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THE CHEMICAL SYNTHESIS OF NUCLEIC ACIDS

by B. F. Vanyushin

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THE CHEMICAL SYNTHESIS OF NUCLEIC ACIDS

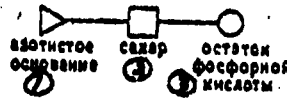
[Following is a translation of an article by B. F. Vanyushin in the Russian-language journal Priroda (Nature), No 4, Moscow, April 1963, pages 29-35.]

Moscow State University imeni M. V. Lomonosov

In recent years a number of new interesting data has been obtained in biochemistry concerning the structure and functional role of many complex biologically important compounds. Science established the fact that the specificity of organisms and manifestation of all basic moments of their life activity are connected first of all with the high-polymeric compounds and are conditioned principally by the characteristics of the structure and metabolism of such highly important biopolymers as proteins and nucleic acids. Therefore, the study of the intricate structure of these polymers, nucleic acids in particular, represents an important and essential prerequisite for the most complete understanding of their biological functions.

All nucleic acids are formed from a large number of separate monomers called nucleotides. Each nucleotide in its turn represents a complex chemical compound. It consists of one nitrous base (adenine, guanine, cytosine, uracil, thymine or for short A, G, C, U, T), one carbon (ribose or desoxyribose), and one radical of phosphoric acid -- all of them combined in a definite manner.

Ribose-containing nucleotides join together and form polyribonucleotide chains of various length. This type of natural chains, usually obtained from cells of various organisms, are called ribonucleic acids (RNA, for short).



Schematic Picture of a Nucleotide

1 -- nitrous base; 2 -- sugar; 3 -- radical of phosphoric acid.

Nucleotides which contain desoxyribose, as a sugar component, form high-polymeric molecules of nucleic acids of a different type. These nucleic acids are called desoxyribonucleic, or DNA for short.

The most intensive formation of nucleic acids takes place in all young and growing bacterial, vegetable, and animal cells, as well as in the cells of specialized secretory tissues which actively produce various proteins. The synthesis of nucleic acids in the organism takes place with the participation of proteins-enzymes which unite various nucleotides and form long RNA or DNA chains. Very recently, only a few years ago, the enzymic synthesis of nucleic acids was reproduced in vitro (in a test tube).

Nucleic Acid "Multiply" in a Test Tube

A prominent American biochemist, Severo Ochoa and associates, isolated from the cells of an azobacterium and *E. coli* a special enzyme which was used in artificially synthesizing for the first time from certain nucleotides non-specific polynucleotides of the RNA type.

An outstanding event was the realization of an enzymic synthesis of specific DNA molecules in the laboratory of Nobel prize winner, Prof Arthur Kornberg. This synthesis represents a polymerization of certain nucleotides on the base of a matrix -- the high-polymeric natural DNA -- which is added to the reaction mixture as a specific catalyst. The newly synthesized DNA molecules are characterized by the same sequence of nucleotides as the original DNA-matrix. This means that the regulated specific arrangement of nucleotides within the polymer is determined by the DNA-matrix, while the special enzyme added to the reaction mixture only induces a mutual union of the nucleotides into one long and continuous chain.

In the same manner, specific RNAs were synthesized in the same laboratory by means of enzymes. As a primer-matrix, also high-polymeric DNAs were used. The resulting high-polymeric RNAs were arranged in strict correspondence with these DNA-matrices.

Thus, the synthesis of high-polymeric nucleic acids has been achieved. But this synthesis is realized on a ready matrix and is carried out by specific proteins-enzymes. How, then, did these primary matrices originate? Did nature wait for the appearance of proteins-enzymes for the synthesis of nucleic acids, or could they have originated independently, in a non-enzymic way? How, in general, did the very first, primitive nucleic acids originate on Earth? All these questions are of great interest to science.

No life can exist without nucleic acids. These unique compounds play an important part in the manifestation of such principal life processes as growth, development, and proliferation. They are connected, in the first place, with the properties of a living organism such as heredity and mutations. Apparently, only with the emergence of nucleic acids could any primitive forms of life originate at all. Therefore, the elucidation of the origin of nucleic acids represents an important part of the great and complex problem which is successfully elaborated in the Soviet Union under the guidance of A. I. Oparin -- the problem of origin of life on Earth.

Indeed, could complex and biologically important compounds such as nucleic acids form in ancient times from relatively simple substances via various chemical reactions, without the participation of specific biocatalyzers -- the proteins N-enzymes? Apparently, the most definite answer to this question can be found in the chemical synthesis of nucleic acids.

The First Time in the History of Chemistry

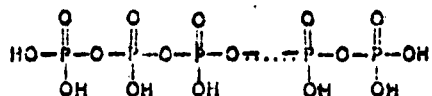
Attempts of chemically synthesizing polynucleotides have been made by a number of chemists. M. Michelson, an Irish scientist, succeeded in condensing isolated nucleotides and obtaining di- and tri-nucleotides. An American scientist, Gobin Coran achieved considerable successes in this respect in the field of organic chemistry; he obtained chemically a product (oligonucleotid) containing about 10

nucleotides. However, no one as yet succeeded in chemically synthesizing a polynucleotide which in its polymerization degree could approach the natural nucleic acids. Let us underline again that the "smallest" natural nucleic acids, the so-called soluble ribonucleic acids, represent relatively short chains consisting of 50-100 nucleotides; in a molecule of infectious RNA of the tobacco mosaic virus the number of nucleotides reaches 6,000.

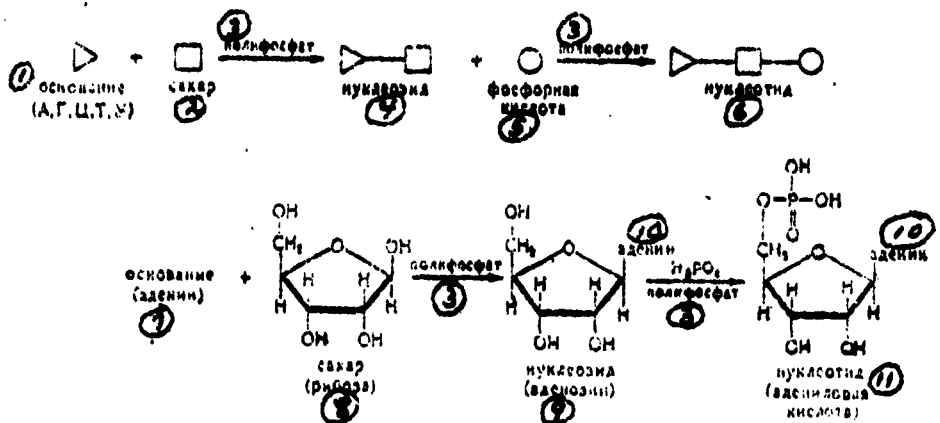
For the synthesis of nucleic acids it is essential first of all to have various nucleotides. They are usually obtained from cells of various organisms, or are chemically synthesized. A chemical synthesis of some nucleotides from their basic parts has been realized in several laboratories in various parts of the world. This synthesis is very complex and of a multistage nature.

Recently a prominent German biochemist, Prof. Gerhardt Schramm and his associates (Tubingen Virological Institute imeni Max Planck) have found a new original and simple method of synthesizing nucleosides (compounds of the type: nitrous base -- glucose) and nucleotides by means of phosphates. A polyphosphate represents a mixture of lineal and cyclic polyesters of phosphoric acid. The polyphosphates form upon dissolving and heating of phosphoric anhydride P_2O_5 in ether and some other neutral solvents.

Polyphosphate



In the presence of a polyphosphate, sugar is attached via a so-called glycoside bond to position 9 of the nitrous base, i.e., a bond is at once established, identical with the one in natural nucleosides. Nucleosides, thus obtained from the adenine base and ribose or desoxyribose sugars, fully resemble the analogous natural substances. In the course of the reaction, the phosphoric acid radical also unites with the formed nucleotides under the effect of polyphosphate, thus resulting in the origin of nucleotides. This completes the entire "construction" of nucleotides, i.e., the elementary ingredients which form the composition of all nucleic acids.



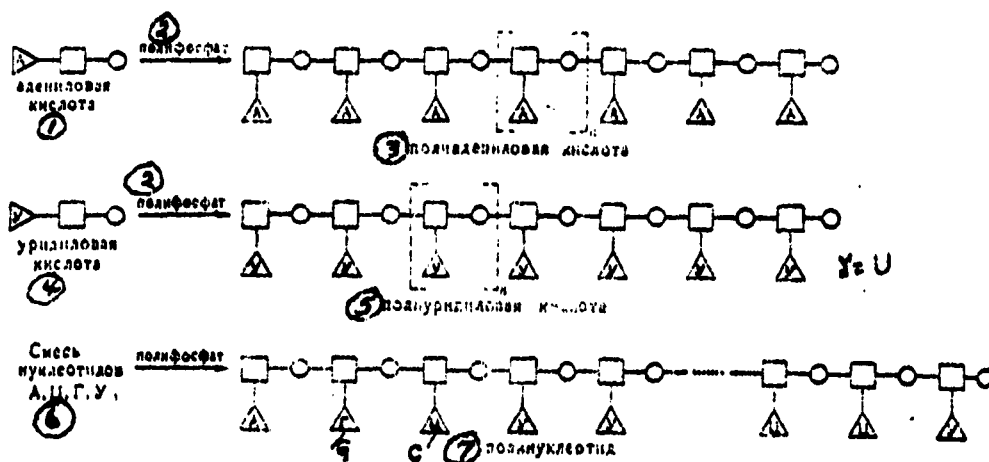
Synthesis of Nucleotides Under the Effect of Polyphosphates

1 -- base (A.G.C.T.U); 2 -- sugar; 3 -- polyphosphate;
 4 -- nucleoside; 5 -- phosphoric acid; 6 -- nucleotide;
 7 -- base (adenine); 8 -- sugar (ribose); 9 -- nucleoside (adenosin); 10 -- adenine; 11 -- nucleotide (adenylic acid).

It was found that polyphosphates not only catalyze the formation of nucleotides, but also "compel" them to become polymerized and form long polynucleotide chains. Upon heating to 50-60° in the presence of a polyphosphate, certain nucleotides form in definite solvents fairly high polymeric products which are analogous to nucleic acids in their physicochemical properties. For instance, from a nucleotide (adenylic acid) a polymer has been obtained with a molecular weight of 21,000, from uridylic acid a polyuridylic acid with a molecular weight of 50,000 has been synthesized. Analogously, polyguanylic acid (molecular weight 28,000), polycitidylic acid (molecular weight 15,000) and polythymidylic acid (molecular weight 18,000) have been obtained.

Thus, for the first time in the history of natural science a chemical artificial synthesis has been achieved of polynucleotide chains with a number of monomers from 50 to 150. These synthetic polynucleotides equal in their length to the natural "soluble" RNAs, or are even somewhat

larger. On photos obtained by means of an electron microscope, the chemically synthesized polynucleotides appear similar to natural RNAs. They are easily hydrolyzed with the ribonuclease enzyme. This attests to the fact that various nucleotides in the chain of synthetic polymers are mutually connected in the same manner as in natural RNAs. Otherwise, these polynucleotides could not have been hydrolyzed by the enzyme which specifically breaks up the so-called phosphodiester bond between 3' and 5' positions of sugars in the two adjacent nucleotides. Upon mixing the synthetic chains of polyuridylic acid and polyadenylic acid, these polymers form a two-chain structure in the same manner as the corresponding polynucleotides synthesized by means of enzymes.

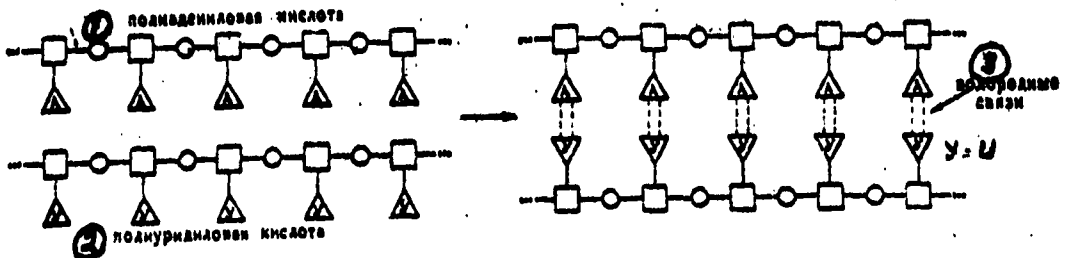


Synthesis of Artificial Polynucleotides under the Effect of Polyphosphates

1 -- adenylic acid; 2 -- polyphosphate; 3 -- polyadenylic acid; 4 -- uridylic acid; 5 -- polyuridylic acid; 6 -- Mixture of nucleotides A.C.G.U.; 7 -- polynucleotide

In contrast to polynucleotides obtained enzymically, the chemically synthesized polynucleotides possess no specific nucleotide sequence. Upon chemical condensation of various nucleotides into a long chain, various nucleotides are apparently combined in a disordered, chaotic manner, whereas in natural nucleic acids the distribution

of nucleotides along the chain is subordinated to definite rules. This constitutes the main difference between chemically obtained polynucleotides and the natural ones.



Formation of Two-Chain Complementary Structure from Polyuridylic and Polyadenylic Acids

1 -- polyadenylic acid; 2 -- polyuridylic acid; 3 -- hydrogen bonds.

Simultaneously with polymerization of nucleotides, the polyphosphates react in a definite manner with amine acids or sugars and induce polymerization also in these compounds, with the formation of corresponding protein-like substances and polysaccharides. The polyphosphate unites with the amine ($-NH_2$) group of the amino acid and activates the latter in such a way that its free carboxyl group ($-COOH$) is capable of entering a reaction with the amine group of another amino acid which results in the formation of a so-called peptide bond, i.e., the basic type of bond between amino acids, characteristic of all natural proteins. In this manner a polypeptide has been obtained consisting of 24 amino acids. From the amino acid of arginine a polipeptide -- polyarginine -- can be produced with a molecular weight of 4000-5000.

In the presence of a polyphosphate in neutral solvents, one can obtain from free sugars high-polymeric polysaccharides in which the sugar radicals are combined lineally. For instance, a polysaccharide has been obtained from glucose with a molecular weight of 50,000. In its physicochemical properties this polysaccharide proved to be similar to the natural polysaccharide -- cellulose -- of the same molecular weight. It has been established, via various analytical procedures, that the glucose radicals are bound in this polysaccharide in the same manner as in the natural polymer (beta-glycoside bond between the

1st and 4th adjacent carbons within the chain of sugars). In an analogous manner, by means of a polyphosphate, polysaccharides of a molecular weight approximating 40,000 have been synthesized from fructose and ribose.

Thus, by means of polyphosphates it is possible to obtain in a very simple manner macromolecules, most important biologically. Protein-like substances are formed from amino acids, polysaccharides -- from sugars, nucleotides -- from nitrous bases of sugars and phosphoric acid, and nucleotides are easily polymerized with the formation of nucleic acids. This discovery of G. Schramm called attention for the first time to polyphosphates as compounds which could have played an important part in the origin of macromolecular compounds on Earth. The data of G. Schramm also compel us to regard in an entirely new light the role of these phosphoric compounds in the life activity of many present day organisms.

Role of the Polyphosphates

Thanks to the works of Academician A. N. Belozerskiy and associates and a number of foreign biochemists, it is known that polyphosphates play an important part in the metabolism of various organisms. These compounds are wide spread among bacteria, seaweeds, and fungi. Polyphosphates are also found in certain varieties of moss, phanerogamia, and insects. Many biochemists regard these compounds as unique "storage cells" of energy, as well as "reserve" depot of phosphorus in the form of phosphoric acid, essential to all vitally important processes which are taking place in the cell. It has been noted that, upon the intensive synthesis of nucleic acids and protein, the amount of polyphosphates in the cell becomes sharply reduced. Apparently the phosphosphates participate in some manner in the biosynthesis of these compounds. It was assumed that the participation of polyphosphates in nucleic acid synthesis consists of phosphorylation of various compounds via the transfer of phosphorus to the general phosphorus carrier -- adenylic acid, and from it to other nucleotides essential to the synthesis of nucleic acids. The discovery by G. Schramm of the catalytic activity of polyphosphates suggests the idea that in the lower organisms polyphosphates may apparently participate directly in the biosynthesis of nucleic acids, by activating in some manner the nucleotides and catalyzing their polymerization. Possibly, an analogous role is played by these compounds also in the synthesis of some proteins. Furthermore, polyphosphates may also

participate in the biosynthesis of various peptides, antibiotics, co-enzymes, nucleotide-peptide complexes, and many other compounds which are playing an important part in the metabolism of various organisms.

The activation of synthetic processes within the cell by means of polyphosphates is undoubtedly a process of more primitive nature as compared to enzymic activation. This perhaps explains the fact that polyphosphates are widespread mainly among lower plants, and that they are not detected among the higher forms, especially among the chordata. Apparently, in the course of evolution, there have developed in organisms instead of polyphosphates new, perfected catalysts -- ferments which ensure more specifically and, energetically, more efficiently the course of various metabolic processes in the cell, including the biosynthesis of proteins, nucleic acids, polysaccharides, and other compounds.

The polyphosphates possibly played an important part in the origin of the initial primitive forms of life. Prior to the emergence of this primary organisms the Earth had an abundance of various organic substances which might have formed from relatively simple inorganic substances. This is attested by the data of many Soviet and foreign investigators who have demonstrated that, under the effect of ultraviolet light, an electric discharge, and other physico-chemical agents, many diverse and fairly complex organic compounds are formed from such simple substances as water, methane, and ammonium; these organic compounds include organic acids, amine acids, and other substances.

Under similar conditions there is also a possibility of formation of bases which enter the composition of nucleic acids. It is known, for instance, that the nitrous base -- adenine -- is formed upon heating of fairly simple organic compounds -- nitril. In the presence of polyphosphates, easily formed from phosphoric acid, not only a synthesis but also a polymerization of the newly-originated organic compounds could have taken place. In particular, under the effect of polyphosphates certain primary polymers of nucleic nature could have originated.

It is perfectly clear that a simple and random condensation of some monomers with the formation of high-polymeric compounds of protein, nucleic, and carbonic nature could not in itself lead to the emergence of a live being. One of the essential conditions for it is the formation of

complex, constantly interchanging, and self-reproducing systems of highly-specific biopolymers.

The Emergence of Primary Biopolymers

The origin of biological macromolecules with a more or less pronounced specific regularity of monomers has apparently been a very complex and extended process which included a gradual step-by-step improvement of numerous very simple and heterogenous molecules of high-polymeric compounds. Apparently, most biologically promising were such systems of macromolecules which provided the quickest and most effective "self-reproduction" of polymers.

In his experiments with enzymic synthesis of nucleic acids, A. Kernberg elicited that the "proliferation" of specific molecules of nucleic acids proceeds on the complementary principle, i.e., in such a manner that the polynucleotide chain, which is synthesized on the matrix of a given nucleic acid catalyst, specifically complements the chain of the nucleic acid matrix and represents in relation to the latter a complementary chain. For instance, the polyadenylic acid chain is complementary to the polyuridylic acid chain. Both these chains complement each other to such an extent that, under certain conditions, they may form a unique and fairly stable two-chain structure.

G. Schramm observed that the complementary principle takes place also in the non-enzymic synthesis of nucleic acids. For instance, chemical polymerization of uridylic acid under the effect of complementary polyadenylic acid is accelerated approximately 10-fold. The addition of non-complementary high-polymeric polyuridylic acid has no stimulating effect on the chemical synthesis of a similar polynucleotide.

Apparently, the principle of complementary and mutually-catalytic matrices has played an important role in the selection and perfection of many macromolecular systems, those of nucleic acids in particular. If one of the chains had a certain advantage as a result of certain physicochemical changes in its structure, this change was reflected in the structure of another polynucleotide chain which was synthesized on it according to the complementary principle. In its turn, the complementary chain had a favorable effect on the initial polynucleotide chain. Presumably, in the course of evolution and as a result of physicochemical mutations

(various physicochemical changes in the nuclear structure) and selection, the primary polynucleotides could have emerged in a more or less definite and regular order and nucleotide sequence. Some of them could, in a sense, turn out to be the precursors of present day nucleic acids.

One must not forget that the role of polynucleotides, as biopolymers, could apparently have been greatly affected by their interaction with the substances of protein nature. For instance, in Schramm's experiments, it has been established that the polipeptide polyarginine has a favorable effect on the chemical synthesis of the polynucleotide -- polyuridylic acid. The possibility is not excluded that also the polynucleotide matrices may catalyze the chemical polymerization of amino acids with the formation of corresponding peptides.

It is still impossible at present to decide as to precisely which systems of high-polymeric compounds could have originated initially, and whether they represented some primitive nucleic acids, or protein-like substances. Today, no investigator will still risk to offer a definite answer to this question. Much in this respect is still within the realm of assumptions and guesses. However, there is one incontrovertible fact -- that the emergence of nucleic acids and proteins, regardless of the order of their origin, represented a powerful stimulus to the perfection of both groups of high-molecular compounds. The interaction of both compounds led to the origin of new and more perfect "self-reproducing" macromolecular systems, which turned out to be the unique "forefathers" of the most important present day biopolymers.

* * *

The discovery by G. Schramm of non-enzymic synthesis of protein compounds, nucleic acids, and polysaccharides by means of polyphosphates is of great scientific importance. It elucidates one of the numerous facets of the process of complication and development of matter, and it reveals one of the possible ways of the origin of important high-molecular compounds, without which the origin of life on our planet would be unthinkable.

In addition to its considerable theoretical value, the data of G. Schramm also represent a practical interest, because they attest to the fact that a simple and easy way has been found of chemical polymerization of various groups

of compounds, using unique "catalyzers" -- polyphosphates -- which can be easily obtained in any chemical laboratory. The possibility is not excluded that the knowledge of the delicate mechanisms of the role of polyphosphates in the synthetic cellular processes will make possible the activation of the facets of metabolism of lower organisms which are of importance to man.

Another mystery of nature has lent itself to the searching mind of the scientist, and it will help in the future in the victory of human mind over the forces of nature.

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