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THE NATURE OF VIRUSES

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A complete understanding of the historical development of living matter, if it is to be in accord with the tenets of dialectical materialism, requires the acceptance of the postulate that organisms which were structurally simpler than the most primitive cells existed for a long time prior to the emergence of the cellular forms of life. This precellular stage of development was, in turn, preceded by the slow evolution of organic substances (11). Can we assume then that precellular types of organisms still exist today? The answer to this question would be entirely conjectural, if we did not know of the existence of viruses, a fact which D. I. Ivanovskiy first discovered in 1892 (6, 7).

The following argument can be made in support of the proposition that viruses represent a type of precellular organisms.

Despite the advanced evolutionary stage of contemporary animal and plant forms, organisms can still be found that are structurally representative of all previously existing forms of life. These organisms range from the Rickettsiae, which stand just on the threshold of cellular organization, to the highest phanerogamous plants and mammals.

In giving rise to new species and classes of plants and animals, the process of evolutionary development did not destroy the previous forms of organisms. On the contrary, paleontological data confirm the fact that all the principal functional types of plants and animals, irrespective of the time when they originated, have their representatives even today. Since evolution proceeds not only by changes from higher to lower types of organisms, but by changes within the species themselves, and since present conditions favor the survival of the most varied types of cellular organisms, including the most primitive, it must necessarily be accepted that precellular forms are no exception to this rule. Consequently they inhabit the earth today in various forms.

Pathogenic viruses originate from the freely living precellular organisms which comprise a specialized type of obligate, intracellular parasite. It must be recognized that there are numerous and diverse precellular organisms which lead a saprophytic existence. One may assume that the study of viruses will lead to the future discovery of freely living ultramicroscopic organisms. However, it is not necessary to consider, as Zil'ber did, that "saprophytic viruses," or more exactly saprophytic precellular organisms, can only live in a protoplasmic medium. In this context, the term "saprophytic" is devoid of all meaning. Precellular organisms may have inhabited media which were replete with organic substances, i.e., the remains of dead animals, plants, or micro-organisms, since their organization as freely living forms was probably more complex than that of viruses. They may have possessed certain enzymatic systems, and ultimately, in the capacity of commensals or symbionts, they may have utilized the biochemical activities of cellular bacteria (3).

The conception which has been outlined above seemed to us to be most closely in accord with the requirements of materialistic biology, a field in which new frontiers in the theory of development have been opened by the works of I. V. Michurin, T. D. Lysenko, and O. B. Lepeshinskaya.

Viruses fill in the gap which existed in the evolutionary scale as it was known to biology prior to the discoveries of D. I. Ivanovskiy.

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We can not agree with Kalina, who not only rejects the possibility that precellular forms still exist today, but assumes, on the contrary, that the development of the earth has already proceeded beyond the point where existence of precellular forms is possible, and that all living beings have now reached a high stage of evolution. It is well known that such a conclusion does not agree with the actual facts. Kalina, in one way or another, associates the origin of all viruses with the filterable forms of bacteria. He writes: "Inasmuch as monocellular microorganisms have existed during every phase of the evolutionary history of life, the development of viruses from them may likewise have been going on during that entire time, and may have been completed just prior to our era. The disintegration of microorganisms within organisms into filterable forms arrested the development of the microorganisms. The phenomena of stabilization occurred at a very early stage -- the stage of precellular forms. The last step in this process was the loss of independent enzymatic systems and development of complete dependence on the metabolism of the host cells. This is the way that viruses were formed."

Evidently Kalina did not express his idea correctly when he wrote: "Monocellular organisms have existed during every phase of the evolutionary history of life."

It is clear to every materialist that the emergence of cells was preceded by the slow evolution of precellular living beings, and, consequently, that there was a long period in the evolutionary history of life when monocellular microorganisms did not exist. This was the period when the precellular, virus-like forms, which inhabited the biosphere of the earth and laid the ground for the evolutionary emergence of the simplest cells, flourished. The more primitive organisms are, the more adaptable they are and the more rapidly can they adjust to changing conditions of life. The bacteria that emerged in the early eras of life are omnipresent even today. They inhabit places where no other forms of life are able to exist.

A high degree of adaptability can also be ascribed to the precellular forms of life, and this in turn compels us to expect that they are widely dispersed in nature. We do not know the properties of contemporary free-living precellular forms, since discoveries in this field are still relegated to the future, but we can discuss the wide range of adaptability of phytopathogenic viruses. In this respect, viruses far exceed pathogenic bacteria. It is a well-known fact that intracellular, obligate parasitism involves a high degree of specialization on the part of the parasites. The host cycle in the case of obligate parasitism is extremely small, and often is limited to a few or even a single species of macroorganisms. Quite the contrary is true of phytopathogenic viruses, where the number of possible hosts is comparatively large. The virus of tobacco mosaic is capable of multiplying in the cells of 236 investigated species representing 33 families; the cucumber mosaic virus, in 191 species representing 40 families; the virus of tobacco necrosis, in 88 species belonging to 27 families; the alfalfa mosaic virus, in 92 species belonging to 28 families; etc.

The host cycle for the enumerated viruses is undoubtedly even higher than these figures indicate, since the experiments were carried out with only a limited number of species. This astounding capacity to adapt to the conditions of life prevailing in phylogenetically distinct species of plants attests to the enormous potential vitality of viruses and compels us to presuppose a similar potentiality in free-living precellular forms. The same conclusions may be reached from a study of the proven capacity of several phytopathogenic viruses to multiply within the organisms of certain insects.

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As far as the advancement of science is concerned, it is more useful to inquire into the existence of free living precellular forms and to try to discover instances of them than to shut off this avenue of approach and represent all the simplest forms of life merely as different manifestations of the more complex cellular forms. Naturally this does not mean, that organisms genetically related to the filterable forms of bacteria have not, due to our inadequate knowledge of the subject, been arbitrarily included among the viruses. It seems to us (a) that it would be inexpedient to impose limitations on the investigations of this matter and (b) that the more widely we utilize various approaches to the problem, the more rapidly we will be able to solve the general enigmas of virology.

A survey of all the multiform precellular organisms, represented by the phytopathogenic and zoopathogenic viruses, reveals their varying degrees of complexity. A number of viruses are composed only of nucleoproteids. These are the simplest forms of life known to science. Other viruses have lipoids as well as nucleoproteids in their composition. Apparently some of the viruses attain a relatively high heterogeneity and are composed of a still greater number of varied substances. This gradation of complexity in the organization of viruses reflects, to a certain degree, the course of the evolutionary process from living nucleoproteids to cellular beings. Notwithstanding the specialization of viruses as obligate parasites, we can still delimit the important role of nucleoproteids as essential components of living systems.

In a number of phytopathogenic and zoopathogenic viruses, the nucleoproteids consist of a single substance. Engels' famous definition of life (19, 20), which states that the simplest organisms are made up of living proteins, can be literally applied to these viruses. In this respect a need has arisen for more precise definitions of the commonly accepted concepts concerning the higher proteins. First of all it should be pointed out that life is a property of definite systems of living matter. Even the simplest of living substances, the nucleoproteid viruses, are known to be complex and heterogeneously organized. It is sufficient to say that their particle weight exceeds many million, and that besides the thousands of various amino-acid residues, each of which is equivalent to a macromolecule or organic substance, their composition includes ribonucleic acid, a complex chemical compound in which purine and pyrimidine bases, carbohydrates, and phosphoric acid are combined.

In addition the virus nucleoproteid is apparently combined with certain metals. It must be remembered that this structure is representative of the complex virus system after it has been isolated from the host organism. We can assume that the structure of the virus particle is even more complex during the period when it is vitally active in the protoplasm of the host, since it then contains water, electrolytes, and possibly a number of protoplasmic organic compounds with which it is combined, forming a kind of an elementary protoplasmic cell.

The results of X-ray analysis show the comparatively high degree of structural complexity of the virus micelle. Bernal (24) points out that X-ray pictures of the virus particles show them to be composed of separate protein blocks, regularly disposed in a three-dimensional arrangement. In his opinion, the structure of a virus micelle corresponds more closely to a protein crystal composed of a number of particles than to an individual protein particle. From what has been said, we can conclude that virus particles are not molecules, even though it is customary to call them molecules. For methodological purposes we must accept the fact that there are not, and can not be, any living molecules, since organisms can not be converted into molecules without losing the property of life or vitality.

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The application of the term "macromolecule" to the virus particle represents a misunderstanding which is based on the old metaphysical practice of considering only the quantitative side of phenomena. When matter changes from a molecular condition into a supramolecular condition, thereby forming living protein, a transition from a quantitative change to a change involving acquisition of a new quality takes place from the dialectical standpoint. While preserving a number of properties which are peculiar to molecules, i.e., the capacity to crystallize, the protein acquires a number of new properties, chief among them being those potential capacities the development of which causes the emergence of life.

Life is a property of a complex system composed of a protein body and a number of other substances and is characterized by a higher, supramolecular level of material development.

Not long ago we were supporting the idea that nucleoprotein viruses are proteins capable of transmitting infections. We assumed that the transfer by various means of certain nucleoproteins from the cells of one species of plant to those of another species could lead to the accumulation of alien proteins, acting as pathogenic viruses.

Recently we have been obliged to abandon this concept. Experiments conducted during the past few years show that no positive results are obtained by inoculating plants with alien proteins. The latest data on the biology of viruses also attest to the contradictions inherent in this hypothesis.

It must be noted that many virologists have adhered to the concept of viruses as chemical substances. Zil'ber (2) in 1946 wrote: "It must be pointed out that viruses are not living microorganisms, but rather high-molecular proteins. They do not develop every time in a diseased plant. They only reproduce in it. These proteins are in no way living infectious agents, although they may possess properties characteristic of them. The essential difference between the two types of infectious agents, bacteria and viruses, consists of the fact that bacteria multiply within the infected organism at their own expense, while the ultra-virus types or heavy proteins are reproduced at the expense of the organism." This quotation is mute evidence of the fact that its author has completely abandoned the concept of viruses as organisms. One can not help but be surprised therefore that in a recent article (5) Zilber remarked that he had always been, together with Ivanovskiy and Gumaleya, a consistent defender of this concept.

It was not by chance that at the 1950 meeting dedicated to the 30th anniversary of the death of D. I. Ivanovskiy (4, 12, 15) many virologists defended the concept of the living nature of viruses. The course of development of Michurinist biology has determined the methodology to be followed in the study of viruses and has given a new direction to the research done by a number of Soviet scientists.

Many significant arguments can be introduced in support of the view that viruses are precellular organisms.

Viruses, like all living beings, are capable of reproduction. Proliferating under the constant conditions of a culture medium, viruses persistently preserve the characteristics of their species, i.e., exhibit the capacity to inherit and transmit species characteristics. At the same time, viruses are extremely adaptable and, in response to specific variations in their environment, are susceptible to directed modification.

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The modification of viruses now being investigated with the use of contemporary research methods represents a process of species formation, and can be widely utilized as an argument in favor of T. D. Lysenko's treatment of the concept of species (10). The numerous strains of viruses described in literature, and obtained in the course of our experiments (16), are actually existing species, which are in antagonistic relationships to each other. In mixed infections, which have undergone a number of passages, some species of viruses replace related species. The invasion of a plant by any one species of virus tends to prevent the accumulation of other related viruses in it. It was established recently that antagonistic relationships can exist between some individual species of viruses, i.e., between the rugose mosaic virus of potatoes and the virus causing virulent tobacco etch [*Myzus persicae*] (21, 22). Such antagonisms between closely related species of viruses have been successfully utilized in obtaining living vaccines against certain human virus diseases. The biological nature of the action of these vaccines was correctly indicated by Lysenko. The use of living vaccines will undoubtedly be applied in the future to the growing of plants.

On the basis of the features enumerated above, we are able to acknowledge the fact that many characteristics typical for organisms are inherent in viruses. The determination of the type of metabolism peculiar to viruses will be of great significance in explaining their nature. The propagation of viruses, in itself, testifies to their high degree of biochemical activity. The use of radioactive isotopes permits us to conclude that virus particles carry on an intensive metabolism, using the substances of the cells which they inhabit. A number of data reported in the literature support the contention that at least part of the substances which enter into the structure of newly forming virus particles is low-molecular material similar to amino acids and polypeptides (24, 26).

In our own laboratories (17), we established the fact that the conditions which facilitate the accumulation of protein hydrolysis products in tobacco leaves stimulate the accumulation of a virus in them when they are subsequently subjected to conditions favorable to the synthesis of proteins. In control leaves maintained under normal conditions at all times the virus accumulated more slowly. It is interesting to note that the introduction of excessive amounts of nitrogen and phosphorus into the nutrient medium of plants, a condition which causes extreme suppression of their growth, not only does not impede the accumulation of a virus in them, but on the contrary accelerates it. This phenomenon is a manifestation of the capacity of the virus to exist independently of the host cell, i.e., the living mass of the virus increases in this case while no corresponding increase occurs in the living mass of the host.

When a host plant is deprived of its source of nitrogen, the proliferation of the virus is suppressed. Nevertheless, the virus continues to increase and gradually attains a high concentration (14) despite the dystrophic condition of the plant, which is accompanied by an over-all decline in vital functions and the initiation of hydrolytic processes. Finally a virus can increase, although slowly, in leaves which have been placed in a dark chamber at a temperature of 34-35° C, i.e., under conditions which normally destroy plant tissue.

It is difficult to imagine a similar increase in normal cell nucleoproteid under such conditions, but the virus nucleoproteids do increase in quantity, and, if a sufficiently virulent species of virus is used, attain a significant concentration in the course of a week. Consequently, viruses, although completely dependent on the metabolism of their hosts, have a biochemistry of their own. This is typical for their species and enables them to increase their own living mass under conditions which deprive their hosts of this possibility. The protein of the virus is differentiated from the proteins of the host by its

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potential chemical capacities, although in some instances it is capable of carrying individual antigen groups which are related to the antigens of the host. In these instances the virus seems to be marked in some way by those exchange reactions in the metabolism of the host, which evidently participate both in the synthesis of the proteins in the host and the formation of new virus particles.

It is interesting to note that a chemical difference apparently has been established between one of the pyrimidine bases entering into the composition of bacteriophage T₂ and the corresponding base of its bacterial host (29). This is the first indication of a specific chemistry of viruses which distinguishes them qualitatively from the chemistry of the host. This may also serve as a demonstration of the synthetic potentialities of viruses and their capacity for assimilation.

Although there has been very little study of the metabolism of viruses, and our knowledge of the physiological conditions attendant upon the reproduction of viruses is still completely inadequate, data exist which convince us that physiological metabolism takes place in viruses, just as it does in cellular organisms. The acceptance of this position is the acceptance of the living nature of viruses, since the first and principal manifestation of life is the process of physiological metabolism. If this process is absent, a protein body can not be considered alive even though it preserves its structure and viability.

Zil'ber does not agree with this position (5). He expresses surprise at my treatment of this question. Evidently he considers any interruption in the vital activity of viruses impossible. The resolution of this question is possible, in my opinion, under two conditions: (a) the availability of a scientifically valid criterion for the presence or absence of life, and (b) strict adherence to this criterion in the evaluation of the various phenomena observed in nature.

The criterion for the presence or absence of life elaborated by Engels (19, 20), which is in accord with the tenets of dialectical materialism, is the basis of Michurinist biology. Life is thought of as the physiological process or metabolism which is carried on by a protein substance in its relationship with the conditions of life. If, however, this relationship is disrupted, if the protein substance is isolated from the conditions of life for a period of time, and the physiological process or metabolism is suspended, can we then say that the substance exhibits life activity? Evidently not, inasmuch as there is no life process. Are we obliged, under these circumstances, to assume that there will be an immediate destruction of such a protein substance, that it will lose its physicochemical structure, or that it will be irreversibly denatured? Evidently not. Is it possible to restore the vital activity of such a protein substance by returning it rapidly enough to the conditions of life? We say yes, on the basis of known virological facts. The conditions necessary to support the life of viruses exist within the protoplasm of the host. Nevertheless, some viruses maintain their viability for extended periods in vitro.

It must be admitted, therefore, that the dependence of the physiological metabolism of viruses on the cells of the host has been exaggerated. Viruses can carry on a reduced metabolism in an aqueous solution. We can deprive a virus, i.e., tobacco mosaic virus, of water and gases, precipitate it in a crystalline form from a saturated solution of ammonium sulfate, or freeze it at very low temperatures, keeping it under these conditions for as long as 10 years, and still many of its particles will preserve their original state, and when introduced into the cells of a susceptible plant will regain their vitality. If we do not wish to depart from our accepted criterion for the presence or absence of life, we must admit that the vital activity of a virus is interrupted when it is isolated from the conditions of life. The virus temporarily loses its

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vitality, while preserving its physicochemical structure and viability for a certain period of time. Naturally such interruptions can not exceed specific time limits. Many viruses are destroyed very rapidly in vitro, and even the most stable viruses are irreversibly denatured with the passing of time. Without metabolism any protein, even virus protein, is doomed to destruction, but the rapidity with which the irreversible denaturation and destruction of proteins takes place depends on the characteristics of their physicochemical structure, and varies within wide limits.

We do not know whether such an interruption of vital activity is peculiar to the nucleoproteids of viruses only, but it is thought that similar interruptions are possible in the case of certain bacteria and their spores. Lembke (26) discovered that a part of the bacteria which had been killed by a high temperature could be restored to life if they were subjected to a pressure of 200 atmospheres, or if a 1/100 M concentration of phenol or glycine was established in the culture liquid. Evidently Boshyan also observed the restoration of bacteria to vital activity (1). Under certain unfavorable conditions, it is possible for the spores of bacteria to lose their metabolism and vital activity, and thus undergo a modification which enables them to preserve their viability for unusually long periods of time.

An irreversibly denatured virus particle is like a dead body. A normal particle which has lost its vitality, but still maintains its physicochemical structure and viability, can not, however, be called a dead body. We are of the opinion that interruption of life activity is a normal property of viruses which plays a significant role in their capacity to adapt themselves to environmental conditions, and must be considered a species characteristic just as anabiosis is regarded as a biological characteristic typical for many species of plants and animals.

A state of physiological quiescence in a complex organism, which possesses large internal resources, merely represents an extreme lowering of the rate of metabolism, while in the case of virus particles, which do not have such resources because of their comparatively homogeneous composition, this condition represents a complete cessation of vital activity. The biological roles of both of these phenomena are evidently identical, and each of them serves to maintain the species. The problem of how nucleoprotein viruses reproduce is also very interesting. Zil'ber, for example (5), is of the opinion that viruses reproduce in the same way as cells. We assume that the reproduction of the virus micelles does not proceed in the same way as that of cells, but does represent a process of neoformation. On this basis we submit that the offspring particles for all practical purposes do not contain any of the substance of the parent particles. Evidence supporting such a possibility can be found in various literature sources (24). It has been shown, for example, with the aid of phosphorus tracer atoms, that the substance of the parent particles of bacteriophages is not actually inherited by the offspring particles.

In the different experiments no regular transfer from the parent to the offspring particles was observed, and the insignificant amounts of phosphorus of parent origin which were detected in the bacteriophage offspring varied by as much as 400 percent. This suggests that in this case the tracer atoms were not directly inherited by the offspring particles, but were acquired by them from the decomposition products of the parent particles. This is supported by the fact that the inactivation of a part of the parent particles by ultraviolet rays or X-rays does not diminish the amounts of atoms of parent origin in the offspring, and also by the fact that in a common mass culture tracer phosphorus originating in parent particles of T₆ bacteriophage is transferred to the offspring of the unrelated bacteriophage T₇.

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How do we envision the neoformation of virus particles? We suppose that the parent particles which are introduced into cells at the time of infection represent centers of biochemical reactions, and, as a result of these reactions, new virus particles are synthesized from the amino acid, polypeptide, and other substances of the cell. The physicochemical connections which exist between the parent particles and offspring particles being synthesized during this process must be regarded as a temporary union, since the offspring particles are formed from the substances of the medium and not from the substances of the parent particles. In this sense, the offspring particles represent a true neoformation.

In our laboratories it was established in experiments with tobacco mosaic virus that aggregates which were evidently paracrystalline in structure were formed in the individual cells within 6 hours after infection. This supports the idea that the accumulation of a virus is brought about by the synthesizing activity of the particles introduced at the time of infection, around which colonies of the same type of virus are formed, and that these colonies are drawn together, to a degree which depends on the magnitude of the intramolecular forces, with the result that paracrystals are formed. Judging by recent investigations (22), the viruses of the polyhedron diseases of insects also proliferate locally. The polyhedron is a protein mass within which the formation of the virus particles occurs.

Another circumstance which obliges us to admit that there is a difference between the reproduction of cells and that of virus particles is the fact that, in view of the occurrence of cyclosis, we are not able to speak of the constant topography of the various parts of protoplasm. On the other hand, as far as we can determine by means of X-ray pictures, the virus particle is a solid structure within which any internal movement similar to cyclosis can not be suspected. This bears witness to the fact that the newly forming virus particles are topographically delimited from the substance of the parent particle and that they must, by reason of the origin of the substances entering into their composition, i.e., of the fact that these substances originate in the medium, represent neoformations. In regard to the external morphological picture of the reproduction of a virus, the fact that it may be connected with linear growth of the aggregates and an increase in their diameters suggests that it might imitate the type of reproduction found among cells (27, 23). Finally, we are very much interested in Talmud's idea (18) concerning the possibility that the new particle, during its synthesis, passes through several rudimentary stages within a space formed by a protein globule of the parent particle. The micrographic investigation of the virus of polyhedron disease, which is reported in the work of Bergold (23), is of great interest in this respect.

To conclude, one of the qualitative differences between a nucleoprotein virus and a cell is the fact that the assimilation products being synthesized by the cell enter into the internal composition of its structure, where they facilitate growth and development which results in the cleavage of the body of the cell, while the assimilation products of a virus particle do not enter into its internal composition, but are merely temporarily affixed to the external surface of the body of the parent particle by chemical bonds. In connection with bacterial cells it must be assumed that only one of the two individual cells formed by cleavage is the offspring. In this case we are inclined to think that a certain degree of polarity is present in the relationship between the parent and offspring cells. In the particles of nucleoprotein viruses this polarity reaches the limit at which the substance of the offspring particle, with the exception of individual atom groupings, contains practically none of the substance of the parent particle.

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It does not follow from this that the parent particle is inactive during the reproductive period. It is undoubtedly engaged in a very active metabolism, thus giving rise to the intensive chemical reactions that occur. Its atomic composition, especially in the side chains, changes constantly. However, I repeat that basically the substance of the parent particle is not inherited by the offspring particle. The qualitatively distinct cellular type of reproduction is probably an evolutionary characteristic acquired as a result of the increased degree of complexity in the organization of living matter.

Naturally, at the present stage in the development of virology, we can not arrive at a single interpretation encompassing all the complex problems which touch upon the nature and origin of viruses. The problem is still subject to discussion, and consideration of it in the literature and at conferences is absolutely necessary. The increased interest in this subject evoked by the discussion of the works of O. B. Lepeshinskaya will make possible further progress in the investigation of viruses, the simplest precellular forms of living matter.

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