VIROLOGY IN THE SECONDARY SCHOOL

By

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CHAPTER I

INTRODUCTION

Due to the increasing knowledge of viruses and at the same time an increasing concern for the cause and effect of these organisms, it is the opinion of the writer that a high school student should be introduced to them with more than a "Viruses are too small to be seen without the aid of an electron microscope."

The purpose of this paper is to give the high school biology teacher, trained in the biological sciences, a guide to the teaching of virology in secondary schools. A background of bacterial principles and concepts is strongly advised before attempting to introduce this material. The two fields are closely related and this paper will refer to bacteria and bacterial procedures, especially concerning the reproduction of viruses.

It is anticipated that this material will be presented in approximately two weeks in the general biology course. Some sections may be omitted at the discression of the instructor without losing the continuity of the unit.

If equipment is available, many laboratory demonstrations and/or experiments may be performed. In the appendix of this report are suggested exercises for this purpose.

CHAPTER II

DEFINITION AND CLASSIFICATION

Viruses may be defined as "submicroscopic entities, capable of being introduced into specific living cells and of reproducing inside such cells only."[1]

At the present time viruses belong to the Order Virales as described in the 6th edition of Bergy's Manual of Determinative Bacteria (1948), and are divided into three sub orders, one each for plant viruses, animal viruses, and viruses infecting bacteria. It is anticipated that eventually the application of Linnean binomials will appear in classifying these organisms. Most virologists feel that more of the fundamental knowledge and structure of viruses is needed before this should be attempted.

In 1953 at the Sixth International Congress of Microbiology, the Virus Subcommittee of the International Nomenclature Committee recommended a uniform system of forming the equivalent of generic names, in which the suffix " virus" was to be used. A group of respiratory viruses have been named "adenoviruses" and the virus causing poliomyletis is referred to as poliovirus. Little confusion has arisen in the past

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[1] S.E. Luria, General Virology (New York, 1953), p.2

from the use of descriptive terms such as smallpox, vaccinia, influenza, and poliomyelitis.

A group of virus isolated from the stool of polio patients has been named Enteric Cytopathogenic Human Orphans (Echo viruses). These organisms cause no known disease. Many viruses have been rescued from this group upon discovery of the diseases they produce.

As the knowledge of the virus is increased so will evolve the system of nomenclature.

CHAPTER III

FUNDAMENTAL PROPERTIES OF VIRUSES

Intracellular Habitat

No evidence of respiratory or biochemical activity exists in a suspension of virus particles free of tissue cells. When such a suspension is inoculated into a susceptible host, the virus particles enter the cells and multiply therein. This multiplication of viruses if affected by nutritional and hormonal factors existing in the host cell.[2] Animals deficient in certain nutritional requirements are less susceptible to virus infections than normal animals. The cortisone hormones have been shown to increase susceptibility to certain viruses by increasing intracellular reproduction.

Size and Shape

The size of virus particles is measured in millimicrons. One millimicron is equivalent to 1/1000 millimeter.

By various means the size of virus particles can be estimated. A virus suspension may be filtered through a collodin membrane, fritted glass, diatomaceous earth, porcelain

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[2] A. J. Rhodes and C. E. van Rooyen, Textbook of Virology, (3rd ed., Baltimore, 1958), p. 12.

or plaster of Paris of known porosity and the filtrates tested for the presence of virus. With the use of the ultracentrifuge it is possible to concentrate virus particles, determine the state of homogenecity of the particles in the suspension from the sedimentation characteristics, and from this, estimate the size of the virus particle. The application of this technique is limited and few viruses have been studied in this way.[3]

The electron microscope has greatly increased the knowledge of size and shape of viruses. With this instrument, viruses have been found to be rectangular or brick shaped such as the vaccinia used for smallpox immunization, circular as in the equine encephalitis, globular as are some of the influenzae or filamentous form as in the tobacco mosaic virus.

Some viruses that infect bacteria (bacteriophages) have a "head," either circular, oval, or hexagon shaped, with a straight or wavy like appendage called a "tail."

TABLE I[4]

APPROXIMATE SIZES OF VIRUSES AND REFERENCE OBJECTS

Structure	Size in Millimicrons	Shape
Red Blood cell	7500	
Vaccinia	210 x 260	
Rabies fixe	125	
Staphlococcus bacteriophage	100	
T2 coli bacteriophage	60 x 80 head 12 x 120 tail	
Tobacco mosaic	15 x 300	
Poliomyelitis	25	
Foot-and-mouth disease	10	

[3]Ibid., p. 14.

[4]Thomas M. Rivers, Frank L. Horsfall, Jr., Viral and Rickettsial Infections of Man, (3rd ed., Philadelphia, 1959) p. 13

Reproduction

The process of heredity, how like begets like, is one of the most fascinating mysteries in biology. Of the many angles from which biologists are studying these phenomena, none are more exciting than the experiments on bacterial viruses or bacteriophages. These organisms reproduce their own kind in a simple, dramatic way.

In the case of the bacterial viruses of the stain T2, which infects the common bacterium Escherichia coli, the hypothetical method of reproduction has been confirmed with the aid of radioactive tracers.[4] The outer layer or "coat" of the virus is protein and has the ability to attach itself (adsorb) to the surface of the bacterium and to react with antivirus serum. The core of the virus is nucleic acid, composed mostly of desoxyribonucleic acid (DNA).

The first stage of reproduction is infection. Here the virus particle attaches itself, by the tail, to the bacterium, and the nucleic acid core empties into the bacterial cell. (Figures 1 and 2). The protein coat remains outside. It is believed that the tail contains an enzyme which destroys (lyses) the cell wall of the bacterium to allow for the passage of the DNA core.

In the second stage, the latent period, the virus DNA within the bacterial cell begins to induce the formation of

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[4] Gunther S. Stent, "The Reproduction of Viruses," The Physics and Chemistry of Life, (New York, 1955), p. 134.

new protein coats. However, the coats contain no DNA, thus they are noninfectious. The original DNA multiplies within the bacterial cell by using as raw materials, the nucleic acids of the bacterium. At this stage, the DNA is also non-infectious.

In the third stage, called the eclipse stage, some of the protein coats contain nucleic acid. They are the first infective particles of the new generation (Figure 2).

The final stage occurs about 30 minutes after the first. The infected bacterium bursts (is lysed) and releases the new generation of virus particles into the surrounding medium (Figure 2). The T2 strain produces approximately 200 infective particles per bacterial cell. The number of progeny produced varies with the type of virus as does the time required for production of the new generation.

After lysis of the bacterial host, the progeny are free to infect new cells of Escherichia coli. Virus particles are specific concerning the cells they infect. A bacteriophage of Escherichia coli can not infect the epithelial cell of a human organism. Perhaps this is because of the nature of the enzyme produced for lysis of the cell wall and subsequent entrance of the virus DNA.

FIGURE I

PARTS OF THE BACTERIAL CELL AND VIRUS PARTICLE

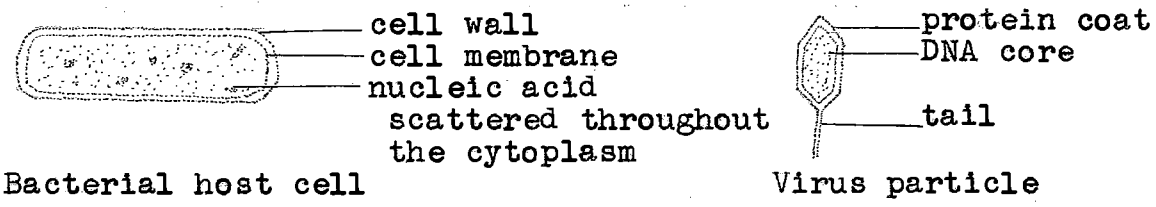
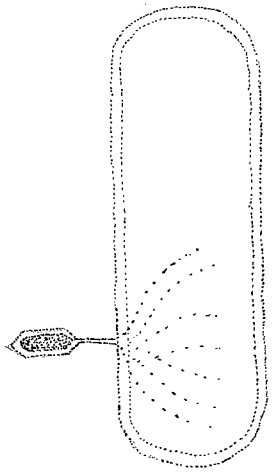
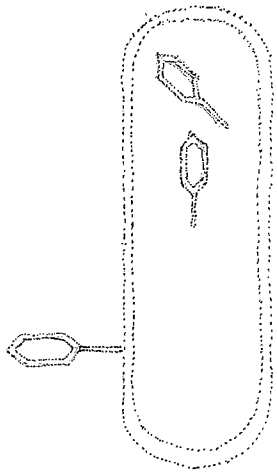


FIGURE 2

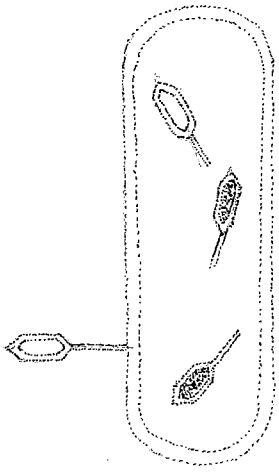
REPRODUCTION OF BACTERIOPHAGES



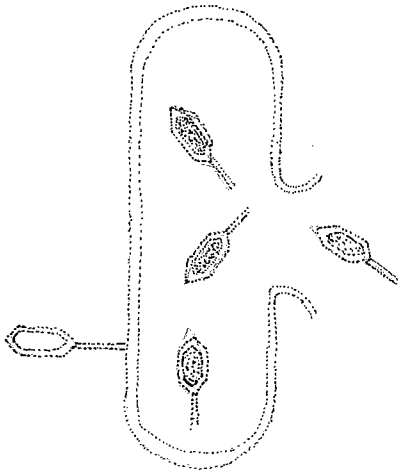
Stage I
(Infection)



Stage II
(Formation of new protein coats)



Stage III
(Formation of new infective units)



Stage IV
(Lysis of cell wall and freeing of virus particles)

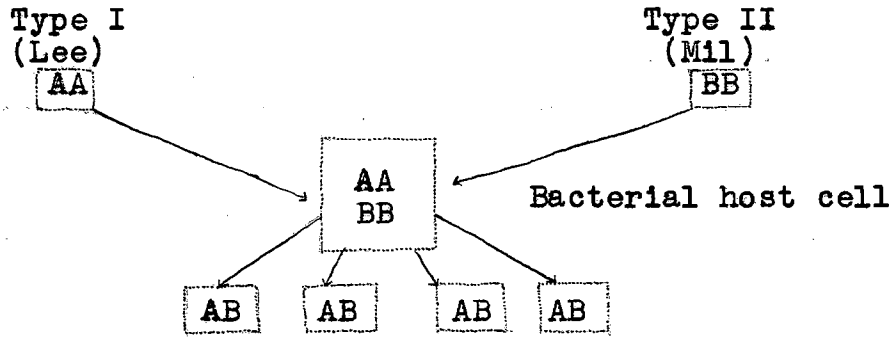
Not all virus cause lysis or bursting of the host cell. The virus may reproduce, but remains inside the host cell. These viruses are said to be lysogenic. Lytic virus causes the bursting of the host cell. If several viruses become attached to a bacterium and proceed to lyse the cell wall, the cell may burst before the DNA of the virus enters. This results in the death of the cell and is termed "lysis from without."

Recombination

Burnet believes genetic interaction occurs when two related virus particles infect a single host.[5] Two influenza virus strains have been inoculated simultaneously into epithelial cells. Studies of the resulting progeny showed them to contain characteristics of both "parent" organisms.

FIGURE III

RECOMBINATION IN TWO STRAINS OF INFLUENZAE VIRUS



[5]Rhodes, p. 25.

Toxins

Few viruses produce toxins (poisons). However, toxin formation has been demonstrated in members of the influenza and pneumonia groups. The toxic factors associated with the influenza virus appears to be present only when large numbers of viruses are injected into experimental organisms. Inoculated mice die early without evidence of virus multiplication.[6]

Cultivation

As stated before, viruses will not grow in a lifeless medium, even if rich in animal protein. The presence of living cells is essential for reproduction. Tissue culture is a technique by which living tissues can be grown outside the living organism. Cells are kept alive for years by repeatedly changing the surrounding nutrient fluid. By this method, nutritional and environmental factors can be more easily controlled than in the living organism. Viruses may be injected into these growing cells. Among the cells used for tissue culture are epthelial cells from rabbit cornea, monkey kidney, human kidney, human amnion, human embryo and HeLa cells.

Viruses will also grow in the cavities, tissues, and membranes of the fertile chick embryo. Growth occurs in the

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[6] Rhodes, p. 23.

cells of these structures and not in the surrounding fluid. It is the cells lining the cavities that are the reproductive areas. Viruses may invade the blood stream to cause "spreading" of the disease to other parts of the body which usually results in the death of the embryo.

A single layer of cells called a monolayer can be produced for the cultivation of viruses or they may be grown in susceptible bacteria or plant and animal tissue of the intact organism.

Purification

A purified suspension can only be obtained by separating virus particles from material derived from the host cell. Purification is desirable in studies to determine chemical structure of the particles. Chemical or physical means are used for this purpose.

Because viruses are nucleoprotein in structure, the addition of certain salt solutions such as amonium sulfate or magnesium sulfate will cause the precipitation of the virus particles as will acetone, alcohol, and lead acetate. By separating the precipitate and filtrate, a high degree of purity may be obtained. Another chemical means of purification is the adsorption-elution technique in which the adsorption of virus on some material is accomplished at a given pH or temperature. The viruses may be freed (eluted) from the adsorbent at another pH or temperature. Bacteria may act as

an adsorbent for some viruses while red blood cells of various animals are used by others. The influenzae virus is adsorbed on chicken red blood cells at 4°C and are eluted at 37°C.[6] The adsorption of tobacco mosaic virus on celite at pH 4.5 is followed by elution at pH 7.[7]

Enzymes have been used to digest protein impurities present in viruses. Immunochemical methods have been used whereby virus are precipitated with specific antiserum, followed by the removal of attached antibody with enzyme action or dissociation.

Chemical procedures may lead to the inactivation of the viruses. In such cases, physical techniques may be employed. Viruses are small enough to pass through filters while the filters will retain impurities. It was from early experiments of this type with bacteria that viruses received the name "filterable viruses."

Virus particles may also be separated from host cells with a centrifuge, hypertonic saline solutions and freezing-thawing techniques.

Chemical Structure

After purification is accomplished, the chemical structure of the virus may be studied. Rabbit papilloma is a

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[6] G. K. Hirst, "The Agglutination of Red Cells by Allantoic Fluid of Chick Embryos Infected with Influenza Virus," Science, Vol. 94, (May 1941), p. 22-23.

[7] W. M. Stanley, "Isolation of a Crystalline Protein Possessing the Properties of Tobacco Mosaic Virus," Science Vol. 81, (February 1935), p. 644-45.

nucleoprotein complex, the nucleic acid component being desoxyribonucleic acid. This is considered one of the simpler animal types. Vaccinia contains DNA, carbohydrate, lipid, cholestrol, biotin, and copper. Influenza virus is a liponucleoprotein complex containing ribonucleic acid (RNA).

In 1935, Stanley reported a crystalized nucleoprotein isolated from the leaves of plants infected with tobacco mosaic disease.[8] Viruses have been crystalized as a salt grain can be crystalized, and have been found to infect a plant. Are viruses living organisms?

Reactions to Chemical and Physical Agents

The virus particle is readily destroyed by heat, due to its protein character. Sterilization of virus infected material may be accomplished by raising the surrounding temperature to 60°C for 30 minutes. At the time viruses may be stored for prolonged periods at -60°C in a carbon dioxide icebox sealed in a screw cap vial or ampule. As a general rule, infectivity of the virus particles exists over a narrow range of pH around the neutral point (pH 7).

The smallpox virus is resistant to drying. Brief exposure to ultraviolet light is an effective immunizing agent. Many viruses are resistant to glycerol and can be stored in a 50% solution of this substance for prolonged

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[8] Ibid., p. 644.

periods in the domestic referigerator. Ether destroys the infectivity of most viruses but poliomyelitis and smallpox are resistant.

Because viruses have no known metabolic process, being entirely dependent on the host cells, they do not respond to sulfa drugs, penicillin, or antibiotics except in a very few cases. The antibiotics interfere with the multiplication of those viruses that are affected by it. Methionine, also used in insecticides, reduces the rate of growth of many viruses.

CHAPTER IV

IMMUNITY

For many decades it has been common knowledge that the individual who recovered from an infectious disease was often resistant to subsequent attacks of that disease. This resistant state, or acquired immunity, was observed to last for many years or perhaps a life-time. The practice of deliberate exposure to the infectious agent in order to produce the disease was practiced then as it is today. Today children are exposed to measles and mumps because the disease is milder in children than adults and lasting immunity is acquired.

Artificial inoculation with an infectious agent of reduced virulence for man to produce a mild infection and consequent protection against naturally occuring diseases began with the work of Jenner in 1796.[9] Jenner noted that milkmaids who had been infected with cowpox (vaccinia) were seldom infected with smallpox. After preparation of a suitable cowpox inoculum (vaccine), a human subject was inoculated. Subsequent tests showed this individual to be resistant to the smallpox virus. The method of introducing into the blood stream either a nonvirulent strain of the disease organism or

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[9] William Burrows, Textbook of Microbiology, (17th ed., Philadelphia 1959), p. 9.

organisms of a related, nonpathological disease, in the case of vaccinia, with the stimulation of antibody protection is called vaccination. This method, devised by Jenner, remains in use today, and has been adapted to the development of rabies prevention (prophylactic).

Toxins, remembering that few virus produce toxins, may be treated with substances, like formaldehyde, to render the toxin inactive. Upon injection of this inactive toxin (toxoid) into the human organisms, antibody production is stimulated to give immunity to that organism.

Active immunity may be acquired through having the disease itself, or injections of toxoids, or use of vaccines. The person's own blood stream produces the antibodies which result in immunity. Active immunity may last for 10 years as in the case of pneumonia, for about a year with influenza or for smallpox about 7 years.[10]

Passive immunity is a state of resistance to infection produced in a normal person by the administration of serum containing antibodies.[11] Such sera are obtained from actively immunized persons or animals. So far as is known, this state of resistance to virus diseases can be brought about only by the injection of serum containing neutralizing antibodies.

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[10] Lorus J. Milne and Margery J. Milne, The Biotic World and Man, (2nd ed., New Jersey 1958), p. 127.

[11] Thomas M. Rivers and Frank L. Horsfall, Jr., Viral and Rickettsial Infections of Man, (3rd ed., Philadelphia 1959), p. 8.

Whether the antibodies introduced into people protect susceptible cells against the entry of virus, whether they act directly on the virus so as to prevent the production of disease, or whether they enhance the destruction of virus by certain phagocytic cells, is not known.[12] Passive immunity is of great practical interest because of its use in prevention and treatment of virus diseases. It should be remembered that such inoculations are only a temporary measure. Young children are often given serum containing measle or mump antibody to prevent the disease at a certain time but not necessarily to build up a lasting immunity.

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[12] Ibid., p.8.

CHAPTER V

METHODS OF TRANSMISSION

The period during which a virus disease is communicable, the infectious period, is variable, but commonly extends from a few days before the onset of recognizable illness to some days afterwards.[13] The virus may reach large quantities before symptoms are expressed. In respiratory diseases, the infectious period extends from two to three days before the onset of the illness to about a week thereafter. The virus may be excreted in the feces for several weeks after the onset of symptoms. Many viruses remain latent (dormant) in the body tissues for prolonged periods. No clinical evidence of infection is usually observed, but only after following the presence of a certain stimulus the virus becomes active and symptoms appear. This condition exists in the case of warts, cold sores, and jaundice.

Direct contact is probably the most obvious means of spreading virus. This is accomplished by the hands, restaurant employees, and indeed by anyone with whom an organism comes in contact.

Indirectly, viruses are spread through droplets and by touch. Using the same clothing, sleeping in the same bed,

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[13] Rhodes, p. 75.

and by such objects as toys, towels, door knobs, gymnastic equipment or clothing soiled with fecal material.

Coughing, sneezing, and noseblowing place large droplets in the air. These organisms are highly concentrated in crowded classrooms, theaters, and stores. School age children have a higher instance of colds than do adults.[14]

Few virus outbreaks have been connected with foodstuffs and milk. Poliomyelitis has been recorded following consumption of infected milk and swimming in lakes and rivers polluted by sewage. It is unlikely that properly chlorinated water can spread virus disease. However, skum not cleaned from a swimming pool offers bacteria and virus protection from the chlorine.[15] It is suspected that viral infections of the skin, mucous membranes, and gastro-intestinal tract have been traced to the swimming pool.

Animal transmission of virus is accomplished by the bite of an infected dog in the case of rabies. Parrots spread psittacosis by infected particles from the mouth or feathers which are inhaled by passers by. The urine of infected mice carries the nervous disease, lymphocytic choriomeningitis. Migratory birds are instrumental in transmitting virus diseases over large land and water masses. Because birds harbor many diseases, importation of foreign species is not often permitted.

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[14] C. H. Andrews, "A decade With the Common Cold," Bulletin of Johns Hopkins Hospital, May 1958, Vol. 103, p. 7.

[15] C. R. Amies, "Surface Film on Swimming Pools," Canadian Journal of Public Health, September 1956, Vol. 47, p. 93.

Flies transfer infection mechanically on their bodies or by regurgitation of ingested material. Flies pick up the infective agents from uncovered human feces and sewage.

Arthropods such as ticks, mosquitoes, and sandflies spread disease when they become infected from human carriers by sucking blood during the infectious period of the disease. Mosquito vectors transmit equine encephalitis, Japanese B encephalitis, St. Louis encephalitis, and yellow fever. Sandfly fever is spread by the sandfly. Ticks spread Colorado tick fever.

In recent years substantial progress in the control of arthropod borne diseases has been achieved by the use of DDT and newer chlorinated insectisides. The World Health Organization has played an active part in many such campaigns. A disturbing feature, however, is that insects, for example, lice and mosquitoes, have a tendency to develop resistance to DDT.

CHAPTER VI

LABORATORY DIAGNOSIS

Collection of Specimens

A great variety of specimens may be submitted for biological examination. Secretions and discharges, as from the eye, skin, or mucosae, should be thinly smeared or spread on a microscopic slide. Vesicle fluid should be collected in a glass capillary tube which is then sealed at both ends. Sputum should be collected in a screw capped jar. Throat secretion is collected by means of a cotton swab, or by having the patient gargle sterile saline or nutrient broth. Venous blood is usually allowed to clot, and the serum used for serologic tests. Stools, urine, and cerebrospinal fluid are placed in screw capped jars or bottles as are autopsy specimens.

Storage and Shipment

Specimens are stored temporarly in domestic refrigerators at 4°C. If tests are not carried out promply, specimens should be placed in an electric deep freeze (-20 to -40°C) or in a carbon dioxide chest (about -60°C) until tested. Likewise, if a specimen is to be shipped to a test laboratory it should be packed around dry carbon dioxide ice in a large vacuum flask and sent by special messenger or "special delivery."

Laboratory Tests

The laboratory tests for the presence of a virus infection are of two general types: (1) isolation tests which establish a diagnosis by direct demonstration of the causal agent, and (2) serologic tests which establish a diagnosis indirectly, by demonstrating a rise in antibody titer during convalescence.

Befor 1952, laboratory diagnosis of virus infections was tedious and time consuming. The tests in general use involved the inoculation of pathologic specimens into animals and chick embryos. Sometimes histological technique was required. Diagnosis could be based on a demonstration of an increase in antibody level but this information is not available until the convalescent period.

In 1956, Enders reported that poliomyelitis virus produces microscopic evidence of infection in fragment tissue cultures and that these changes are specifically inhibited by antibodies. The monolayer types of epithelial cell cultures, prepared from HeLa cells, kidney cells, or human amnions are even more suitable for this type of work. Some viruses produce nuclear or cytoplasmic inclusions and giant cell formation.

Because a certain virus can be isolated from a human organism does not mean that the individual is suffering from that disease since he may be only a carrier. Deciding the cause of the illness is the responsibility of the physician.

The presence of a virus is only a presumptive diagnosis.

Serological tests are of limited value to the practicing physician interested in obtaining quickly a presumptive diagnosis.[16] Various procedures are in common use for conducting serologic tests. Among these are the complement fixation technique, titration of virus neutralizing antibodies from tissue culture cells, pH inhibition or color test of Salk, and hemagglutination inhibition tests.

Host Reactions

Host reaction to a virus is recognized by comparing infected and normal hosts. Virus manifestations may be divided into two main categories: local reactions and generalized or systemic reactions.

Local reactions are those manifestations that involve only a limited number of host cells localized in one area. The limitation in size of the affected area is due to either a localization of the virus in a few cells or to the fact that only a few cells show the effect of the virus present.[17] However, the virus may be present beyond the limit of the recognizable lesion. A virus may remain in a local area because of its limited ability to spread, because of a limited number of susceptible host cells, or because of a balance between host defenses and virus invasiveness.[18]

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[16]Rhodes, p. 110.

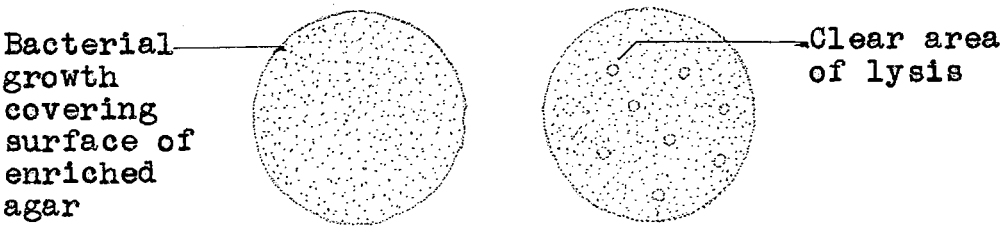
[17]Luria, p. 21.

[18]Ibid.

The local lesion of bacteriophage is called a plaque and is an area of lysis or dissolution in a bacterial layer growing on a solid medium.

FIGURE 4

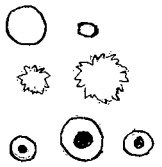
DEMONSTRATION OF A PLAQUE



The appearance of the plaque is characteristic for each phage-host system, and reflects the kinetics of bacteriophage growth and of bacterial growth, and the size of the bacteriophage.[19] Plaques vary in size (measured in millimeters), and margin. Some have smooth margins while others are serrated. Some plaques appear to have a halo.

FIGURE 5

PLAQUE TYPES



smooth margin

serrated margin

halo appearance

On plants, local lesions are seen on the surfaces of leaves. The most interesting type is the necrotic lesion, a black or brown spot which may be from 1 to 10 millimeters in diame

[19] Luria, p. 21.

This lesion appears at the point of entry of some viruses like that of the tobacco mosaic virus. The necrotic lesion results from the rapid death of a group of infected cells, with consequent localization of the virus in a small area.

Plants may also exhibit the starch-iodine lesion. This consists of local areas of altered starch metabolism around the points of entry of the virus. To demonstrate this type of lesion, an infected leaf is treated with alcohol to remove chlorophyll and then with iodine to stain starch (black). The lesions appear as areas lighter than the rest of the leaf, if the leaf has recently been exposed to light. Synthesis of starch is reduced in the infected area.

Lesions found on animals are formed at the primary point of entry or by secondary localization of a circulating virus. Infectious warts found in man may result by virus entry. Exanthemata (redening), the major symptoms of smallpox, measles, and chicken pox are examples of secondary localization.

Generalized or systemic infections produce symptoms in plants that go under a variety of names: chlorosis, mosaic (irregular patches), streak, yellows, ringspot, and leaf roll. They describe the type of deformation or discoloration that appears on the leaves of the infected plant. Lateral buds may proliferate to produce the symptoms of witches' broom. Tumors may be produced throughout the infected plants. Flowers may show symptoms of virus infection in the form of

variegated colors or "breaking." Tulip break, caused by virus, is a condition which has produced a highly appreciated flower. In most cases, the younger parts of the plant show the greatest susceptibility to virus infection.

Silent infections with animal viruses are often encountered. The virus reproduces without giving visual symptoms. In most of these cases the presence of the virus can be traced by the presence of antibodies circulating in the blood. Also, animal virus may require an incubation period (time between infection and appearance of symptoms). The incubation period for measles is 10 to 12 days, for smallpox around 12 days and for mumps 18 to 21 days. This period may include the time needed for the virus to penetrate, to circulate, to localize itself in susceptible cells, and to reproduce to the extent needed for the manifestations to appear.

CHAPTER VII

SUMMARY AND CONCLUSIONS

Due to the increasing knowledge of viruses and at the same time an increasing concern for the cause and effect of these organisms, their fundamental properties, methods of spread, immunity and laboratory diagnosis should be introduced to the high school student.

Viruses are submicroscopical entities, made of protein and threads of nucleic acid, capable of entering into specific living cells, and of reproducing inside such cells only.

Viruses range in size from 10 to 300 millimicrons and possess a variety of shapes: circular, globular, rectangular, and filamentous. Many of the bacteriophages have a body or head of various shapes, and an appendage called a tail. Size and shape of these particles are easily studied by use of the electron microscope.

Purification of the virus particle is necessary for studying the virus free from its host cell. This may be accomplished by many physical and chemical means.

Due to the protein nature of the virus, these organisms are greatly affected by such chemical and physical agents as heat, freezing, pH, ultraviolet light, glycerol, ether, and sulfa drugs.

Virus diseases have been found to be spread directly from organism to organism and indirectly from object to organism. One of the most effective carriers of these particles is the arthropod.

Immunity may be active, passive, or not at all.

Laboratory diagnosis of the virus may be by isolation of the causative agent or serologically by demonstrating the presense of antibodies. Care must be taken in the collection and storage of specimens to be analized.

The causative agent may be grown in the chick embryo, in tissue cell cultures of various kinds, or in an experimental organism. Many procedures are in common use for the conduction of serological tests. These include the complement fixation technique, neutralizing antibodies, pH inhibition or color test of Salk, and hemagglutination inhibition tests.

Host reaction to a virus is recognized by comparing infected and natural hosts. Virus manifestations may be divided into two main categories: (1) local reactions and (2) generalized or systemic reactions. These reactions are expressed differently in the plant viruses, animal viruses, and bacteriophages.

It is the hope of the writer that this paper will stimulate high school teachers to teach virology and others to summarize progress in the field for such use.

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APPENDIX A

THE ELECTRON MICROSCOPE

Matter may be studied in two ways: directly, by the use of ordinary human senses; and indirectly, by the use of planned experiments using large populations and the resulting data analyzed statistically. Direct method can be used to study the detail of matter down to the order of 1/25,400 inches or 1/10,000 centimeters. The largest detail of matter that can be studied by indirect methods is in the order of 1/25,400,000 inches or 1/10,000,000 centimeters. Out of necessity to fill the gap between these two methods, the electron microscope and other related scientific approaches have been developed.

Electron microscopy may be defined as a coordinated direct and indirect study of the structure of matter in the linear dimensional range from one millimicron to one micron. It derives its name from an image reproducing device called the electron microscope.

The electron microscope may be compared to the light microscope in that optical column and the major components are analogous. Each contains a radiation source, condenser lens, specimen holder, objective lens, and a projector lens. The light microscope uses visible light as a source of radiation which passes through air and forms an enlarged image of a specimen by a series of glass lenses. The electron microscope

uses an invisible electron beam as the radiation source which is generated by a high voltage electrical current (approximately 50,000 volts) emitted by a hairpin of tungsten wire called an electron gun. This beam of electrons passes through a tube which is under vacuum to prevent the electrons from scattering. The electron beams are focused on the specimen by magnetic lenses analogous to the glass lenses of the light microscope. The electrons passing through the specimen are then focused on a fluorescent pad. When the electrons hit this pad visible light is given off which one is able to see. The enlarged image of the specimen that one sees is formed by the number of electrons that pass through the specimen. The denser parts of the specimen allow fewer electrons to pass through, thus, resulting in a darker area.

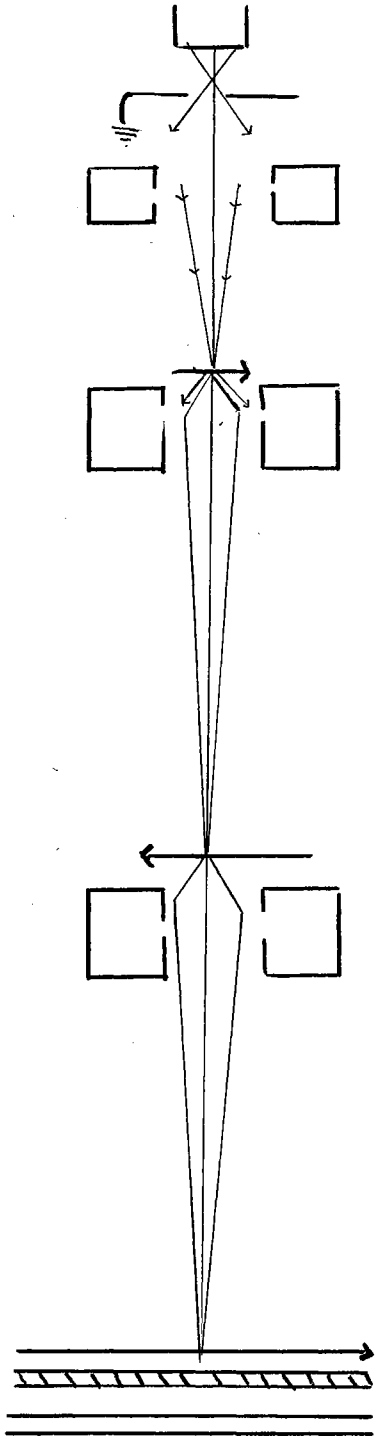
The specimens of the light microscope are placed on a glass slide and stained with various colors to increase contrast. For the electron microscope, the specimen is placed on a small copper grid with many tiny holes which are not visible to the unaided eye. Sometimes thin sheets of clear chemicals are placed over the grid to give additional support. The size of the grid is near that of the diameter of the eraser on a pencil. Because there is no spectrum within the electron beam, one is unable to detect color differences, only differences in density.

With the aid of special machines and various preparatory techniques, many different substances may be studied. The smallest biological life may easily be studied with this

microscope as well as the detailed structure of crystals and metals. One of the most studied biological specimen is that of the tobacco mosaic virus. Depending upon the specimen, a magnification up to 100,000 times can be made. Still greater magnification can be obtained by enlarging the photomicrographs.

(Adapted from Cecil E. Hall, Introduction to Electron Microscopy, New York, 1953, p. 137).

Schematic Diagram of a Typical Electron Microscope



(Adapted from Cecil E. Hall, Introduction to Electron Microscopy, New York 1953, p. 137).

APPENDIX B

BACTERIOPHAGE - PREPARATION OF VIRUS STOCK

PURPOSE:

1. Production of a high titer (10^6 or more) virus stock by inoculating a growing culture of bacteria with a small amount of virus from a previous stock.
2. The virus will multiply inside of the bacteria and destroy them. After a few hours, almost all bacteria will be destroyed and the culture will contain a large number of virus particles and lysed bacteria.
3. A few of the bacteria will resist the action of the virus. These resistant forms arise by mutation of the original strain of bacteria. If the cultures are incubated overnight, the resistant forms grow and the cultures are turbid the next day.

MATERIALS:

1. Virus stock, E. coli phage.
2. Test tubes containing 10 ml. nutrient broth.
3. Suspension of susceptible bacterial host cells 4 to 20 hours old, E. coli B.

PROCEDURES:

1. Inoculate 3 tubes of nutrient broth with a sufficient amount of the bacterial suspension to produce visible turbidity (broth should be at least room temperature or preferably at 37°C.)
2. Add 0.2 ml. of the virus suspension to two of the bacterial suspensions.
3. Omit the virus from one tube to serve as a control.
4. Incubate at once at 37°C. Observe at 30 minute intervals for disappearance of turbidity.
5. Use uninoculated tube of broth as control for readingg the disappearance of turbidity.
6. When the tubes are incubated, both the bacteria and the viruses will multiply, but soon the viruses, which grow faster than the bacteria, will catch up with them. The culture tubes will therefore first become turbid and then clear. The tube which was inoculated with bacteria only serves as a control. Its turbidity will increase continuously while the other two tubes at first show the same amount of bacterial growth and then clear. When they have cleared, they contain a large number of virus particles and a few resistant

bacteria.

7. Refrigerate one of the cleared tubes for Virus Stock.
8. Continue incubation of other tubes over night or longer. The few resistant bacteria in the phage lysed culture will cause that tube to become turbid again due to new growth of resistant mutant strain.
9. Hold this culture (from # 8) for isolation of mutant strain.

(According to Zana Skidmore, Assistant Professor, Department of Bacteriology, Oklahoma State University, Stillwater, Oklahoma, 1960)

PLAQUE COUNTS FOR BACTERIOPHAGE

In order to separate the bacteriophage from non-susceptible bacteria that may obscure its presence, the material being tested is filtered through a bacteriological filter that will retain the bacteria while allowing the phage to pass through in the filtrate. A Selas filter of Number 03 porosity is suitable for this purpose. When milk is being examined the casein must first be removed or the filter will clog. To accomplish this, add to the milk sufficient 10 per cent lactic acid to curdle the milk; mix well; and allow to stand for 30 minutes. Filter the whey first through the filter paper and then through the Selas filter. In many instances the whey from cheese vats may be examined, and in such instances filtration through the Selas filter only is required. A quantitative estimate of the phage in the filtrate can be made by plaque counts or by a limiting dilution technique.

PROCEDURE:

1. Pour 12 ml. of an agar medium (e.g. tomato juice-peptonized milk agar) appropriate for the test culture into sterile petri plate and allow to harden; pour one plate for each dilution of the phage filtrate to be tested.
2. Place 3 ml. of 3.25% $CaCl_2.2H_2O$ in a 16 x 125 mm sterile test tube as the cell diluent.
3. Add 2 drops (0.1 ml.) of an 18 hour phage susceptible culture.
4. Add 3 ml. of phage filtrate diluted to the desired concentration in sterile distilled water.
5. Add 3 ml. of the melted agar medium tempered to 65°C (150°F).
6. Invert the tube 6 times to mix contents thoroughly.
7. Transfer 3 ml. of the mixture to the solidified underlay medium and distribute evenly over the surface by tilting the plates from side to side.
8. Place plates on level surface to solidify.
9. Incubate in an upright position at 30-32° C (86-90°F) for 18-20 hours.

Each clear area, where lysis of the bacterial culture has occurred, is considered to represent one phage particle. Multiply the number of plaques per plate by the dilution of filtrate in the plate to get phage titer per ml. of the original material used.

(Potter and Nelson, Effects of Calcium on Proliferation of Lactic Streptococcus Bacteriophage, I: Studies on plaque formation with a modified plating technique. J. Bact., 64, 105-111. (1952).

APPENDIX C

VIRUS DISEASES OF MAN

Diseases of the Skin or Mucous Membranes

Contagious pustular dermatitis
Cowpox
Foot and mouth disease
Herpangina
Herpes simplex
Herpes zoster (shingles)
Vaccinia
Warts

Exanthemata

Echo infections
Chicken pox
German measles
Measles
Smallpox

Respiratory Diseases

Acute laryngotracheobronchitis
Adenovirus infections
Common cold
Epidemic influenza
Mumps
Ornithosis
Pandemic influenza
Pneumonitis
Primary atypical pneumonia
Psittacosis

Diseases of Genito Urinary System

Herpes
Inclusion cervicitis and urethritis
Lympthogranuloma venereum (LGV)
Warts

Eye Diseases

Epidemic keratoconjunctivitis
Herpetic keratitis
Inclusion conjuctivitis
Newcastle virus conjunctivitis
Pharyngoconjunctival fever
Trachoma

Pyrexias of Virus Origin Spread by Arthropods

Bwamba fever
Colorado tick fever
Dengue
Mengo infection
Rift Valley fever
Sandfly fever
Semliki Forest infection
West Nile infection
Yellow fever
Zika infection

Liver diseases

Infectious hepatitis
Serum jaundice

Coxsackie Infections

Virus (aseptic) meningitis
Bornholm disease
Herpangina
Myocarditis

Neurotropic Virus diseases

Acute anterior poliomyelitis
Acute lymphocytic choriomeningitis
Virus (aseptic) meningitis
Australian epidemic encephalitis ("X" disease, Murray Valley encephalitis)
California virus infection
Coxsackie and Echo virus infections
European tick borne encephalitis
Japanese B encephalitis
Pseudorabies
Rabies
Russian spring summer encephalitis
St. Louis encephalitis

Virus Diseases of Plants

Name of Virus*	Transmission
Abutilon variegation	White flies?
Aster yellows	Leafhoppers
Bean mosaic	Mechanical; seeds; aphids
Corn streak	Leafhoppers
Corn stripe	Leafhoppers
Cucumber mosaic diseases	Mechanical; seed; aphids; cucumber beetles
Elm phloem necrosis	Leafhoppers
Pea mosaic	Aphids
Peach mosaic	Aphids
Peach rosette	?
Peach yellows	Leafhoppers
Potato leaf roll	Aphids
Potato virus x	Mechanical
Potato virus Y	Aphids
Potato yellow dwarf	Leafhoppers
Rice stunt disease	Leafhoppers
Sugar beet curly top	Leafhoppers
Sugar beet mosaic	Aphids
Sugar cane Fiji disease	Leafhoppers
Sugar can mosaic	Aphids
Tobacco mosaic	Mechanical
Tobacco necrosis	Mechanical (soil to roots)
Tobacco ringspot	Mechanical
Tomato bushy stunt	Mechanical
Tomato spotted wilt	Thrips
Tristeza of citrus	Aphids
Tulip break	Aphids
Turnip yellow mosaic	Flea beetles
Wheat mosaic	Mechanical (soil to roots)
Wound tumor	Leafhoppers

* Generally includes name of major host

(Adapted from General Virology, S. E. Luria, 1953, p. 14.)

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