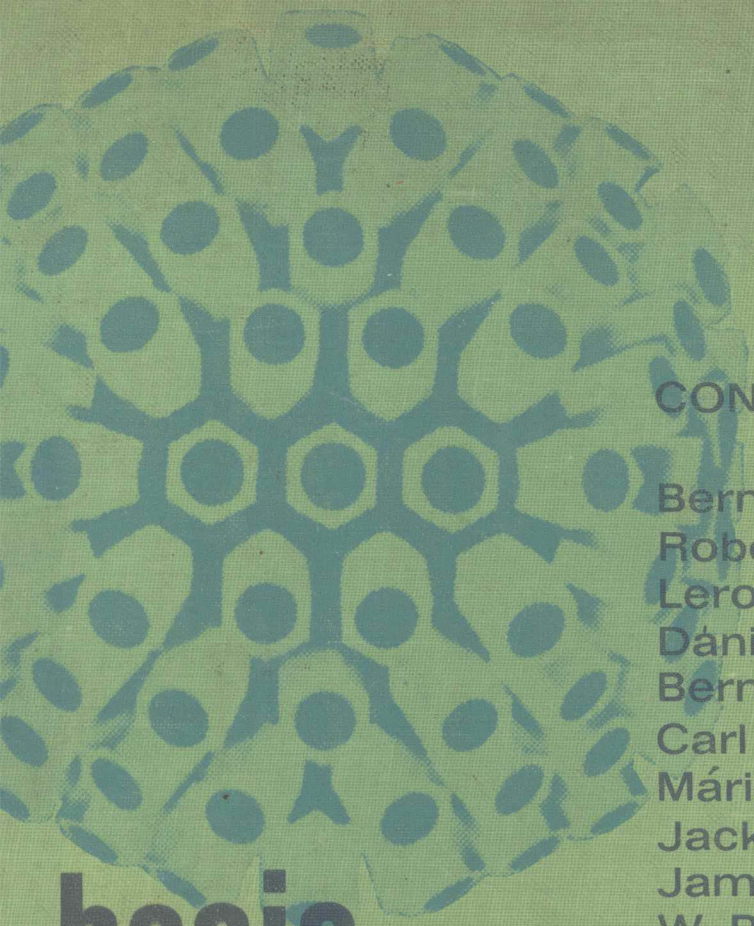

basic medical virology

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BASIC MEDICAL VIROLOGY

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PREFACE

Considering the great amount of literature on the subject of virology, which by some standards may even be excessive, anyone who attempts to add to this bibliographic excess must offer some justification. In the present case, the voluminous virologic verbage itself represents part of the justification. The novice who now approaches the subject can only be overcome with despair and frustration, for the complexity of virology as a science has far outrun the mechanisms to teach it in a logical and orderly fashion.

It is no longer possible to include in a single book all the facets of virology. This volume, then, is directed to a particular audience, and the pieces of the subject that seem appropriate have been dissected out and put together into a format that is intended to be a teaching text. It is intended to be a textbook for students of basic science, particularly those whose ultimate occupation will be the practice of medicine or medical research. In addition to students of medicine and veterinary medicine, it is hoped that some application may be found for graduate students who select virology as a part of their sphere of interest and activity. It must be emphasized, however, that this is not a text of virus diseases, and clinical aspects have been intentionally omitted. Rather, an attempt has been made to bring together into a single book the essential information that is required for a basic understanding of the viral parasites and the mechanisms by which they exert their biologic influences on animal hosts.

In preparing their contributions, the authors were given only one guideline. This was to provide the information that they considered most important for the preclinical student of medicine. Although there may not be universal agreement on the material selected for this purpose, it is believed that a concensus would find it generally appropriate. A result has been that no uniformity exists in the construction of the various chapters, or in the type of emphasis given to a subject. Thus, in some cases the mechanisms of virus synthesis are emphasized, in others the epidemiologic aspects of viruses, and in others factors of host-parasite relationship. It is obviously impossible, because of space limitations, to cover all these aspects in detail for each group of viruses studied, and this method of selection allows the student some concept of the magnitude of the total subject, and hopefully a perspective that would not be achieved by limiting each topic to the same format.

No attempt has been made to orient the material according to species. It has become clear in recent years that species orientation in basic medical science is an invalid concept for either the classroom or research. This is particularly true in virology, and for that matter in microbiology generally, where the majority of emphasis is at cellular and subcellular levels. This does not mean, of course, that the clinical manifestations of cellular damage are of less importance, but rather that understanding of these gross phenomena of viral parasitism has the prerequisite of knowledge of more basic biologic mechanisms. Clinical aspects of virus infections have been covered amply in other texts.

The general format of *Basic Medical Virology* is based on recent and generally accepted concepts of nomenclature and virus classification. Thus, the viruses are

discussed according to the groups that have been formulated according to physical, chemical, and biologic characteristics, such as myxovirus, picornavirus, herpesvirus, and so forth. There are several exceptions to this general pattern, however, for seemingly valid reasons. Some viruses are not yet adequately classifiable into major categories, and so are considered separately, as infectious hepatitis, rubella, and lymphocytic choriomeningitis. Certain viruses are featured separately because they have peculiar biologic or economic significance, such as rabies, hog cholera, measles-distemper-rinderpest, and diseases of fish. Two subjects that are somewhat unique and are considered separately are the arboviruses, which are classified on the basis of vector transmission rather than physicochemical properties, and the oncogenic viruses, which are grouped together purely on a biologic basis. Finally, a series of chapters is included on viruses of lower animal species, primarily for completeness and also because this information is not as readily available or familiar as are similar data relative to the virus diseases of man. In these cases some clinical data have been included for the same reasons.

The advice of a number of colleagues was sought in the preparation of the book, and several have permitted the use of illustrative material. Of particular importance has been the help and guidance of the editorial staff of The Williams and Wilkins Company in resolving the many problems associated with bringing the book to completion.

JAMES E. PRIER

Philadelphia, Pennsylvania
1966

CONTENTS

1

INTRODUCTION

James E. Prier

For that minor group of traditionalists who will proceed to read this book from the beginning, we have provided both a title page and an introduction, two of the least remembered portions of any textbook. In order to lighten the chore of the persistent reader, both have been kept as brief as practical. On the following few pages an attempt has been made to indicate some points of relative importance to medical virology, most of which are discussed in greater depth in subsequent chapters. Perhaps, for the student, some perspective of the entire subject of virology can be achieved before exploring the details of specific topics.

I. The Development of Modern Virology

The history of any area of study or human achievement is highlighted by relatively few events of great significance. In the sciences, these are usually specific discoveries which have been, or at least are reputed to have been, the bases for subsequent exploitation by many, and thus have brought from obscurity a new degree of understanding. Perhaps the great discoveries of the past should be considered with some awe and respect, but it is difficult to view in retrospect with complete objectivity. It must be considered that the factor that has, in many cases, transformed a curious observation into a discovery of great repute, is the development of an idea into a mass of concrete data by the experimentations of many people. Thus, in some instances the real origin of an idea, the accident of discovery, and the touch of genius are not recognized and become lost forever to the annals of scientific history. Even today, as a new thought or discovery bursts forth upon the pages of a modern scientific journal it is difficult to know from whose mind the original germ of creative thinking originated. This difficulty is compounded by the frequently practiced system of multiple authorship of scientific papers, which has the effect of plunging the origins of discovery into a total gray of anonymity.

It is most practical and, perhaps, accurate to discuss the development of virology in terms of general trends and changes in emphasis, rather than in reference to specific personalities. Yet, some consideration must be given to a few outstanding pioneers, partly because of a comfortable nostalgia embodied in the past, and partly because time and the concensus of succeeding generations have placed them in chairs of greatness. Today's heroes of science have not yet been chosen, and, if in some year long into the future this volume should be found in a dust-laden library recess, I hope the peruser of this ancient literature will forgive my negligence in not clothing my colleagues who are destined for greatness in the robes of scientific nobility.

A. DISCOVERY OF VIRUSES

Studies were conducted on infectious diseases caused by viruses long before the

discovery of the etiologic agents. Smallpox immunization was practiced for many centuries before the virus was identified, and Jenner observed the antigenic relationship of two agents, cowpox virus and smallpox virus, without having an experimental basis for theorizing the nature of the causal agents. Pasteur developed a vaccine for rabies in 1884, 14 years before the isolation of animal viruses, although he recognized the cause of the disease as a "living" entity and called it a virus.

It is generally accepted that virology, as a distinct biologic science, had its origins in the isolation of tobacco mosaic virus by Iwanowski in 1892, and of foot and mouth disease virus by Loeffler and Frosch in 1898. These investigators showed that fluid containing infectious capacities would, when passed through bacterial retaining filters, remain capable of inducing infection. The concept of subcellular infectious agents was propounded by Beijerinck in 1899, but the proposal was not met with general enthusiasm immediately.

The identification of the yellow fever virus by Carroll, together with the demonstration of the mechanism of transmission by an arthropod vector, comprised one of the most dramatic episodes in the history of medical biology. Together with W. Lazear, J. Carroll, and A. Agromonte, Walter Reed went to Cuba in 1900 as the Head of the American Army Commission for the study of yellow fever. The self-sacrifice and professional devotion exhibited by these men are unique in medical annals, and by analogy with the events of succeeding generations they seem to represent an attitude and a sense of purpose that are no longer borne by men of science.

The mission of the military commission was to determine the cause and to demonstrate the method of control of yellow fever. Although the disease was rampant in Cuba and the members were aware of the terrible clinical course of the malady, they presented themselves as experimental subjects. Recalling the work of Smith and Kilborne, who demonstrated the ability of ticks to transmit the protozoan of Texas fever of cattle, and the theory of Carlos Finlay and H. R. Carter that yellow fever might be transmitted in a similar manner, but by *Aedes* (*Stegomyia*) species of mosquitoes, this point was put to experimental trial. The officers and enlisted men of the commission permitted themselves to be bitten by mosquitoes that had contacted yellow fever patients. Carroll was the first to become infected, and although he recovered, he died of residual effects several years later. Lazear, while caring for patients in the wards, deliberately allowed a mosquito to feed on his hand. Five days later he became ill, and after a short but violent course was dead. In 1901, Dr. Reed recalled this episode before a meeting of the Medical and Chirurgical Faculty of Maryland in Baltimore. "I can hardly trust myself to speak of my late colleague, since the mention of his name brings back such scenes of anxiety and depression as one recalls only with pain. . . . Filled with an earnest enthusiasm for the advancement of his profession and for the cause of science, he let no opportunity pass unimproved . . . he was suddenly stricken, and, dying, added one more name to that imperishable roll of honor to which none others belong than martyrs to the cause of humanity." In the span of 5 years the cause and mode of transmission of yellow fever was determined, and the effectiveness of mosquito control in limiting the spread of infection was shown in both Cuba and Panama by Gorgas. Many viruses which cause disease in man and animals were isolated during the following 30 years, but by comparison with the yellow fever story these events seemed anticlimactic.

The general technic used for identification of viruses as causal agents of disease in early investigations was quite straightforward. Fluids or tissue suspensions of the spontaneous hosts were passed through bacteria-proof filters, and the filtrates were then injected into susceptible animals. This was a period of rapid progress, for each year saw a new advance in understanding of the etiology of specific, infectious diseases. Enthusiasm for this work was spurred by the influenza pandemic following World War I. In the 1920's, Laidlaw and Duncan

demonstrated that a respiratory disease of dogs, canine distemper, could be prevented by immunization, and increasing effort was exerted in the direction of prevention of virus diseases by the use of biologics, an area of exploration that has not yet reached its peak of activity.

An event in 1915 was a preamble to a series of events that only began to gain momentum nearly 30 years later. This was the discovery by Twort that bacteria were subject to "disease" caused by a virus. At about the same time, the French Canadian, d'Herelle, discovered a bacterial virus that was pathogenic for a coccobacillus that caused an enteric disease of locusts, and another that lysed *Shigella* bacilli. He called the organisms "bacteriophage." Although it was considered at first that bacteriophage was an effective therapeutic agent for bacterial disease, further study indicated that the bacterial viruses are ineffectual for such therapy. In spite of the ineffectiveness of bacteriophage therapy, clinicians continued to apply them for nearly 20 years. Not the least of the results of the discovery of bacteriophages was the creation by Sinclair Lewis of the novel *Arrowsmith*, in which the hero appears to be an Americanized d'Herelle.

The nature of the bacteriophage remained obscure for a number of years. Twort originally described it as a bacterial enzyme, and d'Herelle considered his virus a different factor of bacterial lysis. The observation of a phenomenon, now recognized as lysogeny, resulted in many years of confusion before the real events of the bacteria-virus relationship were elucidated.

As a tool of basic virologic study, the bacteriophage has been extremely useful for determining mechanisms of virus synthesis and cell-virus interactions. The early work of Burnet, Schlesinger, and Delbrück was followed by a vast effort of many investigators, and today nearly every biologic research institution has an active program in some phase of study which utilizes the bacteriophage as a model.

The relative simplicity of bacterial technics as compared with tissue cell procedures is a primary reason for the exploitation of phage systems. Further, as the amount of data relating to the bacterial viruses grew, and the various phenomena associated with virus synthesis became known, greater understanding and definition of the complete living system as a biologic model was possible. The composition of the phage particle as protein and deoxyribonucleic acid (DNA) (rarely ribonucleic acid (RNA)) was discovered early in the 1930's by Schlesinger, and subsequently analysis of both components was accomplished for several different species of bacteriophage. That the DNA is the sole determinant of phenotypic characteristics was shown for bacteriophage of the T2-coli series by Hershey and Chase in 1952.

In addition to mechanisms of replication of DNA and viral protein synthesis, bacteriophage parasitism has been used to study RNA synthesis, enzyme synthesis, virus latency, virus mutation, and genetic recombination.

B. DEVELOPMENT OF ISOLATION TECHNICS

After the initial period of virus discovery through 1930, an expansion of laboratory studies began. Until the application of chick embryo technics to virus propagation by Goodpasture and others from 1929 to 1935, most virus investigations required the use of laboratory animals or the natural host for the detection of the presence of infectious subcellular organisms. The use of chicken embryos as a host for virus assay and identification opened a new vista for virologists. Some viruses could be identified by the production of specific lesions, and compared one with another. It became possible to measure the biologic effects of the cellular parasites with greater accuracy. Also the chicken embryo provided a sensitive method for primary virus isolation.

As the world moved toward a second war, the scientific community was forced to turn its attention to pressing practical matters. For animal virologists this meant the immediate problems of disease control and prevention. There exists, therefore, a brief hiatus in the normal course of virus research, followed by an almost explosive expansion of activity in mammalian virology begin-

ning in 1945 to 1950. The most significant single factor contributing to this postwar revolution was the application of cell culture technics to the study of animal viruses. This technologic advance provided an opportunity for the isolation and identification of viruses that were heretofore unknown, such as adenoviruses, which are capable of laboratory cultivation only in cell culture, where specific cytopathogenic effects can be detected. More recently it has been found that some viruses such as rubella can be propagated in cell culture without cytopathologic effect, but can be detected by subsequent challenge of cells with an indicator virus.

In addition to a substrate for virus isolation, cell cultures provide a method for the identification of a wide variety of virus-host mechanisms. There are few phenomena associated with virus-host cell interactions that have not been explored in cell culture systems.

In addition to laboratory methods for virus propagation such as chicken embryos, primary and line cell cultures, and special strains of animals, a number of other technics have enabled investigators in recent years to pursue many details of virus structure and synthesis. Physical characterization of viruses both in purified suspensions and in intracellular locations has been made possible by analytical centrifugation and electron microscopy. Technics of the latter have been improved by the system of negative staining which was applied by Brenner and Horne (1959). Before this method was used electron microscopic preparations suffered a great deal of distortion and realistic visualization was difficult.

C. NUCLEIC ACIDS

Following the demonstration that the infective potential of tobacco mosaic virus was resident in the RNA fraction, nucleic acids were isolated from several animal viruses. The essential nucleic acid of animal viruses is either RNA or DNA, and both types have been isolated and shown to be infective, thus following the basic principle established by Hershey and Chase for bacteriophage. Since the nucleic acid fraction of the virus particle carries the entire potential or code for the synthesis of the complete organism, it is clear that the mechanism of genetic determination of protein synthesis can be specifically altered by administration of viral nucleic acid. Further, because a single viral nucleic acid molecule can infect a host cell with the resulting synthesis of 100 or more virus particles, the cell genetic mechanism must be induced to replicate the specific viral nucleic acid.

Thus, it would appear that a logical sequence of events for an infection by DNA virus would be the contact of viral and host nucleic acids, followed by specific protein synthesis, in order to provide the enzymes necessary for viral nucleic acid synthesis, and then, as a final step, the synthesis of specific viral protein. Since the cell does not survive the experience of virus synthesis, it appears clear that the support of virus synthesis is accompanied by irreversible alteration of the genetic structure of the host. Although the active alteration of the host DNA by virus DNA at the level of the cell genome can explain the abrupt conversion of the synthesizing capacity of the cell, a dissimilar mechanism must exist for RNA viruses. Clearly, in this case, RNA carries the complete code of virus synthesis, and must in some way act directly as a messenger component for cytoplasmic protein synthesis. The details of virus synthesis are considered further in the following chapters.

D. VIRUS PURIFICATION

For accurate study of the entire virus particle it is necessary to separate it from host cell components. A number of methods of virus purification have been developed in recent years. One which has had increasing use is zonal ultracentrifugation. When the partially purified virus is forced through a solution such as cesium chloride, particles or molecules layer at different strata, depending upon their density. A number of other methods for partial or complete virus purification involve alcohol precipitation, enzymatic digestion of host compo-

nents, and adsorption and elution from erythrocytes or other particles.

E. FLUORESCENT MICROSCOPY

Another method that has yielded important data relative to virus-cell relationships is the immunofluorescent technic first described by Coons. The procedure generally is to conjugate immune globulin with fluorescein isothiocyanate, and then react the globulin with specific antigen. The location of the antigen-globulin complex in tissues or fluids can be identified by visualizing fluorescence with a microscope fitted with a source of ultraviolet illumination. Wherever virus is localized within the cell, provided that the protein coat is present, a locus of fluorescence appears following treatment with fluorescent antibody.

F. METABOLIC STUDIES

The use of radioactive tracers has been a useful tool in examining specific pathways of viral synthesis. Isotopes incorporated into amino acids and precursors of DNA and RNA (thymidine and uridine) have been used in a vast panorama of experiments, and it is almost unfashionable and irreverent not to embrace this particular methodology. It is essential, in using technics of this type, to have a clear understanding of the sequential chemical events of cellular metabolism and intermediate steps leading to virus synthesis. Not only is the selection of an appropriate labeled compound necessary, but proper timing of such experiments is critical in obtaining significant experimental data.

Specific chemical inhibitors or substitutive analogs have been employed to determine the chemical nature of the complex events leading to virus synthesis by infected cells. These include inhibitors of protein synthesis such as puromycin, and purine and pyrimidine analogs. If analogs of uracil or thymine will prevent molecular completion, RNA or DNA synthesis can be inhibited specifically. In the synthesis of DNA, 5-fluoro-2′-deoxyuridine (FUDR) will substitute for thymidine and prevent formation of DNA viruses. Also, 5-bromo-2′-deoxyuridine (BUDR) will act in the same manner. In some cases, inhibition of cellular but not viral RNA can be effected, thus providing an additional method for the analysis of virus-cell interactions.

Autoradiography can be used as a method for localizing intracellular events. Following exposure of cells to radioactive labeled chemicals, the preparation is fixed and covered with a film strip or emulsion. After an appropriate period for film exposure, the preparation is developed and the spots on the film are compared with the underlying intracellular location. For example, if the black exposure spots on the film correspond to a position of the nucleolus in the cell preparation, it can be concluded that the labeled compound has localized in this portion of the cell.

Staining methods for specific substances have been used to determine alterations in enzyme production and for identifying DNA and RNA. For nucleic acid, Feulgen staining identifies DNA, and acridine orange is specific for RNA.

II. Classification of Viruses

As the number of different viruses increased, attempts were made to place them in some logical taxonomic order. Since bacteria were organized in the general pattern of a dichotomous system, early attempts were made to classify viruses similarly. There were, however, insufficient definitive characteristics to utilize such a system adequately, so that it was necessary to relate each agent to a pathologic condition. Thus, the virus causing poliomyelitis was poliovirus, that of canine distemper, distemper virus, and so forth. Some attempt at nomenclature was made in past editions of *Bergey's Manual of Determinative Bacteriology*, but insufficient data were available to administer reasonable order to a classification system.

Viruses have been grouped according to the organ or anatomic system that is affected in the mammalian host. Thus, neurotropic viruses included poliomyelitis, equine encephalitis, rabies, and other agents that produced a clinical expression of central nervous system invasion. Similarly, influenza, canine distemper, and adenoviruses

were pneumotropic agents, and variola, measles, and vesicular viruses were grouped as dermotropic viruses. This crude system of categorizing viruses took no account of characteristics of the agents themselves, and further did not recognize that "tropism" and clinical expression were not necessarily a primary association.

In recent years the accumulation of information on virus particles has provided an opportunity to categorize the various viruses on the basis of characteristics relating to chemical or physical structure or to some specific biologic behavior. The recent text by Andrewes should be consulted for details on virus taxonomy (Andrewes, 1964).

The division of viruses into those containing RNA or DNA results in two major groups. Further, such characteristics as lipid content, pH stability, and size can serve to place them in more definitive groups.

Within the major groups of viruses such as myxovirus, picornavirus, herpesvirus, and adenovirus, antigenic characterization can serve to further separate into strains or types. Influenza virus, for example, is separated into several major groups (types A, B, and C) by the presence of specific antigens which are, however, not shared with other members of the myxovirus. Within the major group or type further separation into strains can be made by identification of additional antigens that are peculiar only to a single strain.

In some cases the antigenic relationship may be so close that partial cross-reactivity exists. For example, antibody produced against measles virus will neutralize both measles and distemper viruses. Antibody against the latter agent, however, will only neutralize in the homologous system. Similar relationships exist between human and canine adenoviruses.

General cytopathogenic changes in cell culture frequently are typical of members within a group. Necrosis, giant cells, vacuolization, and syncytial formations are effects that can be related to one or another virus type. One of the most distinctive changes in cell culture is the formation of intranuclear inclusions such as those associated with synthesis of adenovirus and herpesvirus.

Even more sophisticated methods have been used to distinguish members of a group. Thus, the papilloma viruses (rabbit, bovine, human, and canine), which are grouped together with other oncogenic agents (polyoma and SV_{40}) as the "papova" viruses, can be distinguished on the basis of small differences in particle size and differences in DNA base ratios (guanine-cytosine) (Crawford and Crawford, 1963).

When an unknown virus is examined for the purpose of identification with specified groups, there are several characteristics that may be used for tentative placement. Serologic studies, such as complement-fixation with a group-specific antiserum, cytopathogenic studies in cell cultures, stability in ether and acid, and animal pathogenicity, can serve for preliminary classification. Additional characterization such as nucleic acid type and antigenic structure is necessary before final definition can be justified.

Within groups of viruses, small structural differences in the particle may serve to form a basis for separation into subgroups. Such differences have been used, in part, to distinguish between two groups of myxovirus, the influenza-fowl plague and Newcastle-mumps types (Waterson, 1962).

The influence of metal cations on stability of viruses to heat has been used as a classification basis. Magnesium ions have been studied in regard to their effects on picornaviruses (polio, ECHO), which develop increased heat stability in the presence of such ions. Others, such as adenovirus, herpesvirus, and myxovirus, are more labile to heat when magnesium is present. Additional studies have shown that aluminum ions may suppress growth of some viruses (Wallis and Melnick, 1962).

III. The Virus as a Biologic Entity

Little was learned of the nature of the virus particle during the first three decades following the identification of viruses as filterable agents. It was assumed that the infective fluids contained particles of a high

degree of specificity, and that since they were obligate cellular parasites, they did not contain the enzyme systems that were necessary for maintaining themselves as self-propagating organisms. It was not clear for many years whether the cell simply supplied the essential materials for the virus to use in replicating itself, or whether the host was an active participant in virus synthesis. That the latter is true in mammalian viruses followed the demonstration of the mechanisms of viral synthesis in bacteriophage.

A. PHYSICAL NATURE

The two technics that have led to the elucidation of the physical nature of the virus particle, or "virion" (Lwoff *et al.*, 1959) are electron microscopy and, to a lesser degree, X-ray diffraction. During the latter half of the 1930's and 1940's information began to accumulate on the physical appearance of the virus particles. Compared with current knowledge, these data were relatively gross and only offered a rough estimate of size and shape. In the past decade technics of electron microscopy have been developed that permit physical examination of particles with a comparatively high degree of accuracy. Further, by the combined use of electron microscopy and labeled antibody technic, even more dynamic studies beyond mere physical characterization are possible.

It is now recognized that two basic and separable components of the virus particle exist, nucleic acid and protein. Further, the arrangement of these components within the particle or virion are in a definite pattern for a given virus, but may vary among different viruses. Also, size and shape of the virion are fixed factors for a given virus type. Particles may have a limiting membrane with surface projections, as influenza virus, or relatively smooth single or double membranes as with poxvirus. Other morphologic details have been defined for a number of different viruses.

The internal parts of the virus particle also are distinctive and arranged in a definite order or symmetry. With some viruses, morphologic subunits called capsomeres have been identified. These are the viruses that have cubic symmetry which, unlike those of helical symmetry, require a rather definite geometric arrangement of the subunits. The capsomere may be a monomolecular unit, but some appear to have several protein units. The nucleic acid portion of the particle appears to exist in most cases as a single nucleoid of DNA or RNA in plant viruses or bacteriophage, but there is no data available on this point for mammalian viruses.

B. MUTATION

As the understanding of viruses as genetically complete units became clear, it was obvious that they possessed the important potential of mutation. Although these abrupt and sudden alterations of genetic character undoubtedly occur in the natural life history of viruses, laboratory procedures have allowed more detailed study of the phenomenon of mutation. The basic mechanism of mutation is unknown, but certain agents such as X-irradiation can be used to induce these changes with quantitative accuracy. Alterations of an environment in which the virus is being propagated may favor the selection of some mutants as the dominant type in a mixed virus population. There can be little doubt that the primary site of alteration in the sudden production of a mutant is the nucleic acid, and even in RNA viruses differences can be detected between a mutant and parent nucleic acid (Papaevangelou and Youngner, 1961).

Practical use of mutant virus strains, aside from genetic and other basic studies, has been applied to the development of immunizing agents. These include vaccinia, yellow fever, poliomyelitis, canine distemper, hog cholera, canine adenovirus, and infectious bovine rhinotracheitis viruses. Because of this practical application, more study has been done on poliovirus mutants than other mammalian viruses. The most detailed and diverse studies on virus mutants, of course, have been done with bacteriophage.

In determining the presence of a mutant strain some physical or biologic expression must be present. Since physical differences are minimal, most reliance is placed on an alteration of biologic, or specific biochemical, characteristic(s). Differences in suscepti-

bility to environmental factors, dependence upon specific chemicals for synthesis, and other such characteristics that differ between parent and progeny strains have been referred to as genetic markers. Poliovirus strains may differ by one or several markers, such as ability for growth at high or low temperatures, resistance to or dependence upon the presence of guanidine in the medium, capacity to produce specific pathology in injected animals, plaque size, and other factors.

Growth of viruses in the presence of some chemical compounds that are partially inhibitory may result in the selection of mutant strains that are not inhibited. In other cases, drug-dependent strains have been isolated that do not have previous history of drug contact. Another type of variation among mutant strains concerns the ability of viruses to induce specific enzyme activity in host cells. Herpesvirus is capable of inducing thymidine kinase activity, but herpesvirus mutants occur that lack this inducing capacity (Kit and Dubbs, 1963; Dubbs and Kit, 1964).

The frequency of mutation in situations of natural infection is unknown. The phenomenon apparently does take place, since mutant strains can be isolated from natural infections. A question that has been raised concerning the use of mutants of attenuated virulence as vaccines is whether or not a so-called reverse mutation to a strain of virulence can occur. There is some evidence to indicate that mutations of attenuated virulence are relatively stable and that back mutation may not occur. With poliovirus vaccine strains, some increase in virulence may occur with passage through susceptible hosts, but return to the full virulence of the parent strain has not been demonstrated. Hog cholera-attenuated strains, on the other hand, will return to fully virulent types following back passage through swine. Thus, stability of mutations in viruses is a variable factor and must be defined according to the specific virus and the environmental conditions in which it exists.

Recombination is, in a sense, the hybridization within a host cell of two parent agents of phenotypic identities so that three types of progeny are produced (two parent types and a third type). A phenomenon of this type requires dual infection with two genetically distinct agents, and then incorporation of part of the nucleic acid molecular structure of each into a new basic viral structure. In addition to bacteriophage, recombinants of mammalian viruses have been studied, particularly influenza and poxvirus. It is necessary, of course, that closely related agents be used for the demonstration of recombination. The presence of a recombinant depends upon the development of a phenotypic expression that is distinct from those displayed by parent strains. Such expressions, or markers, may be altered pathogenicity, drug resistance, antigenic composition, or other characteristics that can be measured in a controlled system. It is not known whether such dual infection of cells with the production of new viral offspring occurs in natural circumstances as well as in laboratory systems. If so, it is interesting to contemplate this phenomenon as an explanation for the derivation of new virus types.

C. LYSOGENY, TRANSDUCTION, LATENCY

The well-known phenomenon of lysogeny in bacteriophage-host cell interactions is the primary example of genetic incorporation of virus nucleic acid into the host cell genome. It is becoming increasingly clear that similar mechanisms can occur in mammalian virus-host cell systems. A great interest in this regard has been generated by workers in viral oncology, since a sequence of events of this sort offers a logical explanation for the peculiar biologic phenomenon of tumor induction by virus. This subject is discussed in more detail in succeeding chapters.

Less attention has been given to the theoretical possibility of transduction as a mechanism in mammalian virus-cell interactions. This reaction, which was discovered by Zinder and Lederberg (1952) in bacteriophage infections, involves the transfer of genetic information from one host cell to another by a virus. In brief, the virus accepts genetic characteristics from one host cell into the viral genome, and then donates these genetic data into the genome of a recipient cell. The mechanism, therefore, differs from transformation in which in-

formation is transferred from one bacterial cell to another by DNA alone. Transduction is, in essence, an "infective heredity," and it is interesting to consider the implications of a hypothetical reaction of this type in a mammalian virus system *in vivo*.

The influence of viral nucleic acid in the pathogenesis of naturally occurring disease is difficult to evaluate. Without proof, it might be suggested that viral nucleic acid can be freed from protein and infect cells. This might explain the progress of such diseases as herpes stomatitis in the presence of circulating antibody. On the other hand, such transmission of infective nucleic acid must either be rapid and between adjacent cells, or it must be under the influence of some protective factor to prevent enzyme inactivation. A further explanation might be offered, which is that a mechanism exists for the production of enzyme inhibitors during the infective process.

A phenomenon that is of increasing significance is the mammalian host-virus relationship called latency. Although there is an obvious interest in the viruses that cause proliferation rather than cell death, latency has been considered in other types of virus-cell reactivity.

Lysogeny in bacteria-bacteriophage systems is a clearly defined event. It is not yet certain that a completely analogous system occurs in mammalian cell-virus systems, but the term "virogeny" has been applied to the lysogenic-like state. This implies that a mammalian virus exists as a provirus in the living cell. In other words, the virus, or at least a part of it, has been incorporated into the genome of the cell. Such a phenomenon has been suggested to be part of the mechanism involved in the transformation of normal fibroblasts to tumor cells by Rous sarcoma virus and other viruses.

The implication associated with mechanisms of this sort is that viruses may assume a dual role in some cases, that of the etiologic agent of acute disease and, in addition, an insidious hidden passenger of otherwise normal cells, a relationship that might on some subsequent day manifest itself in a manner unrelated to the initial acute syndrome. Thus a virus, theoretically at least, might either cause an acute respiratory disease on the one hand, or, on the other, cause a chronic condition characterized by either cellular proliferation or degeneration.

Latency in mammalian virus infections is not necessarily concerned with the incorporation of viral nucleic acid into the genome of the host, and extrachromosomal latency is conceivable.

A latent infection may mean little more than a chronic virus infection in which the host reaction is maintained below the clinical level for a period of time. In this sense, adenovirus may be a latent infecting agent for it apparently produces minimal damage for long periods of time, but can be recovered from affected tissue.

Although virus infections are usually considered as causes of acute clinical episodes of relatively short duration, there is increasing evidence that it may become necessary to credit them with more diverse capacities. In this regard, two animal diseases, Aleutian mink disease and scrapie of sheep, are of particular interest, for they illustrate mechanisms of disease production by viruses that could typify other host-virus mechanisms. Aleutian disease has the clinical and pathologic characteristics of an autoimmune disease, and at one time was thought to be transmitted as a recessive genetic factor, since it was peculiar to the offspring of certain matings. It was found eventually to have a virus etiology, in spite of its chronic nature, and its pathologic identity with the so-called collagen diseases. Scrapie, which is discussed in more detail in a succeeding chapter, is a virus disease that is characterized by a chronic degenerative lesion of the central nervous system. Natural transmission can be achieved by direct contact, or by congenital infection. The time period from initial contact of virus to the development of signs is usually very long. The analogy between these diseases and some chronic degenerative processes in other species, including man, can be clearly indicated, and the possibility that they, too, may have causal viruses is an intriguing postulation.

Immune tolerance is a relatively new aspect of the host-virus relationship. The classic example is the virus of lymphocytic choriomeningitis (LCM) in mice, although