \tag{III-11}$$

or that it dies out:

$$p_{10}(t) = \frac{\mathscr{F}t}{1+\mathscr{F}t}. \tag{III-12}$$

Summing Eq. (III-11) for all $k \geqq 1$ and combining with Eq. (III-12) yields

$$\sum_{k=0}^{\infty} p_{1k}(t) = \frac{1}{1+\mathscr{F}t} + \frac{\mathscr{F}t}{1+\mathscr{F}t} = 1 \tag{III-13}$$

in agreement with Eq. (3) in Table 9.

For any $k \geqq 1$, $p_{1k}(t)$ as a function of t will pass through a maximum at

$$\mathscr{F}t = \frac{k-1}{2}. \qquad \text{(III-14)}$$

Here p_{1k} will reach the value

$$(p_{1k})_{max} = \left(\frac{2}{ek}\right)^2 \qquad \text{(III-15)}$$

(e = base of natural logarithms). If we ask, for instance, at which time and for which k will the maximal p_{1k} be equal to $1/N$, we obtain

$$k = \frac{2}{e}\sqrt{N} \qquad \text{(III-16)}$$

or

$$t = \frac{1}{\mathscr{F}}\left(\frac{\sqrt{N}}{e} - \frac{1}{2}\right). \qquad \text{(III-17)}$$

Let us look at $p_{1k}(t)$ as a function of k. For $\mathscr{F}t \gg 1$ we obtain asymptotically

$$p_{1k}(t) \to \frac{e^{-k/\mathscr{F}t}}{(\mathscr{F}t)^2} \quad (k \geqq 1). \qquad \text{(III-18)}$$

This distribution, i.e. $p_{1k}(t)$ as a function of k (log. scale) at given t_1, is shown for $t_1 = N/\mathscr{F}$ in Fig. 7. As is seen, the probabilities are almost independent of k — $p_{1k}(N/\mathscr{F}) = 1/N^2$ — until k reaches the order of magnitude of N, where it decays exponentially. At the same time we have the probability of extinction

$$p_{10} = 1 - 1/N. \qquad \text{(III-19)}$$

With

$$\int_0^\infty e^{-k/N}\, dk = N \qquad \text{(III-20)}$$

we may approximate the probability distribution to a constant $1/N^2$ in the range $k = 1$ to N, jumping to the value $1 - 1/N$ at $k = 0$. Thus, starting with a single copy at $t = 0$, there is an approximately equal chance for any degree of amplification k from 1 to N. Since at the beginning ($t = 0$) we had N different single copies, the chance that one of them has amplified to any number $> N/2$ is already 50%, $\left(\text{i.e. } N \sum_{k=N/2}^{\infty} p_{1k} = 0.5\right)$ at $t = N/\mathscr{F}$. At the same time most of the other copies have died according to $p_{10} = 1 - 1/N$.

The expectation value $\varepsilon = \sum_{k=0}^{\infty} k p_k$ (p_k according to Eq. (III-11)) for each species again remains "one", independent of time, whereas the mean variance for each species increases, e.g. for $\mathscr{F}t = N$ to

$$\sigma_1^2(t = N/\mathscr{F}) = 2N. \qquad \text{(III-21)}$$

We note in conclusion:

The individual information content narrows down to only a few, or even one, highly redundant information carriers before the total information content is extinguished. Such selection behavior, caused by the autocatalytic nature of the formation process, represents a typical case of "survival of the survivors". There is no other criterion for selection than the outcome, i.e. the fact of survival, occuring among a group in which each individual had an equal chance of surviving.

This type of purely "stochastic" survival at a steady state is unrealistic since the quality factor can never be exactly equal to one. It should be distinguished from the Darwinian type of selection, for which optimization criteria of survival can be formulated.

Case 2. In the second example put forward in § III.3 we have to distinguish qualitatively the solutions for the total population and for single individuals. For the total population we may set $\mathscr{F} = \mathscr{R}$ (without distinguishing any species). The behavior of the total population, therefore, will be exactly the same as described above, i.e. complete extinction for times exceeding the order of magnitude $N^2/\mathscr{F}$.

For any single species k, however, we have to set $\mathscr{F}_k < \mathscr{R}_k$ because of $\mathscr{Q}_k < 1$, again assuming that all individual species are degenerate with respect to $\mathscr{F}_k$, $\mathscr{R}_k$ or $\mathscr{Q}_k$. Then Eq. (14) in Table 10 shows that p_{10} approaches 1 for $t \gg 1/(\mathscr{R}_k - \mathscr{F}_k)$ according to

$$p_{10}(t) \to 1 - (1 - \mathscr{F}_k/\mathscr{R}_k)\, e^{-(\mathscr{R}_k - \mathscr{F}_k)t}. \qquad \text{(III-22)}$$

The expectation value for each copy decays from 1 to 0 as

$$\varepsilon_i(t) = e^{-(\mathscr{R}_k - \mathscr{F}_k)t} \qquad \text{(III-23)}$$

and the mean variance approaches

$$\sigma_1^2(t) \to \frac{\mathscr{R}_k + \mathscr{F}_k}{\mathscr{R}_k - \mathscr{F}_k}\, e^{-(\mathscr{R}_k - \mathscr{F}_k)t}. \qquad \text{(III-24)}$$

Each individual species will die out. No species will survive for any length of time comparable to the lifetime of the "survivors" in the first example. Instead there is a steady drift of the information content due to error production. The system during its lifetime — i.e. during a time smaller than $N^2/\mathscr{F}$ — will scan a large amount of information without stable reproduction of any given copy for a time appreciably exceeding $\frac{1}{\mathscr{R}_k - \mathscr{F}_k}$. However, as mentioned before, the set as a whole will have a similar fate as that in case 1.

Case 3. Stable and predictable selection can only be found if a finite variation of rate parameters exists. However, even here we have a range of "uncertainty" for selection. First, for $\mathscr{F} > \mathscr{R}$ we obtain an expectation value for the growing population:

$$\varepsilon_i(t) = i e^{(\mathscr{F} - \mathscr{R})t} \qquad \text{(cf. also Eq. (III-23))}$$

in agreement with the deterministic theory. Moreover, for large i at $t = 0$ the fluctuations are within a factor of $\sqrt{i}$ (i being the initial value) of the expectation value. Let us now look at an equilibrated distribution with $W_m = \bar{E}$. Those species which have a finite

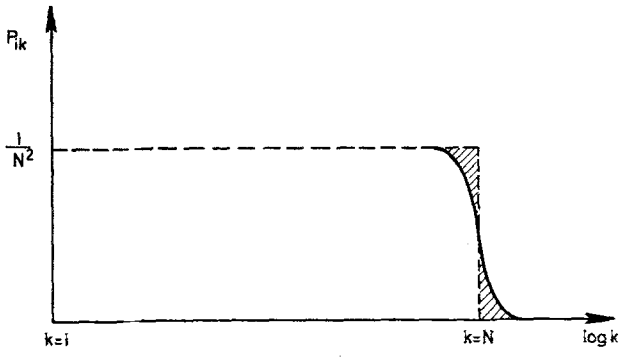


Fig. 7. Probability distribution for "survivors" at $t_1 = N/\mathscr{F}$, according to Eq. (III-18)

"selective advantage" $W_{m+1} > W_m$ have a finite chance, but no certainty, of being selected. According to Eq. (14) in Table 10, the chance of the species dying out if k copies are present at $t=0$ is

$$p_{k0}(t) = \left[1 - \frac{\mathscr{F}_m - \mathscr{R}_m}{\mathscr{F}_m - \mathscr{R}_m \exp\left[(\mathscr{R}_m - \mathscr{F}_m)\, t\right]}\right]^k \quad \text{(III-25)}$$

which for $t \to \infty$ approaches

$$\lim_{t\to\infty} p_{k0}(t) = \left(\frac{\mathscr{R}_m}{\mathscr{F}_m}\right)^k. \quad \text{(III-26)}$$

This result includes an exact answer to the last of our questions in Section III.3. If a mutant $(m+1)$ with selective advantage, i.e. $W_{m+1} > W_m$ occurs in a previously equilibrated population (e.g. $W_m = \bar{E} = 0$), the one initially present copy may still finally die out with a probability $\mathscr{R}_m/\mathscr{F}_m$. Its chance of survival is $1 - \mathscr{R}_m/\mathscr{F}_m$, and this chance will increase as the redundancy (k) of the mutant copy increases. However, there is no real "point of no return" in the *linear* model. The probability of extinction for $\mathscr{F}_{m+1} > \mathscr{R}_{m+1}$ according to Eq. (III-26) will decrease steadily with increasing k and reach zero only asymptotically for large k.

However, we may define a certain probability threshold value as a "point of half-return" $k_{\frac{1}{2}}$ according to

$$\left(\frac{\mathscr{R}_m}{\mathscr{F}_m}\right)^{k_{\frac{1}{2}}} = 0.5 \quad \text{(III-27)}$$

or a corresponding "relaxation" point $k_{1/e}$

$$k_{1/e} = \frac{1}{\ln(\mathscr{F}_m/\mathscr{R}_m)} \quad \text{(III-28)}$$

yielding for $(\mathscr{F}_m - \mathscr{R}_m) \ll \mathscr{R}_m$

$$k_{1/e} = \frac{\mathscr{R}_m}{\mathscr{F}_m - \mathscr{R}_m}. \quad \text{(III-29)}$$

Under this condition $\lim_{t\to\infty} p_{k0}$ could be expressed as

$$\left(\frac{\mathscr{R}_m}{\mathscr{F}_m}\right)^k \to e^{-k\frac{\mathscr{F}_m - \mathscr{R}_m}{\mathscr{R}_m}}. \quad \text{(III-30)}$$

Table 11 gives some values for relaxation points $k_{1/e}$ for various $\mathscr{F}_m/\mathscr{R}_m$. *It is seen that small selective advantages only rarely have a chance of survival and of becoming dominant, as predicted by the deterministic theory.* This fact further underlines the undeterministic nature of selection processes. If a stochastic expression could be given for the rate of appearance of one single specified mutant, it would have to be multiplied by $(1 - \lim_{t\to\infty} p_{10})$ in order to yield an expression for the probability of its macroscopic appearance.

The conclusions just drawn are restricted to linear growth systems. The calculations are being extended to modifications introduced by the condition of constant forces or fluxes as well as to (real) nonlinear growth systems which will turn out to be of special interest with respect to the "nucleation" of living systems (cf. Part VI).

The stochastic treatment, which is essentially based on Bartholomay's linear birth and death model and which is being extended to true steady states implied by the selection criteria, provides some important modifications of the deterministic phenomenological theory of evolution. It not only emphasizes the nondeterministic nature of the elementary processes but also demonstrates quite clearly that certain statements derived from the deterministic theory have to be modified before they correctly describe the essential features of evolutionary processes.

Table 11. *Stochastic threshold for the survival of a mutant with selective advantage* $\mathscr{F}_i > \mathscr{R}_i$

$\mathscr{F}_i/\mathscr{R}_i$	$(\mathscr{F}_i - \mathscr{R}_i)/\mathscr{R}_i$	$k_{1/e}$
2	1	1.44
1.3	0.3	3.82
1.1	0.1	10.5
1.01	10^{-2}	10^2
1.00001	10^{-5}	10^5

IV. Selforganization Based on Complementary Recognition: Nucleic Acids

IV.1. True "Selfinstruction"

The theory of selection—although of a more general nature—has so far been discussed in detail only for simple quasilinear systems. By "quasilinear" we may denote any system described by Eq. (II-32), e.g. for negligible back-flow terms written in the simple form

$$\dot{x}_i = k_0(W_i^0 - \bar{E})\, x_i \quad \text{(IV-1)}$$

in which the "selective value" W_i is represented by a constant. We note that neither the original rate equation for unconstrained growth nor its final form resulting from superposition of selection strains can be a truly linear differential equation. The "formation" term of the original equation contains the "stoichiometric function" $f_i(m_1 \ldots m_\lambda)$—i.e. a function of the (in general variable) concentrations of the energy-rich monomers, the exact form of which depends on the mechanism of the template-instructed polymerization process—on which the (autocatalytic) x_i term is superimposed. Only if the energy-rich monomers are buffered according to the condition of constant overall organization can the selective value be regarded as a constant for a simple "selfinstructed" process. By the same condition, however, we introduce into the differential equation a concentration-dependent (i.e. x_i-containing) function $\bar{E}$. Thus, "quasilinear" can only refer to the term $W_i^0 x_i$, meaning that W_i^0 does not further depend on x_i or any x_k (being a variable in the system of rate equations).

Under these conditions Eq. (IV-1) describes the simplest case of true "selfinstruction", where the formation of a specific sequence i is instructed by the template i itself.

How can we imagine the occurrence of such a type of selfinstruction—*as a general phenomenon*—in nature? We know, of course, many specific autocatalytic processes where a certain reaction product feeds back on its own generation. However, here we are asking for more: *any* product of the polymerization process, i.e. any specified sequence, should instruct the formation of *its own* replica.

As a simple example, we might consider the formation of polyriboadenylic acid (poly-r-A) at low pH. Below

pH 4 poly-r-A is known [66] to form a double-stranded helical structure by specific pairing among the protonated adenine residues. Unlike the Watson-Crick structure, this double helix involves parallel-oriented strands (i.e. both strands running parallel from the 3′ to the 5′ end). Furthermore, the protonation of the bases essentially neutralizes the negative charge of the phosphate groups in the backbone so that this structure is more stable at low ionic strength. Otherwise this helix behaves like the Watson-Crick structure. This can be concluded from the detailed thermodynamic and kinetic studies carried out in our laboratory by D. Pörschke [67]. A similar phenomenon can be observed with poly-r-C, where pairing requires the presence of both the protonated and unprotonated species so that double (and triple) helix formation is restricted to a quite narrow pH range.

In any case, one could well imagine a "selfinstructing" template which directs the reproduction of an *identical* replica, and such a system is described by rate equations of the type of Eq. (IV-1) with constant selective values W_i^0. Let us analyze the parameters $\mathcal{A}_i$, $\mathcal{Q}_i$ and $\mathcal{D}_i$ which determine the selection and selforganization behavior of the system in some more detail.

The exact form of the *amplification factor* $\mathcal{A}_i$ depends on the mechanism of template-instructed polymerization. Several mechanisms have been discussed on the basis of stochastic models by J. Gibbs [68].

The simplest model would attribute a constant and uniform time interval to the inclusion of each digit into the polymeric chain. If reproduction of each chain is finished before a new one can be started, the time constant of reduplication must show a straightforward chain length dependence. It is proportional to ν (the number of digits in the chain) if the process occurs far from equilibrium, but approaches a ν^2-dependence if the polymerization process reaches equilibrium. Here the probabilities of the reaction either preceeding or reversing become equal, thus the reaction will resemble a simple linear diffusion process (where the time of propagation is proportional to the square of distance). For the present application we may disregard systems close to equilibrium and thus exclude chain length dependences stronger than linear.

On the other hand, there are mechanisms which yield an appreciably weaker chain length dependence:

a) The process may be cooperative and thus require a certain time (and length) for nucleation, which may turn out to be appreciably longer than the time constant of "propagation". Then—up to a certain "cooperative length"—the time of reduplication would be determined by the nucleation time and hence be independent of length. Such a cooperative behavior, for instance, is found for base recognition in helix formation of oligo-r-A, where the "nucleation length" involves 3 and the "cooperative length" about 30 base pairs (at room temperature).

b) A new chain may be started at both the template and the replica before the latter is finished. This phenomenon is well known for enzymic single-strand template reading (e.g. by ribosomes or RNA polymerases) and may well be expected for non-enzymic single-strand reproduction. As J. Gibbs has shown [68], quite familiar "traffic jam" problems may occur in such a multiple reproduction process. Contrary to case a), chain length dependence would be found here only for relatively short sequences and would disappear above a certain length.

If we combine the two cases a) and b), we may encounter a quite weak (less than linear) chain length dependence which only slightly—if at all—favors the reproduction of shorter sequences. There may be, however, quite drastic differences in rate for different long-range sequence orders, since internal chain folding and loop formation might produce regions of widely differing template effectiveness. Furthermore, if competition includes the use of different energy-rich monomeric digits, present at different concentrations, the most abundant digit will be greatly favored (via $\mathcal{A}_i$ as well as $\mathcal{Q}_i$) and may lead to quite uniform sequences. If enzymes are involved[1], recognition (like "nucleation") may involve specific regions of the template sequence (possibly involving both ends, as in the case of $Q\beta$-replicase, cf. Part VII).

The influence of the quality factor Q_{i0} imposes more drastic restrictions on chain length than does the amplification factor, as a consequence of the power relationship

$$Q_{i0} = \prod_{k=1}^{\lambda} q_k^{\nu_{ik}} \qquad \text{(IV-2)}$$

where the index k refers to the different digits $1 \ldots \lambda$. If the single digit recognition factors q_i were uniform, the simple model discussed in Table 8 (cf. Part II.5) would apply.

As was shown there, the maximum number of digits which can be reproducibly copied is restricted by the uncertainty of single-digit recognition and also (weakly) depends on the "spread" of rate coefficients. As a consequence, a single-digit quality factor of 0.99 (i.e. an error rate of 1%) will restrict reproducible sequence formation to digit numbers ν_i of the order of magnitude of 100 (or even less, if the "spread" $\mathcal{A}_i - \bar{\mathcal{A}}_{k \neq i}$ and $\mathcal{D}_i - \bar{\mathcal{D}}_{k \neq i}$ approaches zero).

The single-digit quality factor q can be expressed by the free energies of pair interaction if recognition is an "equilibrated" process. Let us consider such a step of selfinstructed digit inclusion:

$$\boxed{i} + i_m \underset{k_D}{\overset{k_R}{\rightleftharpoons}} \boxed{i} \ldots i_m \xrightarrow{k_P} \boxed{i} \ldots i_p. \qquad \text{(IV-3)}$$

$\boxed{i}$ represents the template digit, i_m the energy rich monomeric and i_p the included polymeric digit; k_R, k_D and k_P denote the rate constants of the steps of pair recombination, pair dissociation, and digit inclusion (polymerization). "Equilibrated" recognition means

$$k_P \ll k_D. \qquad \text{(IV-4)}$$

Measurements described below show that cooperative base paring (i.e. propagation of a "nucleated" region) occurs with rates as high as 10^6 to 10^7 sec^{-1}, and that k_D can be assumed to be larger than 10^5 sec^{-1} (GC) or 10^6 sec^{-1} (AU).

1 Enzyme-catalyzed processes may be included in the discussion of "quasi-linear systems" as long as the enzyme (e.g. a replicase) represents a constant "environmental factor", i.e. is not part of the "evolving" system (cf. Part VII).

Assuming "equilibrated recognition", we can write

$$q_i = \frac{[\text{probability for pair } ii]}{[\text{sum of probabilities for all pairs of } i]} = \frac{m_i K_{ii}}{\sum_{k=1}^{\lambda} m_k K_{ik}} \qquad \text{(IV-5)}$$

where m_i or m_k are monomeric digit concentrations, and K_{ii} or K_{ik} the corresponding (cooperative) pair stability constants. If equilibration of recognition is not complete, the stability constants may be replaced by suitable steady state constants. The stability constants K_{ik} could just as well be expressed by the free energies of pair formation:

$$K_{ik} = \exp(-\Delta G_{ik}/RT). \qquad \text{(IV-6)}$$

Only if the concentrations of all the monomeric digits are buffered to the same value, do the m_k terms drop out and the simple relation

$$1/q_i = \sum_{k=1}^{\lambda} \exp[(\Delta G_{ii} - \Delta G_{ik})/RT]. \qquad \text{(IV-7)}$$

holds.

As will be seen, it is quite difficult to produce single-digit q-factors which appreciably exceed 0.99 for any enzyme-free recognition process (corresponding to differences of about 3 kcal/mole in ΔG_{ik}).

Finally, the decomposition factors $\mathscr{D}_k$ are of less importance with respect to the present discussion. The formation rate of selected species has to exceed the decomposition rate ($W_i > 0$). As far as $\mathscr{D}$-factors are concerned, they show—for enzyme-free processes—similar tendencies to the other factors, i.e. they do not favor long chains. Unless a protective macromolecular coat is formed, long chains will hydrolyse more easily than shorter ones.

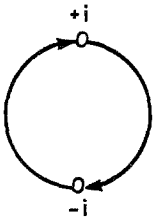
There are further points which may come up again in the discussion of other mechanisms. However, they are of minor importance in comparison to the following conclusion about the evolution of "self-instructing" code systems.

It is highly improbable that all digits are present from the beginning with similar abundances. If, on the other hand, one digit—e.g. an adenine nucleotide—is present in large excess, its inclusion is so strongly favored in the competition that very uniform polymeric sequences (e.g. poly-r-A) would dominate. Such uniform sequences have no coding capacities, hence such a system, apart from other shortcomings (cf. below), would offer very little advantage for any further evolving selforganization.

Nowadays we do not find in nature the simple "self-instructing" code. In principle it could have existed, but if it did, it was at a great disadvantage with respect to a system using "complementary instruction". Such a system, in the presence of even one dominating digit of high abundance, would immediately start to collect another, namely the complementary digit, and thus build up mixed systems, which are a prerequisite for the generation of a code.

IV.2. Complementary Instruction and Selection (Theory)

The simple form of Eq. (IV-1) cannot be used for the treatment of "complementary instruction". The copying process represents an alternation between the "positive" and the "negative" copy, which will be denoted by $+i$ (for the plus strand) and $-i$ (for the minus strand). The reaction collective ($\pm i$) can be represented by a cyclic graph



Each such collective is described by two rate equations:

$$\begin{aligned} \dot{x}_{+i} &= k_0(\mathscr{A}_{+i}\,\mathscr{Q}_{+i}\,x_{-i} - \mathscr{D}_{+i}\,x_{+i}) \\ \dot{x}_{-i} &= k_0(\mathscr{A}_{-i}\,\mathscr{Q}_{-i}\,x_{+i} - \mathscr{D}_{-i}\,x_{-i}) \end{aligned} \qquad \text{(IV-8)}$$

and we have two eigenvalues λ as solutions of the characteristic equation:

$$\begin{vmatrix} -(\mathscr{D}_{+i}+\lambda) & (\mathscr{A}_{+i}\cdot\mathscr{Q}_{+i}) \\ (\mathscr{A}_{-i}\cdot\mathscr{Q}_{-i}) & -(\mathscr{D}_{-i}+\lambda) \end{vmatrix} = 0, \qquad \text{(IV-9)}$$

$$\lambda_{1,2} = -\frac{\mathscr{D}_{+i}+\mathscr{D}_{-i}}{2} \pm \sqrt{4\mathscr{A}_{+i}\mathscr{Q}_{+i}\mathscr{A}_{-i}\mathscr{Q}_{-i} + (\mathscr{D}_{+i}-\mathscr{D}_{-i})^2}. \qquad \text{(IV-10)}$$

These eigenvalues are attributed to certain "normal modes" of reaction [69] represented by concentration parameters y_i which are linear combinations of the x_{+i} and x_{-i}. One of the λ-values is always negative, the other can be positive if

$$\mathscr{A}_{+i}\mathscr{Q}_{+i}\mathscr{A}_{-i}\mathscr{Q}_{-i} > \mathscr{D}_{+i}\mathscr{D}_{-i}.$$

The relation between the x- and y-variables can be written in vectorial form:

$$\vec{y}_i = \boldsymbol{M}_i\vec{x}_i; \quad \vec{x}_i = \boldsymbol{M}_i^{-1}\vec{y}_i, \qquad \text{(IV-11)}$$

where the matrix $\boldsymbol{M}_i^{-1}$ is the inverse of $\boldsymbol{M}_i$, and $\boldsymbol{M}_i$ is determined from the components of the eigenvectors. If we consider for simplicity the (more transparent) case of a uniform decomposition term $\mathscr{D}_{+i} = \mathscr{D}_{-i}$ (which for most experiments at constant forces and adjusted fluxes represents a good approximation) the matrices are

$$\boldsymbol{M}_i = \begin{pmatrix} 1 & -\sqrt{\frac{\mathscr{A}_{+i}\mathscr{Q}_{+i}}{\mathscr{A}_{-i}\mathscr{Q}_{-i}}} \\ +\sqrt{\frac{\mathscr{A}_{-i}\mathscr{Q}_{-i}}{\mathscr{A}_{+i}\mathscr{Q}_{+i}}} & 1 \end{pmatrix}; \qquad \boldsymbol{M}_i^{-1} = \frac{1}{2}\begin{pmatrix} 1 & +\sqrt{\frac{\mathscr{A}_{+i}\mathscr{Q}_{+i}}{\mathscr{A}_{-i}\mathscr{Q}_{-i}}} \\ -\sqrt{\frac{\mathscr{A}_{-i}\mathscr{Q}_{-i}}{\mathscr{A}_{+i}\mathscr{Q}_{+i}}} & 1 \end{pmatrix}. \qquad \text{(IV-12)}$$

Within the same approximation the eigenvalues become

$$\lambda_{1,2} = \pm\sqrt{\mathscr{A}_{+i}\mathscr{A}_{-i}\mathscr{Q}_{+i}\mathscr{Q}_{-i}} - \mathscr{D}_i. \qquad \text{(IV-13)}$$

The physical interpretation is that each reaction cycle is assigned two real eigenvalues representing the temporal behavior. One of these eigenvalues will always be negative. It describes a relaxation process of "equilibration" between the formation of the plus and the minus strands:

$$y_{1i}(t) = y_{1i}^0 \exp[-(\sqrt{\mathscr{A}_{+i}\mathscr{A}_{-i}\mathscr{Q}_{+i}\mathscr{Q}_{-i}} + \mathscr{D}_i)\,k_0 t]. \qquad \text{(IV-14)}$$

It decays to a constant ratio of x_{+i} and x_{-i}. The second eigenvalue is positive if the average formation term

$\sqrt{\mathscr{A}_{+i}\mathscr{A}_{-i}\mathscr{Q}_{+i}\mathscr{Q}_{-i}}$ exceeds the decomposition term $\sqrt{\mathscr{D}_{+i}\mathscr{D}_{-i}}=\mathscr{D}_i$ [analogous to a positive W_i^0 in Eq. (IV-1)]. It represents the autocatalytic growth property of the $(\pm i)$ collective (for $\mathscr{D}_{+i}=\mathscr{D}_{-i}\equiv\mathscr{D}_i$)

$$y_{2i}(t)=y_{2i}^0\exp\left[(+\sqrt{\mathscr{A}_{+i}\mathscr{A}_{-i}\mathscr{Q}_{+i}\mathscr{Q}_{-i}}-\mathscr{D}_i)\,k_0 t\right]. \quad \text{(IV-15)}$$

This is the important part of the solution with respect to selection. The matrices $\boldsymbol{M}_i$ and $\boldsymbol{M}_i^{-1}$ allow us to convert the solutions from "normal" to actual concentration variables and vice versa, e.g. for $\mathscr{D}_{+i}=\mathscr{D}_{-i}$

$$\begin{aligned} y_{1i}&=x_{+i}-\sqrt{\frac{\mathscr{A}_{+i}\mathscr{Q}_{+i}}{\mathscr{A}_{-i}\mathscr{Q}_{-i}}}\,x_{-i};\\ x_{+i}&=\frac{1}{2}\left[y_{1i}+\sqrt{\frac{\mathscr{A}_{+i}\mathscr{Q}_{+i}}{\mathscr{A}_{-i}\mathscr{Q}_{-i}}}\,y_{2i}\right];\\ y_{2i}&=\sqrt{\frac{\mathscr{A}_{-i}\mathscr{Q}_{-i}}{\mathscr{A}_{+i}\mathscr{Q}_{+i}}}\,x_{+i}+x_{-i};\\ x_{-i}&=\frac{1}{2}\left[-\sqrt{\frac{\mathscr{A}_{-i}\mathscr{Q}_{-i}}{\mathscr{A}_{+i}\mathscr{Q}_{+i}}}\,y_{1i}+y_{2i}\right]. \end{aligned} \quad \text{(IV-16)}$$

The "equilibrium" ratio of x_{+i}/x_{-i} follows from $y_{1i}\to 0$ for $t\to\infty$.

$$\frac{\bar{x}_{+i}}{\bar{x}_{-i}}=\sqrt{\frac{\mathscr{A}_{+i}\mathscr{Q}_{+i}}{\mathscr{A}_{-i}\mathscr{Q}_{-i}}}. \quad \text{(IV-17)}$$

For equal formation rates of the plus and the minus strands, this ratio is equal to one. If growth starts from the equilibrium ratio (at $t=0$) only one solution (i.e. the growth solution) is observed ($y_{1i}^0=0$). A more common case (cf. the $Q\beta$-phage experiments described in part VII) is to start with one copy, e.g. the plus strand ($x_{-i}^0=0$). Then both solutions contribute to the temporal change of each species until a constant ratio is reached and the growth solution prevails.

Introducing now the selection strain of constant overall organization, we obtain for each collective

$$\begin{aligned} \dot{x}_{+i}&=k_0\left[\left(\mathscr{A}_{+i}\mathscr{Q}_{+i}\frac{x_{-i}}{x_{+i}}-\mathscr{D}_{+i}\right)-\bar{E}\right]x_{+i}\\ \dot{x}_{-i}&=k_0\left[\left(\mathscr{A}_{-i}\mathscr{Q}_{-i}\frac{x_{+i}}{x_{-i}}-\mathscr{D}_{-i}\right)-\bar{E}\right]x_{-i}. \end{aligned} \quad \text{(IV-18)}$$

It is immediately seen that these equations belong to the general type discussed in Part II, since the ratio x_{+i}/x_{-i} or its inverse does not vanish with t.

We may denote this ratio x_{+i}/x_{-i} by z_i. A differential equation for its temporal change can immediately be derived from (IV-18)

$$\begin{aligned} \dot{z}_i&=\frac{\dot{x}_{+i}x_{-i}-\dot{x}_{-i}x_{+i}}{x_{-i}^2}\\ &=k_0\left[\mathscr{A}_{+i}\mathscr{Q}_{+i}+(\mathscr{D}_{-i}-\mathscr{D}_{+i})\,z_i-\mathscr{A}_{-i}\mathscr{Q}_{-i}z_i^2\right]. \end{aligned} \quad \text{(IV-19)}$$

Integration yields a somewhat lengthy expression

$$z_i(t)=\frac{\bar{z}_i\,(z_i^*+e^{-\alpha_i t})-2\beta_i\,e^{-\alpha_i t}}{z_i^*-e^{-\alpha_i t}} \quad \text{(IV-20)}$$

with

$$z_i^*=\frac{z_i^0+\bar{z}_i-2\beta_i}{z_i^0-\bar{z}_i};\qquad \beta_i=\frac{\mathscr{D}_--\mathscr{D}_+}{2\mathscr{A}_-\mathscr{Q}_-}$$

and

$$\alpha_i=k_0\sqrt{4\mathscr{A}_+\mathscr{A}_-\mathscr{Q}_+\mathscr{Q}_-+(\mathscr{D}_+-\mathscr{D}_-)^2}$$

which for $t=0$ reduces to the initial ratio z_i^0 and for $t\to\infty$ to the equilibrial ratio

$$\bar{z}_i=\sqrt{\frac{\mathscr{A}_+\mathscr{Q}_+}{\mathscr{A}_-\mathscr{Q}_-}+\beta_i^2}+\beta_i. \quad \text{(IV-21)}$$

Insertion of $z_i(t)$ into (IV-18) yields an inhomogeneous differential equation which can be integrated.

The general behavior of a system with complementary instruction is analogous to that of a "selfinstructing" system as discussed above. Each information-carrying collective now consists of two components and can be represented by a two-component column vector $\vec{x}_i=\begin{pmatrix}x_{+i}\\ x_{-i}\end{pmatrix}$. The former quantity $W_i^0=\mathscr{A}_i\mathscr{Q}_i-\mathscr{D}_i$ now is replaced by a matrix

$$\begin{pmatrix}-\mathscr{D}_{+i} & \mathscr{A}_{+i}\cdot\mathscr{Q}_{+i}\\ \mathscr{A}_{-i}\cdot\mathscr{Q}_{-i} & -\mathscr{D}_{-i}\end{pmatrix}.$$

This matrix has two eigenvalues, one of which represents equilibration of the $(\pm i)$ collective, whereas the other describes the competitive growth property of the collective. Hence this eigenvalue and its corresponding normal mode (y_{2i}) enter the selection equation. After equilibration of the collective, we may replace the normal mode y_{2i} simply by the sum $x_{+i}+x_{-i}=y_i^*$, both terms of which are proportional to y_{2i}, and then write the selection equation at constant forces in the common form

$$\dot{y}_i^*=k_0(W_i^0-\bar{E})\,y_i^* \quad \text{(IV-22)}$$

with

$$W_i^0=\sqrt{\mathscr{A}_{+i}\mathscr{Q}_{+i}\mathscr{A}_{-i}\mathscr{Q}_{-i}}-\mathscr{D}_i,$$

(assuming $\mathscr{D}_+=\mathscr{D}_-=\mathscr{D}_i$)

and

$$n\cdot\bar{E}=\sum_{k=\pm1}^{\pm N}E_k\,x_k;\qquad E_k=(\mathscr{A}_k-\mathscr{D}_k)$$

where summation is extended over all $+$ and $-k$.

Selection equilibrium is obtained for $W_i^0=\bar{E}$ yielding, again under the assumption $\mathscr{D}_{+i}=\mathscr{D}_{-i}$,

$$\frac{\bar{y}_{2i}}{n}\,\frac{(1+\sqrt{\mathscr{A}_+\mathscr{A}_-/\mathscr{Q}_+\mathscr{Q}_-})}{2}=\frac{y_i^*}{n}=\frac{W_i^0-\bar{E}_{k\neq i}}{\bar{E}_i-\bar{E}_{k\neq i}} \quad \text{(IV-23)}$$

with

$$\bar{E}_i=\frac{E_{+i}\sqrt{\mathscr{A}_{+i}\mathscr{Q}_{+i}}+E_{-i}\sqrt{\mathscr{A}_{-i}\mathscr{Q}_{-i}}}{\sqrt{\mathscr{A}_{+i}\mathscr{Q}_{+i}}+\sqrt{\mathscr{A}_{-i}\mathscr{Q}_{-i}}}=\frac{\bar{z}_iE_{+i}+E_{-i}}{1+\bar{z}_i}.$$

(The expression for $\mathscr{D}_{+i}\neq\mathscr{D}_{-i}$ has the same general form, but W_i^0 and E_i have to be calculated according to Eq. (IV-10) and the complete transformation matrices $\boldsymbol{M}_i$ and $\boldsymbol{M}_i^{-1}$.)

It is seen that selection occurs, even if for one of the copies (e.g. $-i$) the E-value is smaller than $\bar{E}_{k\neq i}$, as long as

$$W_i^0>\bar{E}_{k\neq i}.$$

The different collectives i_i again compete for selection. In the absence of any further coupling among different i_i, only one collective i_m (together with its "comet tail" of errors), i.e. the one with the highest selective value $W_m^0>0$ (or a degenerate group), will survive. The selective value of the collective contains the geometric means of the $\mathscr{A}_m\mathscr{Q}_m$ parameters of both the $+$ and the $-$ strands. This is an interesting result, showing that the reproduction parameters of both strands are of equal importance. (Note that any arithmetical means would be equivalent to a "rate-limiting" term.) In the present case the slower component grows to a higher stationary concentration level and thereby attains the rate of the faster component.

The important feature of "complementary instruction" is that, even in presence of a large excess of one sort of digits, the system always has to accumulate at least two different digits which then occur in the selected species with almost equal abundance. Due to error copying, the system will then always form

mixed sequences. This is a prerequisite for the generation of any code, which may gain a "meaning" (i.e. "representing" valuable information) as soon as any of the mixed sequences shows a selective advantage with respect to its own reproduction. As will be seen later, binary code systems have certain advantages at the very beginning, without precluding transition to a higher (e.g. quaternary) code form whenever this offers advantages. Due to the competitive nature of single ($\pm i$) ensembles, the amount of information which can be stored is limited to the capacity of one single carrier class [i.e. a master copy and its (reproducible) "comet tail" of error copies] or a degenerate group. Hence, length restrictions essentially imposed by the quality factors $\mathcal{Q}_{+i}$ and $\mathcal{Q}_{-i}$ are of importance. Information about the complementary recognition of the nucleo-bases can be obtained from experimental data.

IV.3. Complementary Base Recognition (Experimental Data)

IV.3.1. Single Pair Formation

Complementary instruction is based on exclusive pair formation between A and U or G and C, respectively. What is the basis of this exclusive interaction which guarantees exact reading, translation and amplification of genetic messages throughout nature, from phage to man?

A biochemist's answer would be, of course: "specific enzymes", whereas a physical chemist might prefer to say: "specific forces". Both would be right, because we know some of the enzymes quite well, and we also know that there is specific interaction, such as the hybridization of complementary strands, in the absence of enzymes.

The specific complementary pair structures, as proposed by F. H. C. Crick and J. D. Watson in their epochal paper [1], are shown in Fig. 8. However, a glance at Fig. 9 immediately reveals that hydrogen bonding as such is not sufficient to explain nature's obvious choice. First, there is a difference in the geometry of the different pairs (cf. Fig. 9) so that the isomorphic structure of the two Watson-Crick pairs will definitely be of advantage with respect to the formation of uniform double-stranded structures involving all four nucleotides, and especially with respect to the evolutionary adaptation of a common polymerizing enzyme. On the other hand, there is a possibility that evolution started with a two-digit code, e.g. A and U. Furthermore, before enzymes were specifically adapted, there may have been some type of codon-anticodon interaction with other choices of "complementarity". These questions can be answered by suitable experiments.

Some of these experiments have already been done. For instance, we can provide quantitative data on free energies of single pair formation for the different combinations of nucleobases. A disappointing result was obtained when such measurements were tried using aqueous media as the solvent: none of the pairs appears to be stable. Even at the highest concentrations of the fairly readily soluble nucleotides, no single pair formation could be detected. We also know why: if H-bonding between the polar groups such as NH, NH_2, OH and CO: or N: is the only source of stabilization, then the polar H_2O molecules would compete far too strongly by

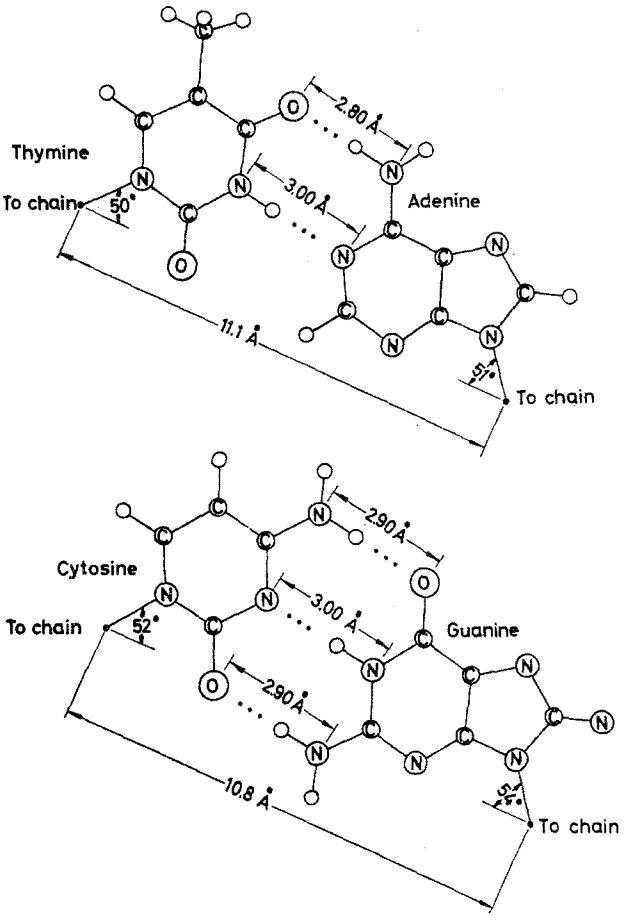


Fig. 8. The complementary base pairs

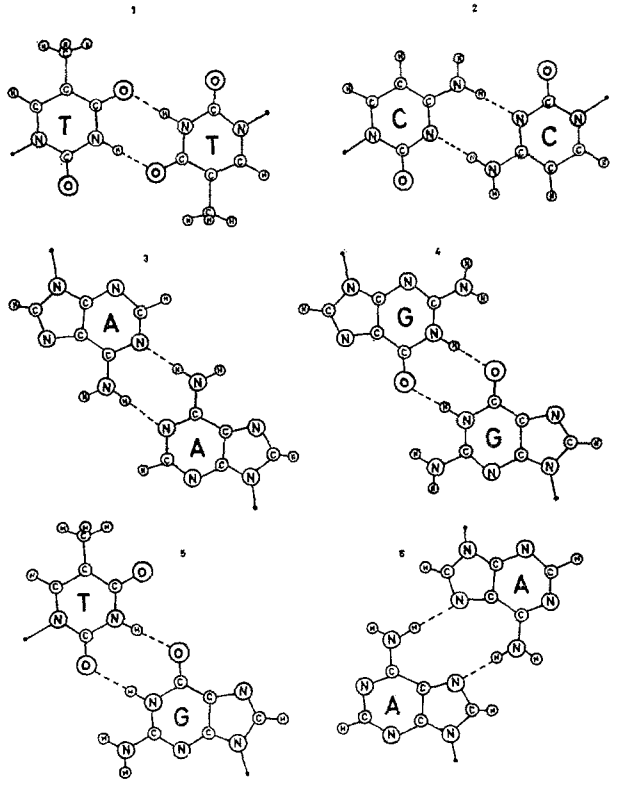


Fig. 9. "Non-complementary" base combinations

Table 12. *Stability constants of base pairing.* $K_{Ass}[M^{-1}]$ *at 25 °C (2'-3'-5'-O-substituted ribonucleosides in non-polar media)*

CCl_4 / C_6H_6	U	A	C	G
U	45 / 15	550	< 50	$< 10^3$
A	150	22 / 8	< 50	$< 10^3$
C	< 28	< 28	50 / 28	$> 10^4$
G	$< 1.2 \cdot 10^3$	$< 1.2 \cdot 10^3$	$3 \cdot 10^4$	$\sim 10^3$ / $1.2 \cdot 10^3$

solvating any exposed dipolar group. Hence a nonpolar solvent should be of advantage for experiments on *relative* stability.

Measurements have been made in various nonpolar solvents using nucleosides which can be made soluble in these solvents by substitution of nonpolar groups at different positions on the ribose (without interfering with the potential of the bases for H-bond formation). The most extensive work of this kind, in particular infrared studies of various substituted nucleosides, was done by A. Rich and coworkers [70] of M.I.T. Similar studies have also been reported by E. Küchler and J. Derkosch of Vienna University [71]. Dielectric studies of pair formation were carried out in our laboratory by T. Funck [72] partly in collaboration with R. Hopman and F. Eggers. They also determined kinetic parameters from relaxation measurements [73].

There is general agreement among all the results. The complementary pairs AU and GC are the strongest when compared to the alternatives. Table 12 shows some values for stability constants determined by dielectric measurements. As is seen, AU is at least ten times more stable than either AA or UU, whereas GC is the favored pair among all G and C combinations. The GC pair is much more stable than the AU pair. Since G also shows considerable "selfpairing", it is not possible to determine stability constants of any hetero-pair of G other than GC. It should be noted that certain pairs can form in different ways and that the equilibrium constants given in Table 12 represent overall values.

All pairs form very quickly. The rate constants indicate that every encounter leads to pair formation and that the lifetimes of the pairs in general are less than one microsecond.

The pronounced relative stabilities of AU and GC are probably due to one strongly polarized hydrogen bond. The only XH ... Y combination of this type—common to AU and GC (but not to the competitors)—is the H-bond between the fairly acidic NH group (pK ~9.5) and the ring nitrogen (pK ~4.5). The pronounced stability of such a bond can be understood on the basis of quantum mechanical theory. Thus, this important prerequisite of code formation is in principle explained by physical theory.

In short, the data show clearly preferred pair formation between those bases we now call "complementary". "Recognition" is a very fast process, but as a consequence absolute stabilities are quite low—so low that pairs do not form to any detectable extent in polar media. Such low stabilities could not account for any very accurate recognition such as would be required for code reading in longer sequences. In order to learn more about base recognition in sequential code reading, one has to study the cooperative interactions occurring in oligo-or polymeric species.

IV.3.2. Cooperative Interactions in Oligo- and Polynucleotides

It has already been emphasized that in aqueous media single base pairs are too unstable to be detected by present techniques. On the other hand, it is well known that complementary polymeric strands form quite stable double helical structures, which "melt" only at high temperatures. The shapes of the melting curves indicate a strongly cooperative behavior. The source of cooperativity is a relatively strong "stacking" interaction between adjacent base pairs and also chelate effects resulting from the "freezing" of degrees of freedom upon helix formation. It is obvious that these cooperative effects greatly enhance the "instructive" abilities of nucleic acids.

A straightforward way to study the cooperative phenomena is to build up the polymer step by step starting from the mononucleotide and to study the associated thermodynamic and kinetic properties as they change with increasing chain length. A great advantage is the relative simplicity of thermodynamic and kinetic analysis of the conformational changes of oligomeric species, especially their "all or none" type of base pairing behavior. The difficult part of such work on oligomeric species is the preparation of the material, requiring polymeric samples to be degraded, separated, collected and purified. Various groups in our laboratory (as well as in others[1]) have been engaged in such work for several years.

The first kinetic studies were done on oligomeric adenylic acid (chain length 2 to 10 digits) by D. Pörschke in his Diplomthesis [66, 67]. As has already been mentioned, oligo- and poly-r-A form double-stranded helical structures in the acidic range (pH ~4) and this presented a good model for our first studies. D. Pörschke [75] later extended the work to the double and triple helix formation between oligo-r-A and oligo-r-U at neutral pH (chain length up to 18). The work included thermodynamic studies (phase diagram, melting curves by UV spectrophotometric observation) as well as kinetic investigations using flow and relaxation (T-jump) techniques.

Extension of this work to GC-oligomers met with great difficulties, due to the aggregation of these species to more complex structures. S. K. Podder [76] during his postdoctoral years at Göttingen was able to study the pairing of a tetramer

GpGpGpC GpGpGpU
CpCpCpG CpCpCpG

as well as that of GpG and GpGpG with poly-r-C.

G. Maass and a joint group from the laboratories at Göttingen and Braunschweig-Stöckheim (D. Riesner, R. Römer, S. Coutts) in collaboration with a group in H. G. Zachau's laboratory at Munich studied the melting behavior and conformational kinetics of *t*-RNA's [77] (cf. Fig. 2) as well as of fragments of known sequence [78], which they obtained by reproducible splitting of the native molecule according to a method described by H. G. Zachau [79].

Very valuable information about specific pairing of various sequences was obtained in P. Doty's laboratory at Harvard University from studies with oligomeric copolymers [80, 81] such as

ApApApApUpUpUpU ApApApGpUpUpU
UpUpUpUpApApApA; UpUpUpCpApApA;

ApApApGpCpUpUpU
UpUpUpCpGpApApA

1 E.g. R. Baldwin (Stanford), D. Crothers (Yale), N. Davidson (Pasadena), P. Doty (Harvard), J. Fresco (Princeton). B. Zimm (La Jolla).

and also with various oligomers when combined with exposed complementary sequences of *t*-RNA molecules (cf. Table 13). During visits to Göttingen two of P. Doty's coworkers, F. Martin and O. C. Uhlenbeck, cooperated with D. Pörschke on further thermodynamic and kinetic studies of the above-mentioned oligomers [82]. The results and (preliminary) conclusions of all these studies can be reviewed as follows:

Helix-Coil Equilibria

Under conditions where double-stranded helices form, the "melting" curves show a straightforward correlation with chain lengths, i.e. an increase of slope with increasing chain length. The reciprocal "melting temperatures" (i.e. the temperatures of half-transition from helix to random coil) yield a straight line if plotted vs $1/(\nu-1)$ (ν being the digit number of the maximum number of base pairs in the double-stranded helical structure). The slope of this line is proportional to $1/\Delta H$; the intercept represents $1/T_\infty = \frac{\Delta S}{\Delta H}$, the reciprocal melting temperature of the "infinitely long" helix. "Infinitely" means large compared to the "cooperative length" which includes about 30 base pairs. Hence, T_∞ can be determined from long chain polynucleotides. In order to achieve this result one has to correct for single-strand stacking, the extent of which differs at the different melting temperatures (as can be determined by experiments with single-stranded polymers). For short chain lengths, the transition from helix to random coil can be represented by an "all or none" process. If $\sigma_i s$ denotes the stability constant for each base pair adjacent to a continuous sequence of $(i-1)$ pairs—with the understanding that the "nucleation parameter" σ approaches one above a certain number n referred to as the "nucleation length"—we can write for the equilibrium constant of the "all or none" transition involving $\nu > n$ base pairs

$$K_\nu = \bar{\sigma} s^\nu; \quad \bar{\sigma} = \sigma_1 \sigma_2, \ldots, \sigma_n. \qquad \text{(IV-24)}$$

The kinetic data (see below) indicate that the nucleation length n at room temperature is 3 for AU and 2 for GC helices, but also approaches 3 for GC helices at temperatures > 50 °C. This is equivalent to saying that σ_n approaches values close to one for $n \geqq 3$. The melting curves then indicate that for $n \geqq 3$ there is a constant (and negative) increment of ΔH_s and ΔS_s associated with each base pair, according to the Van't Hoff relation

$$\ln s = -\frac{\Delta H_s}{RT} + \frac{\Delta S_s}{R}. \qquad \text{(IV-25)}$$

The fact that the corrected $1/T_m$ is proportional to $1/(\nu-1)$ rather than to $1/\nu$ indicates that a constant increment cannot be present for $n \leqq 3$, that, in particular, the nucleation parameter $\bar{\sigma}$ shows a temperature dependence opposite to that of s, so that $\bar{\sigma} s$ is almost temperature-independent ($\Delta H_{\sigma s} \sim 0$). Although $\bar{\sigma} s$ is *not* the stability constant of the first base pair—this would be $\sigma_1 s$— the physical interpretation is that the essential part of ΔH_s results from stacking interactions (note the relatively high stacking increment of ΔH for each single strand) rather than from hydrogen bond formation between the complementary bases. The ΔH-value for the first base pair thus would be relatively low. This pair is very unstable ($\sigma_1 s < 1$) due to competition with solvent molecules (cf. the values of the constants in Table 13). For longer chain length, the "all or none" model does not apply. The partition function of the system then has to be evaluated by statistical methods, as was done for a "staggering zipper model" by J. Applequist and V. Damle [83]. Experimental data were evaluated according to this model by D. Pörschke with the help of a computer program [84].

Kinetics and Mechanism of Base Pairing

With the help of kinetic data it is possible to elucidate the mechanism of cooperative base pairing. For short helices the "all or none" type of transition is clearly confirmed. The relaxation spectrum consists of only one time constant representing a second-order "all or none" mechanism, i.e. the recombination of both (complementary) single strands to a double helix with a maximum number of base pairs. Only for longer chain lengths is an additional spectrum of first-order time constants ($\tau \ll 1$ μs) observed, which represents changes in open-ended or staggered conformations. The second-order process is characterized by high rates of recombination which, however, are clearly below the limit of diffusion control, indicating a nucleation threshold (k_R: 10^6 to 10^7 M^{-1} sec^{-1}). The values of these rate constants are almost the same for all chain lengths above 3 (but below the cooperative length). They show a small but significant temperature dependence which, for AA (pH ~4) as well as for AU (neutral range, $T < 50$ °C) and also for GC helices at higher temperatures ($T > 50$ °C), is represented by *negative* apparent activation energies.

The absolute values of these "activation" parameters allow a quite straight-forward assignment of nucleation lengths. If the formation of the first base pair were rate-limiting[1], the activation energy (for the encounter process) should be positive. Since we know that the first pair is not stable ($\sigma_1 s < 1$ means that the pair dissociates more rapidly than it forms), we would have expected a *nucleation barrier*. If only the first pair represents this barrier, i.e. if the "zippering reaction" propagates as soon as a stable two-pair nucleus forms, the rate constant would be given by $k_R = \sigma_1 s\, k_{12}$, k_{12} being the (first-order) rate constant for the formation of the second pair (next to the first one). Since the activation energy increment of k_{12} must be positive and σs has an almost zero ΔH increment, $\sigma_1 s$ includes at best a small negative value of ΔH), the total apparent activation energy would turn out to be around zero, but by no means close to the experimentally observed value of -10 kcal/mole (e.g. for AU oligomers). This relatively large negative value is consistent only with the expression $k_R = \sigma s^2 k_{23}$ describing a process in which formation of the third pair (k_{23}) represents the rate-limiting step. Thus a paired base triplet represents the stable nucleus for AU sequences. We might then assign to the rate constant of propagation (k_{23}) values of the order of magnitude of 10^7 to 10^8 sec^{-1}, in agreement with the observed relaxation spectrum with time constants $\lesssim 1$ μsec for open-ended configurations. GC oligomers at room temperature, due to their appreciably higher stability constants "s", can form stable "two-pair nuclei". Their recombination rate constants show positive activation energies, as is to be expected from the (in comparison to AU) higher energy parameters. In melting curves these are "masked" by the (also higher) single-strand stacking parameters.

The results are confirmed by the independently determined rate constants of dissociation. Due to microscopic reversibility we must have

$$k_D = \frac{k_{23}}{s^{\nu-2}} \quad (\text{since } k_R/k_D = \bar{\sigma} s^\nu). \qquad \text{(IV-26)}$$

With $s^{\nu-2}$ in the denominator, the activation energies are positive, involving the large increment $(\nu-2)\Delta H_s$.

The expected values and a corresponding decrease of the absolute values of k_D with increasing chain length (amounting to orders of magnitude) are indeed observed.

IV.3.3 Conclusions About Recognition

Table 13 compiles the data obtained from equilibrium and rate studies with oligo- and polymeric *ribonucleotids*. The most interesting effect is the preference for the triplet, however, not just for the logically obvious reasons, i.e. the prerequisite for the coding of more than 20 symbols, but rather due to *mechanistic coincidences*. Codons with less than three digits would be very unstable (at least for A and U). Codons with more than three digits, especially for G and C, become too "sticky". The life time of a condon-anticondon pair should not exceed milliseconds so that enzymes with corresponding turnover numbers can adapt optimally. The same type of optimization between stability and rate is always found for enzyme–substrate interactions. Any gain in stability means a lowering of complex dissociation rates; these have to match the turnover numbers in order not to become the rate-limiting steps for the turnover.

1 Only those processes are observed which lead to complete helices with the maximum number of base pairs.

Table 13. *Stability constants for pairing of base triplets and quadruplets (tri- and tetranucleotides) with exposed regions (preferably anticodons) of t-RNA according to P. Doty et al.* [80, 81]. $K_{Ass}[M^{-1}]$ *was measured in aqueous solution of 1.0 M NaCl, 10 mM* $MgCl_2$ *and 10 mM phosphate at pH 7 and 0 °C. K-values* $<400\ M^{-1}$ *are not distinguishable from "no association"*

f-met-*t*-RNA AA[UAC]UC	K_{Ass}	tyr-*t*-RNA AA*[AU*G]UC [a]	K_{Ass}
AUG	1200	UAC	700
(regular codon)	± 200	(regular codon)	
AUGA	13500	UACA	90000
AUGU	1400	UAU (3′-wobble)	700
AUGC	900	UAUA	37000
AUGG	1000		
GUG (5′-wobble)	1200	phen-*t*-RNA [a]	
GUGA	9800	AY[AAG*]UC*	
GUGU	1000		
		UUC (reg. codon)	900
		UUCA	10000
		UUU (3′-wobble)	300
		UUUA	1000

A* = N(6) dimethyl-A; U* = pseudo-U (ψ); G* = 2-O-methyl-G; C* = 2-O-methyl-C.

[a] Private communication by O. C. Uhlenbeck.

Table 14. *Compilation of average equilibrium and rate parameters of cooperative base pairing, obtained from relaxation studies with oligo-ribonucleotides (chain length 3 to 18) in aqueous media. Data are extrapolated to 0 °C, and refer to pH 7 and ionic strength of 0.05 M (Na⁺ cacodylate) for* AU *and of 0.1 (phosphate buffer pH 7.2) for GC. Measurements by D. Pörschke* [66, 67, 75, 84] *and S. K. Podder* [76]

Cooperative pair	σ [M^{-1}]	s	ΔH [a] [kcal/mole]	k_R [M^{-1} sec^{-1}]	$\Delta H^{\#}(k_R)$ [kcal/mole]	k_{23} or k_{12} [sec^{-1}]
...A... ...U...	$\sim 10^{-3}$	10	−11	10^6	−9	10^7
...G... ...C...	$\sim 10^{-3}$	100 to 200	−15	10^6	+5 to +7	10^7

[a] Extrapolated for "unstacked" single strands. (The actually measurable values which refer to stacked single strands, are appreciably lower.)

Note: Only orders of magnitude are given for rate and equilibrium constants, since precise values refer to special pair combinations (cooperativity) and depend strongly on experimental conditions (ionic strength etc.).
Lifetimes of different regions can be estimated with the help of k_{23} (or k_{12}) and s (or σs^N resp.).
The data in Table 13 refer to pairing, mainly at the anticodon, possibly involving also other exposed regions. The values are higher than those from Table 14, probably due to steric stabilization of the anticodon loop. σ-values therefore cannot be extrapolated from these data. Mispairing within a paired region should yield lower stability constants (within "noise level") than indicated by the constants for terminal mispairing given in Table 13.

A further interesting feature of the triplet may be noted. If one derives stability constants for complementary triplets from obligomeric double helices with chain length longer than $\nu = 4$, one arrives at values which are noticeably lower than those directly determined for base triplets or quadruplets (cf. the values in Table 14 for interactions of base triplets and quadruplets with exposed regions of *t*-RNA, as determined in P. Doty's laboratory). The same holds for the activation parameters of GC pairing starting with a doublet pair (cf. the positive values). Apparently, short exposed regions show higher stacking interactions since the base pairs are free to arrange for the most favorable stacking overlap. Part of this interaction energy will be lost by extension of this region to a twisted helical structure in which the arrangement of the base pairs is more constrained. (Similar "steric" restrictions hold for the "wobble" pair GU, which is effective at only one end of the triplet.)

If we use the data in Table 14, we might arrive at some conclusion about the q-values, i.e. the single-digit quality factors which determine the accuracy of complementary instruction. Using Eq. (IV-5), modified for complementary interaction, e.g. for AU (A being the template element):

$$q_{AU} = \frac{m_U K_{AU}}{\sum_{X=AUGC} m_X K_{AX}} \qquad \text{(IV-27)}$$

we obtain values which, even for optimal conditions, may on average hardly reach a value of 0.99. An exact determination would require still more knowledge about stability constants of "misfits" within a complementary region as well as of different nearest neighbor combinations. For AU the values are very likely appreciably lower than 0.99, for GC they might—under special conditions—be somewhat higher GC being always at least 10 to 50 times more stable than AU (depending on nearest neighbors). Such values represent upper limits. They require equilibration of the complementary recognition prior to the inclusion of the digit into the polymeric chain, and they are related to concentrations of monomeric digits which are all buffered to about the same values. These conditions are not likely to be found in nature. We may conclude that $\mathcal{Q}$-values will already noticeably depart from one for relatively short chain lengths. According to Eq. (II-45), for reproducible formation of a code carrier, $\mathcal{Q}$ has to remain above a certain threshold value. Thus reproducible formation of nucleic acids with specified base sequences, without catalytic help, was possible only for relatively short chains, probably not exceeding 30 to 100 digits (with $q = 0.99$ we obtain $\mathcal{Q} \approx 1/e$ for $\nu = 100$, cf. Table 8). Another difficulty is the *mechanism of reduplication.* Low temperatures are required for a certain accuracy of recognition between complementary bases. At these temperatures the double helices formed are stable. Thus, strong temperature gradients or fluctuations are necessary to dissociate the helix as is required for repeated template action of a given strand. This would not allow large differences in rates (as are also found in present enzyme-instructed replication processes) to occur as a consequence of individual secondary structure.

If finally we raise the question:

Can nucleic acids organize a selfreplicating and further evolving unit without catalytic help?

Our answer must be:

Due to their complementary interactions each collective consisting of a positive and negative strand has the inherent property of selfinstruction. Under favorable conditions they could have selected single collectives with specified sequences. However, these sequences—if they occur repro-

ducibly at all—represent a very low information content ($\nu < 100$). Since different collectives have to compete with each other, such a system (without selfinstructing catalytic help) would not be able to organize itself to any type of correlated function.

V. Selforganization Via Cyclic Catalysis: Proteins

V.1. Recognition and Catalysis by Enzymes

Before we ask whether proteins can form selfinstructive systems, we should note certain of their properties:

1. As was shown in the introduction (Part I), with twenty classes of amino acids one can form an immense variety of sequences (cf. Table 3), of which only a minor fraction could ever form by chance. On the other hand, there is no property of self- or complementary instruction inherent to the amino acids. Wherever anything approaching such a property may seem to be present, e.g. as in "pleated sheet"- or β-structures stabilized by $(+-)$-salt bridges, or in other simple regular structures with complementary arrangement of certain amino acids such as in collagen, it is a unique consequence of a very specific arrangement but not an inherent property of the digits as was the case for nucleic acids. Such specific "instructive" arrangements lack the very important property of mutagenicity. When an error occurs, they would not be able to reproduce the error copy. Moreover, the strong tendency of polypeptide chains to undergo specific spatial folding is a great hindrance for any straightforward copying process.

2. *Spatial folding*, on the other hand, is the basis of the ability of proteins to recognize specific structures. The catalytic properties of enzymes are another consequence of this unique feature, provided the recognizing groups also possess concerted catalytic functions. As an example, the active center of the enzyme chymotrypsin is shown in Fig. 10. The precise spatial arrangement of the functional groups was only recently revealed by X-ray structure analysis [85]. It provides a wonderful example for the dependence of function on a sophisticated structure in which groups of quite distant sequential location are brought together into a precisely fixed spatial arrangement. The enormous diversity of specific recognition sites is also clearly demonstrated by the large variety of antibodies, the binding capacity of which involves any haptenic group, even though it may not have been in contact with the antibody during evolution. Furthermore, it has been shown in laboratory experiments that random synthesis of polypeptides involves a large variety of catalytic functions of sometimes quite high specificity (cf. a resemblance to chymotrypsin function in random polypeptides [86]). These products do not form reproducibly. Even if certain functions do occur reproducibly, they are carried out by quite different and unrelated structures.

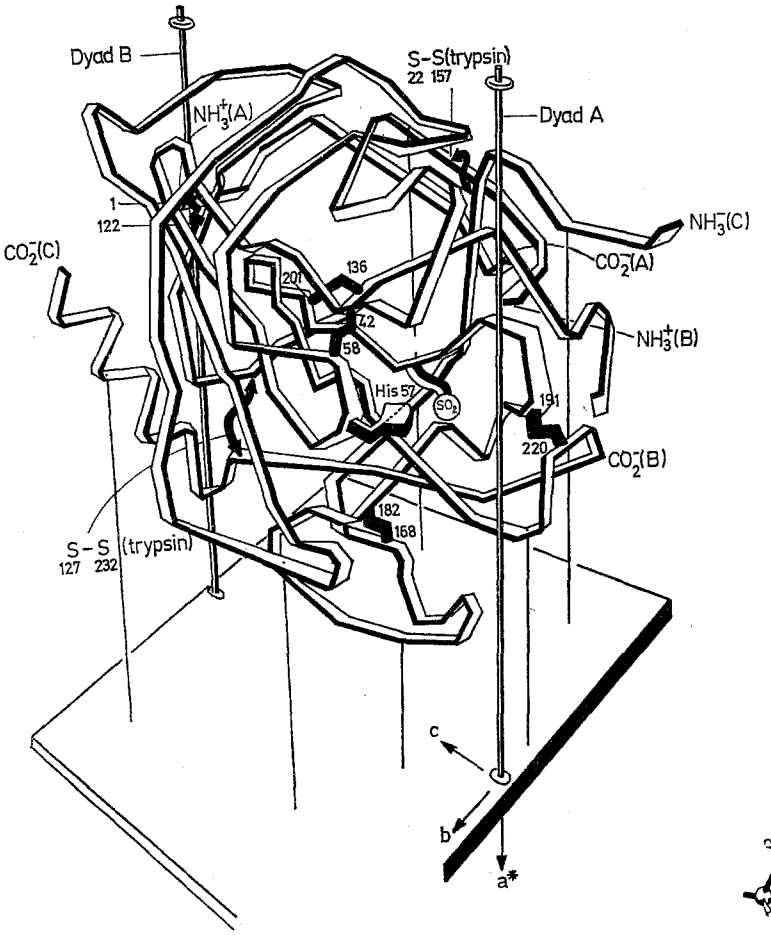


Fig. 10a

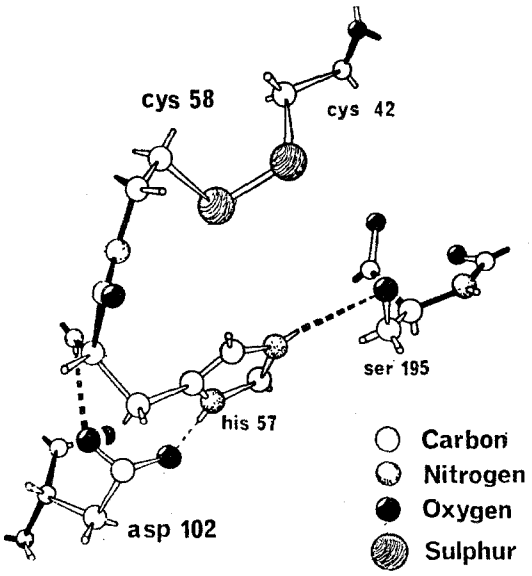


Fig. 10b

Fig. 10a. Schematic drawing representing the conformation of the polypeptide chains in α-chymotrypsin. Reproduced from D. M. Blow, J. J. Birktoft, B. S. Hartley [85]

Fig. 10b. The conformation of a few amino acid side chains in the active center of α-chymotrypsin, demonstrating that "recognition" by proteins is a unique result of spatial folding and not any "inherent" property of the digits. (Reproduced from [85])

3. *Specific recognition* of macromolecular species is restricted to short sequences or spatial (tertiary) structures of relatively little extension. For instance, certain enzymes (e.g. papain [87]) degrade peptides of specific amino acid sequences, whereas other enzymes synthesize specific sequences. F. Lipmann [88] and his group have recently shown that gramicidin S, a cyclic decapeptide, is completely synthesized by an enzyme without any encoding by nucleic acids. This enzyme, with a molecular weight of 280000, is admittedly a complex system, consisting of several subunits. Anyway, it acts very precisely as a "protein template", linking together the ATP-activated amino acids into the precisely defined sequence

D–Phe–L–Pro–L–Val–L–Orn–L–Leu,

which remains thioester-linked to the protein until two such completed pentapeptides cyclize to form the gramicidin S molecule. Thus we must bear in mind that specific, and very precise instruction for the formation of proteins can be given by proteins alone, without the help of a nucleic acid code. Such instruction, however, is restricted to relatively short sequences (e.g. pentapeptides). Nevertheless, with this property one could conceive of an enzymic network which produces oligopeptides and links them together specifically in a number of steps until complete protein molecules occur, possibly with catalytic function for their own reproduction.

4. Such a network can appear as a highly controlled machinery. Since enzymic function results from precise steric arrangements of particular groups, the enzymic properties can change drastically as a consequence of conformation changes, triggered by the binding of inducers or by interaction with other protein structures. Models for such control of enzyme functions were first proposed by F. Jacob and J. Monod [9]. Specific mechanisms were derived by J.-P. Changeux, J. Monod and J. Wyman [89], as well as by D. E. Koshland, G. Nemethy and D. Filmer [90], and have been tested by kinetic studies with various enzymes (e.g. glyceraldehydephosphate dehydrogenase, as studied by K. Kirschner [91], using relaxation techniques). It is possible to show that these enzyme systems may possess all the properties known for electronic control devices [67]. Hence, whenever a selfreproducing network evolves, it may include highly sophisticated control functions.

V.2. Selforganizing Enzyme Cycles (Theory)

V.2.1. Catalytic Networks

With the properties outlined in paragraph 1 in mind, we may construct a "catalytic network" (cf. Fig. 11). Certain proteins are present which have the ability to catalyze the condensation of a limited number of amino acids into chains of specified sequences (e.g. defined pentapeptides); other such "enzymes" recognize given terminal sequences of these oligopeptides and link them up further, so that finally defined chains of any length may occur. The enzymes involved in such catalytic functions are usually polyfunctional. They may recognize specified sequences belonging to different peptide chains (of various lengths), but they are still selective, depending on the particular tertiary structure of the substrate and the availability of the recognition site which must not be hidden in the spatially folded peptide chain.

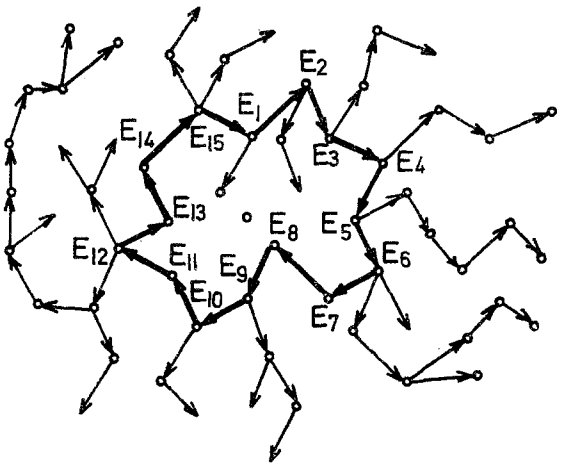


Fig. 11. Catalytic network of proteins, including a closed loop: $E_1, \ldots, E_{15}$

Let us now assume that each catalyst in this network is made with the help of another catalyst. The activation of a given catalytic control function comes about by certain chain combinations or cleavages. Similar processes are very well known today, e.g. the activation of trypsin or chymotrypsin from trypsinogen or chymotrypsinogen, respectively, via enzymic cleavage of a peptide bond close to one end of the chain. In this way a widely branched catalytic network, as shown in Fig. 11, may result. At least some of the enzymes have to be polyfunctional (cf. branches) in order to make this network selfreproductive, because each enzyme requires more than one enzyme for its own reproduction. For instance, if sequences of at most 5 amino acids can be recognized, production of a chain of 80 peptide bonds requires at least 5 enzymes to increase the degree of polymerization to 80, i.e.

$$\overset{E_1}{\smile} 5 \overset{E_2}{\smile} 10 \overset{E_3}{\smile} 20 \overset{E_4}{\smile} 40 \overset{E_5}{\smile} 80.$$

The larger the extension of the network, the higher is the chance of finding a closed loop. Only such a ring closure makes the system autocatalytic and hence guarantees selfreproduction. If the loop is large enough, all auxiliary functions, such as production of a great variety of oligopeptides and precursor chains, can easily be located in the branches.

V.2.2. The Selfreproducing Loop and Its Variants

For the moment we will concentrate on those enzymes which represent the closed loop and number them $E_1 \ldots E_n$, in analogy to the organic chemist when he looks for the "chromophor" in a complex aromatic structure. Let us represent this loop by the cyclic graph given in Fig. 12. The differential equations for the reaction rates would be in general nonlinear. However, for simplicity we may consider a linear approximation corresponding to the previously treated case of buffered substrate concentrations. Even if this is not as realistic as in the case of monomeric digits—the substrates for the cyclic path in the

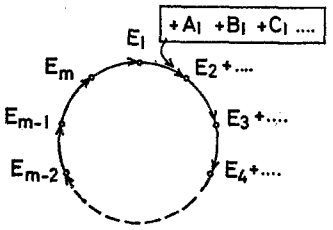


Fig. 12. Graph representation of a catalytic cycle

network involve mainly polymeric precursors—such conditions could in principle be achieved. Moreover, the essential conclusions also apply to the nonlinear case.

The system of rate equations represents a generalization of the systems (8) or (18), given in Part IV, respectively. For each cycle with m members we have in absence of selection constraints:

$$\begin{aligned} \dot{x}_1 &= \mathscr{F}_1 x_m - \mathscr{R}_1 x_1 \\ \dot{x}_2 &= \mathscr{F}_2 x_1 - \mathscr{R}_2 x_2 \\ &\vdots \\ \dot{x}_m &= \mathscr{F}_m x_{m-1} - \mathscr{R}_m x_m \end{aligned} \qquad \left| \begin{aligned} \mathscr{F}_i &= k_0 \mathscr{A}_i \mathscr{Q}_i \\ \mathscr{R}_i &= k_0 \mathscr{D}_i \end{aligned} \right. \qquad \text{(V-1)}$$

With the rate coefficient matrix

$$\begin{pmatrix} -\mathscr{R}_1 & 0 & \cdot & 0 & \mathscr{F}_1 \\ \mathscr{F}_2 & -\mathscr{R}_2 & \cdot & \cdot & 0 \\ 0 & \mathscr{F}_3 & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & 0 \\ 0 & \cdot & 0 & \mathscr{F}_m & -\mathscr{R}_m \end{pmatrix} \qquad \text{(V-2)}$$

the characteristic equation yields for the eigenvalues m roots

$$\prod_{k=1}^{m} (\mathscr{R}_k + \lambda) = \prod_{k=1}^{m} \mathscr{F}_k . \qquad \text{(V-3)}$$

Since all $\mathscr{R}_i$ and $\mathscr{F}_i$ are positive numbers, we obtain a polynomial of m-th degree in λ, in which all terms containing λ^i for $i \geqq 1$ have positive coefficients. The constant term ($\sim \lambda^0$), however, reads

$$\Pi\, \mathscr{R}_k - \Pi\, \mathscr{F}_k . \qquad \text{(V-4)}$$

It is negative for $\Pi\, \mathscr{F}_k > \Pi\, \mathscr{R}_k$. In this case the polynomial contains one reversal of sign, i.e. all terms except the last are positive. According to the Cartesian rule, one finds *one* positive and $(m-1)$ negative (possibly complex) eigenvalues.

In analogy to Eq. (IV-13), we obtain with the special assumption $\mathscr{R}_1 = \mathscr{R}_2 \ldots \mathscr{R}_m \equiv \mathscr{R}$ for the positive eigenvalue:

$$\lambda = +\sqrt[m]{\prod_{k=1}^{m} \mathscr{F}_k} - \mathscr{R} = \hat{\mathscr{F}} - \mathscr{R} . \qquad \text{(V-5)}$$

In the absence of decomposition (i.e. all $\mathscr{R}$ terms equal to zero) the solutions simply reduce to roots, containing the absolute value of the geometric means $\hat{\mathscr{F}}$ multiplied by the values of the m-th unity root, according to

$$\begin{aligned} e^{i k 2\pi/m} &= \cos(k\, 2\pi/m) + i \sin(k\, 2\pi/m) \\ k &= 0, 1, \ldots, (m-1) \end{aligned} \qquad \text{(V-6)}$$

After "equilibration" (i.e. after decay of the $(m-1)$ normal modes which are associated with the negative eigenvalues), the population densities of the different members of the cycle approach constant proportions. These "equilibrial proportions" are (for the above condition of uniform $\mathscr{R}$):[1]

$$\frac{\bar{x}_{i-1}}{\bar{x}_i} = \frac{\sqrt[m]{\prod_{k=1}^{m} \mathscr{F}_k}}{\mathscr{F}_i} = \frac{\hat{\mathscr{F}}}{\mathscr{F}_i} , \qquad \text{(V-7)}$$

$$\frac{\bar{x}_i}{\sum_{k=1}^{m} \bar{x}_k} = \frac{1}{1 + \frac{\mathscr{F}_{i+1}}{\hat{\mathscr{F}}} + \frac{\mathscr{F}_{i+1}\mathscr{F}_{i+2}}{\hat{\mathscr{F}}^2} + \cdots + \frac{\mathscr{F}_{i+1}\mathscr{F}_{i+2}\cdots\mathscr{F}_{i+m-1}}{\hat{\mathscr{F}}^{m-1}}} = \frac{1}{1 + \sum_{s=1}^{m-1} \prod_{k=1}^{s} (\mathscr{F}_{i+k}/\hat{\mathscr{F}}^s)} . \qquad \text{(V-8)}$$

The indices in Eq. (V-8) run cyclically, i.e. $i+k = i+k-m$ for $i+k > m$ ($k = m-i+1,\ m-i+2 \ldots m-1$). The physical interpretation of the obtained result is: *Each cycle has one normal mode representing autocatalytic growth of the whole "collective" and $(m-1)$ normal modes representing relaxational phenomena, i.e. "equilibration" within the cycle. Thus, a closed catalytic loop is equivalent to a system with self- or complementary instruction.*

Before we go to analyze further the selection behavior among competitive cycles under selection strains, we may consider different types of reaction networks to find out more about the prerequisites for selection.

Two conditions seem to be necessary prerequisites for the selective self-reproduction of a reaction network:

1. the system has to involve a "closed loop" of reactions;
2. the couplings among reaction states have to be catalytic.

In order to illustrate the first condition we may consider a general open (quasi-linear) reaction chain

$$\begin{matrix} & & + \text{ reactants} & & \\ & \overset{\vec{V}}{\Longrightarrow} & A_1 \to A_2 \to \cdots \to A_{m-1} \to A_m & \Longrightarrow & \\ & & + \text{ products} & & \end{matrix} \qquad \text{(V-9)}$$

Again, for simplicity we assume that apart from the chain of species A_i all further reactants participating in the reaction are present at high (and hence buffered) concentrations, so that they have not to be considered explicitly. At constant reaction flow $\vec{V}$, the different states will fill up to stationary levels and the whole system will finally "overflow". The system will "produce" the different states if conditions are favorable but it will not "reproduce" itself, i.e. the production rates of given states are independent of their population. The system—even if some or all of the A_i are catalytically active, which means that they participate in the reaction without being consumed—lacks the important property of autocatalytic growth.

Now let us consider a cyclic system like that in Fig. 12 in which, however, the E_i are not catalysts but simple reaction products and partners. The system may have some in- and outflow, and all partners (A_i, B_i, C_i, ...) except the E_i are buffered again. Such a cycle will reproduce itself. However, as long as none of the E_i is a catalyst, the system will approach a stationary state in which each E_i after its production will be consumed again. Several such cycles will not compete

1 For $\mathscr{R}_1 \neq \mathscr{R}_2 \neq \cdots \mathscr{R}_m$ the expressions are much more involved, but can easily be derived by recursion formulas starting from the identity

$$1 = \frac{x_m}{x_1}\,\frac{x_1}{x_2} \cdots \frac{x_{m-1}}{x_m}$$

and the "equilibrium condition":

$$\mathscr{F}_1 \frac{\bar{x}_m}{\bar{x}_1} - \mathscr{R}_1 = \mathscr{F}_2 \frac{\bar{x}_1}{\bar{x}_2} - \mathscr{R}_2 = \cdots \mathscr{F}_m \frac{\bar{x}_{m-1}}{\bar{x}_m} - \mathscr{R}_m .$$

with each other but rather assume a stationary distribution. Such cycles do exist in biological systems, and the different reaction steps are usually also catalyzed by enzymes. However, *the enzymes* do not reproduce themselves via the particular cycle but rather are maintained by some other "circuit" which is a part of the selfsustaining cycle of the whole living entity.

The mathematical treatment may illustrate this behavior further. In absence of other decomposition reactions, the non-catalytic transformation of E_i into E_{i+1} yields the same rate expression for the disappearance of E_i as for the appearance of E_{i+1}. Thus, in the matrix of rate coefficients (V-2) all $-\mathscr{R}_i$ would be replaced by $-\mathscr{F}_{i+1}$.

Then the characteristic equation yields only $(m-1)$ eigenvalues which are all negative

$$\left(\text{e.g. for } \mathscr{F}_1=\mathscr{F}_2\ldots\mathscr{F}_m\equiv\mathscr{F}\text{: } \lambda=\mathscr{F}(\sqrt[m]{1}-1)\right.$$
$$\left.\text{or for } m=4\text{: } \lambda_1=0;\ \lambda_2=-2\mathscr{F};\ \lambda_{3,4}=-\mathscr{F}(1\pm i)\right).$$

Note, that for the non-cyclic reaction the term in the upper right corner of the matrix [cf. Eq. (V-2)] would be missing. For the above example, the characteristic equation would reduce to $(1+\lambda/\mathscr{F})^m=0$, yielding $\lambda_{1,2,3,4}=-\mathscr{F}$.

The essential condition for selective selfreproduction is that the reaction partners A_i of the cycle are not consumed by the reaction, i.e. that they are catalysts. However, the cycle still maintains its competitive, selfreproductive properties when not all the steps in the cycle are catalytic. In other words, it is not necessary that at each stage the product of the reaction catalyzes the next step without being consumed. *Actually, the whole cycle need produce only one such catalyst in order to become autocatalytic.* This is easily seen from the above treatment [cf. the matrix (V-2)]. In the non-catalytic cycle we would have to replace all $-\mathscr{R}_i$ by $-\mathscr{F}_{i+1}$, because the species disappear by the same reactions by which their products appear. If, however, the first species in the cycle, and only this one, represents a catalyst E_1 formed from E_m, but not consumed in the formation of E_2, then the upper left term $(-\mathscr{R}_1)$ in the matrix Eq. (V-2) becomes zero.

Here we obtain

$$\lambda\prod_{k=2}^{m}(\mathscr{F}_k+\lambda)=\prod_{k=1}^{m}\mathscr{F}_k \qquad \text{(V-10)}$$

which indeed has a positive real eigenvalue, e.g. for $m=2$ and $\mathscr{F}_1=\mathscr{F}_2\equiv\mathscr{F}$

$$\lambda(\mathscr{F}+\lambda)=\mathscr{F}^2$$
$$\lambda_1=\frac{\mathscr{F}}{2}(\sqrt{5}-1) \qquad \text{(V-11)}$$
$$\lambda_2=-\frac{\mathscr{F}}{2}(\sqrt{5}+1).$$

V.2.3. Competition Between Different Cycles: Selection

Given a number of different independent (uncoupled) catalytic cycles with each cycle "internally equilibrated", the system is then characterized by the normal mode y_{i+} which belongs to the positive eigenvalue. Since all other normal modes have decayed in the equilibrated system, we can replace y_{i+} by $y_i^*=\sum_{k=1}^{m_i}x_{ik}$. The selection equation at constant forces for each cycle then assumes the common form

$$\dot{y}_i^*=k_0(W_i-\bar{E})\,y_i^* \qquad \text{(V-12)}$$

where the selective value W_i is given by the positive eigenvalue of the cycle (e.g. for $\mathscr{R}_{i1}=\mathscr{R}_{i2}\ldots\equiv\mathscr{R}_i$)

$$W_i=\hat{\mathscr{F}}_i-\mathscr{R}_i \qquad \text{(V-13)}$$

and $\hat{\mathscr{F}}_i$ according to Eq. (V-5). Selection can occur only if $\hat{\mathscr{F}}_i>\mathscr{R}_i$ or—for different $\mathscr{R}_{ik}$—if $\hat{\mathscr{F}}_i>\hat{\mathscr{R}}_i$, where the symbols $\hat{}$ characterize the geometrical means.

In analogy to Eq. (IV-23), we could also calculate the concentrations of the selected species. We realize that independent cycles behave like individual self-instructing species or complementary collectives, as long as the original reactions can be described by systems of linear equations.

The cycles, however, do not need to be independent of each other. Due to branching (i.e. polyfunctional behavior of certain enzymes) they may be linked together, as shown for example in Fig. 13. If we write down the rate equations for all species regardless of which cycle they belong to, but order them appropriately according to their membership of a cycle, we can easily derive their properties from the matrix of rate coefficients, as shown in Fig. 13.

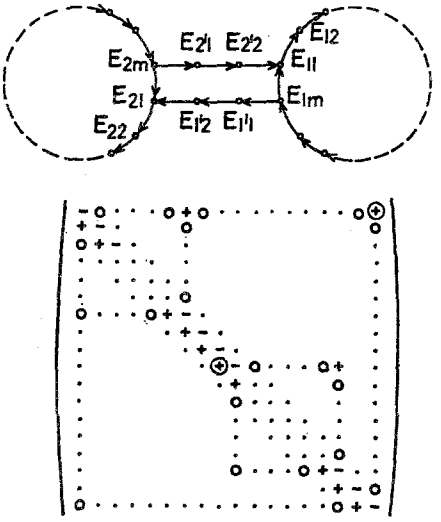


Fig. 13. Graph representation of cycles coupled by a feedback loop. The system involves three cycles, i.e. cycles 1 and 2 plus the "big" loop involving both cycles. Due to the feedback, the cycles do not compete for selection but rather stabilize each other. The matrix of the rate coefficients of the total system reflects the reaction behavior. The columns represent species, the rows reactions. The two encircled points ⊕ represent the coupling points of the loops (i.e. via the reactions catalyzed by $E_{2/2}$ and $E_{1/2}$). If these elements were zero, the matrix could be separated and the two resulting matrices would represent two independent (competing) cycles. The framed regions represent the three closed loops. Diagonals outside the frames represent straightforward branches

A typical property of these systems is that the selected species (max. W_i) carries along with it all coupled branches and cycles, provided the coupling originates in the selected cycle. The branching of a cycle may be a great disadvantage if it does not carry any function in favor of the reproduction of the cycle, because it represents useless ballast (or even unfavorable function) which restricts the availability for reproduction and thus may lower the selective value W_i. We shall refer to these branches as "parasitic".

V.3. Can Proteins Reproduce Themselves?

The results quoted in the preceding paragraphs suggest a negative answer to this question.

There are two properties of proteins which—at first glance—seem to make them even more suitable than nucleic acids for starting a selforganization:

1. the appreciably higher precision of recognition of certain substrates gained with the help of their tertiary structure, and

2. the higher information content involved in a multiple-step cycle (with branches), as compared to the information capacity of a single chain of restricted length.

The disadvantage is that one single protein cannot reproduce a longer chain from single digits and, more specifically, that specified recognition is not an inherent property of "any" chain but rather a unique property of "certain" chains only, or a rare coincidence of special functional properties of different species. As a consequence, proteins which catalyze their own reproduction via specific cycles will not automatically reproduce their mutants resulting from error copying, even if these were to offer advantages. Since there are advantages as well as disadvantages, we have to analyze the above question in more detail.

It has been shown that independent catalytic cycles are "selfinstructive", similar to self- or complementary instructing single species such as nucleic acids (for which this is an inherent property, in contrast to proteins). Although we do not at present know of the existence of any such selfsustaining protein network in nature—except for the mentioned biosynthesis systems of certain antibiotics such as gramicidin S (for which the enzymes are genetically encoded)—we could at least conceive of their artificial construction.

How great is the probability that such cycles can form by themselves? If we look only at the function of catalyzing peptide bond formation among different amino acids, we may say that out of a random population of sequences a certain fraction always will show such a catalytic activity. The whole process of formation of protein-like substances without specification of the sequences, therefore, is already autocatalytic. This will turn out to be an essential prerequisite for the evolution of living entities and it is important to note that it can occur without instruction by nucleic acids.

For evolutionary behavior, however, unspecified autocatalytic growth is not sufficient. The system can improve only by utilizing selective advantages and that requires specification of sequences. In Part I it was shown that the probability of finding a coincidence of several exactly specified sequences is certainly much too low to be of any significance. On the other hand, only optimally adapted enzymes (as we find them nowadays) are represented by unique sequences. The system may well start with much less than optimum performance and, for this, specification of relatively few strategic positions of a sequence may be sufficient. A specific function may occur—to a much larger extent than with present enzymes—for a relatively large class of different sequences which have only a limited number of positions in common.

Let us assume the presence of a sufficiently large number of functional proteins which catalyze formation of other proteins from precursors. We represent each of these proteins by a lattice point and draw a connecting line between those points which are catalytically coupled. Each point is assumed to have the same a priori probability p of being a target for the catalytic activity of another given point. A continuous loop including k lattice points therefore has the probability p^k. There are $\frac{n!}{k(n-k)!}$ possible k-membered closed loops. The expression $\frac{n!}{(n-k)!}$ represents all possible complexions differing by selection and sequence of elements (i.e. variations of n elements in classes of k without repetitions). Since the arrangements are cyclic, these variations are k-fold degenerate (beginning and end of a cycle is arbitrary for any of the k-positions such as DABC and ABCD etc.). Hence the probability for the occurrence of any k-membered cycle is

$$P_k = \frac{p^k n!}{k(n-k)!}. \qquad \text{(V-14)}$$

For large n and $(n-k)$ we may apply Stirling's formula

$$n! \simeq n^n e^{-n} \sqrt{2\pi n} \qquad \text{(V-15)}$$

and obtain for the maximum value of P_k $\left(\text{according to } \frac{\partial P_k}{\partial k} = 0\right)$

$$p = \frac{1}{n-k_m} \exp\left\{\frac{2n-3k_m}{2k_m(n-k_m)}\right\} \qquad \text{(V-16)}$$

suggesting that k_m is close to n for any $p > \frac{e}{n}$, whereas it will approach one for $p < 1/n$.

The conclusion then is that for large populations n (for which we may find $p \gg 1/n$) large loops will form, with many cross-links involving almost all catalytically active proteins.

The above procedure, assuming uniform a priori probabilities for the existence of a catalytic coupling, may be questionable, since it is a drastic simplification of an otherwise quite complicated situation, a more precise description of which would require more detailed information than is at present available. There may well be specific correlations of certain sequences with certain reproductive functions, but they will not be an inherent property of the structure as for nucleic acids, where a given sequence will always induce the reproduction of its own kind. For proteins this a priori correlation between sequence and reproductive function is lacking, so that the conclusions about reproductive loops remain qualitatively valid. Each coupling, of course, is characterized by specific rate parameters, as expressed in the selectiv values W_i. The probability for the existence of a specific coupling will decrease as W_i increases. Thus, one should actually use a probability distribution function $p(W)$ and specify the probability $p(W)\,dW$ of finding a coupling with a selection value between W and $W + dW$. Such a specification would be required if different loops—characterized by different selective values W_i—were competing for selection. Then each loop with a high W_i value will be linked to many (more extended) loops with lower selectiv values without being able to "escape" them via selection.

One of the major disadvantages of simple reproductive loops is, that they cannot select against parasitic couplings of lower efficiency.

There is another even more severe disadvantage of catalytic cycles with respect to evolution: Assume there exists an independent cycle which is selected against competitors:

This cycle (Fig. 14) could evolve by producing mutants via imprecise reproduction of an enzyme involved in the cycle. Let E_1' be such a mutant. Then it is not sufficient that E_1' is just a "better" catalyst than E_1, because it would reproduce $E_2 \ldots E_n$ and thus lead again

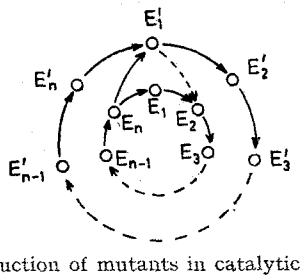


Fig. 14. Reproduction of mutants in catalytic cycle

to E_1 rather than to E_1'. In order to improve, the cycle has to undergo a chain of specific mutations $E_1' \rightarrow E_2' \ldots E_n' \rightarrow E_1'$, i.e. it has to form a new specific cycle. The probability that a chain of *specified* events will occur is, indeed, much lower than the probability of the occurrence of "any" cycle with k members (in the above picture: p^k as compared to P_k according to Eq. (V-14)).

The catalytic networks have so far been described by linear rate equations, although under general conditions (e.g. in absence of buffering of certain reactants) the system would be nonlinear. Moreover, special nonlinear control effects may be superimposed onto the behavior described by the rate equations (V-1). The question arises whether consideration of nonlinear effects would qualitatively change the essential conclusions. We shall certainly observe a qualitative change in the nature of the solutions (see, for instance, the next paragraphs). *However, the main conclusion that systems cannot evolve because of the lack of an inherent self- or complementary type of instruction is even stressed for nonlinear catalytic networks, where again reproducibility is the result of a coincidence of unique macromolecular sequences. The system cannot easily utilize "selective advantages" because it is loaded with too much "information of low selective value".* The "linear" approximation of the selection behavior is—in this respect—quite representative.

VI. Selfordering by Encoded Catalytic Function

VI.1. The Requirement of Cooperation between Nucleic Acids and Proteins

The results of our studies with nucleic acids and proteins as prototypes of information-processing molecules cause us to arrive at the following conclusions:

1. *Nucleic acids* provide one important prerequisite for selforganization, namely complementary instruction, as the basis of "inherent" selective selfreproduction and code formation using an even-numbered (e.g. binary or quaternary) digit system. The recognition power is not so high as to allow the accumulation of a large—and still reproducible—information content in single chains. This would require relatively high free energies of interaction for the single complementary digits in the chain which, in turn, would make the code "sticky" and difficult to read at reasonable rates. Thanks to the cooperativity of digit interaction, codon–anticodon recognition can be quite selective and still be processed within microseconds. Due to complementary interaction within one strand, characteristic individual single strand structures (targets for enzymic recognition) can form. Catalytic abilities, if present at all, are too weak to bring about a coupling among competing information carriers, and hence can not lead to an appreciable increase of the information content.

2. *Proteins*, on the other hand, possess just this property, i.e. an enormous functional and recognitive diversity and specificity (cf., for instance, the recognition power of antibodies). Via catalytic couplings they may link together many information carriers and thus build up a very large information capacity. Recognition, however, is not an inherent property of the sequence elements (i.e. the amino acid residues) but rather a special coincidence of residue interaction in the spatial structure of the active site. This structure can reversibly change and modify the recognition (and catalytic) power, thereby providing control properties (e.g. by "allosteric" activation). Proteins may also show general autocatalytic behavior and selection via cyclic couplings. However, this is not an "inherent" property of all protein molecules, but rather a unique property of a particular species. Hence, proteins cannot easily utilize selective advantages occurring in ("phenotypic") mutants and therefore lack an essential prerequisite for evolution which nucleic acids are able to provide.

3. *"Linear" reaction systems* cannot combine all the properties necessary for the nucleation of a self-organizing system. If the information carriers are competitively selfreproductive, the selected information capacity is limited to that of one single species. They require catalytic couplings to enlarge their information capacity. On the other hand, if reproduction is brought about only by a (linear) cyclic catalytic coupling, it may involve a large information content but the system cannot select against "parasitic" branching. Only nonlinear systems (for further arguments, see below) provide all the properties needed to start selforganization and allow further evolution to a level at which the system can escape the special prerequisites of its origin. (The level of sophistication is such that it could not be reached by random assembly with any finite probability.)

4. A combination of *complementary instruction with catalytic coupling* will lead to nonlinear selection behavior. We have to find the simplest way of coupling the functions of nucleic acids and proteins in order to reproduce a type of evolutionary behavior which can lead to the structure and functions of the living cell. We should not pretend to explain the historical path of evolution. All we can try to do is to state the minimum prerequisites and obtain some insight into the physical principles of the evolutionary process. Independent of its particular structure, the system has to utilize the code-forming properties of nucleic acids as well as the catalytic capabilities of proteins. It thus requires the nucleation of a translation machinery. So we have to deal with two questions which are not independent of one another.

a) How can *a code and a corresponding translation machinery* originate? Nowadays this machinery involves adaptors in the form of t-RNA molecules and recognition enzymes as represented by the aminoacyl synthetases.

b) How can such a system, represented by an ensemble of nucleic acids and proteins, organize itself into a stable selfreproducing, further evolving unit?

Let us start with the latter question, since the answer to this is involved in the solution of the first problem.

VI.2. A Selfreproductive Hyper-Cycle

VI.2.1. The Model

We consider the simple model depicted in Fig. 15. It consists of a number of nucleotide sequences (or better (±) collectives) I_i of limited chain length.

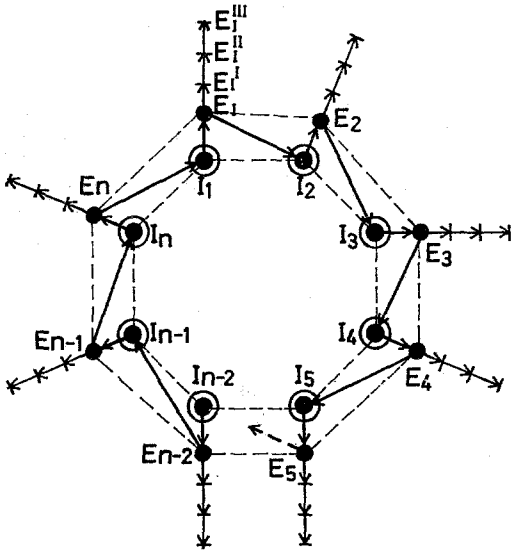


Fig. 15. The selfinstructive catalytic hypercycle
The I_i represent information carriers, i.e. complementary single strands of RNA. Each small cycle indicates the selfinstructive property of the I_i collective involving the two complementary strands. The E_i (encoded by I_i) represent catalytic function. Each E_i branch may include several functions (e.g. polymerization, translation, control), one of which has to provide a coupling to the information carrier I_{i+1} (e.g. enhancement of the formation of I_{i+1} by specific recognition). The trace representing all couplings must close up, i.e. there must be an E_n which enhances the formation of I_1. The hypercycle is described by a system of nonlinear differential equations.

These do not have to provide more information than for one or two catalytically active polypeptide chains, denoted by E_i. The cycle around I_i is a graph representation of the complementary instruction power of the nucleotide collective, each consisting of a "positive" and a "negative" strand which mutually reproduce each other. They do it preferably with the specific catalytic enhancement provided by the preceding polypeptide chain E_{i-1}. This polypeptide is coded for by the nucleotide chain I_{i-1}. The presence of a translation system ensures sufficiently precise translation from I_i to E_i. Only part of the information stored in each I_i has to be used for coding the enhancing function in favor of formation of the next information carrier; other parts may be left for the coding of general enzymic functions such as translation, polymerization, control functions etc. Furthermore, each E_i which has the specific enhancing function for formation of the next information carrier may (but does not have to) be a specific polymerase (as, for instance, $Q\beta$-replicase, cf. Part VII). It may just as well be a specific inducer (or derepressor) acting on a general polymerase. It is important that the whole "hyper-cycle" be closed, i.e. that there is an E_n which feeds back on I_1. Thus the system represents a "cyclic hierarchy" in which many cyclic (complementary) nucleotide collectives are linked together by an enzymic "hyper-cycle". This secondary loop closure is important, since otherwise the different I_k would not cooperate but compete and thus select against each other.

This system has the following properties which are discussed below in more detail:

1. Each cycle—like the systems in Parts IV and V—has autocatalytic growth properties.

2. Independent cycles compete for selection.

3. As a consequence of nonlinearity, selection will be very sharp, possibly resembling "all or none" behavior, if singularities are involved.

4. With these selective properties, the system will be able

a) to utilize very small selective advantages (which have to occur to a stochastically significant extent) and

b) to evolve very quickly. A selected system will not tolerate the nucleation of independent competitors, thus code and chirality will be universal.

5. The cyclic coupling will provide an information capacity which is adapted to the requirements of the system. Nevertheless, the replication length of the single code unit (cf. ν_{max} according to Table 8) will be small enough to ensure reproducibility.

6. The system can evolve, i.e. improve, by utilization of selective advantages. "Genotypic" mutations, i.e. alterations in I_i, can be immediately utilized by E_{i-1} and do not have to await a correlated series of mutations in order to propagate through the cycle, as was necessary for "linear" catalytic cycles (cf. Part V). Selective advantages can become effective via repression, derepression or promotion.

7. The system selects against parasitic branches if these have selective values smaller than that of the members of the cycle. Parasitic branches with higher selective values will not allow the cycle to nucleate if they are present from the beginning. However, if they appear after the cycle was nucleated, they will have no chance of growing, as a consequence of the nonlinear selection behavior. A cycle can reduce the number of members by constriction, if this presents any selective advantage. For coupled cycles the conditions for simultaneous existence are quite restricted.

8. There is only one type of branches which can co-exist with the cycle, i.e. a branch whose selective value exactly matches that of the cycle. An exact matching would be possible only if branch and coding region I_i inside the cycle make use of the same promotor located in E_{i-1}. This will automatically lead to a gene and operon structure of the code system. Within the branches the system can evolve functions of general utility (e.g. polymerases, a translation system, control factors, metabolic enzymes).

9. The system, after nucleation, has soon to escape into a compartment. Only compartmentalized systems can utilize functional branches (brought about by mutations) exclusively to their own advantage (and thus also allow evolution of the branches). By the same mechanism, the system is saved from any pollution caused by unfavorable branch mutations.

10. A system enclosed in a compartment may "individualize" by linking its code units into a stable chain, e.g. with the help of an (evolving) ligase, and reproduce the total chain as an individual unit. In such a chain (which will be cyclic if ligases are involved) genes resulting from a given unit I_i should be localized in neighboring positions. However, the

message for the coupling factors occurring in I_{i-1} can be situated at a quite distant position.

The two last points certainly do not represent intrinsic properties of the cycle. They show that the cycle is not a "dead end" with respect to further evolution. It is able to utilize any advantages which will bring it to a level of sophistication resembling that in living cells. Or, in other words, only those systems which managed to compartmentalize and individualize finally had a chance to survive.

VI.2.2. Theoretical Treatment

The theory of the cyclic system described above has been worked out in cooperation with Peter Schuster. The numerical evaluation has been achieved with the help of computer programs. The work will be published in detail elsewhere [92]. Only a few preliminary results are summarized in this paragraph.

Let us first consider a simple limiting case of nonlinear rate equations which provides an instructive insight into the type of solutions to be expected. If the proteins E_i are in quasi-equilibrium with their code units I_i, we do not have to consider explicitly their formation rates and can assume the proportionality of their concentrations to y_k, the representative concentrations of the instructive code units I_k (which here are treated simply as "self instructive"). If, furthermore, neither of the concentrations (E_{i-1} and I_i) is present in large excess, so that the concentration of the complexes between E_{i-1} and I_i can be assumed to be proportional to the product of both concentrations (corresponding to the second-order range of the Michaelis-Menten mechanism), the rate equations for the formation of code units—in the absence of selection strains—can be written as

$$\dot{y}_i = (\mathscr{F}_i + \mathscr{F}_i' y_{i-1} - \mathscr{R}_i) y_i. \qquad \text{(VI-1)}$$

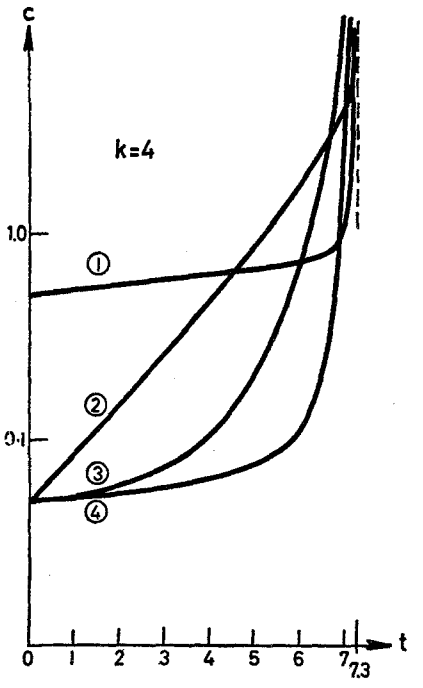


Fig. 16. The growth of a 4-membered hypercycle
(4 information carriers I_i encode 4 coupling enzymes E_i.) At $t=0$ I_1 is assumed to be present in 10fold excess of I_2, I_3 and I_4. The formation rates for all 4 members are represented by simple second-order terms: $x_i x_{i-1}$. Decomposition is neglected. The time axis is reduced and refers to a rate constant $k=1$ (or $t=kt'$). The singularity occurring at $t=7.3$ leads to a very sharp selection if several competing cycles are involved. (Calculations by P. Schuster [92].)

(All energy-rich monomeric digits are again assumed to be buffered. The "backflow" terms are also neglected.)
The solution of this equation—similar to Eq. (II.21)—has a singularity at finite t (provided that $\mathscr{F}_i + \mathscr{F}_i' y_{i-1} > \mathscr{R}_i$). An example is shown in Fig. 16. It is interesting to note that oscillations may occur for cycles with three or more members. Such oscillations appear even more pronounced in the presence of selection strains (cf. Fig. 17). Two-membered cycles do not show any oscillation; for three members oscillations occur, although they are still damped; for four and more members the damping disappears. If we start from the time-independent averages (corresponding to internal equilibrium), the oscillation builds up upon small perturbation. The time-independent averages can be obtained from the stationary solution (internal "equilibrium")

$$\frac{\langle y_i \rangle}{\sum_k \langle y_k \rangle} = \frac{1 - \sum_k \frac{b_{i+1} - b_k}{b_k} \Big/ \sum_k \langle y_k \rangle}{a_{i+1} \sum_k \frac{1}{a_k}} \qquad \text{(VI-2)}$$

where the sum extends over all k members of the cycle, $a_i = \mathscr{F}_i'$ and $b_i = \mathscr{F}_i - \mathscr{R}_i$ according to Eq. (VI-1). The symbol $\langle\,\rangle$ denotes the temporal average of the oscillating concentrations.

With increasing number k of code units, the oscillatory behavior shows up in the form of waves with characteristically shaped spikes which run around the cycle. The shape of these spikes depends on k as well as on relative rates of formation and decomposition of individual code units (cf. Figs. 17 and 20). The selection behavior of competing cycles depends not only on the average rate parameter of the cycle but also on relative rates of single steps and their distribution among the differents steps, as well as on k, the number of code units in a cycle (Figs. 21–23). To bet on the result of such a competition turns out to be as difficult and—almost—as exciting as betting on a horse race. It is beyond the scope of this paper to discuss the details of the reaction mechanisms (which can be found elsewhere). The same is true for the behavior of coupled cycles, the properties of which resemble, in many ways, social behavior.

A more complete solution has to take into account the following complications:

1. The system E_i, although coupled to I_i, has its own characteristic growth rates. Thus we need two sets of concentration variables, x_i for the enzymes E_i, and y_i for the code system I_i.

2. The reduplication rate of the code carriers is not simply proportional to the product $x_{i-1} y_i$. The Michaelis-Menten approximation (in which substrate is assumed to be in large excess of enzyme) is not satisfactory. The concentration of complexes between E_{i-1} und I_i, denoted by z_i, has to be calculated using the law of mass action. It can be represented in the form

$$z_i = \sqrt{x_{i-1} y_i}\, \mathrm{tg}\frac{\alpha}{2} = \frac{x_{i-1} + y_i + K_i^{-1}}{2}(1 - \cos\alpha) \qquad \text{(VI-3)}$$

with

$$\sin\alpha = \frac{2\sqrt{x_{i-1} y_i}}{x_{i-1} + y_i + K_i^{-1}} \qquad \text{(VI-4)}$$

where x_{i-1}, y_i refer to the total concentrations of E_{i-1} or I_i respectively (regardless of whether they are free or complexed) and K_i to the stability constant of complex formation between E_{i-1} and I_i.

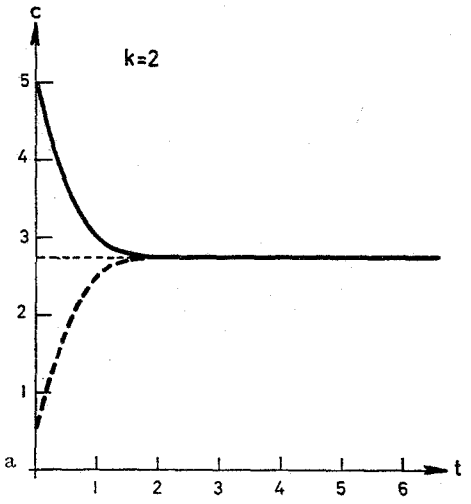
3. The formation rate of I_i involves two terms, a linear one and a term proportional to z_i. There is always some reproducible formation of I_i without the *specific* help of E_{i-1}. If only the second-order term were involved, nucleation of the cycle would be a highly improbable process.

4. The coupled systems of rate equations can be formulated as[1]

$$\begin{aligned} \dot{y}_i &= (\mathscr{F}_{I_i}' - \mathscr{D}_{I_i}) y_i + \mathscr{F}_{I_i}'' \cdot z_i \\ \dot{x}_i &= \mathscr{F}_{E_i} \cdot y_i - \mathscr{D}_{E_i} \cdot x_i \end{aligned} \qquad \text{(VI-5)}$$

with z_i according to Eq. (VI-3).

1 A distinction of the concentrations of free $[(y_i - z_i]$ or $(x_i - z_i)$ respectively] and of bound species (z_i) would not change the general form of these equations, since terms proportional to both y_i (or x_i) and z_i are involved.



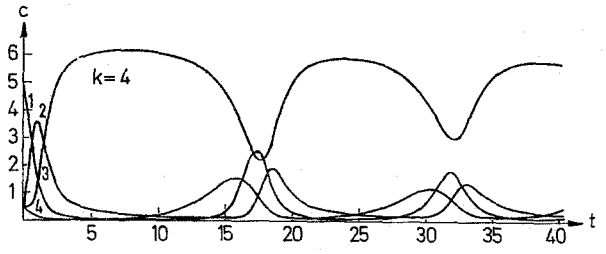
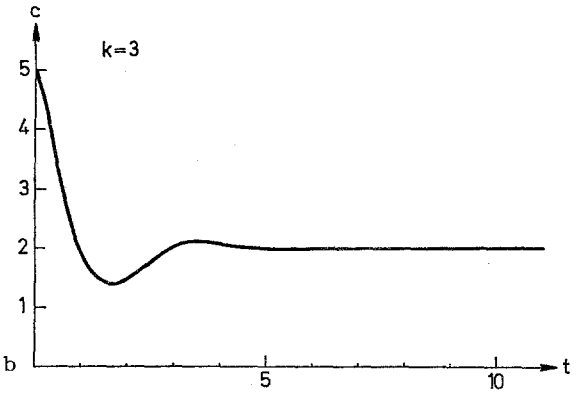
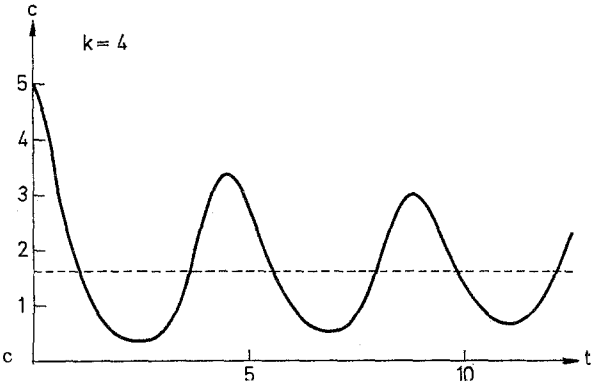
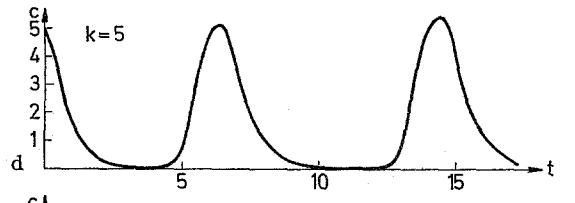
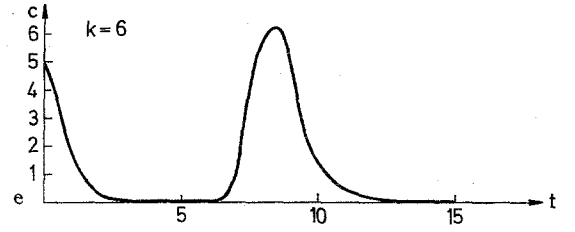


Fig. 20. Stationary oscillation in an equilibrated 4-membered hypercycle with unsymmetrical rate distribution. The formation rate constant of the 4th member is 10 times smaller than that of all other members (cf. [92])









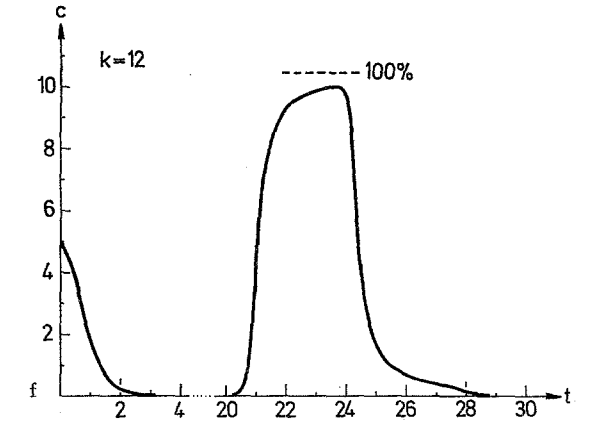


Fig. 17. Solutions describing the selection of k-membered hypercycles under the constraint of constant overall organization ($k=2$, 3, 4, 5, 6, 12). The reaction system again is described by a simple second-order formation term—identical for all members—(cf. Fig. 16) as well as by a first-order "removal" term to maintain the condition $\sum_k x_k = \text{const}$. The solutions are shown for one member only. The "equilibrium" value is constant for $k \leqq 3$. For $k=3$ the approach to selection equilibrium is represented by a damped oscillation, whereas for $k>3$ stationary oscillations occur. This can be shown by starting from a constant distribution and introducing a small perturbation at $t=0$. The oscillation then builds up. (Calculations by P. Schuster [92])

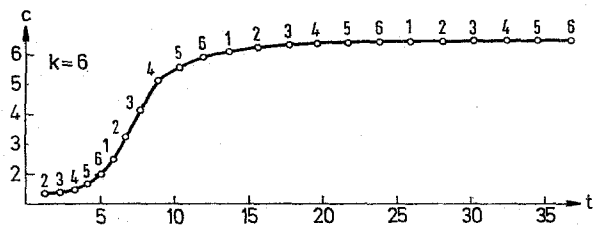


Fig. 18. The migration of the amplitude in an oscillating hypercycle ($k=6$), which builds up from a constant and stationary distribution at $t=0$; $x_1^0=1.35$; $x_{k>1}^0=1.25$ (cf. [92])

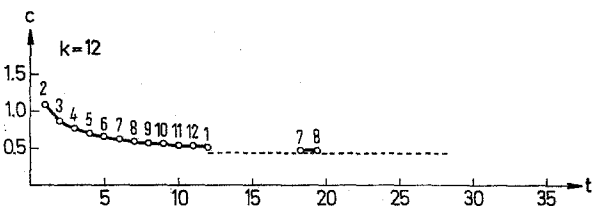


Fig. 19. For comparison: the decay of amplitudes in a linear catalytic cycle (as treated in Part V). Under selection constraints the concentrations always decay to a constant stationary level (cf. [92])

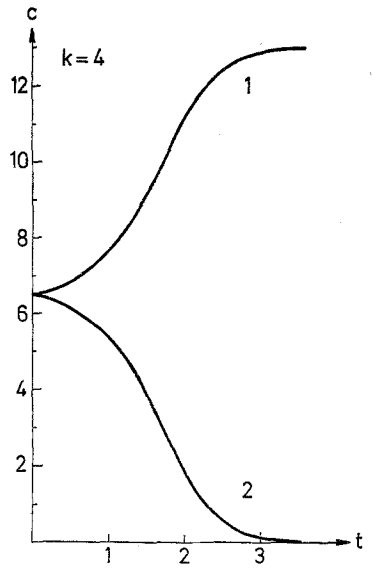


Fig. 21. Competition between two 4-membered hypercycles, one of which is at a disadvantage of 10% difference in the formation rate constants, i.e. $\mathscr{F}'_{11}=\mathscr{F}'_{12}=\mathscr{F}'_{13}=\mathscr{F}'_{14}=1.0$; $\mathscr{F}'_{21}=\mathscr{F}'_{22}=\mathscr{F}'_{23}=\mathscr{F}'_{24}=0.9$ (cf. [92])

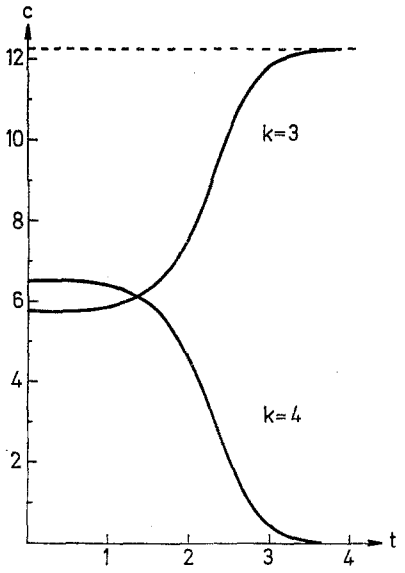


Fig. 22. Competition between a 3- and a 4-membered cycle, having the same individual rate parameters but differing in initial concentrations. The 3-membered cycle "wins". 3-membered cycle: $\sum_k x_k^0 = 5.76$; 4-membered cycle: $\sum_k x_k^0 = 6.50$. Note that each species of the 4-membered cycle is present at about 15% lower concentration than each species of the 3-membered cycle (cf. [92])

The solution (obtained for the selection constraint of constant overall organization) shows a second-order range as discussed above, with quite analogous properties, which holds for

$$x_{i-1}+y_i \ll K_i^{-1}.$$

If one of the concentrations exceeds K_i^{-1}, the solutions become quasi-linear and the oscillations disappear, but this occurs generally in a range where the system has already undergone sharp selection while passing through the nonlinear range. For further details cf. [92].

The conclusions of the theory with respect to the selective behavior of competing cycles can be summarized as follows:

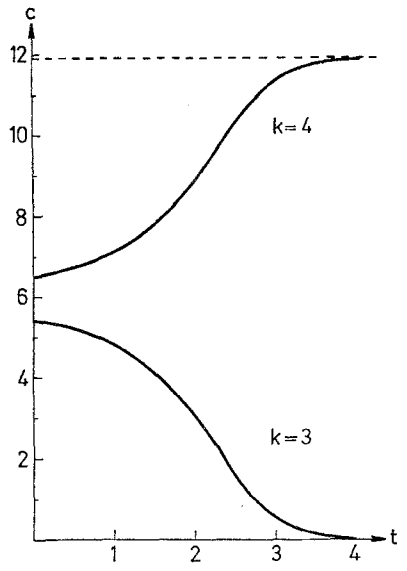


Fig. 23. Competition between a 3- and a 4-membered cycle, having the same individual rate parameters, but differing in the initial concentrations. 3-membered cycle: $\sum_k x_k^0 = 5.4$; 4-membered cycle: $\sum_k x_k^0 = 6.5$. Note that here the 4-membered cycle wins, though each of its species is still present at a lower initial concentration than each of the species of the 3-membered cycle (cf. [92])

Under selection constraints different hypercycles will compete for selection. Only one system will survive; it is characterized by the highest value function, which has a quite complicated form and can be expressed in terms of rate and quality parameters as well as concentration averages of the members. The concentrations of single members may oscillate as long as the system is passing through the nonlinear range. Selection is very sharp and thus accounts for the uniqueness of code and chirality. Whenever a cycle starts by choosing a certain code and translation machinery (cf. next paragraph)—and it has to do this in order to reproduce its functional features—the sharp selection behavior will bring about the universal utilization of this particular code, since new cycles cannot coexist after a stable hypercycle has evolved. The same is true for chirality. Once the polymerizing functions have happened to prefer a given stereospecific configuration, they will continue to do so and evolve it to perfection which requires uniform stereospecificity. Although both configurations have "a priori" an equal chance, the one which, due to fluctuations, happens to be present at the moment of nucleation will then always be preferred as a consequence of nonlinear amplification. For nonlinear systems with "all or none" selection behavior, only one type can win. This is not necessarily true for those functions which are not involved in the nonlinear coupling. For instance, oligopeptides which are not made by the biosynthetic machinery with encoded function can just as well utilize *d*-amino acids (cf. gramicidine S, as mentioned in Part V).

Nonlinearity is also the reason for selection against parasitic branches, which cannot grow after a stable hypercycle has been nucleated—unless they are part of a unit encoded by the cycle. The oscillation be-

havior of the cycle adds further features—especially with respect to the sharpness of selection. Reproduction of the various members occurs in the form of waves running around the cycle and—as seen in Fig. 21—selection is complete after a few such revolutions of waves. This behavior makes it difficult for coupled hypercycles to coexist—unless very specific types of couplings occur (cf. [92]).

The origin of a hypercycle depends on the presence of translation machinery (cf. VI.3) involving a nucleation procedure based on the same type of statistics as shown for the linear cycles in V.3. (cf. Eqs. (V-14) to (V-16)). However, unlike the cyclic protein networks, the hypercycle *can evolve* and therefore adapt to optimal function. First, it is not branched like the linear network discussed in Part V. Second, it can utilize selective advantages occurring inside the cycle—as far as they represent advantages with respect to the reproduction of a genotypic mutation. Such evolution may also include constriction of the cycle to an optimum size. This size has to provide a sufficiently large information capacity, including all the auxiliary functions such as polymerization, translation and control (later also more complex metabolic functions). There is a definite advantage in linking different units I_k into a collective of relatively large information capacity. The single unit has to include not more than two functions: *recognition* in order to provide the cyclic coupling of the E_k system and (occasionally) an *auxiliary function* (i.e. for translation or polymerization). If all the functions represented in the cycle had to occur in one continuous sequence of nucleic acid, a very high recognition accuracy (i.e. a highly adapted enzyme function) would be required right from the beginning, otherwise the total information content could not be reproduced in a stable form; it would drift away until all its useful information has been lost.

On the other hand, whenever the cycle has developed a sufficiently precise recognition system, the occurrence of a ligase, which links the different units into one reproducible chain, offers a definite selective advantage. This may also be the moment when the evolution of DNA structures offers advantages as compared to single-stranded RNA. The "individualization" of the hypercycle—which thereby becomes a truly "self-reproductive" system—has to be seen in connection with "compartmentalization". Neither "individualization" nor "compartmentalization" are inherent properties of the hypercycle—as are, for instance, the other properties mentioned above. However, where they occur after nucleation, they may offer a selective advantage and therefore are inevitable evolutionary consequences of the hypercycle. The advantages lie in the utilization of mutations. If a mutation—especially in the auxiliary function—turns out to be of advantage, it will be utilized by the whole "disperse" system and therefore does not favor specifically the reproduction of the mutant—unless it occurs within a compartment. If it does so, it will favor that particular system which after individualization will also select against its precursors. The same is true for unfavorable mutations which—if they occur in a compartment—will affect (or even destroy) only their particular compartment and thus disappear, whereas otherwise they would pollute the whole system.

Suitable compartments could be coacervates, as first described by A. I. Oparin [93], or microspheres consisting of lipids, or protenoids with mainly hydrophobic side-chains, which have been shown by S. W. Fox [94] and others to form spontaneously under conditions of random condensation of amino acids, corresponding to primordial conditions. The occurrence of lipid microspheres is quite common, as various authors have shown. They can be formed reproducibly and used in laboratory experiments [95–97].

VI.3. On the Origin of a Code

The possibility of the existence of a hypercycle as described in VI.1 and 2 rests obviously on the presence of a code and translation machinery. Such a code would be required for any model utilizing a correspondence between the inherent capability of instruction associated with nucleic acids and the functional potential of three-dimensional protein structures. The problem of the origin of a unique code (whose existence is a fact) is therefore of a more general nature than the question of the existence of any particular model for a selfsustaining catalytic hypercycle.

Does the present table of the genetic code (cf. Table 2 in Part I) provide any clue to its origin? This problem has been thoroughly discussed by a number of authors, and especially in a monograph by A. Woese [98]. The following clues may be recalled:

1. All the facts known today indicate that the code is universal.

2. It is unlikely that the present triplet code evolved from a precursor code utilizing doublets or singlets. As Francis Crick has clearly pointed out [99], a change of codon size would result in the complete loss of the information so far collected—unless the spacing of codons in the message is conserved, or a simple translation is provided from the old into the new codon sequence. This does not preclude single digits in different positions of a codon from having a different weight with respect to their information content.

3. An explanation for the pronounced degeneracy of the code with respect to the third position in the codon (3′ end) is provided by Crick's wobble hypothesis [20]. It refers to some degeneracy in the complementarity of bases at the 5′ end of the anticodon and the 3′ end of the codon

anticodon (5′)	*codon (3′)*
G	U, C
C	G
A	U
U	A, G
I	A, U, C

leading to the equivalence of U and C as well as A and G (or A, U and C) in the third position of the codon.

4. The middle position in the codon triplet seems to exert some preference in determining the nature of the amino acid (hydrophobic, polar or charge type). This regularity was recognized as being of possible significance for the origin of the code based on some specific nucleotide-amino acid interaction [100].

5. The code seems to reflect an optimization principle, as was recognized and formally treated by I. Rechenberg [101]. For most codons the change of only one out of the three digits results in a minimal change in the nature of the amino acid and may thus also reflect the regularity with respect to the middle position. Four classes of amino acids again were specified: hydrophobic, polar, positively and negatively charged. One may furthermore add functional correlations, such as certain acid or base functions of amino acid side chains, as well as structural correspondences such as the phe-tyr similarity, etc. Any optimization principle would be of special significance for a random start of a code (cf. below) and should influence the different choices in the nucleation process. It would also provide some invariance of the information content of a base sequence (in terms of amino acid classes) with respect to an overlapping reading of the code (which at the stage of poorly adapted enzymes might have happened quite frequently).

6. The eight codons consisting of A and U digits represent a much larger variety of functions than the eight GC triplets (cf. Table 2, Part I). It was F. Lipmann [102] who first emphasised this fact and its possible relation with a simple origin of the code. In support of this view are:

a) The probably very high abundance of A as compared to U, C and G under primordial conditions—resulting in a higher abundance of AU as compared to GC pairs.

b) The higher stability of the GC as compared to the AU pair, which allows GC later to substitute for AU whenever it is of selective advantage.

c) The recently found [103] relatively large AU content of ribosomal RNA from mitochondria and chloroplasts which may not have been exposed to high selection pressure—according to the hypothesis that both cell organelles represent precaryotic inclusions in eucaryotic cells.

d) Finally, a pragmatic argument from a statistical point of view: nucleation of any code is easiest when the number of digit classes is as small as possible.

How could a defined assignment between amino acids and codons or anticodons come about?

No doubt, the simplest explanation would be a specific interaction between the two sets of digits. Numerous models have been proposed [104], ranging from single codon-amino acid interaction, through amino acid fitting into a cleft between the complementary (double) strand of the corresponding codon–anticodon sequence, to finally amino acid recognition by the spatially folded structure of a large adaptor molecule, i.e. the t-RNA precursor [35] or to preferred stabilities of such aminoacyl-*t*-RNA precursors[1]. The value of any such model depends entirely on the presentation of appropriate experimental evidence, and this is very scarce so far.

1 L. Orgel, private communication.

It is obvious that specificity of amino acid-nucleotide recognition could be greatly enhanced if the interaction were not limited to the single digits, i.e. the amino acid and the codon or anticodon region. One of the reasons why *t*-RNA is a relatively large molecule could be either to provide sufficient characteristic tertiary structure for recognition by some enzyme, or to make use of its extended structure for recognition like an enzyme. Both cases will be discussed in connection with "random" models. The difficulty is still how to prevent random assignment, since all permutations of the anticodon region could occur in an otherwise unchanged adaptor structure.

It is pointless to develop any model which rests solely on a hypothetical interaction, as yet experimentally unidentified. For a unique start, quite distinctive interactions (q close to 1) would be required anyway, and it is very doubtful whether inherent interactions strong enough for a direct anticodon amino acid assignment are generally present at all[2]. We are therefore justified in asking:

Could, in absence of specific interactions, a unique code assignment also start from a random combination of amino acids with anticodons?

Any specific interaction between a codon (or any inherently codon-linked structure) and an amino acid—whenever it is present—may enhance the probability for an otherwise undirected start of translation. Again, what we are interested in is not so much a particular (speculative) model as an estimate of possibilities for a random start (or nucleation) of possible precursors of the presently known adaptor recognition system. In other words, we want to know how complex a system could nucleate with finite probability and start unique translation assuming that no intrinsic interaction would bias the choice.

Let us assume only those interactions for which evidence can be presented. For instance, we know that *t*-RNA or similar structures can be quite specifically recognized by three-dimensional protein structures. We know also that amino acids can be activated (e.g. by ATP) and linked to a nucleotide sequence, but we do not know of any specific and inherent amino acid-anticodon assignment which would work satisfactorily without the help of enzymes.

The simplest model for a "random" start of translation is based on essentially equal a priori probabilities for the assignment of amino acids to codons or anticodons. Thus, any of the amino acids a, b, c... may a priori couple to any of the adaptors A, B, C..., the assignment a—A, b—B, etc. being only the final outcome (to which the nomenclature has been adapted in retrospect). Three models may be mentioned to which this supposition applies and which are therefore subject to the same general treatment.

1. The amino acid is recognized by the tertiary structure (e.g. a cleft) of a polynucleotide resembling the *t*-RNA precursor. The anticodon is localized at an exposed loop; but it may not be involved (or determinant) in the recognition and fixation of the amino acid at the adaptor, so that a priori any triplet could have coupled to the given amino acid.

2. The same model as 1) but with the amino acid replaced by an oligo- or polypeptide (2-nd adaptor), the terminal amino

2 There is a further point: if such inherent interactions existed between amino acids and codons, one might suppose that enzymes should have evolved, utilizing this interaction and now allowing reverse reading, i.e. from protein to nucleic acid (contrary to the "central dogma" of molecular biology). Although there are enzymes which allow a reversal of transcription (i.e. RNA→DNA), no evidence exists (and it also is hard to imagine) that such a reversal of reading could occur for translation, although it should have been of advantage if it had existed.

acid of which is to be activated. Such an interaction of a polynucleotide with a polypeptide could be much more intimate and distinctive than with a single amino acid, but again, for a given interacting polypeptide and polynucleotide structure, any terminal amino acid and exposed anticodon could be substituted.

3. A model like 2. in which, however, polypeptides have enzymic function (as amino-acyl synthetase precursors), recognizing specifically a free amino acid (or oligopeptide) which is to be linked to a given adaptor structure, again allowing for any possible anticodon amino acid combination.

In any of these models we have λ digits, i.e. the amino acids $a, b, c \ldots$, which have to be translated into the codons $A, B, C \ldots$ with the help of adaptors A', B', C' (complementary to $A, B, C \ldots$). There are λ^2 possible assignments, e.g. for $\lambda = 2$: aA', aB', bA', and bB'. Different assignments are possible since A', for instance, is assumed to be a class of adaptors all of which have in common only the anticodon of A, but otherwise can interact quite differently with different amino acids or activating enzymes. It is assumed that any assignment has equal a priori probability. If we consider now a volume element[1] in which λ such assignments are present, the probability (P) of finding a given set is proportional to the reciprocal of the number of all possible sets, which is the number of "variations with repetition" of λ^2 elements in groups of λ: $V_r = \binom{\lambda^2+\lambda-1}{\lambda}$. This allows also for those combinations in which all assignments are the same, e.g. a-A', or those in which a given adaptor is linked to many different amino acids: a-A', b-A', c-$A' \ldots$, or vice versa; in short, any population of assignments is allowed. This is a fairly extreme (and possibly not quite realistic) assumption, but it was made in order to get a lower limit for the probabilities, so that any deviation can only strengthen the argument.

Among all the assignments there are $\lambda!$ unique ones, for which a given amino acids is linked to only one (anti-)codon and vice versa. Thus the probability of finding a volume element with any *unique* assignment is

$$P_{\mathrm{Ass}} \sim \frac{\lambda!}{V_r} = \frac{(\lambda!)^2\,(\lambda^2-1)!}{(\lambda^2+\lambda-1)!}\,. \qquad \text{(VI-6)}$$

Such a volume element will start—possibly with some catalytic help—to translate nucleotide sequences uniquely into amino acid sequences, but only as long as the particular "fluctuation" of assignment in the volume element persists. In order to stabilize this type of translation, we must find among the *nucleotide sequences* those which after translation reinforce the use of the same code. Only such an ensemble of nucleotides would represent a stable and reproducible source of information for the code and translation machinery (consisting of a particular set of adaptors and activating enzymes). In order to be selected against other competing systems, especially those which are not unique and thus always include some nonsense reproduction, it has to form a selfenhancing hypercycle as described in the two first paragraphs of this part[2].

The probability of finding the set of nucleotides which enhance a particular translation function is based on the same prerequisites as the probability of finding the set of proteins carrying out this function. The polynucleotides, if somehow translated, represent a set of random sequences of polypeptides; thus we have to start from the same assumptions as for the finding of λ specific "coordinators"—however, without the degeneracy $\lambda!$, because the system now has to reinforce one particular (out of $\lambda!$ possible) unique assignments. If random nucleotide sequences were present at a similar concentration level as random polypeptides, the probability of finding the particular set in the defined volume element would again be given by the reciprocal number of "variations with repetitions" of λ^2 elements in groups of λ.

The joint probability is then essentially given by

$$P \sim \frac{(\lambda!)^3\,[(\lambda^2-1)!]^2}{[(\lambda^2+\lambda-1)!]^2}\,. \qquad \text{(VI-7)}$$

illustrated by the following examples:

λ	2	4	8	20
P	$2 \cdot 10^{-2}$	$1.6 \cdot 10^{-6}$	$4 \cdot 10^{-16}$	$5 \cdot 10^{-50}$

The joint probability also contains a factor describing the relative concentration ratio of polynucleotide and polypeptide sequences. There are further quite unrealistic assumptions involved—such as equal "a priori" probability for any sequence—which restrict the use of this formula to an estimate of some rough figures.

There is one important point: the procedure is to find the probability of the existence und reproduction of a certain *function* (i.e. amino acid—codon assignment) among a random population of polypeptides, but not to find the probability for the coincidence of certain *sequences*. The function can be represented by quite a large number of different polypeptide sequences, so large that one really has a good chance of finding them among any random population (cf. experiments by S. W. Fox [94] and coworkers which showed the simulation of chymotrypsin function in any randomly synthesized set of polypeptides). Let us call this probability p—whatever its special form may be. Then the same probability of possessing the same *function* (after translation) applies to a population of random sequences of polynucleotides (of equal concentration). In the first case we have still $\lambda!$ unique choices for assignments, whereas the second choice has to coincide with the first one. The joint probability thus becomes $\lambda!\,p^2$. What was asked for was a coincidence of *functions* not of *sequences*. If we had asked for the probability of finding a nucleotide sequence which after translation resembles exactly the polypeptide sequence (which started the particular translation), this probability would be as low as 10^{-130} (for 100 amino acids of 20 classes), reconfirming E. Wigner's argument discussed in Part I.

However, there are enough pitfalls in such estimates to discourage us from going into further detail until more experimental evidence is available about the catalytic function of randomly synthesized polypeptides. The main argument, that a certain catalytic specificity is not a unique function of one or very few given sequences, but rather occurs quite frequently among any random population of sufficiently large size, can be tested by experiment (cf. Part VII). Even without such data, we can estimate for which degree of complexity a unique translation starting from random fluctuations becomes completely improbable.

The conclusions with respect to the probabilities for a random start of translation are as follows (cf. also the numbers following Eq. (VI-7)): It seems quite easy to start a binary translation system, but two digits (or classes of digits) on the functional side would not be sufficient to provide enough specificity. Four classes of digits have been proposed as a minimum for the start of an optimization procedure in the evolution of the code. The nucleation probability for a 4-digit translation is still of a reasonable magnitude. The value for an 8-digit code is perhaps around the limit of what seems feasible with realistic concentrations within the dimensions of the earth and within the time available for early evolution (which is—probably—considerably less than 10^9 years ($\sim 3 \cdot 10^{16}$ seconds)). An 8-digit code would be provided by the AU system. Furthermore, 8 amino acids

1 The size of this volume element is adjusted to the condition of finding λ assignments. There are, of course, many more polypeptide and polynucleotide sequences present in this volume element. They have, however, no function in fixing amino acids to adaptors. There is one problem: the assignment of a given sequence has to be specific, or better: the specific assignments have to be determinant. Otherwise a given combination will not be unique and a large "noise" level will be superimposed.

2 One possibility is that adaptors, from the beginning, were quite extended nucleotide structures possessing dual functions: 1. acting as specific adaptors by carrying an anticodon loop and being specifically recognized by activating enzymes; 2. representing with their sequences specific information (I_i) for the enzymes E_i which are members of the hypercycle. However, at this stage no definite conclusion can be reached about the complexity of the nucleating system; it may also include short sequence adaptors which have a high a priori abundance.

would be sufficient for the building of any type of functionally specific sequences. It is not even necessary to start with only 8 amino acids, but rather with 8 (or less) classes of functionally related species. The same is true for the instructional code, which could start with degenerate classes and later evolve further according to some optimization procedure [101]. We may therefore conclude that it does not seem at all impossible that the particular code which we find nowadays started from a random fluctuation, so that we do not have to suppose a highly specific (direct or indirect) amino acid-codon interaction. If this is true, any independently evolving system (e.g. "somewhere" in the universe, or at "some time" in the laboratory) could utilize a different code, but it would be based on *similar principles*. Furthermore, the present code—originally—may not have been the only one; however, *universality is guaranteed by the sharp nonlinear selection procedure.*

On the other hand, it should be emphasized that the only correct statement at this time is: "We cannot exclude ...". Therefore the only meaning of the above estimations is, to find out from which degree of complexity on a random start becomes too improbable. There is a good argument—raised by L. Orgel—that there was a stepwise or continuous approach to the formation of a translation system starting with one or two preferred adaptor—amino acid assignments rather than a nucleation of a whole (e.g. 4 or 8 letter) dictionary. Wherever such "intrinsic" correspondences are present, they will increase the probability of nucleation of a selfreproducing functional network.

We may finally ask: How can we physically understand such a *"random start"* of translation?

It is again a consequence of the value criterion of selection theory, which is reflected by Prigogine and Glansdorff's principle of nonlinear irreversible thermodynamics. Whenever we have a selforganizing system with selection behavior—as defined by certain properties of the reaction system and by specification of external constraints—the occurrence of a new species or ensemble of higher selective value (by fluctuation or mutation) will cause an instability, i.e. a breakdown of the former steady state and a build- up of a new steady state which is dominated by the species or ensemble having the higher selection value. The "breakthrough" of the new species is subject to certain limitations and can be described correctly by stochastic theory.

We conclude:

Nucleic acids provide the inherent prerequisite of selforganization. However, they require a catalytically active coupling factor of high recognition power in order to build up a high information capacity. "Information" acquires its meaning only by functional correlation. Any fluctuation in the presence of potential coupling factors leading to a unique translation, and its reinforcement via the formation of a catalytic hypercycle, offers an enormous selective advantage and causes a breakdown of the former steady state of uncorrelated selfreproduction.

As a consequence of such instability, the nucleation of this functional correlation (we may call it the origin of life) turns out to be an inevitable event—provided favorable conditions of free energy flow are maintained over a sufficiently long period of time. The primary event is not unique. Universality of the code will result in any case as a consequence of nonlinear competition.

VII. Evolution Experiments

A theoretical model is worth only as much as its capacity for experimental testing; a general theory is valuable to the extent that it guides such work and defines clear and reproducible conditions for comparative studies. A good experiment, then, decides among possible alternatives, usually by exclusion of the incorrect ones.

Test-tube experiments on evolution are still scarce, because the tools as well as the objects, i.e. well-defined molecular species, have only become available during recent years. An ingeniously straightforward and conceptually simple model experiment of this kind has been conducted by S. Spiegelman and his group. Since it is representative of the kind of experiment suggested by the theory developed in this paper, it will be discussed in more detail. (For a survey of literature cf. [105].)

VII.1. The Qβ-Replicase System

The $Q\beta$ story begins with a claim which—at the time it was made by S. Spiegelman [106]—did not find many subscribers among his fellow biochemists. The claim was that the phage $Q\beta$ uses a specific replicating enzyme which recognizes exclusively $Q\beta$-RNA. In answer to all the scepticism, a highly purified and well characterized enzyme was presented, which was able to reproduce infectious viral RNA in cell-free media. The fact that it was indeed the cell-free synthesized RNA which contained all the instructions was demonstrated in a classical experiment [107]. The cell-free solution was subjected to a serial dilution process (allowing sufficient time for reproduction at each step) the final product of which contained less than one in 10^{15} of the initial natural phage templates, i.e. not even one single copy, and yet the sample was as infectious as the original one. Furthermore, the use of a temperature-sensitive mutant excluded the possibility that anything other than the RNA molecule was the source of information. The carrier of this information is the plus strand only; hence, reproduction of infectious copies requires an induction period in which complementary (non-infectious) minus strands have to be accumulated. Reproduction of a whole population can even be initiated by one single template copy and can thus lead to a *clone* of uniform descendents. This was demonstrated in an experiment (cf. R. Levinsohn and S. Spiegelman [108]) where a simple solution was diluted and distributed among test tubes. Synchronized initiation of synthesis then led to an identification of the tubes which received zero, one, two or more copies of the template—strictly according to a Poisson distribution. Recognition by the enzyme involves regions which are distributed over the whole molecule, including both ends, as has been shown by "chopping" experiments: neither of the two halves of a strand is accepted by the replicase. Since both

the plus and the minus strands have to be replicated by the same enzyme, a certain symmetry is to be expected with respect to complementary regions within a strand. Any "internal" complementarity of one strand will be reflected in the complementary copy as a mirror image and thus should be symmetrically arranged in the 3'- and 5'-halves.

This very interesting structure problem can be resolved by sequence analysis, which is under way (and partly completed) in the laboratory of C. Weissmann [109] at the ETH, Zürich. Some sequence work was also done by Spiegelman's group (cf. [110]), who showed that there is indeed some complementary resemblance between the 3'- and 5'-ends. The 5'-terminus of the plus strand is:

pppGpGpGpGpApApCpCp ...

The minus strand also terminates with pppG at the 5'-end and involves a longer sequence of purines. This implies that the 3'-end of the plus strand includes regions complementary to its 5'-end, since—due to complementarity with the minus strand—it must be rich in uridine and cytosine and also terminate with C.

The discovery of the $Q\beta$ system may turn out to be of fundamental as well as of practical importance. The "unsuspected structural diversity and subtlety" [1] of individual RNA molecules explains how selection forces came into play in the interaction of nucleic acids with proteins and directed precellular evolution. Practical applications may include the use of specific recognition sites a) in combination with degenerated non-infectious RNA which can interfere with phage infection, or b) as specific inducers of RNA synthesis in other correspondingly modified systems.

VII.2. Darwinian Evolution in the Test Tube

The availablity of purified $Q\beta$-replicase led to the performance of a series of most intriguing experiments in which a molecular species is exposed to selection constraints by "serial transfer" and thus becomes subject to "evolution in the test tube". A typical experiment starts with a standard reaction mixture (cf. [112]):

0.25 ml sample solution at pH 7.4 (10^{-1} M Tris HCl with $MgCl_2$ ($2 \cdot 10^{-2}$ M), EDTA ($3 \cdot 10^{-3}$ M), 200 mμ-moles each of ATP, UTP, GTP, CTP, (^{32}P labelled in the α-P of UTP, such that 4000 cpm correspond to 1 μg of synthesized RNA), 40 μg of $Q\beta$-replicase (purified by CsCl and sucrose centrifugation). The procedures for base composition and sedimentation analysis, as well as various assays of enzymic activities, are described in detail in Refs. [111] and [112].

The experiment then consists of initiating synthesis by incubation with the viral RNA (here the temperature-sensitive mutant ts—1) and a series of dilutions effected by the transfer of 0.02 ml of the reaction mixture to 0.25 ml of fresh standard solution after specified periods of synthesis. The first reaction was initiated by 0.2 μg of the ts—1 RNA, which was incubated at 35 °C for 20 min. The incubation time then was reduced from 20 min (transfers 1–13) to 15 min (transfers 14–29), 10 min (transfers 30–38), 7 min (transfers 39–52) and finally to 5 min (transfers 53–74), where a final product was reached. At each transfer 0.02 ml of the mixture was used for counting, and another 0.02 ml served to prime the reaction mixture in the next tube. At transfers 0, 8, 14, 29, 37, 53 and 73 some of the product RNA was also taken to prime production for sedimentation analysis on sucrose. The product analysis provided the following clues concerning the replicated RNA molecules (cf. Fig. 24): Infectivity was lost after the 4th transfer. The molecular weights of the RNA templates decreased more or less steadily during the transfers until at the 75-th stage a constant end product was obtained which had eliminated about 83 percent of its original genome content. Of the 3600 nucleotide residues present in the parental copy, only 550 were retained. Concomitantly with the decrease of molecular weight an increase of the rate of ^{32}P incorporation is observed, so that at the 74-th transfer the inclusion rate per nucleotide is 2.6 times that of the original synthesis rate. This is directly demonstrated by a study of the kinetics of nucleotide incorporation under saturation conditions. Fig. 25 shows an example: the rate increase in the linear region is paralleled by a shortening of the induction period (in which the level of minus strands has to be increased to the "equilibrial" ratio of plus and minus strands). A further rate increase together with a decrease in length to 180 nucleotide residues could be observed under special conditions of selection constraints. This fraction of "mini-monsters" is also being studied in L. Orgel's laboratory at La Jolla [113].

At first glance the results of these experiments seem to reflect merely some trivial "evolutionary" response to the given injunction: "to multiply as rapidly as possible". The RNA molecules, liberated from the requirement of being infectious, adjust to such "paradise" conditions by throwing away all infor-

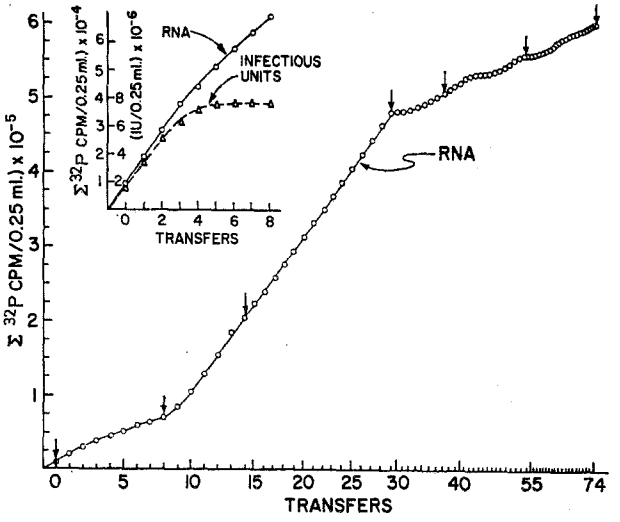


Fig. 24. Serial transfer experiment with $Q\beta$-RNA carried out by D. R. Mills, R. L. Peterson and S. Spiegelman [112]. The experiment is described in the text. The arrows above transfers (0, 8, 14, 29, 37, 53 and 73) indicate where 0.01–0.1 of product was removed and used to prime reactions for sedimentation analysis on sucrose. Incubation times were 20 min (transfers 0 to 13), 15 min (transfers 14 to 29), 10 min (transfers 30 to 38), 7 min (transfers 39 to 52), and 5 min (transfers 53 to 74). The results show that biologically competent RNA ceases to appear after the 4-th transfer. (Reproduced from [112])

1 Quotation from S. Spiegelman.

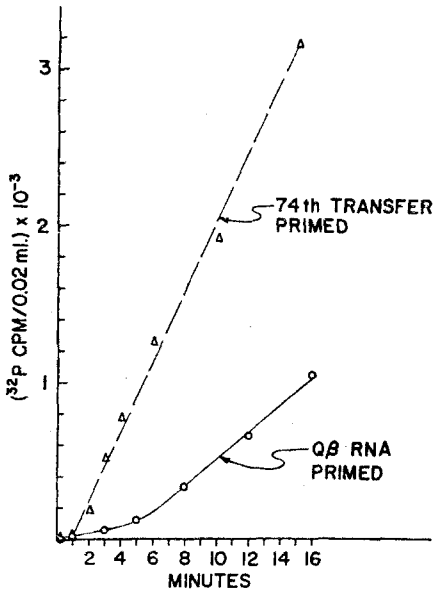


Fig. 25. A comparison of the kinetics of synthesis of the 74-th variant and the original ts-$Q\beta$ RNA. Two 0.25 ml standard reactions (as detailed in the text), one primed with gel-purified single-stranded variant RNA (74-th transfer), and the other primed with ts-$Q\beta$ RNA (both above saturations), were initiated at 35 °C. Aliquots of 0.02 ml were drawn at the times indicated and assayed for incorporation of ^{32}P-UTP. Data are represented as cpm/0.02 ml. (Reproduced from D. R. Mills *et al.* [112]

mation which is not necessary for fast replication. However, they cannot just abbreviate the replication process by breaking off the cycle before termination, because the recognition site involves various parts of the molecules, especially both ends. Actually, the compression of time intervals between transfers does not require such behavior. The reaction is not quenched as a consequence of the transfer and an enzyme molecule which has started a replication process at a template is more likely to finish its job than to fall off and to look for a new copy.

In fact, what is provided by the serial transfer experiment is an approximation of the condition of "constant reaction forces" or constant "overall organization".

The concentration conditions of the original standard reaction mixture—at least as far as the energy rich monomers ATP, UTP, GTP, CTP are concerned—are restored with each transfer, and thus the affinity of the overall formation is, on average, kept constant. With compression of the time period between single transfers, it is possible to compensate for the increased speed of replication. However, a certain drift of the steady state was still present in Spiegelman's experiment, as can be seen from Fig. 24. It is due more to the intuitive choice of selection constraints, which was perfectly justified within the more qualitative scope of these experiments. A more precise determination of selection constraints for the maintenance of a steady state under constant conditions may affect the evolution rates and will therefore be required when quantitative evaluations are intended.

The qualitative conclusion is that the system will always favor the species with the highest selection value. Under the *"paradise" conditions* of the test tube experiments, infectivity is not a prerequisite but rather represents a hindrance to fast reproduction. This example shows clearly that, while selective value is always determined by the rate and recognition parameters $\mathscr{A}$, $\mathscr{Q}$ and $\mathscr{D}$, these can be quite dramatically changed by varying environmental conditions. In subsequent papers, S. Spiegelman and his coworkers described the isolation from test tube experiments of a whole variety of mutants which had adapted to different secondary changes in the selective forces. Among the properties which can be built into the variants, is resistance to interfering analogues of the normal ribosidetriphosphate or inhibitors such as ethidium bromide. It was also possible to select for species with increased molecular weight by fixing the enzyme to a membrane and thus favoring (longer) chains of greater stickiness. Experiments involving "starvation" of one of the bases (C) did not yield C-deficient mutants; instead, the enzyme was able to adjust its efficiency of incorporating C to the changed conditions. All these experiments document the enormous structural and functional variability and adaptability of single-stranded RNA and its possible significance in early evolutionary processes.

VII.3. Quantitative Selection Studies

There is much important information which can be deduced from quantitative evolution experiments. Although more work has to be done on this, it is possible to extract some further information from the data so far published.

"Selective advantage" is always related to the reproduction of the whole species or ensemble. If the reproduction rate depends on the length of the chain, a simple loss of unnecessary information, resulting in a shortening of the chain—without increase of the "intrinsic" reproduction rate (related to the single digit)—may already represent "selective advantage". However, such a chain length dependence would be absent under saturation conditions where the rate also becomes independent of template concentration. The term "saturation" refers to conditions where practically all enzyme molecules E are bound to the templates I_i, i.e. $\sum_k x_{EI_k} \approx x_{E_0}$. The apparent advantages which these conditions offer to kinetic studies were utilized by Spiegelman in his rate studies. If the speed of reproduction is independent of template concentration, the number of template molecules (assayed for ^{32}P incorporation) increases linearly with time (cf. Fig. 25). The presence of an induction period—as seen in Fig. 25—indicates a difference in rate and (or) binding parameters of the plus and minus strands. If we denote the plus and minus strands by I_+ and I_-, respectively, and the concentrations by x, the rate equations referring to the conditions described in the legend of Fig. 25 may be simply written

$$\begin{aligned} \dot{x}_{I_+} &= \mathscr{F}_+ \, x_{EI_-} \\ \dot{x}_{I_-} &= \mathscr{F}_- \, x_{EI_+}. \end{aligned} \qquad \text{(VII-1)}$$

EI_+ or EI_- denoting the enzyme template complexes. A procedure analogous to that of Michaelis and Menten leads to

$$x_{EI_+} + x_{EI_-} = x_{E_0} \frac{K_+ x_{I_+} + K_- x_{I_-}}{1 + K_+ x_{I_+} + K_- x_{I_-}} \qquad \text{(VII-2)}$$

(E_0 refers to the total amount of—bound and free—enzyme).

K_+ and K_- are stability constants for the enzyme template binding or their steady state analogues, respectively. If the solutions are primed solely with plus strands, the initial slope refers to incorporation into minus strands only:

$$\text{"initial slope"} \sim \mathscr{F}_- x_{E_0}. \qquad \text{(VII-3)}$$

After accumulation of a sufficiently large number of minus strands, a constant "equilibrium" ratio $\bar{x}_{I_+}/\bar{x}_{I_-}$ will be approached.

With

$$d/dt(\bar{x}_{I_+}/\bar{x}_{I_-}) \approx 0$$

or

$$\dot{x}_{I_+}/\bar{x}_{I_+} \approx \dot{x}_{I_-}/\bar{x}_{I_-} \qquad \text{(VII-4)}$$

one may deduce, using Eqs. (VII-1):

$$\bar{x}_{I_+}/\bar{x}_{I_-} = \sqrt{K_+ \mathscr{F}_+ / K_- \mathscr{F}_-} \qquad \text{(VII-5)}$$

or for the final slope referring to ^{32}P incorporation into plus and minus strands:

$$\text{"final slope"} \sim \frac{\mathscr{F}_+ \sqrt{K_- \mathscr{F}_-} + \mathscr{F}_- \sqrt{K_+ \mathscr{F}_+}}{\sqrt{K_- \mathscr{F}_-} + \sqrt{K_+ \mathscr{F}_+}} x_{E_0} \qquad \text{(VII-6)}$$

yielding for $K_+ = K_-$ a proportionality to

$$\sqrt{\mathscr{F}_+ \mathscr{F}_-} \cdot x_{E_0}$$

or for $\mathscr{F}_+ = \mathscr{F}_- \equiv \mathscr{F}$ to

$$\mathscr{F} x_{E_0}$$

(cf. the results of IV.2, which, however, do not refer to enzymic reproduction in the saturation range).

The conclusions with respect to the experimental data are that the plus strand forms more rapidly than the minus strand (each using the complementary strand as a template). The difference in slopes may be explained by differences in $\mathscr{F}$ and K (but not by differences solely in K). If the differences are solely due to the rate parameter, then $\mathscr{F}_+$ may be as much as 100 times as high as $\mathscr{F}_-$. A quantitative evaluation would, however, require more detailed experimental evidence. The final variant (after 74 transfers) shows a higher mean rate parameter. The increase in the final slope is 2.6-fold (as compared to the final slope of the original $Q\beta$-RNA). Although there is good reason to believe that most of this change is due to an increase in the rate parameters, it is not possible to make an exact evaluation of the single $\mathscr{F}$ and K values from the experimental data presented. The reduction of the induction period may be due to an increase in rates as well as to an initial presence of plus and minus strands. (Note that no induction period should be found if both strands are initially present at their "equilibrium" ratio). The data presently available are insufficient to justify the conclusion that the 2.6-fold increase in slope (which definitely means an increase in the average single-digit copying rate) indicates a 15-fold increase in the reproduction rate of individual variant RNA molecules (as compared to original $Q\beta$-RNA). Apart from a more detailed evaluation of rate data, it would have to be proved that shortening of the molecule is linearly reflected in a decrease in its total reproduction time, which is unlikely if more than one enzyme can simultaneously read the template, and which also would depend on concentration conditions. Nevertheless, there is an appreciable increase in the reproduction rate of individual molecules, otherwise the incorporation of ^{32}P—as shown in Fig. 25—would not have increased with the number of transfers, despite the 4-fold shortening of the incubation period.

It should also be emphasized that the above evaluation is based on the simplest possible steady state model for enzyme-template interaction and was given only in order to demonstrate the possibilities of obtaining further information from quantitative studies of rates and mechanisms.

One additional remark should be made with respect to the *selection mechanism*. One may argue from the discussion in Part II that, in the saturation range, the selection mechanism breaks down. The process will not be "autocatalytic" any more if $\dot{x}_i$ is not proportional to x_i, but constant. This would only be true if we refer to the replication of a uniform kind of primer. As soon as several competing sequences I_i are present, each of which can form a complex with the enzyme, characterized by a stability constant

$$K_i = \frac{x_{EI_i}}{x_{I_i} x_E} \qquad \text{(VII-7)}$$

we obtain

$$\sum_k x_{EI_k} = x_{E_0} \frac{\sum_k K_k x_{I_k}}{1 + \sum_k K_k x_{I_k}} \qquad \text{(VII-8)}$$

or

$$x_{EI_i} = x_{E_0} \frac{K_i x_{I_i}}{1 + \sum_k K_k x_{I_k}}$$

and

$$\dot{x}_{I_i} = \frac{x_{E_0}}{1 + \sum_k K_k x_{I_k}} \mathscr{F}_i K_i x_{I_i} \qquad \text{(VII-9)}$$

which reduces for uniform $K_i \equiv K$ to

$$\dot{x}_{I_i} = \frac{\mathscr{F}_i x_{E_0} x_{I_i}}{x_{I_i} + K^{-1} + \sum_{k \neq i} x_{I_k}}. \qquad \text{(VII-10)}$$

The physical interpretation is that, even in the saturation range now defined by $\sum_k K_k x_{I_k} \geqq 1$, a defined mutant I_i appearing in a small number of copies is reproduced according to an exponential selection mechanism ($\dot{x}_{I_i} \sim x_{I_i}$), which is valid until the selected species reaches a concentration level corresponding to a dominance: $x_{I_i} \gtrsim K^{-1} + \sum_{k \neq i} x_{I_k}$.[1]

VII.4. "Minus One" Experiments

Evolution experiments—the prototype of which is the above described $Q\beta$-experiment—can indeed give a quantitative account of evolutionary processes at the molecular level. Numbers, however, only have a meaning if well-defined and reproducible reaction conditions are chosen and constant constraints are applied. If the molecular process involves complex reaction patterns with cooperation of several simultaneously evolving species, the mechanism may become hopelessly complex.

How would one have to conduct test-tube experiments in such a case?

There is a possibility which I like to call the "minus one" approach. "Minus one" refers to a type of music record (known as "Music Minus One") in

[1] For simplicity a "selfrecognition" mechanism is treated here. The principal result for mechanisms of complementary recognition is analogous.

which the work, which normally requires n players, is actually recorded by only $(n-1)$ musicians. The missing part is supposed to be supplied (in his own home) by a single musician, usually a dilettante who enjoys playing in a big orchestra.

The proposed evolution experiment follows this principle (as did Spiegelman's $Q\beta$-experiment). All species but one are provided in their final form. The missing one, whose evolution from random precursors is to be traced, should be varied for each successive experiment (the number of which must exceed the number of species present to allow also an analysis of the couplings, since cooperation is not simply a sum of single processes). The total rate of evolution of such a system may then be estimated from a composite of all the data.

It is obvious that these experiments require uniform and reproducible reaction conditions, where constant constraints are to be maintained, otherwise data would not be comparable. One could imagine the construction of an automatically controlled machine in which the concentrations of monomers, polymers and enzymes are kept at constant levels by steady regulation (steady dilution or defined serial transfer) relayed by automatic assays of ^{32}P (and/or other label) incorporation. A separate system for maintaining concentration levels of monomers, RNA polymers and enzymes could be effected with the help of semipermeable walls, made of millipore filter material etc. The most interesting—but difficult—part of such experiments would be the inclusion of cell-free protein synthesis.

We may finally conclude that it does not seem to be impossible to test the various models for the origin of the code and the evolution of the molecular translation machinery by such test-tube experiments.

VIII. Conclusion

VIII.1. Limits of Theory

What the Theory Does Explain

is the general principle of selection and evolution at the molecular level, based on a stability criterion of the (non-linear) thermodynamic theory of steady states. Evolution appears to be an inevitable event, given the presence of certain matter with specified autocatalytic properties and under the maintenance of the finite (free) energy flow necessary to compensate for the steady production of entropy. The theory provides a quantitative basis for the evaluation of laboratory experiments on evolution.

What the Theory May Explain

is how to construct simple molecular models representing possible precursors of "living" cells. Four such models have been examined, of which only one could be shown to fulfil all the requirements for evolution into the present state of cellular life.

What the Theory Will Never Explain

is the precise historical route of evolution. The "never" is a consequence of the stochastic nature of the processes involved and the tremendously large multiplicity of possible choices. This also applies to predictions of future developments beyond certain time limits. Hence: "Whereof one cannot speak, thereof one must be silent" [114].

VIII.2. The Concept "Value"

When I gave these lectures at the Weizmann Institute, my friend Shneior Lifson asked me: "New concepts usually bring about a new constant. What is yours?"

To answer this question, let us first make a distinction between two kinds of concepts. One I shall call "new physics", the other a "new" but derivable "concept".

Only twice so far have we experienced the introduction of "new physics". It revealed the two fundamental natural constants: Planck's number, as manifested in the uncertainty relationship of quantum mechanics, and light velocity, which was only raised to the rank of a fundamental natural constant by relativity theory. Such might well happen a third time, since—as Heisenberg once joked—we have after all c-g-s system. "New physics" means the abandonment of the general validity of previously accepted fundamental principles required by experimental facts which, although obtained under clear and defined conditions, are in disagreement with the conclusions of theory.

On the other hand, the second kind of "new concept" does not invalidate any principle so far accepted; it deals only with a new aspect and may be derived from known principles. Again, there are certain experimental facts which are unexplained, but due rather to lack of insight or experience than to the violation of any fundamental principle. An excellent example is provided by the statistical concept of thermodynamics, introduced by Boltzmann, which was conceived after the realization that matter consists of molecules and atoms to which the known laws of Newtonian mechanics (later substituted by quantum mechanics) should be applicable. The only problem was the large number of particles (e.g. 10^{24}), each of which required a specification of three space and three momentum coordinates. The great breakthrough came with the introduction of statistical methods which allowed the derivation of distribution functions and the characterization of macroscopic states by "averaged" quantities, such as temperature. (The averaging rules, later implied by quantum theory, turned out to be even simpler than in classical theory.) It was immediately realized that this concept required the introduction of a new, but derivable, quantity which expresses how much "information" is lost by the procedure of averaging over all states (Z) among which energy can be distributed. This *quantity characterizing the "lack of knowledge" is entropy* and its physical meaning is expressed by Boltzmann's relation, which (for a micro-canonical ensemble) can be written in the simple form:

$$S = k \ln Z.$$

If entropy describes the "lack of information" due to the representation of Z microstates by one (averaged) number, then the same type of relation can be used to describe "information", as long as "infor-

mation" is characterized by one specific choice out of Z possible choices of equal a priori probability (Eq. (I-2)).
Similarly, if microstates of different a priori probabilities are involved, the average (normalized) information content can be described in analogy to Boltzmann's H-function by Shannon's formula (Eq. (I-4)).
The discrepancy of signs in such a conceptional definition of "entropy" and "information" was realized from the beginning. P. G. Tait, a close friend of Kelvin, in a paper published in 1868 expressed his discomfort with Clausius' choice of a positive sign for entropy, which he considered in fact of "negative" quality (see Ref. [16], p. 116).
Boltzmann's constant as it appears in Eq. (VIII-1) is not a fundamental natural constant. Its physical meaning results from the historical concept of temperature or heat. It could just as well have been adjusted to Shannon's concept of information and as such be represented by a dimensionless number such as 1/ln 2. On the other hand, that with entropy a "new concept" was introduced, becomes obvious with the axiomatic foundation of thermodynamics given by C. Caratheodory and others [115, 116].
The concept which selection theory is dealing with is of a similar nature, and this provides an answer to the question raised by Shneior Lifson. An understanding of the basic principles of evolution as self-organization at the molecular level does not require "new physics", but rather a derivable principle which correlates macroscopic phenomena with elementary dynamical behavior. The concept is expressed by introducing a value parameter, to be associated with the concept of information. However, *any* specified state among the ensemble can represent "information", rather than only one or a certain number of defined states. The introduction of what is in practice a continuously varying value parameter, associated with each informational state, allows us to develop *a general theory which includes the origin or selforganization of ("valuable") information*, thereby uniting Darwin's evolution principle with classical information theory and—after applying this concept to molecular selforganization—providing a quantitative basis for molecular biology.
Both the selective value and the average excess production are derivable quantities which involve the dimension of time, even if they are reduced to a dimensionless form by the introduction of some general rate constant (k_0).

What is their physical meaning?

Let us consider a macromolecular chain, built of a sequence of at least two kinds of monomeric digits. All possible sequences are assumed to have exactly the same energy content (which for any realistic case, of course, could only be a more or less valid approximation). By thermodynamic standards, all these states are indistinguishable or "degenerate". Their formation from monomeric digits, for a given class of uniform length, as well as their decomposition into energy-deficient fragments, are characterized by uniform overall affinities. However, if the reaction mechanisms include different individual intermediates, the rates need not to be uniform. There are three phenomenological parameters which characterize the "selective value" of each individual sequence with respect to its reproduction: the rates of formation and decomposition, physically determined by their "free energies of activation", and a quality factor which can be related to possible branching of the reaction in the intermediate state (at which instruction occurs). All other possible (environmental) influences are secondary, in that they can act only through these three parameters. At steady state, a defined combination of these three factors, depending on the particular constraints, determines the selective value.

VIII.3. "Dissipation" and the "Origin of Information"

The Prigogine-Glansdorff principle [43] provides the link between selection theory and thermodynamics (of irreversible processes).
A steady state at constant flow is characterized by minimum entropy production. If we plot (internal) entropy as a function of time, we must obtain a linear dependence (cf. Fig. 26). The steadily produced entropy could, for instance, be measured via a compensating heat flow in a thermostat, while the reaction system is kept at constant internal conditions. Such a steady state is dominated by a "selected" sequence (or collective) corresponding to

$$\bar{P}=W_{\max} \qquad \text{(cf. (II-37))}.$$

Now let us assume a (stochastically significant) fluctuation consisting of the production of a mutant which has a higher selective value than the previously selected copy. This is equivalent to a negative variation in the entropy production (i.e. an increase in average rate associated with a decrease in the overall affinity of the degenerate class). According to Prigogine and Glansdorff, such a negative fluctuation must lead to a breakdown of the existing steady state, which cannot be maintained if the external flows are kept constant. Thus, in thermodynamic theory, evolutionary behavior at constant flow is characterized by the occurrence of instabilities. What has really happened, if we compare the two selected species after restoration of the steady states, is a change in "valued" information which is reflected by increased order. In the diagram (Fig. 26), a negative fluctuation of entropy production is indicated by a deflection of the curve towards a smaller slope, which, due to the instability, amplifies until the new steady state is approached. Since external flows are kept invariable, and since the mutant copies have the same affinities as their precursors, the original slope will be restored when the new steady state is attained. The resulting constant difference in the absolute values of S_i (cf. the distance between the parallel solid and dotted lines) exactly equals the entropy difference, which is due to the increased internal order, as represented by the degree of organization (i.e. the fraction of digits in organized form). There is no violation of the second law. However, the thermodynamic description does not reflect, that almost uniform populations (or some of their important constituents) have to be completely exchanged in order to produce new (more "valuable") information and thereby decrease the internal entropy.

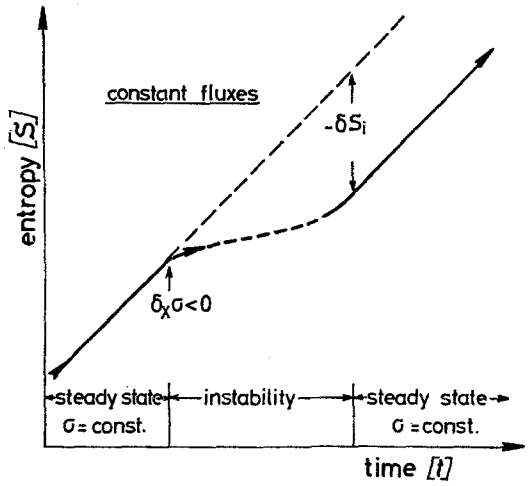


Fig. 26. Entropy time diagram for a selection process at constant overall flows of digits. The occurrence of a mutant exhibiting a selective advantage $W^F_{\text{mutant}} > W^F_{\text{master copy}}$ corresponds to a negative fluctuation of entropy production causing an instability (i.e. breakdown of the steady state). The former master copy dies out, whereas the mutant grows to a dominant level. Since both species have the same energy content, both steady state slopes (representing entropy production σ) are equal. The constant difference between entropy curves at steady state reflects the increase of order as expressed by the increase of overall organization

The balance becomes more involved if we allow the different sequences to have different free energies and thus different affinities for the formation and for the decomposition processes. Wherever this results in a change of the selective values, it will be reflected in the evolutionary behavior. It will also appear in the entropy balance at constant flow since the overall and individual affinities no longer agree. However, the decisive quantity for selection is still the selective value, if the appropriate selection constraints for competing sequences are represented by constant overall concentration of polymers and monomers rather than "constant average value of affinities". Under these conditions of buffering, there is no shortage of free energy and hence any economization is of little importance with respect to selection. It is quite clearly seen here that the concept of selection theory goes beyond that of irreversible thermodynamics. It is the individual *information* content and its "value" with respect to reproduction which is of interest, not the unspecified entropy balance. The above example of degenerate sequences was chosen in order to demonstrate that "selective value" as the driving force for evolution is a new variable which is linked to, but which goes beyond the present scope of irreversible thermodynamics. With respect to the utilization of free energy, especially in more complex reproductive systems, one further remark should be made.

One often reads that the guiding principle of evolution is economization of the use of free energy. This is not universally true. Where free energy is supplied in excess, the system will utilize any selective advantage regardless of how "costly" it is. However, if the use of energy becomes a secondary constraint by influencing the selective value, then the system will respond accordingly. The use of *information* associated with a high "*selective value*", rather than economization with respect to the consumption of free energy, is the decisive factor in evolution.

"Information" in this context (i.e. applied to a self-organizing system of matter) is more than simply a "structural correlate of function". It serves to specify a certain amount of detail lost by the averaging procedure of statistics. It refers to single, phenomenologically distinguishable states of an ensemble in which the total number of possible states may be in large excess of the number of states which actually are (or can be) populated. Such information is of significance only if it is able to preserve itself in the dynamical process of formation and decay. It may imply the existence of previous information from which it derived, but if we trace it back to its origin, we would have to say that "primary" information represents function for its own reproduction and variation to a state of higher "security" expressed by higher redundancy. Then it may also include secondary "non-reproductive" information as an instructive correlate of some function, limited in extent and time. "Selective value" characterizes the executive property of information carriers to evaluate their chance of survival and to preserve the most stable (or fittest) state.

VIII.4. The Principles of Selection and Evolution

The larger the content of information, the more there is a justification for separating the two processes:

1. Selection among populated alternative states.
2. Evolution of the selected states.

Both processes merge into one if the structural capacity is so low that *all possible* alternative states are populated. However, the number of possible complexions is usually tremendously large compared to the number of states which can be populated, and only under those conditions will the concept of information turn out to be a useful one. The total information content of the human genome, for instance, is stored in about 10^{10} nucleotides, but evolution has brought about the choice of one or a few out of the $4^{10^{10}}$ possible complexions (which include, of course, certain degeneracies).

Some difference may exist between the most favourable conditions for selection and evolution, and a compromise will be required for optimal performance, just as selectivity itself is a balance between precision (with consequent "stickiness") and dynamical flexibility.

Selection at constant selection strains is a process in which the average productivity is optimized by approaching the highest selective value among a population of information carriers present at a given environment.

The process is characterized by the optimization principle

$$\bar{P} \text{ or } \bar{E} \rightarrow W_m$$

which for nonlinear systems may be replaced by the more general integral relationship (II-34). W_m represents a *relative* maximum among a population of competitors. $\bar{E}$ approaches this value in the presence of certain constraints (optimization procedure).

The process of selection usually includes an economization with respect to the information content. In a

fixed environment, those sequences which require the lowest number of digits in order to include all the necessary information (i.e. those which do not carry any useless information) will usually show the highest selective value, i.e. where $\mathcal{A}_i$ (formation rate), $1/\mathcal{D}_i$ (lifetime) and $\mathcal{Q}_i$ (precision parameter) are as large as possible. A minimum threshold of accuracy (e.g. $\mathcal{Q}_{\min}$ as defined by Eq. (II-45)) is required in order to preserve the information of the selected state.

For optimal selection, the required precision of information transfer has to be adjusted to the amount of information to be transferred.

On the other hand, evolution is fastest if $\mathcal{Q}$ is as small as possible, but since is is based on selection, $\mathcal{Q}$ must be above the threshold $\mathcal{Q}_{\min}$. The variability decreases with increasing $\mathcal{Q}$ and this will always favor the evolution of systems for which $\mathcal{Q}$ is close to $\mathcal{Q}_{\min}$.

Evolution represents a further optimization procedure under certain constraints imposed by the selection criteria.

For instance, in a "linear" system with constant value parameters subjected to the extreme constraint of constant overall organization, the evolutionary route is restricted to a monotonic increase of W_m for all subsequently selected species:

$$W_{m1} < W_{m2} < \cdots < W_{\text{opt}}.$$

This excludes all evolutionary routes which pass through a minimum of W_m, among which one may find several which would lead to higher final values. The constraints, whatever they are, reduce the number of choices and thereby most probably prevent the best choice. One may conclude that—for a system with a large and unsaturated information capacity—the inequality

$$W_{\text{opt}} < W_{\max}$$

generally holds, characterizing a finite difference which Jacques Monod [117] may refer to as the difference between the "is" and the "ought". $W_{\max}$ represents an absolute maximum whose approach would require the initial presence or availability of "all" information. If one constructed an "information" space, the coordinates of which represent all possible "informations", the evolutionary variation of the system point would describe a fundamentally non-ergodic trajectory indicating the inevitability of the evolutionary process. This defines a privileged direction of time which applies to all living systems. It is connected with, but appears to be even more pronounced than the unidirectional increase of entropy for any irreversible process.

For non-linear systems it is not generally true that the absolute value of W_m has to increase during evolution. The occurrence of internal couplings among the information carriers is equivalent to a change of environment. Furthermore, secondary changes (e.g. pollution) may cause a universal reduction of all the selective values. Then evolution is still characterized by a sequence of W_m values, each of which characterizes a species or collective with optimum performance; this sequence, however, need not be monotonic. It may also decrease due to an environmental change, but then it will usually force the system to evolve towards a larger information content. To survive a change in the environment, or to change the environment to its own advantage requires additional information. Thus:

Evolution may involve an increase in selective value as well as utilization of a larger information content.

At least at higher levels of molecular organization, the second influence will prevail, because the possibilities of couplings in a complex system become so numerous that a large number of mutations may bring about a general reduction in value which can only be compensated for by the occurrence of mutants which are able to cope with the changed environment. The terms "good" and "evil" assume a meaning as soon as single information carriers start to interact and thereby mutually increase or diminish their "values".

Evolution at the molecular level may be considered a game in which the intelligence of the player is replaced by a selective "instinct" for advantage among randomly occurring events. Therefore game theory, as introduced by John von Neumann [118], which in recent years has been developed to a high level of sophistication [119], is the key to any further generalization of evolution theory[1].

VIII.5. "Indeterminate" but "Inevitable"

The fact that "selection" and "evolution"—in a certain analogy[2] to "equilibrium" in thermodynamics—can be characterized by extremum principles allows a physical foundation and a quantitative formulation of Darwin's principle. In this form the principle does not refer simply to a historical path, but rather to a physically deducible law which governs the general process of selforganization of matter. The evolution of higher forms of life, especially of "intelligent" forms of control, however, will require additional principles to be taken into consideration. Therefore, we confine ourselves here to the realm of molecular biology, i.e. to selforganizing processes at the molecular level.

The fact that we have a physical assessment of the concept of "value" may modify our interpretation of Darwin. In modern biology, it is a widely accepted view that Darwin's principle expresses—to use C. H. Waddington's words [124]—merely "a truism or tautology". Gunther Stent [125] in his book "The Coming of the Golden Age" writes: "As everybody now knows, survival of the fittest is nothing but the tautology: survival of the survivors, and hence in this connection 'unfit' represents not an objective scientific but a subjective value judgement".

Such a statement could only be correct if "value"—or whatever we may call it—were to represent simply the outcome of an otherwise completely indeterminate event. It is therefore important to subject

1 Specific methods for the mathematical treatment of optimization problems in molecular biology were proposed by I. Rechenberg [101]. A treatment of general evolutionary phenomena from the point of view of game theory was proposed by R.C. Lewontin [120] and F.E. Wartburton [121]. M. Kimura [122] and D.M. MacKay [123] emphasized the fact that "information" may originate from "noise".

2 Note the difference with respect to a) "optimization" and "maximization", b) the lack of microscopic reversibility at steady state and c) the limited use of total differentials.

the value concept, as introduced with the (deterministic) phenomenological theory, to a stochastic analysis. This has to be done separately for "selection" (among a given information content) and evolution (which utilizes the selection procedure in order to approach an optimum value). The basic concept of stochastic analysis was treated in Part III. One of the results obtained there is of great significance with respect to the above question. It deals with the precise reproduction ($\mathcal{Q}=1$) of a variety of n different sequences, all being degenerate in W_i. The stochastic analysis shows that such a system—due to its autocatalytic behavior—will always (or with high probability) narrow down to the information content of one sequence which, however, will group up to a redundancy of n copies. This represents a true case of "survival of the survivors" because there is no way to predict *which* template will survive—they are all physically indistinguishable; it is just a fluctuation which amplifies as a consequence of the inherently reproductive mechanism.

However, this example represents an unrealistic, singular case, since $\mathcal{Q}$ can never equal exactly one. If we still maintain the condition of complete degeneracy and overall balance of production and decomposition, the selective value of each individual sequence must become negative ($\mathcal{F}_i=\mathcal{D}_i$, but $\mathcal{Q}_i<1$) and hence *no* selection of stable information can occur among completely degenerate information carriers. Only if the selective values of different information carriers show a certain distribution, will *stable* selection for the species with maximum W_i occur at steady state.

Again this process is not completely deterministic, but the fluctuation limits decrease with increasing number of selected copies, as for any selfregulating stochastic process. Since selection usually starts from small numbers or even from one single mutant copy, fluctuation phenomena are of great significance.

Closely related to the problem of complete degeneracy treated above is the phenomenon of "random drift". It occurs when several species produced by "neutral" mutations [126] are degenerate with respect to their selective values. In the literature it is often referred to as "non-Darwinian" evolution [127]. Its occurrence had been neglected in earlier estimates of evolutionary rates based on sequence analysis of proteins from species at different phylogenetic levels. Significant as they are with respect to such estimations, it seems a little inappropriate to call this phenomenon "non-Darwinian". "Neutral" mutants and their "random drift" are, of course, well within the scope of the more abstract selection concept outlined in this paper.

More severe restrictions of determinacy are introduced by "error copying" in the reproduction process, or by other kinds of mutation on which the optimization procedure of evolution is based. Although the different digit positions are not completely equivalent with respect to mutations, and the resulting mutants are still related to their master copies, there is little if no connection between "cause" and "effect" of mutation, so that the whole process appears to be random. (Also, the elementary physical processes leading to mutations are intrinsically indeterminate due to their quantum-mechanical nature [54]. The superimposed autocatalytic selection processes filter out and amplify the mutants of high selective value, thereby reducing indeterminacy as far as the value principle is concerned. However, indeterminacy persists with respect to the individual copy choice and is mapped macroscopically. As a consequence, it is impossible to trace back the precise historical route or to predict the exact course of future development beyond certain time limits. Indeterminacy, furthermore, is what makes it impossible to reach an absolute maximum of value.

At higher levels of evolution, especially in population genetics, the most promising approach to selection was to start from the very fact of survival. It was the success of this approach (associated with the names of R. A. Fisher [26], J. B. S. Haldane [27] and S. Wright [28][1]) that has led some biologists to the tautologistic reinterpretation of Darwin's selection principle.[2]

Nevertheless, if we can relate survival to a physically objective "value" (which is a quite specific combination of rate and interaction parameters), the selection principle does not represent a *trivial* tautology or truism. Any principle, after its logical content has been elucidated, may appear to be more or less obvious, since logic represents an uncovering of tautologies or correspondences.

We may furthermore conclude that the evolution of life, if it is based on a derivable physical principle, must be considered an *inevitable* process despite its indeterminate course (cf. below). The models treated in Parts IV to VI and the experiments discussed in Parts IV, VI and VII indicate that it is not only inevitable "in principle" but also sufficiently probable within a realistic span of time. It requires appropriate environmental conditions (which are not fulfilled everywhere) and their maintenance. These conditions have existed on earth and must still exist on many planets in the universe. There is no temporal restriction to the continuation of the evolutionary process, as long as energy can be supplied. Thus any predictions of "inherent" temporal limitations will finally depend on our knowledge about the availability of cosmic energy sources and hence be linked intimately with problems of cosmology (to which no definite solution can, as yet, be offered).

The abstract formulation of the selection and evolution principle in the present paper does not, of course, involve the assumption that evolution actually took place under the extreme and abstract constraints of steady state. The analogy to equilibrium thermodynamics has already been stressed. The abstract treatment of Carnot's cycle, which refers to the optimal efficiency of a steam engine, has brought about an understanding of the principles of (equilibrium) thermodynamics, and yet—no steam engine has worked or could ever work under equilibrium conditions. Progress in evolution would be extremely slow (and there are many sociological implications) if extreme constraints were always maintained.

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1 The term "selective value" has been adopted from their work.

2 The work of V. Volterra [49] and A. J. Lotka [53] deserves to be mentioned. It is related to a formal mathematical treatment of specific problems of competitive growth and "struggle", but has less connection with the general problem of the generation of "information" in macromolecules.

However, these conditions reveal the principle, allow us to analyse models, show us how to do reproducible experiments, and may finally lead us to a reconstruction of certain evolutionary events.
We are now ready to comment on the final question:

VIII.6. Can the Phenomenon of Life be Explained by our Present Concepts of Physics?

A simple "yes" may bring us into a difficult position, because we may be asked to prove this answer, e.g. by complete induction. It may be wiser to turn the question round and, whenever anybody claims that physics does not offer any explanation of life, to let him prove it, or better, to disprove the claim by giving just one counter-example.
Do we have a counter-example? This may depend very much on whether we agree about the definition of life.
A. I. Oparin [128] once proposed the following list of properties:

metabolism,
selfreproductivity,
mutability

as a basis for the definition of the word "living".
This definition could be fulfilled by a machine like J. von Neumann's "selfreproducing automaton" [129]. Such a machine would certainly have a "metabolism". In an environment of electric plugs or oiltanks, it could find enough "food" and perform any type of work. The feature of the automaton, of course, is its ability not only to reproduce itself according to a program, but also to reproduce the program, thus enabling any descendent machine to reproduce mistakes which might lead to advantageous "genotypic" mutants. Would we say such a robot was alive? Probably not, because it needs man to start it, and therefore we would call it "artificial".
Other examples are the reproductive macromolecular cycles treated in Parts IV to VI. They are able to start by themselves, but we would not attribute the quality of "being alive" to anything less sophisticated than the catalytic hypercycle treated in Part VI, which is characterized by a list of some ten properties (cf. VI.2), including the three put forward by A. I. Oparin. The existence of such a cycle depends only upon:
a) certain chemical properties of matter, as detailed in Parts IV to VI, which, at least in principle, can be explained by quantum-mechanical theory; and
b) the presence of certain physical conditions, which we have every reason to assume existed on earth.
We have to conclude that no "new physics" is required for the foundations of biology, but we see at the same time how little we have gained by this conclusion. The step from a single macromolecule to a catalytic hypercycle or a "living" cell is certainly less dramatic than the transition from the single cell to a selfconscious and intelligent human being. To understand the various steps involved in this transition will probably require just as little "new physics" but as many further (derivable) "concepts" as were required for the first step.
As Wittgenstein said fifty years ago [130]:
"Die Lösung des Problems des Lebens merkt man am Verschwinden dieses Problems".

IX. Deutsche Zusammenfassung

Die vorliegende Arbeit ist zugleich Übersichtsartikel und Originalmitteilung. Sie wendet sich an Physiker und Biologen. Für den Physiker mußte sie die Zusammenfassung einiger — dem Biologen (oder Biochemiker) wohlbekannter — Tatsachen bringen um zu zeigen, von welchen Voraussetzungen eine Theorie der Lebenserscheinungen auszugehen hat, und warum ganz bestimmte und nicht andere Modelle diskutiert werden. Dem Biologen andererseits soll gezeigt werden, daß die Lebensvorgänge von physikalischen Prinzipien kontrolliert werden, die sich einer quantitativen Formulierung nicht entziehen. Manche dem Physiker geläufige Ansätze und Lösungen, oft auch der „Durchsichtigkeit" halber vorgezogene Näherungen, werden daher ausführlicher diskutiert.
Im Brennpunkt steht die (im Schlußkapitel explizit gestellte) Frage:
„Ist die Biologie durch die Physik — in ihrer gegenwärtigen Form — begründbar?"
Die Antwort, sofern sie sich überhaupt in einem Satz zusammenfassen läßt, müßte lauten: Bei den bisher hinreichend untersuchten biologischen Vorgängen und Erscheinungen gibt es keinerlei Hinweise dafür, daß die Physik in ihrer uns bekannten Form nicht dazu in der Lage wäre, wenngleich auch — wie in den makroskopischen Erscheinungen der unbelebten Welt — einer Beschreibung im Detail Grenzen gesetzt sind, die nicht im Grundsätzlichen sondern allein in der Komplexität der Erscheinungen begründet sind. Ebensowenig wird damit ausgeschlossen, daß die uns geläufigen wesentlichen Prinzipien der Physik sich in den Lebenserscheinungen in einer besonderen, eben für diese charakteristischen Form äußern. Zu nennen sind hier vor allem das — für die Theorie der Informationserzeugung charakteristische und physikalisch ableitbare — Wertkonzept, das den Optimierungsprozeß der Evolution beherrscht, oder die diesem Vorgang eigene zeitliche Vorzugsrichtung, die in den Stabilitätskriterien der thermodynamischen Theorie irreversibler Prozesse ihren Ursprung hat und die Evolution zu einem grundsätzlich „unabwendbaren" Ereignis macht.
Natürlich liegen die wesentlichen Aussagen im Detail.
Die phänomenologische Formulierung des Evolutionsprinzips wird im Teil II vorgenommen. Darwins Prinzip erscheint als ein an bestimmte physikalische Voraussetzungen gebundenes ableitbares Optimalprinzip, nicht etwa als ein der Biosphäre allein zugrunde liegendes irreduzibles Phänomen. Es ist durch das Stabilitätskriterium von Prigogine und Glansdorff an die thermodynamische Theorie stationärer Zustände angeschlossen. Begriffe wie Selektionsspannung und Selektionswert lassen sich bei Annahme definierter dynamischer Bedingungen (z. B. konstanter „Flüsse" oder „Kräfte") physikalisch objektivieren und quantitativ formulieren. Das hier zum Ausdruck kommende Wertkonzept liefert die Grundlage einer Informationstheorie, die eine Beschreibung der Informationserzeugung einschließt. „Information" ist hier eine in der dynamischen Theorie der Materie begründete „molekulare" Eigenschaft, „bewertet" durch die Fähigkeit sich selbst zu reproduzieren. Sie vermag insbesondere energetisch entartete Zustände voneinander zu unterscheiden und kennzeich-

net damit eine „primäre" Selbstorganisation der Materie nach rein funktionellen Gesichtspunkten.

Im Teil III werden die Einschränkungen untersucht, denen die phänomenologischen Ansätze aufgrund der Unbestimmtheit der Einzelereignisse unterliegen. Die stochastische Theorie liefert eine Begründung für das „Mittelwerts"-verhalten der phänomenologischen Ansätze im Falle großer Teilchenzahlen. Sie führt aber weit über die Aussagen der phänomenologischen Theorie hinaus. Da evolutionäre Entwicklungen durchweg ihren Ursprung in Einzelereignissen haben, die durch den Wachstumsprozeß „verstärkt" und damit makroskopisch „abgebildet" werden, ergibt sich eine im Vergleich zu abgeschlossenen, im Gleichgewicht befindlichen Systemen wesentlich stärker hervortretende Unbestimmtheit sowohl der individuellen Strukturen als auch des „historischen" Ablaufs der Ereignisse. Allerdings räumt die Theorie auch mit einem unter Biologen verbreiteten, auf eben diese „Unbestimmtheit" der Einzelprozesse Bezug nehmenden Vorurteil auf, nämlich der Ansicht, daß das Darwinsche Prinzip, formuliert als: „survival of the fittest" lediglich die triviale Tautologie: „survival of the survivor" beinhalte. Eine solche Interpretation wäre nur dann gerechtfertigt, wenn „fittest" als Zufallsergebnis allein durch die Tatsache des „survival" bestimmt wäre. Dieser Fall ist singulär und unter natürlichen Bedingungen kaum zu realisieren. Er würde einmal eine vollkommen präzise, d.h. absolut fehlerfreie Reproduktion zur Voraussetzung haben, zum anderen aber auch eine vollständige Entartung aller Selektionswerte, d.h. eine Ununterscheidbarkeit der dynamischen Eigenschaften der konkurrierenden Spezies. Dann könnte sich — aufgrund der autokatalytischen Reproduktionsmechanismen — eine zufällige Schwankung so verstärken, daß es zu einer Selektion kommt, ohne daß man in irgendeiner Weise das Ergebnis hätte voraussagen können. Alle tatsächlich ablaufenden Reproduktionsprozesse sind aber wegen der Endlichkeit der Wechselwirkungsenergien mit einer gewissen Fehlerrate behaftet. Darüber hinaus unterscheiden sie sich im allgemeinen hinsichtlich ihrer Selektionswerte. Dann aber ist die Selektion immer durch einen Optimierungsprozeß gekennzeichnet, wobei der Begriff „fittest" einem Wertmaximum unter einschränkenden Nebenbedingungen in Form von Ungleichungen, d.h. einem Optimum zugeordnet ist. Schwankungen spielen eine große Rolle, da die zur Selektion anstehenden vorteilhaften Mutanten zunächst in Form einer einzigen Kopie auftreten. Solche Schwankungen fallen um so stärker ins Gewicht, je kleiner der die Auswahl begünstigende „selektive Vorteil" ist.

In den Abschnitten IV bis VI werden konkrete Reaktionsmodelle behandelt. Es läßt sich zeigen, daß die *Entstehung „lebensfähiger" Strukturen* (etwa Vorläufer einzelliger Mikroorganismen) an besondere Bedingungen des Selektionsmechanismus geknüpft ist, die weder von den Nukleinsäuren noch von den Proteinen allein erfüllt werden. Der dem Selektionsmechanismus zugrunde liegende Reproduktionsprozeß muß für sich allein bereits nichtlinearer Natur sein. Sowohl Nukleinsäuren als auch Proteine können sich nach einem „quasilinearen" Reaktionsmechanismus reproduzieren. Dabei entstehen jedoch Systeme, die im Falle der Nukleinsäuren zu wenig, im Falle der Proteine aber zu viel Information enthalten. „Zu wenig" bedeutet, daß die verschiedenen miteinander konkurrierenden Sequenzen nicht in der Lage sind, genug Information für die Codierung selektionsbegünstigender Funktionen reproduzierbar anzusammeln. „Zu viel" Information heißt dagegen, daß die Wahrscheinlichkeit für eine sich selbst begünstigende Mutation zu klein wird, oder daß das System sich nicht von einer Vernetzung durch „parasitäre" Kopplungen befreien kann. Dagegen ist es möglich, daß ein System, das sowohl Nukleinsäuren als auch Proteine enthält, die funktionellen Vorteile beider Stoffklassen für eine stabile Selektion ausnutzt. Die vorteilhaften Eigenschaften sind:

a) die inhärente Selbstinstruktivität der Nukleinsäuren, mit deren Hilfe sich nicht nur jeder einmal ausgebildete Informationszustand, sondern auch jede weitere Veränderung reproduzieren läßt, sowie

b) die enorme funktionelle Kapazität (spezifische Erkennung, Katalyse und Regelung) der Proteine, die zur Kopplung und Korrelation einzelner Reaktionsschritte beim Aufbau geordneter Funktionseinheiten unerläßlich ist.

Die aus einer solchen (nichtlinearen) Kopplung zwischen Nukleinsäuren und Proteinen resultierende Hierarchie von Reaktionscyclen zeigt bereits wesentliche Merkmale eines „lebenden" Systems auf und ist für eine weitere Evolution bis zur lebenden Zelle „offen". Die Entstehung eines solchen sich reproduzierenden „Hypercyclus" hängt von der Ausbildung eines eindeutigen Code-Systems ab. In der zweiten Hälfte von Teil VI werden die Voraussetzungen für die Entstehung eines Codes mit eindeutiger Zuordnung diskutiert.

Im Teil VII schließlich sind Evolutionsexperimente beschrieben, wie sie zuerst von S. Spiegelman mit $Q\beta$-Phagen ausgeführt wurden. Die in den Teilen II und IV entwickelte Theorie liefert die Grundlage zur Ausführung reproduzierbarer Messungen und deren quantitative Auswertung.

Die Ergebnisse der vorliegenden Arbeit lassen sich etwa folgendermaßen zusammenfassen:

1. Die detaillierte Analyse der Reproduktionsmechanismen von Nukleinsäuren und Proteinen bietet keinerlei Anhalt für die Annahme irgendwelcher nur den Lebenserscheinungen eigentümlichen Kräfte oder Wechselwirkungen. Das für die Evolution lebender Systeme charakteristische Selektionsverhalten tritt bereits auf dieser Stufe als eine speziellen Reaktionssystemen inhärente Materieeigenschaft in Erscheinung.

2. Jedes durch Mutation und Selektion erhaltene System ist hinsichtlich seiner individuellen Struktur unbestimmt, trotzdem ist der resultierende Vorgang der Evolution zwangsläufig — also Gesetz. Das Auftreten einer Mutation mit selektivem Vorteil entspricht einer Instabilität, die mit Hilfe des Prinzips von Prigogine und Glansdorff für stationäre, irreversible thermodynamische Prozesse als solche erklärt werden kann. Der Optimierungsvorgang der Evolution ist somit im Prinzip unausweichlich, hinsichtlich der Auswahl der individuellen Route jedoch nicht determiniert.

3. Schließlich zeigt es sich, daß die Entstehung des Lebens an eine Reihe von Eigenschaften geknüpft ist, die sich sämtlich physikalisch eindeutig begründen lassen. Die Vorbedingungen zur Ausbildung dieser

Eigenschaften sind vermutlich schrittweise erfüllt worden, so daß der „Ursprung des Lebens" sich ebensowenig wie die Evolution der Arten als einmalig vollzogener Schöpfungsakt darstellen läßt.

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