THE PHYSICS OF VIRUSES

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CHAPTER ONE

THE NATURE OF VIRUSES AND THEIR RELATION TO PHYSICS

Introduction

Viruses are small objects which can produce the most devastating changes in certain living organisms. A bacterial virus which invades a bacterium of over a thousand times its size can in thirteen minutes have totally changed the functioning of the bacterium, and instead have produced three hundred replicas of itself. An animal virus, like small pox or influenza, can modify the total metabolism of a whole large animal, changing its mean temperature drastically, and producing physiological reactions requiring major changes in very large numbers of functioning cells.

The viruses themselves vary in size from 4,000 Å diameter for *psittacosis* to 100 Å diameter for foot and mouth disease. This last virus probably does not contain more than ten separately recognizable molecules, yet its net results on large animals are no more mild than many much larger and more complex viruses.

It is primarily because viruses possess in a remarkable degree one essential feature of life—the ability to reproduce—that they are exciting to study. Although a very great share of this ability resides in the host organism, the fact remains that a relatively simple and, in general, inert object can precipitate the formation of hundreds of like objects in a matter of minutes. How does it achieve this and what essential physical characteristics enable it to do so? These are the challenging questions of virus research.

Overwhelmingly the greatest part of virus research has been nonphysical in character. It has been concerned with virus-host

relationship, symptomatology, virus classification, growth of viruses in different species to produce attenuated viruses, immunological properties of viruses, chemical contents, and genetics. A very valuable and impressive body of knowledge has resulted. Nevertheless, the contribution of physics has not been small and is rapidly growing. The size and shape, degree of hydration, absorption spectra, and thermal properties of viruses are all being studied with very interesting results. Physics is beginning to tell something about the viruses themselves. Furthermore, viruses are so small that they represent one of the desirable extremes of study. If, indeed, the processes of the cell are by the present known laws of nature, by electrical and quantum-mechanical forces, by the processes of the molecular theory, it ought to be possible to see the action of these in the processes of virus multiplication. A very valuable start in this direction has been made by Puck, Garen, and Cline, who have shown the role of electrostatic forces in virus attachment to bacteria.

Therefore, even if the past direction of virus research has been primarily biological and chemical, it looks as though physical experimentation and theoretical speculation on viruses is well worth while. The aim of this book is to collect together the knowledge which bears on the physics of viruses in the hope that it can serve as a starting point for still more penetrating studies.

Because it is hoped that physicists and physical chemists will read this book, and because a summary is often a means of clarification, a brief outline of virology, slanted to suit our needs, is here given.

Outline of Virology

The first and most important fact about viruses is their apparent intimate concern with some host. So far, all attempts to secure virus multiplication on any kind of medium other than an intact organism have failed. This failure does not mean that all future attempts will fail, but it does mean that, whereas a plant will grow if supplied with water, air, light, and a few

inorganic nutrients, a virus seemingly needs a more complex and accurately balanced form of medium. It seems likely that the *physical distribution* of the material of the host cell, proximity to membrane or cell wall, etc., may be of importance. This is, as yet, unproven but it seems likely.

Because of this concern with a host, viruses are classified by their host. Thus we have tobacco mosaic virus, or an *E. coli* bacteriophage, or rabbit papilloma virus. There is, as yet, no definite and accepted classification of viruses, partly because no very definite means of distinguishing similar viruses exists.

All viruses, therefore, operate by invasion of a cell. This is a process which in itself merits much study. Once inside the cell, the virus is instantly concerned with the whole metabolic (chemical and energy turnover) process of the cell, which apparently shifts its function to serve the purpose of the virus, starting a process which ultimately yields a highly multiplied number of viruses. There is evidence from bacterial viruses that after invasion the virus ceases to have its normal structure and becomes something much less visible, and only later, in the multiple stage, does it become apparent in its normal condition.

Virus Multiplication

The unique nature of the bacterial host has enabled detailed studies of virus multiplication to be made. These studies probably apply to other viruses, but it must be remembered that much of our ability to generalize about viruses comes from the fact that we have, so far, made detailed studies on only a few. The process which occurs in a bacterial virus is the following. The virus first attaches, probably at the outset by electrostatic forces, but subsequently by a chemical surface action. No measurable multiplication takes place for a period known as the *latent period*, which is 13 min for T-1 *E. coli* bacteriophage, 26 min for T-2 coliphage, and 60 min for M-5 *B. megaterium* phage. At the end of this time, lysis, or dissolution, of the bacterium occurs, and a number, varying from 50 to 1,000, of new virus particles are released. This process is called a *burst*, and the number the *burst size*. Both the latent period and burst

size are to some extent dependent on the previous history of the virus. A virus which has been dried and heat treated has a longer latent period and a smaller burst size.

Various agents can be used to estimate the number of virus particles present at any stage of this process. The larger phages can be seen as scattering centers in the ordinary microscope. The number of these drops from one, at the original moment of invasion, to zero for the first 95% of the latent period. In the last 5%, many scattering centers are seen, and these correspond to the number of virus particles released. Or the cellular processes can be stopped with proflavin, the cells forcibly burst open by pressure, and the contents examined with the electron microscope. The results of this kind of study show that, at about 85% of the latent period, incomplete virus particles are present—doughnut heads in place of barrel-shaped heads, for example. Thus, virus assembly would appear to be a relatively late and correspondingly rapid process. Similar conclusions can be reached by bombarding infected *E. coli* with X-rays at various stages of the latent period. Only toward the end of the latent period does a multiple-hit type of survival curve become apparent, again indicating that virus assembly is a late process. This process of assembly is not understood—the reader must see that it represents a tremendous intellectual challenge.

Size and Shape of Viruses

This will be the subject of detailed consideration later. The shortened list in the table on page 5 gives an idea of the range of sizes covered by viruses. The unit used is the Ångstrom, 10^{-8} cm, which is the experimental unit used in describing atomic and molecular sizes.

The objects we deal with, therefore, vary in diameter over a factor of 45, and in mass by a factor of roughly 10^5. One might, therefore, be on guard regarding treating viruses as all alike, and no attempt will be made to do so here.

Not nearly enough is known of the true shape of viruses. They seem to be constructed, very broadly speaking, out of spheres and rods. Only for four plant viruses—tobacco mosaic, southern

Virus	Diameter or appropriate size (Å)
Psittacosis	4,500
Vaccinia	2,100 × 2,600
Herpes simplex	1,500
Influenza	1,150
T-1 bacteriophage	500; tail 1,200
Southern bean mosaic	310
Bushy stunt	260
Lansing polio	250
Foot and mouth disease	100
Tobacco mosaic	2,800 × 120
Transforming factor r → III pneumococci	3,400 × 85

bean mosaic, tobacco necrosis, and tomato bushy stunt—are the shapes in solution or suspension accurately known. Of these, the first consists of long rods, 2,800 Å, or more, in length, with probably a hexagonal cross section 120 Å across. The other three are very accurately spherical. Electron micrographs of dried viruses abound, and, from these, three shapes seem to cover all observed classes: roughly spherical; rod-like; or sperm-like, with nearly (but not accurately) spherical heads and quite long tails. It is very unwise to suppose that we know enough to generalize as yet.

Mutation of Viruses

Viruses are apparently capable of definite mutation. Various means for recognizing these exist, for example, in terms of the symptoms they produce or in terms of the range of host in which they will multiply. There are supposedly 50 types of tobacco mosaic virus.

Virus mutations are found in various ways. The usual mutagenic agents are effective on viruses, notably ultraviolet light, chemical mutagens, and thermal action. The application of genetic methods of study to viruses is perhaps the most powerful presently available technique. A multiple infection of a bacterium with two different mutants of a bacterial virus yields differentiatable progeny from which many genetic facts can

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be deduced. Thus T-2 phage has three linkage groups, and at least 15 distinguishable genes. This will be discussed in more detail later.

Mutual Interference of Viruses

Two wholly unrelated viruses, which operate in two different classes of cell, seem to be capable of separate multiplication, so that a joint infection of a large organism containing both types of cells will yield some of each in the end. However, if two viruses can grow in the same cell, they interfere with one another (unless they are very closely related) so that from a mixed strain only one will develop. However, in a multicellular organism there may be "victory" by one virus in one cell and the other in the other cells. Thus a mixed strain of two viruses may stay mixed after an infection, but the properties of the two are likely to be changed. Very closely related strains, such as two mutants of the same virus, will give a mixed yield. This applies to very similar viruses in some cases, such as T-2 and T-4 *E. coli* bacteriophage. T-1 and T-7, which are morphologically distinct, always mutually interfere.

Virus Attenuation after Multiple Passage

A very important feature of applied virus research is the production of attenuated strains which have lost their virulence for one host, and maintain the ability to multiply harmlessly. Such a virus can produce full immunological protection against a later invasion by a dangerous virus and is obviously a very desirable agent in disease control.

The procedure used is to try to find secondary hosts, notably chick embryos, which will survive enough to permit serial passages. This is kept up, and every so often the virus is tried in the original host. Very remarkable results are found. In some cases, after several hundred passages, the desired strain is produced. In other cases, although a virus which is not pathogenic in the new host can be developed, a full return to virulence after one passage in the old host is observed.

It is hard to be very definite as to what this process actually

is. According to the present all-pervading genetic doctrines, the attenuation is the selection of a particular virus strain from a mixture. Thus, any fresh virus preparation is thought of as perhaps containing thirty or so different strains. By serial passage through a well-chosen host, the proportion of these can be so altered that the virus is predominantly of a virulent strain. This way of explanation is not necessarily what one would choose *ab initio* from the facts, inasmuch as modification can still be observed after 300 serial passages.

Chemical Composition of Viruses

In order to determine this, quite pure preparations are necessary. This is relatively easy in the case of larger viruses, but very hard for smaller. So no great claim to precision should be made. A table, taken from Stanley and Lauffer (1948), of the elemental composition, and of the proportion of protein, lipid, and nucleic acid, is given below. It is nearly true that only animal viruses contain any lipid.

Virus	C	H	N	P	S	Protein	Nucleic acid	Lipid
Southern bean mosaic	45.6	6.5	17	1.9	1.3	79	21	—
TMV	51	7.6	16.6	0.6	0.2	94	6	—
Tomato bushy stunt	48	7.7	16.1	1.4	0.6	83	17	—
Rabbit papilloma	50	7.2	14.8	0.9	2.2	89.5	9	1.5
Influenza	53		10.0	0.9		67	5	23
Newcastle disease	51.8		9.9	0.9		67	6	27
Vaccinia	33.7		15.3	0.6		83	5.6	4
T-2 phage	42		13.5	5.0		50	45	2

In the case of tobacco mosaic virus (TMV) and cucumber virus, an analysis of the amino acid content of several strains has been made. This is given in the table on page 8. (See Knight 1947.)

It can be seen that the differences between the TMV strains are not marked.

It is remarkable that 90% of the surface amino groups on tobacco mosaic can be acetylated without affecting the infec-

Amino Acid Content of Tobacco Mosaic Virus

Amino acid	TMV	Masked	Green aucuba	Yellow aucuba	Cucumber virus 3	Cucumber virus 4
Alanine	5.1	5.2	5.1	5.1		6.1
Arginine	9.8	9.9	11.1	11.2	9.3	9.3
Aspartic acid	13.5	13.5	13.7	13.8		13.1
Cystine	0.69	0.67	0.60	0.60		0.0
Glutamic acid	11.3	11.5	11.5	11.3	6.4	6.5
Glycine	1.9	1.7	1.9	1.9	1.2	1.5
Isoleucine	6.6	6.6	5.7	5.7	5.4	4.6
Leucine	9.3	9.3	9.2	9.4	9.3	9.4
Lysine	1.47	1.95	1.45	1.47	2.55	2.43
Phenylamine	8.4	8.4	8.3	8.4	9.9	9.8
Proline	5.8	5.9	5.8	5.7		5.7
Serine	7.2	7.0	7.0	7.1	9.3	9.4

tivity. It seems as though something involving arrangement, rather than chemical content, is of great importance.

Virus Serology

Viruses are excellent antigens, and, accordingly, the serological behavior of viruses gives us an excellent method of study of viruses, particularly as regards their surface properties. To illustrate the potency of viruses as antigens, a preparation of infectious sap of a plant virus, which has in no sense been thoroughly purified, is antigenic primarily because of the virus and not because of the unremoved cell constituents also present.

It is unfortunate that we do not yet know how antibodies are produced in response to antigens. It is not a simple physical process, for antibodies can continue to be formed after the original antigen molecules have disappeared. Yet there seems to be an underlying simplicity about the end results, for the result of injecting antigen into an animal is to produce molecules in the blood serum which are clearly related to the original antigen molecules and have for them a strong and specific affinity. Both the affinity and its specificity can be used in virus study, and a very great branch of virus research proceeds along serological lines.

The technique is as follows. As pure a preparation as is practical is injected into the serum-producing animal (for example, a rabbit or horse) in three doses a few days apart. At the end of three weeks, the animal is bled. The serum is separated by spinning out the blood cells, and for crude purposes can be used as an antiserum. It is usual to purify the antiserum further by adding specific substances known to be present in the original preparation, for example, plant sap from healthy plants, or bacterial debris from broken-up bacteria in the case of bacterial viruses. These cause precipitation of the antibodies specific to them and leave a more specific antibody to the virus itself.

Two standard methods of measuring serological combining power, or affinity, are in general use. The precipitin method relies on the observation of a visible precipitate after the addition of antibody to virus; the neutralization of infectivity utilizes the fact that, after combination, the infectivity is removed or reduced.

Virus serology will be taken up in much more detail later. It can be said now that viruses have of the order of 1,000 antigenic units on their surface; that several different types of unit exist per virus; and that the units differ in size, ranging from the equivalent of eight or ten amino acid side chains, to whole protein molecules. The parts of a bacterial virus which cause attachment to a bacterium do not seem to be active antigens, although this can not be asserted too vigorously.

Hemagglutination

An interesting property of a fair number of animal viruses, such as influenza, Newcastle disease, vaccinia, and mumps, is that of causing red blood cells to clump together, or agglutinate. This is in some cases due to substances called agglutinins, which are capable of separation from the virus, and in other cases due to units in the virus itself. An interesting feature of some of these viruses is the property of self-elution, by which is meant that after a while the virus will remove itself from the red cell and agglutination will cease. After this process has occurred, the red cells will not longer agglutinate.

This is interpreted, at present, as follows. The red cells are supposed to have receptors on them which can attach to the virus. The self-eluting virus attaches at a point which is enzymatically active on the receptor as substrate. The enzyme then slowly converts the receptor into some noncombining form, which, therefore, ceases to be bound by the virus, and so the process of elution can take place. Such red cells may resuspend, but they can no longer be acted on by the same virus to agglutinate.

Purified Virus Preparations

In the case of the plant viruses, notably tomato bushy stunt, tobacco necrosis, and southern bean mosaic virus, purified preparations which can be crystallized into crystals of definite form have been prepared. The procedure is elaborate, although one difficulty is simply in starting from enough infected plants to give a high yield. Tobacco mosaic virus forms small, visible crystalline aggregates.

No crystalline preparations of animal or bacterial viruses have, at the time of writing, been prepared. The concentration of bacterial viruses can be raised to about 10^{13} per cm^3 by careful growing and fractional centrifugation. However, physical studies on bacterial virus are still somewhat limited by the lack of a pure preparation.

Virus Assay

Of predominant importance in virus assay is the use of dilution and some all-or-nothing effect, like producing the proper symptoms in a host, or an agglutination of red blood cells. Any given sample containing virus is diluted in an appropriate suspension medium (buffer or broth for example) by known amounts, e.g., in factors of 10, and each dilution is tested for the required effect. If a dilution of 10^6-fold, for example, will still produce symptoms, this number is often used to describe the *virus titer*. To a physicist this may seem to be a very crude measurement. Actually, for many purposes, only the logarithm of the virus titer has any significance, and when this is so, the accuracy is

not so bad. Lack of accuracy did not prevent the founders of modern physics from measuring the charge on the electron, or Avogadro's number, accurately enough to move forward fast in that subject. The physical study of viruses is today historically where atomic physics was in 1900.

This dilution technique is often all that is available for animal virus work. For bacterial viruses a very powerful method is available. The idea used exploits the fact that virus infection of a bacterium results in dissolution, or *lysis*, of the bacterium, producing a clear solution. If agar jelly, nutrient medium, and bacteria are mixed together and poured into a thin layer on a glass petri dish, the result will be a whitish, cloudy growth of bacteria. If a *small* number of virus particles are added to a similar mixture and it is poured, then, after about 8 hr, the cloudy growth will appear, but around each virus there will be a clear spot, or plaque, which is readily seen. The number of these plaques is, then, an exact count of the viruses. This is a method of tremendous sensitivity, comparing very favorably with any of the techniques of physics. As will be seen later, many elaborations of this means of assay can be devised. Of course, to cover a wide range, dilution must also be employed, but this can be done quite accurately.

For plant viruses, a method which is rather similar in character, but unfortunately not so simple or accurate, is available. This is the method of local lesion counting. Some host plants are so sensitive to virus infection that, as the virus spreads, the plant is locally killed, and the infection then ceases because it has been over-efficient. The killed patches, or local lesions, are then easily seen and counted. The difficulty in the method lies in plant variability and in the serious problem of cell invasion. Virus will not penetrate a plant cell until it is damaged, and the damage must not be too severe or the cell dies. The technique used is to rub a little fine Carborundum powder over the leaf and then follow with a smearing of the virus solution to be assayed. Half leaves can be used for comparison. In addition, plant viruses show a marked tendency to aggregation. The theoretical basis for lesion counting is discussed by Bald (in

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