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INFECTIOUSNESS OF VIRAL DEOXYNUCLEIC ACIDS

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by

I. P. Kok

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13. ABSTRACT

In DNA- containing viruses, as in RNA-containing ones, the nucleic acid possesses the complete genetic information necessary for synthesis of enzymes of the vegetative stage and construction of the specific amino acid sequence in the protein and nucleotides in the nucleic acid of the viral progeny.

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INFECTIOUSNESS OF VIRAL DEOXYNUCLEIC ACIDS

The genetic importance of nucleic acid was revealed in the middle of our century as the result of the well known research of the active beginning in genetic transformation, begun in 1944 by Avery, MacLeod, and McCarty, in revealing the role of components of viral particles in the reproduction of T_2 phage, carried out by Hershey and Chase in 1952, and, finally, the discovery of the infectiousness of RNA of tobacco mosaic virus by Gehrer and Schramm in Germany and Fraenkel-Conrat in the U.S.A. in 1956.

At the present time, rather extensive materials have already been accumulated on the infectiousness of viral nucleic acids. There are reviews in the literature devoted to the biological and physicochemical properties of viral RNA, and also summaries of work on DNA, in which there is superficial mention of the infectiousness of nucleic acids of DNA-containing viruses /1, 30, 37, 61, 62, 80, 92, 108, 110, 134/.

Infectious DNA has been studied less thoroughly than transforming DNA since it is difficult to isolate DNA in the undamaged state. This is especially true of DNA of large phages, in which the molecular weight of nucleic acid exceeds 100 million. While in transforming cells can possess a portion of the bacterial DNA molecule, in the majority of cases infectiousness is associated with the integrity of the full DNA molecule. The infectiousness of DNA-an index of its nativeness - is successfully used in research on various aspects of DNA structure and its functions.

The present review is an attempt to systematize our knowledge on the infectiousness of DNA of animal and bacterial viruses. In the table characteristics are given of

Characteristics of Viruses and their DNA

Viruses	Form of Viruses	Dimensions of Viruses & Heads of Phages, m μ	DNA Content %	Molecular Wt. DNA $\times 10^{-6}$	No. of Helices in DNA	Form of DNA in Virus particles
Smallpox Group Smallpox vaccine Rabbit smallpox Fibroma & Microsome of Rabbits	Parallelo-piped	220×220×260	3—8	80—150	2 (and 1)	—
Herpes Group Herpes Simplex, Pseudorabies	Capsid-icosahedron	Capsid-100 in envelope 180-200	4	68 50	2	—
Macrophages *E. coli*: T_2, T_4; *B. subtilis*: SP 3; SP 8; SP 82; SPO 1; SP 50; φ-1; φ-25	Head & Tail	95×65	40—50	100—130	2	Not successfully isolated in circular form (T_2)
Adenoviruses	Icosahedral	60—85	10—30	15—23	2	Circular (Types- 2, 7, 12)
Medium Phages *E. coli*: T_1, T_7; λ; *B. subtilis*: φ-29; *H. influenzae*: HP 1, S 2; mycophage D 29	Head & Tail	45—55	40—50	25—33	2	Linear form (λ)
Papova Grp. Polyoma, Papilloma SV40	Icosahedral	40—60	8—16	3—8	2	Circular
Viruses of Nuclear Polyhedrosis (Mulberry, Oak, Silkworm)	Rodshaped	280×40	12—15	1,7—3	2	Circular
Small phages *B. subtilis*: 2C *E. coli*: φX174; M_{20}; *l*φ-1; *l*φ-3; *l*φ-7; *l*φ-9; *d*φ-3; *d*φ-4; *d*φ-5	—	—	—	2	2	—
M_{13}; fd	Icosahedral	25	25	1,3—1,7	1	Circular
	Filamentous	800×6—8	10—12	1,3—1,7	1	Circular

viruses and their DNAs, whose infectiousness was studied in various laboratories. The numbers expressing the DNA content in virus particles and the molecular weight of the DNA reflect only the order of magnitude of these number. Moreover, these numbers were not determined on all viruses of each group. The DNA content in virus particles depends on their degree of purity and the presence among them of incomplete or empty particles, in which there is little or no DNA. The molecular weight of DNA, especially in large molecules is difficult to determine. For greater precision, one characteristically uses very different methods, which do not always give compatible results. Disruption of molecules at the time of isolation, and also their aggregation in several cases increases the difficulty of obtaining precise figures, not to mention the limitations of the methods themselves.

Scheme of Research on Infectiousness and Oncogenicity of Viral DNAs

Before reporting the results of the study of infectiousness and oncogenicity of various DNA's of animal and bacterial viruses, it is expedient briefly to decide on an overall scheme of all experimental work in this field. Detection of infectiousness in preparations of viral DNA and of evidence that DNA is the active principle in these preparations, can be broken down into a series of steps.

Deproteinisation of Initial Viral Material. DNA preparations are isolated from suspensions of virus particles which are purified to one extent or another or from cells infected with viruses. The phenol or phenol-detergent method of DNA extraction are usually used. A summary of these methods and series of reviews /30, 108, 110, 134/ makes it possible not to discontinue them.

Chemical and Physicochemical Characteristics of DNA Preparations Obtained necessary for identification of isolated substances, determination of impurities in it, characteristics of DNA structure (double stranded, single stranded, intact, disrupted, etc.). In a series of works the determination of activity precedes fractionation of the preparation.

Detection of Virus or Viral Infection, for example a cytopathic effect or malignant transformation of cells after addition of DNA preparations, and identification of virus particles formed by very different virological methods.

Research on the Nature of Infectious Origin in DNA preparations including the usual treatment of these preparations

with DNAase, RNAase (free from contamination with DNAase), proteases, antiviral sera, heated at a temperature which inactivates whole virus (DNA is more stable). If the DNA is infectious, only DNAase, even in very low doses, inactivates the preparations. In order to completely exclude the possibility of an effect of viral impurities in the preparations, the infectiousness of DNA was tested on cells which were not infected with intact virus.

Elimination of Activation of Latent Viruses. In order to control the possibility of activation by DNA preparations of latent viruses found integrated with the host genome, DNA was added to cells isolated from healthy cells and tissues; moreover, cells or whole organisms not containing latent viruses were used as recipients.

Not all works utilized all possible evidence of DNA activity. In revealing the infectiousness of DNA; however, one or another of the methods of identification of the active principle were used in all of them. If viruses studies were arranged in the table in order of decreasing size, the further presentation would first look at mammalian viruses, then - insect, and then - bacterial, while in each group it will be possible to keep chronological succession in the review of detection of infectiousness of viral DNA.

Research on Infectiousness and Oncogenicity of DNA of Animal Viruses (Mammalian and Insect)

Viruses of the Papova Group

This group includes polyoma, shope papilloma, cattle papilloma, human papilloma, SV_{40} vacuolizing virus, etc. These are formed in the cell nuclei, where crystal-like accumulations form. These small viruses possess infection and oncogenic properties. Upon infection of cells of even one clone, they cause either the infectious process and synthesis of virus particles, or transformation of cells into tumors. It is possible that the oncogenic properties (integration by the cell genome) of these viruses are associated with the fact that their DNA has known portions of nucleotide sequence which is complimentary to the nucleotide sequence of the DNA of the cell-hosts. This was found, for example, for polyoma virus DNA /40, 153/ and Shope papilloma /153/.

The virus from which infectious DNA was first successfully isolated was the SE polyoma virus. This occurred

in 1959 in Bendich's laboratory in the U.S.A. /69/. DNA preparations were extracted by the phenol method from unpurified virus suspension, and also from mouse embryo tissue cultures infected with the virus. DNA solutions at a concentration of 3-30 μg/ml possessed a cytopathogenic effect and caused reproduction of specific infectious virus. DNAase treatment destroyed the activity of the preparation, RNAase had no effect on them. Nucleases did not act on the virus.

The infectiousness of polyoma virus DNA was then demonstrated in a series of experiments. A method was introduced for quantitative determination of the activity of DNA preparations according to the appearance of infectious centers on a monolayer of cells in culture, which made the work easier. After addition of the DNA preparations, virus was detected by the appearance of infectious centers on monolayer cells, /53, 149, 151/, formation of specific virus incorporation and antibodies to the virus [120] or development of tumors in the hosts [28, 121] and transformation of cells in culture /55, 88/. All of these were characteristic for polyoma virus.

The infectiousness and oncogenicity of DNA was shown not only for polyoma virus of the usual strain, but also for a variance of the virus giving small infecticus centers [55], and for strain No. 13 of the virus /28/. Well reproduced biological effect, associated with reproduction of virus after addition of DNA preparations from polyoma virus, made it possible to study the time of appearance of viral DNA in an infected cell /84/, the interaction of the virus genum and the genum of the cell upon its infection /53/, and also the nature of the infectious material, the connection between DNA structure and its biological function, (cf below).

In 1960 Ito extracted DNA from tissues of papilloma and purified virus of shope papilloma, solutions of which were rapidly injected in the skin of domesticated and wild rabbits /17, 99, 101/. After 2-6 weeks a change in the cells at the site of the injection developed, which was also observed upon injection of intact virus. The results of the experiment of Ito et al were confirmed by other investigators /93/. From the cells changed under the effect of DNA, typical virus particles were isolated /60/. In several cases transformation to carcinomas, developing from injection of papilloma virus or DNA, from which infectious (oncogenic) DNA could sometimes be isolated in turn, /17, 102/. Immune antiviral serum or RNAase had no effect on the activity of shope papilloma virus DNA preparations; DNAase did not remove their effect either.

As has recently appeared, bovine papilloma virus can be detected by specific changes (transformation) of cultures of cells of calf embryo skin or mouse embryos, and also by the appearance of tumors upon injection into newborn hamsters. This permitted testing of biological activity of DNA preparations from virus particles. Viral DNA in this case also possessed oncogenicity, since it led to transformation of cells in vitro and to the development of fibromas and fibrosarcomas in hamsters over the course of 6 - 10 months in 50% of the recipients, /52/. Neither DNA from healthy tissues, nor viral DNA treated with DNAase were active.

In 1962 Gerber isolated DNA from concentrated suspension of SV_{40} virus and other authors did so from cultures of monkey kidney cells infected with this virus; this DNA caused synthesis of virus and cytopathic effect upon infection of monkey cells sensitive to the virus /51, 82/. The synthesis of virus in cells in this case was not the result of activation of latent virus, since the DNA from healthy cells did not cause its formation, as in the preceeding experiments with DNA from bovine papilloma virus. The virus appearing after addition of DNA to the cells was identical to the original in all properties.

Complete exclusion of the possibility of activation of latent SV_{40} virus upon addition of DNA was observed in experiments of Swedish authors, who in 1963 showed the sensitivity to viral DNA of cells of mouse embryos, bovine and pig kidneys and lungs in tissue culture, which are not infected with virus and do not contain it in the latent state /68/. Upon addition of viral DNA, cells of these cultures undergo transformation, which is easy to detect. A similar method of determining SV_{40} activity by transformation of fibroblasts, which is also used for detection of polyoma virus, made it possible to show the activity of DNA by the acceleration of the growth of cells, appearance of large colonies, whose cells produced tumors in hosts /49/. SV_{40} DNA also induces synthesis of SV_{40} T-antigen in cells/50/. Thus, in all viruses tested of the papova group DNA proved to be infectious and oncogenic. This was shown both in mammalian cells sensitive to whole virus, and in those resistant to it. DNA activity was removed by DNAase, but not by other enzymes or virus antibodies.

It should be noted that the biologically active DNA was extracted only from virus suspension and from cells and

tissues from which it was possible to obtain free virus particles, but not from those in which complete association of viral and cell genomes had occurred. From polyoma cells not transformed by the virus, not liberating virus, neither oncogenic nor infectious DNA was isolated /149/. This concerns carcinoma cells, caused by shope papilloma virus [93], and concerns transplanted fibrosarcoma and ependymoma, induced by SV_{40} virus /83, 130/.

Viruses of the Smallpox Group

This group includes the largest known viruses: smallpox vaccine virus, smallpox virus, fibroma and myxoma of rabbits and others. Despite many attempts over a long period of time infectious DNA has not been obtained from these viruses. However, as has been shown by research of Kilham, Joklik et al, in 1950-1960, the Berry-Dedrick, already observed 30 years ago, may be explained by the activity of DNA of heated myxoma firus [92]. This phenomenon, as is known, is included in the restoration of infectious properties of heat inactivated myxoma viruses upon injection into rabbits in a mixture with intact fibroma virus. Probably, the DNA of heated virus, injected together with the fibroma virus into cells, also reproduces virus particles. Free myxoma DNA virus was not obtained in the infectious state.

The first attempt to isolate infectious DNA from smallpox vaccine virus also ended unsuccessfully /123, 139/. As explained /123, 124/, phenol with deoxycholate in the cold freeze 10-20% of the DNA from purified virus, while only single stranded. DNA is isolated by this method from certain deficient virus particles which sediment more slowly during centrifugation, and which are also distinguishable from heavy ones by electron microscopy. The basic mass of the viral DNA is double helical and is found in the heavy particles. It can be obtained from a virus suspension by treatment with mercaptoethanol and pronase - a protease with a wide spectrum of activity, obtained from streptomyces griseus.

Neither double helical nor single helical kept DNA in these experiments were infectious. The use of hot phenol or phenol with dodecylsulfate for the infection of DNA from the virus or from cells infected with virus also did not lead to liberation of active DNA /58/.

Infectious DNA was only obtained from viruses of the smallpox group in 1964 in two laboratories: Abel and Trautner in Germany and Babbar in India. In the experiments of these authors, DNA from smallpox virus or smallpox vaccine virus

caused the formation of pustules (pox) in chick embryo cells spontaneously [38] of only after controlled passages through choriallantoic membrane (CAM) /41/. In the latter case upon addition of only DNA isolated in CAM no pustules formed even when obtaining DNA from a virus suspension with high titer (0.5 . 10^{12} PSU/ml). In experiments by Abel and Trautner DNA was liberated from the virus with the help of enzymes (cf below), and in Babbar's experiments, by the phenol method, the details of which we do not describe.

Virus of Herpes Group

This group includes herpes simplex virus, pseudorabies virus and other. In our laboratory, I. P. Stasevskoy has undertaken to attempt to isolate infectious DNA from herpes virus. DNA was isolated 48 hours after infection with virus from mouse brain by the phenol-detergent method. In all four DNA preparations were isolated from brain tissue, and 0.03 ml of this suspension contained 10^4 LD_{50} of virus. About 20-80 µg of DNA preparation was injected in mice intracerebrally (in all 52 mouse-recipients were used). In not one case was the development of herpetic encephalitis observed. Control mice, which were injected with a suspension of brain infected with virus, all died from encephalitis. DNA was not active when tested in a culture of A-1 cells either: no cytopathic effect was noted. There have been no successful attempts to isolate viral DNA from culture fluids containing virus or from pseudorabies virus (Bucharest strain) precipitated by centrifugation /103/. Extracts with DNA were added to chick embryo cell cultures.

Thus, infectious DNA has not as yet been isolated from viruses of this group. It is probable that the titers of the original viruses were insufficient for good yield of viral DNA, and was too little for infection of the cells.

Adenoviruses

As is known, there are about 30 types of viruses in the adenovirus group - both infectious and oncogenic (Types 7, 12, 18). According to the data of Green et al, the nucleotide composition of DNA from tumofacient adenoviruses differs from the composition of infectious DNA with lower DC content, which more closely approximates the composition of mammalian DNA. The nucleotide sequence in DNA of viruses of the types 12 and 18 is rather close, but between the DNA of these viruses and of viruses types 2 and 4 there is less relationship. Infectious DNA was isolated from three types of adenoviruses in

1961 in the laboratory of Portocala in Rumania /125/. The culture fluid from amniotic cells infected with adenovirus, served as material for extraction of DNA. Biological effect of DNA preparations was revealed in the cytopathic effect. The activity of the preparations was supressed by DNAase, but not RNAase. Identification of the virus obtained after addition of DNA to the cells showed that according to serological properties it belongs to adenovirus type 3.

During reproduction of adenovirus type 3 in mice - the carriers of Ehrlich ascites carcinoma, the Rumanian investigators obtained strains of the virus with new properties: AE_1 and AE_2. DNA extracted from these viruses caused a cytopathic effect upon infection of cell cultures. Viruses formed after addition of DNA did not differ from the original strains of AE_1 and AE_2 in serological properties /131/.

Thus, the properties of adenovirus which change during its propagation in ascites cancer cells, were transmitted to later generations of virus particles with the help of viral DNA. AE_2 virus was shown to be carcinogenic. This causes the formation of tumors when injected into hamsters. DNA preparations extracted from these tumors possess even greater carcinogenicity than the virus itself, /132/ . The authors of these works assume that AE_2 virus is a harbor of adenovirus type 3 and a hypothetical virus which causes Ehrlich carcinoma, and that the AE_2 virus transforms normal cells to tumorous ones as is observed in viruses of the papova group.

Insect Viruses

Insect viruses belong both to the RNA and to the DNA types. The infectiousness of phenol preparations of RNA of virus particles was studied only in the case of the virus of cytoplasmic polyhedrosis of Dasychira (Dasychira pudibunda L.) [112]. The biological activity of this DNA, observed upon injection of RNA preparations into gypsy moths (Lymantria dispar L.), also inoculated with whole virus, disappeared after 3 hours of incubation at 37°C, and also at 50°C for 10 minutes upon addition of RNAase which does not affect the intact virus.

Of all the remaining viruses affecting insects, the infectiousness of the nucleic acids has been determined in the group of DNA-containing viruses of nuclear polyhedrosis of Lapidoptera: mulberry and oak silkworms. Polyhedral proteins (approximately 95%) and virus particles (approximately 5%) enter into the composition of nuclear polyhedrons -

crystalline bodies of inclusions having dimensions 0.5 -15 μ and forming in the cell nuclei. Among the small number of other substances in the polyhedrons are DNA, detected in them by L. M. Tarasevich in 1946 /32/ and which have been studied in several laboratories, among them ours /25/. The origin and function of this polyhedral RNA is unclear; not more than 1% is found in polyhedrons, and sometimes it is not detected at all.

The DNA of nuclear polyhedrosis virus of mulberry silkworm is double helical /25, 119/ , probably circular /20, 35/ and has a low molecular weight: 1.7-2.2 million /25, 119/ or somewhat higher, approximately 3-5 million /36/.

Nuclear polyhedrosis virus of the oak silkworm served as the first subject for study of the infectiousness of DNA among all the animal and bacterial viruses. It was on this in 1954 that S. M. Gershenzon demonstrated the possibility of reconstruction of virus from nucleic acid and protein /2/ . Both of these components were separately not infectious for silkworm larvae. If this solution of DNA extracted by the salt method from polyhedrons and the polyhedral proteins were mixed in various volumes and incubated together for 7 hours, and then injected into insects, typical virus infection, readily diagnosed by the appearance of polyhedrons in the cell nuclei, appeared in the recipients.

In 1958 at the Fourth International Biochemical Congress in Vienna, Bergold presented the results of his experiments in the study of biological activity of phenol preparations of DNA from purified nuclear polyhedrosis virus of mulberry silkworm /47, 48/. Infectiousness of DNA was not detected either in his experiments or in the research of Chinese authors who tested the activity of 6 DNA preparations from polyhedrons /81/.

The first report of infectiousness of DNA preparations from nuclear polyhedrosis virus were made at the First All Union Biochemical Conference /26/. It was shown that DNA preparations extracted not from purified virus, but from the tissues of caterpillars infected with the virus, caused the development of nuclear polyhedrosis in larvae of the mulberry silkworm. Further research has established that the fundamental infectious principle in these first preparations of DNA was virus specific RNA (cf below), and the low DNA activity was explained by disruption of its structure at the time of extraction /21, 35/.

Infectious DNA was successfully obtained by the use of RNAase treatments of tissue homogenates and careful extraction of the preparations by the phenol-detergent method /7, 18, 21, 22/. The molecular weight of these preparations, representing DNA of infected cells with addition of DNA virus, was several million. Disruption of the DNA structure led to a reduction of biological activity of the preparations, expressed in per cent causing polyhedrosis in recipients, from 58 to 37 /31, 35/. Complete disruption of the DNA by heating for one hour at 100°C destroyed the activity of the preparations. DNA from healthy insects, despite data of other authors /154/, did not cause nuclear polyhedrosis in a single case against a background of viro, average or high polyhedrosis, spontaneously caused in recipients as a result of activation of latent virus /20/. The activity of infectious DNA preparations was reduced exponentially by dilution /7/. Upon storage of DNA under asceptic conditions at 4 or 37°C its activity fell slowly, still remaining at a rather high level after 16-24 hours. Even at very low concentrations (0.1-5 µg/ml) DNAase reduced or completely destroyed the infectiousness of the preparations /20/. Heating the preparations at 87° for 15 minutes, which inactivates whole virus, did not noticeably reduce the activity of the DNA preparations /7/. The infectious DNA had a wider circle of affected hosts than the intact virus: it caused formation of polyhedrons in cabbage butterfly /6, 20/ - and insects belonging to Lepidoptera, which is not sensitive to the virus.

The biological activity of these preparations is explained by the presence of infectious viral DNA. It expressly causes infection, and not activation of latent virus, since the recipients in the experiment were larvae of the mulberry silkwork, in which the addition of various substances does not cause activation of latent virus; DNA caused polyhedrosis in cabbage butterfly, in which there is no latent virus, and, finally, DNA of healthy insects was not active.

The disruption of infectious DNA in experiments with preparations of DNA from infected insects was confirmed by the results of research on the biological activity of DNA of polyhedrons obtained with the help of alkaline phenol [25], and also from purified virus particles /25, 119/. The results of our experiments, partially published, showed that acid phenol did not liberate DNA from polyhedrons, recovering only polyhedral RNA in aqueous solution. Upon treatment of these polyhedrants with phenol at pH 8.2-8.6 both nucleic acids are liberated, while the infectiousness of their mixture is suppressed by the action of DNAase, but not RNAase. DNA from purified virus is readily destroyed

by making the phenol alkaline after treatment of the virus of pronase.

DNA preparations from insects stricken with polyhedrosis and from polyhedrons, which were infectious in our experiments in all insects, did not cause formation of polyhedrons in tissue cultures of mulberry silkworms /29/. In all (together with the experiments of which the results have been published) 15 series of such experiments were conducted. The appearance of polyhedrons in the nuclei of gonad or hemolymph cells was not noted either upon addition of DNA or upon addition of purified virus or in polyhedrons washed from hemolymphs. It is not clear whether in general nucleic acid or viruses penetrate into the cells under culture conditions. They are infected only by diluted infectious hemolymphs containing both polyhedrons and also virus particles and, probably, some other factor promoting infection of the cells in cultures.

Research on Infectiousness of DNA of Bacterial Viruses (Phages)

Small Phages of Intestinal Bacilli

After discovery of the infectiousness of polyoma virus DNA, ϕX174 phage DNA, which is infectious for protoplasts, was isolated in the Sinheimer's laboratory in 1960, and the interaction of the DNA and cells were studied, /90/. Infectious DNA has also been isolated in other laboratories from this phage /11, 74, 95, 136, 150/ and from its mutant /34/. DNA preparations from a suspension of phage particles was infectious not only for protoplasts and spheroplasts of E. coli strains sensitive to the phage, but also to other strains of intestinal bacilli and related bacteria not infected by intact phage. Moreover, bacteria without membrane in general were infected only by DNA preparations, and not by whole phage particles, which immediately excluded the possibility of activity of phage contaminants in the DNA preparations. Among the phage particles only the heavy ones are complete (114 f); and these particles in the DNA extracted from them were infections, while the more slowly sedimenting particles (70 f) were not active and their DNA was also not infectious /74/.

DNA preparations were also successfully extracted from bacteria infected with phage, and produced typical virus infection /98/. The questions of infectiousness of replicative DNA of this phage, detected in infected cells, will be considered below.

The detailed study of infectiousness, physicochemical properties of ϕX174 phage DNA, and the conditions of its penetration into cells has permitted the use of DNA of this phage in a series of studies as a standard for the comparison of properties of other infectious nucleic acids /12, 34, 144/, for testing the effect of various chemical agents on nucleic acids, for example carcinogens /97/, for studying the process of reactivation of DNA after its irradiation with ultraviolet light /129/. Signs of infectiousness of ϕX174 phage DNA have served as an indicator in determining the molecular weight of this DNA by radiosensitivity /85/and have also permitted investigation of the formation and method of replication of nucleic acid during synthesis of ϕX174 phage in the cell /67/.

Infectious DNA preparations have also been extracted from several other small related phages of icosahedral form. The infectiousness of M_{20} phage DNA was demonstrated in Hofschneider's laboratory /96/. B. N. Il'yashenko et al have isolated infectious DNA from phages recently isolated from lakes near Moscow, such as 1ϕ -1, 1ϕ -3, 1ϕ -7, 1ϕ -9, dϕ -3, 3ϕ -4, and dϕ -5 /16/. DNA from 1ϕ -7 phage has been studied in greater detail. It has been demonstrated that upon its addition to spheroplasts of bacteria sensitive and nonsensitive to whole phage, synthesis of phage particles occurs /12-14/. Infectious DNA of this phage has been used for study of the effect of ultraviolet light on DNA and protection from this radiation/8/.

Active, infectious DNA has also been obtained from filamentous phages. The isolation of M_{13} phage (as well as the spherical phage M_{20}) was begun with the observation of infectiousness of its DNA. The phage was isolated from sterile smears formed during infection of spheroplasts of the male strain E. coli F^+ (which only infect filamentous phages) by phenol preparations of nucleic acids from cell lysates treated with samples of stagnant water /96/. DNA has also been isolated from another filamentous phage - fd- discovered in Hoffmann-Berling's laboratory. Although the intact phage infected only whole E. coli F^+ cells, DNA preparations after deproteinization with phenol infected spheroplasts of female E. coli F^- cells /94/. Intact filamentous phages, like other phages in general, do not infect spheroplasts.

Large and Medium Phages of Intestinal Bacilli

All of these phages which served as subjects for isolating DNA and testing its infectiousness cause the usual

infectious process in cells. Only λ phage belongs to the temperate ones, capable of uniting its genome with the genome of the host.

After Hershey and Chase showed in 1952 that the DNA, (and not the protein coat), is injected into the bacteria during phage infection, and infectious RNA was isolated from TMV in 1956, a series of investigations were carried out on the possibility of obtaining infectious DNA from large T-even phages. It was shown that T-even phages treated with urea infect the cells of bacteria without membranes, and not intact cells. However, these preparations were sensitive to the action of trypsin. As has been explained, the active principle in these preparations (π factor) is the damaged phage particle and not the DNA. /106, 110/. It may very well be that the unsuccessful attempts to isolate infectious DNA from T_2 phage particles by the phenol method failed because of the difficulties in obtaining linear DNA molecules in an undamaged state /10, 115/. The biological activity of DNA preparations isolated from T_4 phage has been demonstrated as yet only in genetic recombination between purified phage DNA and phage treated with urea during infection of spheroplasts /30/. DNAase treatment of the DNA preparations, as usual, reduced their activity, and trypsin has no effect on them. This phenomenon has been termed "phage transformation". It occurs upon incorporation of fragments of DNA isolated from the phage into DNA genetically distinct from the phage.

While there are phages of the T-series from which infectious DNA was successfully isolated after unsuccessful attempts /10/, it has only been isolated from T_1 and T_7 phage. In research by Evans et al, a preliminary report which appeared in 1962 has demonstrated the infectiousness of DNA from T_1 phage /56/. Upon comparison of its activity in infectiousness with DNA from λ phage it was revealed that T_1 phage DNA is 10 times less active and less stable upon storage. Infectious DNA was also obtained from T_7 phage particles in the same laboratory /57/.

The most infectious DNA from small phages has been studied in φX174 phage, and from average phages, from phage λ. Even in 1960 in Kiser's laboratory /106/ biological activity was detected in DNA isolated from one of the variants of λ phage - the defective phage λ dg: with its help one can transfer portions of the DNA of a cell of one host to the DNA of another, auxotrophic in Gal (the phenomenon of transduction). This activity appeared only in the presence of

"helper" - phage, which causes the phenomenon of competence in the bacteria-recipients. In Kiser's experiments λ dg phage DNA penetrating into the cell was practically entirely incorporated in the genome of the bacteria, transforming it to prototrophic (GAL+) and lysogenic for λ dg phage. In subsequent work Kiser showed /105/ the infectiousness of DNA of this λ phage in the same system with "helper". The detection of reproduction of λ phage here was carried out on strains of bacteria-indicators, which are not sensitive to progeny of "helper"-phages. Kiser's method was then used in further research on the structure of infectious phage DNA (cf below).

The usual infection of spheroplasts of E. coli with the help of λ phage DNA was first established in Evans' laboratory in 1961. In this case DNA preparations were isolated from suspensions of virus particles, and maintained its infectious properties for at least three days /115/. The fact of the infectiousness of λ phage DNA for spheroplasts was confirmed recently by other investigators /5, 13/, and it has been shown, in addition to previous demonstrations of the infectiousness of DNA alone, that antiphage serum does not affect the activity of phage DNA. DNA isolated from cells lysogenic for λ phage, was not infectious /70/.

λ phage DNA was also infectious for spheroplasts of E. coli cells of strains which are not infected by phage, but the yield of phage is higher from cells of the specific host /73/. This is also observed in the case of DNA of other viruses and phages, for example φX174 phage /11, 12/. This may depend on differential permeability of DNA in various cells, destruction of DNA in "foreign" cells and a decrease in the yield of virus particles in the cell. Consequently, the specificity of virus-cell interaction is insured not only by proteins of the phage particles, which are necessary for adsorption and penetration of the phage into the cell, but also, probably, to some extent on the structure of the phage DNA itself. The nature of this specificity is not clear, and it is possible that methylation plays some role here.

λ phage DNA under known conditions causes the formation of phage particles in a cell-free system. In all experiments on extracellular synthesis of components of virus particles after addition of viral RNA or DNA, which it is not possible to consider here, only in the research of Kokhran, which he reported in 1964, was formation of whole TMV particles demonstrated with the help of electron microscopy /27/, and an experiment in Evans' laboratory showed clear evidence for synthesis of whole phage particles in a cell-free system

/114/. Infectious λphage DNA was added to a system of E. coli cells strain 3350/λ destroyed by various methods. Whole bacteria and spheroplasts of this strain are not infected either by intact phage or by phage DNA under the conditions in which the experiments were carried out. The amount of phage synthesized in this system was determined by the formation of infectious centers on E. coli strain W-1485, which is not sensitive to phage DNA. At the time of incubation of λphage DNA with cell homogenates, the appearance of material which is infectious for W-1845 cells was observed. If only phage DNA was synthesized, and not whole phage, the material could not have infected the cells. Thus, synthesis of virus components and their assembly into whole virus particles may also occur in cell-free systems, which is interesting from the theoretical point of view and, possibly, is also applicable for obtaining virus in vitro.

Phages of Hay Bacilli

As has been demonstrated in various laboratories, DNA preparations from transducing phages SP 10, S 1 /117, 142/ and PBS X [135] carry a portion of the DNA of host-cells. It is interesting that in contrast to the temperate transducing λphage, the SP 10 phage DNA has no portion which is complimentary to bacterial DNA /54/. In DNA preparations isolated from the phage there are fragments of cellular DNA, which are not permanently associated with the phage DNA and which can be separated from the latter by gradient centrifugation /117/.

In 1962 Romig first isolated infectious DNA from a B. subtilis phage from a suspension of SP 3 phage /128/. The DNA was infectious for whole cells, competent for genetic transformation. The capacity for transformation and infection of the phage DNA preparations were demonstrated parallely. The question of competence has been well clarified in the literature /13, 30/ and we will not touch on this here. Infectious DNA was soon isolated successfully from large phages SP 8 and SP 82, which are close in size to T_2 phage /43, 89/. Green obtained DNA preparations from SP 82 phage, which are not sensitive to pronase and which retain infectiousness for six months at 4°C in the presence of chloroform /89/. Infection of cells was not achieved at low DNA concentrations. The author concludes that 3-4 DNA molecules are required for formation of one infectious center. It is possible that here, as in the case of another large phage SPO 1, when fragments of phage DNA were active during multiple infection of competent cells /118/, DNA molecules damaged during extraction complement each other, as on a template,

and synthesis of complete phage DNA may occur. A mixture of DNA from two different phage mutants was used for infection and work with SPO 1 phage. It was demonstrated that recombination of two or more DNA fragments is involved in the formation of intact phage particles. Infectious DNA was also obtained from other large phages of B. subtilis, such as ϕ-1 and ϕ-25 /127/. Infectious DNA was also isolated by Foldes from SP 50 phage which infects cells of hay bacilli, which are somewhat smaller in size than T_2 phage but larger than T_1 phage /78/. All of these were infectious stages. Active DNA has not been isolated from the temperate phages of the SP series /77/. From the medium phage ϕ-29 of hay bacilli /127/, and also from No. 2c phage, infectious DNA preparations have been extracted with DNA of molecular weight of 2 million /122/, which is not surprising since cases of infectious DNA with very high molecular weights have been brought to light.

Phages of Other Bacteria

Although infectious DNA has not yet been obtained from temperate phages of hay bacilli, yet temperate phages of hemophilus influenzae has served as a source for biologically active DNA. The phages HP 1 and S 2 are close in form and size to coli phages T_1 and T_5 respectively. DNA from a suspension of HP 1 phage [91] and from S 2 phage /87/ were infectious for competent H. influenzae cells. The DNA was inactivated by DNAase, but was not affected by trypsin, to which the whole phages are sensitive. The infectiousness of DNA of S 2 phage was very high and was 10-30% as active as intact phage. DNA isolated from lysogenic bacteria (for HP 1 phage), upon addition to nonlysogenic cells free from prophage close formation in them of phage particles /91/. One could suppose that prophage DNA can be isolated in the infectious state. However, as was shown in λ phage, in viruses of the papova group or insect viruses (cf above), neither prophages nor proviruses have yet been isolated in the infectious form in a single case. It is possible that in H. influenzae with prophage HP 1 a portion of the cells already contain phage particles entering by means of activation of latent phage, and also freed active DNA from them.

Infectious double-stranded phage D-29 DNA from mycobacteria infected whole mycobacterium smegmatis cells, forming infectious centers. The relationship of active DNA preparations to DNAase and trypsin was as usual, but upon treatment with RNAase approximately 5-fold reduction in infectiousness was observed /147/. It is not yet clear how RNAase (50 μg/ml) forms an inactive complex with DNA.

Formation of Virus Particles Upon Addition of Infectious DNA to Cells of Very Distantly Related Hosts

As we have already said, infectious viral DNA has a wider circle of susceptible hosts than whole virus. This was also demonstrated for infectious RNA /1, 110/. However, until very recently the infectiousness of nucleic acids was tested on strains not sensitive to virus, but related to the natural strains and types of host cells. Abel and Trautner first demonstrated in smallpox group viruses that passaging of viral DNA through competent cells of hay bacilli increased the yield of infectious material /38/.

By more detailed consideration of experiments and infectiousness of DNA from animal viruses for bacterial cells, it should be noted that the deproteinization of viruses (Utrecht strain, mutants of rabbit smallpox and Brighton strain of cowpox) was carried out with a complex of enzymes isolated by the authors, by "stripping" the virus under natural conditions during its entry into the cell. Preparations obtained in this way were purified in a series of cases by density gradient centrifugation. These preparations were incubated with bacterial cells which were then disrupted, the homogenated dialyzed and chick embryo fibroblasts were infected with them. The activity of the preparations was determined by estimating the pustules formed. It has been shown that after passage through B. subtilis the infectiousness of preparations, which are inactivated by DNAase in controlled experiments but not by viral antiserum, and which are not precipitated by centrifugation at 60,000 g, increases by three orders of magnitude. During centrifugation in a density gradient the basic mass of these infectious preparations corresponded in properties to viral DNA and not to virus. If transforming DNA from hay bacilli cells was added to the bacteria in addition to these preparations, its infectiousness dropped. This phenomenon is usually observed during infection of competent cells by phage DNA. Thus, the results of all these experiments indicate that the infectious principle in the preparations was viral DNA.

The activity of a contaminant of whole virus particles in this DNA could not explain the results obtained, since the cells which were infected by subviral matter, were not sensitive to virus.

In bacterial cells infected with DNA preparations virus particles were synthesized which were identified by a series of traits. The possibility that DNA added to the bacterial cells was retained in them and that this caused infection of

fibroblasts, was excluded by experiments indicating complete insensitivity of the yield of virus from bacterial cells to DNAase, its precipitability at 60,000 g and inactivation by antiserum to the virus.

Synthesis of vaccine virus was also obtained in spheroplasts of intestinal bacilli in experiments of Babbar et al /41/. In this case DNA was isolated by the phenol method. The supernatant solution, collected after centrifugation of spheroplasts infected with viral DNA and then disrupted, caused the formation of pock marks when applied to sensitive animal cells. If an increase in the amount of viral DNA or formation of protective protein coat around the DNA had not occurred in the spheroplasts, then the infectiousness of the homogenate of infected protoplasts would not have been detected, since even introduction only of DNA isolated from virus into chick embryos did not cause the appearance of pock marks. In these experiments Indian authors did not investigate the nature of the infectious principle, which was formed in the protoplasts. To them this could be either free viral DNA in large amounts synthesized in protoplasts or virus particles which formed in them.

The experiments by Abel and Trautner on the synthesis of animal viruses in bacterial cells has aroused great interest in a series of similar investigations. Thus, Bayrauther and Romig, using the experimental scheme of these authors with competent hay bacillus cells have shown that polyoma virus DNA after passaging in bacterial cells yields virus which form both infectious centers and foci of transformed cells on a monolayer of mammalian cells /43/. Calculations have shown that one infectious center appears for each DNA molecule added to the bacteria. One may conclude that the number of viral DNA molecules did not increase, and each of them only received a protein coat in the bacterium. However, it was shown in the experiments that not every DNA molecule infect a bacterium, and that bacteria synthesize more than one polyoma particle per DNA molecule added, although the authors have not obtained direct evidence of this. In these experiments there was most rapid synthesis of many new polyoma DNA molecules, synthesis of informational viral RNA, virus protein and formation of whole virus particles.

The synthesis of another animal virus in bacterial cells was demonstrated by Ben-Gurion and Ginzburg-Tietz. In this case encephalomyocarditis virus RNA was used as the infectious material /45/. Not only nucleic acids of animal viruses, but also of viruses of plants and bacteria caused formation of virus particles in cells which are foreign for them. We will

not consider here the experiments on replication of infectious TMV RNA - a virus of higher plants - in chlorella cells [141], but we will consider the formation of phage particles in tobacco leaves.

In Sander's experiments /133/ a solution of DNA extracted from filamentous phage fd, was smeared on the leaf and then after known time intervals homogenates centrifuged from cell fragments were prepared from infected leaves, added to a culture of whole bacterial cells, and then the number of infectious centers formed was calculated. After infection of the leaves the number of plaque forming units in the extract first increased, and then decreased, with various rates depending upon the time of year the experiments were carried out. Analysis indicated that synthesis of phage in the leaves occurs first, and then, inactivation of phage particles, the extent of which was associated with the physiological condition of the plant. Upon addition of intact phage to the leaves it very rapidly disappeared from them and no increase in the activity of the extract was observed. Phage particles synthesized in tobacco leaves were characterized by infectiousness for intact E. coli F^+ cells, the type of infectious centers, serological properties, determined by phage protein and sedimentation rate in density gradient. The appearance of phage protein in leaves infected with deoxyribonucleic acid, if fd phage replication proceeds as in $\phi X174$ and M_{13} phages, indicates complimentary whole DNA is synthesized on the single stranded DNA molecules added to the plant cells, and this whole DNA which is already in the structure of the replicative form serve as templates for the formation of messenger RNA. The synthesis of phage proteins, which occurs with the help of mRNA, can only be explained by the development of all the synthetic phage systems in the leaf cells.

Although even the synthesis of DNA of the virus particles in plant cells was not directly demonstrated in Sander's experiments (these results could be explained, as in the experiments of Abel and Trautner, by the formation of phage particles from the same DNA molecules added to the leaves, since the concentration was 10^{13} of phage equivalent per ml), this is not grounds for refuting it. In any case, there are no major objections to the formation of virus-specific components of DNA-containing virus in the plant cells, although in nature these viruses are not detected in plants. In tobacco cells there are no fd phage "stripping" enzymes, and therefore the whole phage is not infectious to them. Only added phage DNA caused the synthesis of virus particles in them. However, the addition of free fd phage DNA to the

cell did not always lead to the formation of phage. In his article Sander presents unpublished results of experiments which demonstrated the noninfectiousness of this DNA for chick fibroblasts or KV cells in culture. It is possible that in cells from vertebrates rapid inactivation of the added phage DNA or phage being synthesized occurs.

Circular Structure of Functioning Viral DNA

As was first demonstrated in Sinsheimer's laboratory, only circular DNA molecules of small viruses are infectious. Thus, ϕX174 phage DNA may be separated by ultracentrifugation for the most part (60-90%) into a fast component S_1 and a slow one - S_2 - the product of a single nick in the chain of the molecule from fraction S_1. Only component S_1 possesses biological activity. The differential sensitivity of these two forms of single stranded phage DNA to exonucleases, which cleave the nucleotide only from the ends of the molecules permitted the cyclic structure to be assigned to the infectious DNA molecules of fraction S_1, and the molecules of fraction S_2, nicked at the "joint", - linear /75, 76/ . The resence of circular single stranded molecules in the DNA preparations of ϕX174 phage or the 1ϕ as in 7-phage closely related to it, was demonstrated by electronmicroscopy /79, 33 /.

The thermal stability of double stranded DNA from viruses of the papova group depends on the circular structure. Heating infectious DNA from polyoma or shope papilloma virus at 100°C for 10-20 and even 30 min., followed by rapid quenching, leads to loss of secondary structure in the linear DNA molecules, and in the transforming DNA, to a sharp reduction of activity, which does not cause a reduction in infectiousness /93, 100, 152/. The resistance of infectious DNA from nuclear polyhedrosis virus to heating at 100°C probably indicates its circular structure /20/.

Upon fractionation of polyoma virus [illegible] density gradient centrifugation its two forms were observed: the rapidly sedimenting-F, and the slow one - S. The circular and linear structures, respectively, were assigned to them /72/. Both of these forms proved to be infectious /66, 71, 72/ ; however, this was shown only in qualitative research. The importance of the circular structure in infectiousness of viral DNA is still unclear. The results of further research with the help of electron microscopy showed /148/ that both forms of DNA - F and S - are closed in the circle, but, probably, the first

of these is twisted more compactly, and therefore it sediments more rapidly during ultracentrifugation, and the second is less compact as the result of a nick in one of the two DNA strands in the double helical circle. In this case the second strand closed in a circle insures retention of the circular structure of the damaged molecule. Both forms cause both transformation of cells and also appearance of infectious centers. Introduction of the method of qualitative analysis of the infectiousness of DNA has permitted detection of a difference in activity of the F- and S- forms of polyoma DNA /55/. It has been shown that the F- form is 10 times more active than the S- form. Heating has little effect on the infectiousness of the F- form, but significantly decreases the activity of the S fraction. If one considers that both of these fractions are circular and react differently on heating, one may make a conclusion about the activity of the circular hole DNA molecule of fraction F, and the appearance of the S fraction in preparation is associated with damage of a portion of the molecule at the time of extraction, which leads to a nick in one of the DNA strands at the "joint", which joins the linear molecule in a circle. The partial untwisting of the double stranded molecule, of which one of the helices remained in the circular form, makes a loop structure. The degree of infectiousness of the S form probably depends on the number of damaged molecules in this fraction, on contaminants of the F-fraction in it and on the possibility of restoration of the DNA structure upon entry into a sensitive cell. The stability of infectious polyoma virus DNA upon entry into an organism is attributed to this cyclic nature /44/. DNA has even been isolated from the blood of animals some time after infection with DNA preparations, in which the infectious properties were retained; exonucleases had no effect at all on the circular DNA.

Native DNAs from virus particles of shope papilloma, human papilloma, and SV_{40}, are probably also double stranded closed structures, which are still twisted. Shope papilloma virus DNA may be separated into two components which have sedimentation coefficients of 28 S and 21 S. After dialysis the 28 S fraction gradually converts to a form which sediments like the 21 S /63/. DNA in this virus is also in the form of circles /109/, which may change their sedimentation rate upon damage. It is also probable here, as in the case of polyoma virus DNA, that both components are circular. Upon gradient centrifugation an alkaline CsCl the DNA, very similarly to the preceding human papilloma virus, separates into three components: a mean fast one and two slow ones /64/. As is known, an alkaline solution, which denatures the DNA, the DNA molecules which already have defects are

damaged. Moreover. the results of the effect of DNAase on viral DNA indicated here that in virus DNA preparations there are usually two components: a compact circular one and a loose circular one, and under the influence of alkali in DNA with a disrupted structure there are still nicks and the circular loose structure is converted to a linear one: the appearance of three components in the experiments of Crawford and several other authors /55/ depended on this.

Dna from SV_{40} virus has been less well studied in this regard. Infectious DNA preparations from this virus used in Black's experiments /49/, were centrifuged in a density gradient in Crawford's laboratory. It was shown that they contain fast and slow DNA forms. Here, as in the case of shope papilloma virus DNA, during storage the fast form converts to the slow one /65/.

While circular DNA is found in small virus particles and it is infectious in this form, it has not been revealed whether this is true in large phages. Deep investigation of the structure of biologically active DNA molecules from λ phage have led, as in the case of other viruses mentioned earlier to the discovery of various forms of viral DNA. This DNA, isolated from phage particles in the form of long linear molecules, may under specific conditions be sealed into a circle by coupling its ends, which have short single stranded complimentary portions. All research on the infectiousness of various forms of DNA has been carried out in the Kaiser system (cf above). It has been shown that the infectiousness of DNA is lost upon enzymatic nicking of the molecule /156/, and also upon sealing of the ends of the linear form with the formation of circles or upon construction of single stranded circular portions before the double helical regions by DNA-polymerase /107, 140/. Infectiousness was established by the effect of polymerization of the product by exonuclease III, which removes nucleotides added in the presence of polymeres. Consequently, on the one hand λ phage circular DNA is not infectious, but on the other hand damage of the ends which does not permit formation of circles, also destroys the activity.

Coupling and uncoupling of the ends of the DNA molecules is a reversible process. Uncoupling of the ends of the molecules may also occur during heating, but at a lower temperature than the "melting" temperature of all double helical DNA structures. The low activity of λ phage circular DNA molecules sharply increases by 20 or more times upon heating of the DNA preparations in the range 55-70°C, where only the double helical structures melt, joining the molecule into a circle. It is

interesting to note here that heating of infectious T_7 phage DNA at a temperature below the melting temperature increases its activity by 3-8 times, but complete separation of the helices of this DNA destroyed its activity /57/.

It is possible that only linear λ phage DNA molecules with undamaged single stranded ends can penetrate into the cell (in the Kaiser system!) and insure the infectious process. However, the biological activity of DNA of this phage in the cell is associated with its circularity, for which it probably also needs coupling portions. In any case, in a cell infected with the phage one can detect a form of virus DNA which is similar in sedimentation rate to the circular undamaged double helical molecule /155/.

Immediately after infection of the cell with λ phage, the synthesis of phage DNA begins, but its infectious properties have not been detected. Infectious DNA appears only immediately before assemblage of the virus particles /70/. Thus, in the case of infection of a cell with λ phage there is an eclipse, not only for the phage, but also for phage DNA.

As has been shown in experiments with chloramphenicol, which inhibits protein synthesis, conversion of the noninfectious form of DNA to the infectious one is associated with the synthesis of the last protein. An interesting phenomenon is the eclipse phase of infectious DNA, when the replication of viral DNA occurs, i.e. it is functioning, but at that time is not infectious, and may explain the fact that at that time it is found in the circular form. The last protein, probably an enzyme, converts the circular form to the linear one, which is already infectious in the Kaiser system. However, this is only one of the possible explanations of this phenomenon. Research is being continued in this direction.

There are other facts, indicating the circularity of viral DNA, functioning in the cell. Without dwelling here on the rather well studied replicative form of ΦX174 phage DNA, since this property will be considered below, we will present certain data concerning the T-even phages. With the help of genetic methods it was demonstrated long ago that DNA from these phages is circular (as the λ phage DNA and E. coli chromosome are circular). Judging from the properties of DNA in complex with protein, which is observed in cells infected with T_4 phage /111/, DNA of this phage in the process of replication probably actually has a circular form. However, DNA from phage particles has not yet been isolated in this form. Only after renaturation of denatured T_2 phage DNA were circles found in its structure by

by electron microscopy /146/. It is still unclear whether the structure of the DNA in the virus particles is circular, but it is difficult to isolate in this form, or a linear molecule with ends which may unite.

Infectiousness of Intermediate Forms of Nucleic Acids, Which do not Enter into the Structure of the Virus Particles

As is known from the work of Sinsheimer and Spiegelman et al, and also of other investigators, single stranded circular ϕX174 phage DNA in bacterial cells forms the so-called replicative form, consisting of one strand of DNA of the phage particle and one strand of complimentary DNA. Neither the replicative form nor the complimentary DNA strand enters into the structure of subsequent generations of virus particles synthesized. However, this complimentary strand serves as a template for the synthesis of messenger virus-specific RNA in the infected cell and therefore is still called a coding strand.

In the presence of levomycetin, circular double stranded replicative form DNA may be isolated from cells infected with the phage. This form may be separated from the phage single stranded DNA by centrifugation and a density gradient or by column chromatography on methylated albumin. Both the phage and the replicative DNA have proved to be infectious /4, 13, 46, 59, 67, 104, 126, 138, 143/ ; however, the replicative form was less active (by 10-100 times).

Under specific conditions centrifugation of the replicative form of the DNA gives two fractions. The major one, designated I, consists of 80-100% of the total amount and sediments more rapidly than fraction II. Both have proved to be infectious in seroplasts /104/. Small doses of DNAase converted the circular double stranded DNA fraction I to fraction II, i.e. fraction II is the first product of degradation of native DNA. It behaves similarly to the loose circular structure of damaged DNA from papova group viruses. However, here separation of the DNA strands of fraction II by heating led to production of circular single stranded DNA, possessing higher activity than the original form of the DNA. Upon action of various factors, leading to single mix in the native replicative ϕX174 phage DNA, selection of the damaged strand did not occur: the nick may occur both in the phage and in the coating helices. Upon heating of such a damaged DNA native circles form both in the phage and coating strands.

In experiments of Il'yashenko et al it was possible to unite the coating strands in the inactive form and to show their infectiousness /15/. Consequently, the coating strand of the DNA may play a role in replicating strands. Actually, it was recently shown by other methods that in cells infected with ϕX174 phage "propagation" of the replicative form of the DNA first occurs by semi-conservative means, i.e. both strands serve as templates for synthesis of subsequent molecules of the replicative form, and then even before formation of phage particles massive synthesis of phage DNA (entering into the structure of the phage) occurs on this replicative form by conservative means /67/, as, probably, does the synthesis of messenger viral RNA.

In connection with the fact that the coating strand of the replicative form of the DNA is infectious, one must also recall Sinsheimer's research devoted to obtaining in vitro a DNA-RNA hybrid stable to RNAase on a ϕX174 phage DNA template in the presence of a mixture of the four nucleoside-triphosphates and RNA-polymerase. This double stranded hybrid was shown to be infectious, while after calculation in one molecule it was approximately 10 times less active than the free circular single stranded phage DNA, and approximately as active as the double stranded replicative form /137/. It was also active in the role of template for the synthesis of RNA, but less so than single stranded DNA.

In Spiegelman's laboratory the formation of the DNA-RNA hybrid was carried out under the control of electron-microscopy. It was shown that the circle of single stranded DNA-template is gradually converted to the DNA-RNA hybrid structure which also has a circular form /42/. Despite detailed investigation of the complimentary RNA strand, its composition and structure, no one has attempted to test its infectiousness. Since this RNA is analygous to complimentary DNA taking the place of the phage replicative form, if template synthesis of DNA is possible on RNA, then one must expect that the circular form of the complimentary RNA will be infectious.

We will now consider research on the possibility of synthesizing DNA on RNA as a template and detection of intermediate forms of nucleic acids of animal viruses. As was shown in 1960 in our experiments, RNA preparations isolated from tissues of the mulberry silkworm, infected with DNA-containing nuclear polyhedrosis virus, proved to be infectious. When these were introduced into larvae of the mulberry silkworm or cabbage butterflies which are insensitive

to the virus, typical nuclear polyhedrosis developes, which is infectious for silkworm, but not for the butterfly. It was demonstrated that infectious DNA-containing virus particles are synthesized on the added RNA. Study of the properties of the RNA preparations has shown that their activity is destroyed by the effect of heated RNAase (freed in this way from traces of DNAase) /21/ and is not reduced by treatment with DNAase /3/. These properties were also observed in RNA preparations from a large wax moth infected with the DNA-containing virus /9, 23-25/.

The infectious RNA which we obtained in experiments with insects, is viral in origin, since the addition of RNA from healthy insects did not cause viral disease (and therefore activation of latent virus). The infectious RNA was separated from cellular RNA by chromotography on a column with calcium phosphate /26/. The infectious peak was sensitive to RNAase but not to DNAase /25/.

This virus-specific infectious RNA is probably polycistronic messenger RNA which is complimentary to the virus DNA, and may be separated from insect cells infected by virus. It is possible that it is not mRNA of the virus, but some other RNA, which is complimentary to the DNA, and which plays a role in the replication of viral DNA.

Infectious RNA has not been isolated from mammalian cells infected by DNA-containing viruses. We have attempted to obtain this RNA from brain tissue infected with herpes virus. Not one out of five RNA preparations caused the development of encephalitis after intracerebral injection of these mice /24/. Similar results were obtained earlier by other authors /39/. Similar results were also found in experiments with adenovirus /24, 116/ or smallpox vaccine virus /116/.

No RNA was detected in rod-shaped nuclear polyhedrosis virus particles; they contain only DNA. However, there is RNA in nuclear polyhedrons (ef above). Assuming that this RNA could represent virus-specific RNA, we extracted it from the polyhedrons by the phenol method and tested its infectiousness. We did not detect activity. Only after increasing the lower case pH of the phenol, when the DNA begins to be liberated from the polyhedrons, did infectiousness appear in the extracts from the polyhedrons. The lability of the RNA in alkaline medium and the reduction in infectiousness of the nucleic acids from the polyhedrons by DNAase, but not by RNAase, did not permit detection of infectious RNA in the polyhedrons, if there is any there /25/. It is

difficult to evaluate the report made by L. M. Tarasevich in 1964 on the infectiousness of RNA from polyhedrons obtained by extraction with weakly alkaline solution /32/, since data on the removal of the virus particles from the extract is absent.

Experiments with DNA-containing viruses from insects has shown the infectiousness of virus-specific RNA, which does not enter into the structure of the virus particles, the synthesis of virus DNA on this RNA, and thus the possibility of transmitting genetic information from RNA to DNA. This process, like all template syntheses in general, is enzomatic in character. In what form the virus-specific RNA may be templates for the assembly of nuclear polyhedrosis virus (or what intermediate forms of it) - single stranded or double stranded - has not yet been studied. In experiments by Tamm et al /86/ the enzomatic synthesis (with the help of DNA-polymerase) of DNA on RNA of reovirus occurred only on a double helical template, as in the case of synthesis of polydeoxyribonucleotides on polyribonucleotides in the experiments of Lee-Huang and Cavalieri /113/.

In the literature there is one demonstration of the in vivo synthesis of DNA on RNA. In experiments by Temin virus-specific DNA was detected in cells infected with Rous sarcoma virus /145/. This DNA was complimentary to virus RNA. It is quite possible that having isolated this intermediate DNA, one could detect its infectiousness, the ability to cause the synthesis of RNA-containing virus. Temin assumes that this DNA enters into the genome of the cell, permitting the virus to enter a latent state. If this is so, then it will not be possible to isolate DNA in the infectious state at this stage of development of the virus, when the DNA has already entered the composition of the DNA of the cell. In any case, as was observed in relation to the latent λ phage, the latent nuclear polyhedrosis virus or the latent oncogenic viruses, infectious viral DNA cannot be separated from closely associated virus DNA and cellular DNA.

Conclusions

In DNA-containing viruses, as in RNA-containing ones, the nucleic acid possesses the complete genetic information necessary for synthesis of enzymes of the vegetative stage and construction of the specific amino acid sequence in the protein and nucleotides in the nucleic acid of the viral progeny.

At the present time infectious DNA has been obtained from more than 30 different mammalian, insect, and bacterial viruses, both from very small, simple and from very large, complexly structured viruses. The molecular weight of infectious DNA varies from 1.3 to 130 million. It is true that a very long DNA molecule is damaged during extraction, and the infectious process only occurs in the case of entry of several of these simultaneously in the cell accompanied by the process of recombination.

For infectious DNA, as for RNA, the circle of susceptible hosts is wider than for intact viruses. Viral nucleic acids cause the formation of virus particles not only in cells which are not sensitive to virus, genetically related to the natural virus hosts, but also in very distant ones: reproduction of animal viruses may occur in bacteria, from phages in plants, etc. This holds the promise of preparation of vaccines with the help of addition of infectious nucleic acids to nonspecific cells of the virus.

All the stages of synthesis of complete virus particles-reproduction of nucleic acid, synthesis of viral protein, assembly of the virus particles - may also occur in cell-free systems, in which infectious nucleic acids are introduced.

Investigation of the structure and function of DNA with the use of the infectiveness of DNA as an index of the nativeness of the molecule has led to the conclusion on the necessity of circular structure of DNA at the time of its reproduction and synthesis of mRNA on it. In small viruses single stranded and double stranded DNA are found in the circular form in the virus particles.

The detection of the infectiousness of intermediate forms of nucleic acid of the vegetative stage of viruses is also very interesting. These forms do not enter into the structure of the virus particles being formed. In the case of ϕX174 phage - this is the replicative form of the DNA and is the complimentary or coating strand of the DNA, in the case of nuclear polyhedrosis virus - this is the virus-specific RNA. The infectiousness of the latter indicates the possibility of transmitting genetic information from RNA to DNA /19/.

The field of research on infectious and oncogenic DNAs is expanded all the more. Much of what has been presented in the present review, was discovered very recently, has still not been affirmed scientifically and at times is

hypothetical in nature. It is possible that a portion of the material is already obsolete at the moment of release of this review. Nevertheless, it is quite necessary from time to time to examine the past and to draw inferences on material accumulated.

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