LUDWIK GROSS

Oncogenic Viruses

ONCOGENIC VIRUSES

by

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Introduction

THIS monograph was written with the purpose of reviewing animal tumors caused by oncogenic viruses. It would be difficult to define precisely a virus-caused neoplasm. With only few exceptions, however, such a definition would refer to tumors that could be transmitted in the laboratory by inoculation of filtrates.

At first, only very few tumors could be transmitted by filtrates. More recently, however, the number of tumors transmissible by cell-free extracts has been increasing at an accelerated pace. It is now quite apparent that most of the chicken tumors, and the great majority of mouse tumors, are of viral origin.

It would be rather difficult to assume that malignant tumors in other species are of different origin. It is thus quite possible, perhaps even probable, that malignant tumors in various animal species, including humans, are also caused by viruses.

It is immediately realized that objections will be raised at this point. It is true that cell-free transmission of many fowl and murine neoplasms has been demonstrated. Such tumors are unquestionably caused by transmissible, submicroscopic agents. This does not necessarily imply, however, that all other tumors are caused by similar submicroscopic agents, or that oncogenic viruses are indispensable causative factors in the induction of tumors.

Sufficient experimental data are not yet available to answer many of the fundamental questions referring to the etiology of tumors in animals or in man. It is helpful, however, to be guided by a working hypothesis, provided that facts are used as guideposts.

The interest in oncogenic viruses has increased considerably during the past few decades, and particularly during the last ten years. Many new and unexpected observations have been made, particularly in the fowl leukosis complex and in the field of murine tumors.

The use of newborn animals for inoculation of oncogenic viruses, the propagation of some of such viruses in tissue culture, the visualization of oncogenic agents with the aid of the electron microscope, have contributed considerably to the acceleration of the pace of progress in experimental cancer research. These are fascinating times in tumor research. New observations are being reported almost every few months.

The information concerning filterable, transmissible, oncogenic agents is scattered, however, in different journals, and printed in different

languages. The bibliography has been accumulating at such a rapid rate that it becomes difficult to keep abreast of latest developments.

In this monograph an attempt has been made to review the known oncogenic viruses, to discuss the current status of experimental approach to virus-caused neoplasms, and to list the most important bibliographical references.

It would be beyond the means of a single author to review adequately all data referring to oncogenic viruses as a group. With even best intentions, the author is obviously better informed on some aspects of this problem, than in other fields. Furthermore, limitation of space precludes entering into greater detail.

This monograph could be compared with a sketchbook, outlining in general terms the problem of tumor viruses in experimental cancer research. The reader interested in a particular field should be able to find more detailed information in references which are included at the end of each chapter. An effort was made to include the most important references; each was checked individually from the original publication.

L. G.

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CHAPTER 1

General Considerations

THE EXPERIMENTAL INOCULATION OF COMMUNICABLE DISEASES

General Considerations

The question whether or not a disease is communicable is obviously of fundamental importance, and should be clarified, if possible, before any further study of the disease has been made. There are, however, no particular criteria to indicate, *a priori*, that a disease is caused by a transmissible agent. In fact, in many instances, when studying a new and obscure disease, the investigator may be confronted with symptoms that appear to develop "spontaneously" in one individual or another. At first, it may be very difficult, if not impossible, to trace the mode of transmission of the hypothetical pathogenic agent. And yet, the disease may be communicable.

Whenever the communicability of an obscure disease was suspected, one of the first experiments in the laboratory consisted of an attempt to transmit such a condition by artificial inoculation to other hosts. Actually, this was an attempt to duplicate experimentally, at will, the suspected natural transmission of the obscure disease.

It was common knowledge a long time before the microscope and the world of microbes were discovered, that smallpox, and probably also some other diseases of man, could be transmitted by artificial inoculation. The same was known of certain communicable diseases of animals, such as foot-and-mouth disease of cattle. Gradually it has been learned that the great majority of the known communicable diseases could be transmitted by inoculation, from one host to another.

Successful transmission of a communicable disease from one host to another was possible only when certain experimental conditions were met. These conditions varied for different diseases. Until such requirements were determined, some of the infectious diseases could not be transmitted in the laboratory.

Many difficulties may confront the investigator. In principle, the causative agent is to be transmitted to a susceptible host. It may not be an easy task, however, to recover the pathogenic agent in sufficient quantity from the diseased host, unless the epidemiology of the particular agent is known. The causative agent may be present in the blood of the diseased

host or in the diseased tissues at certain periods of the disease only; in some instances difficulties may be encountered when attempts are made to recover the agent at the time when the disease is already fully developed.

In order to reproduce the disease, the causative agent must be introduced into a susceptible host. This may be readily accomplished in cases where the pathogenic agent has a broad range of infectivity, such as the virus of hydrophobia, which can infect a large variety of species. Many pathogenic agents, however, have a narrow range of hosts. Thus, in the study of certain obscure diseases, hosts of the same species must be employed for the initial inoculation. Furthermore, certain pathogenic agents have to be inoculated into very young, perhaps only into newborn hosts*.

THE FILTERABLE VIRUSES

With the progress of bacteriology, the identification, isolation, and selective, *in vitro*, propagation of various pathogenic microorganisms, became a laboratory routine. It was soon realized, however, that in the case of many obviously communicable diseases, such as smallpox, hydrophobia, or foot-and-mouth disease, no microbes could be visualized in the diseased tissues with the aid of a microscope; yet, inoculation of extracts prepared from such tissues into susceptible hosts reproduced characteristic symptoms of the same disease. It was quite apparent that such extracts may contain microorganisms so small as to be invisible to the human eye, even when examined with a powerful optical microscope. Only the recent advent of the electron microscope made it possible to visualize the smallest pathogenic agents, the viruses.

Pasteur tried vainly to find the microbe of hydrophobia in the brain or spinal cord of infected dogs (Pasteur *et al.*, 1884). When asked by an apparently impatient member of the Academy of Sciences† in Paris (who must have been impressed by Pasteur's successive and rapid discoveries of various microbes): " ... will there ever be a microbe of hydrophobia?", Pasteur replied in 1884: " ... one is tempted to believe, that ... this is a microorganism infinitesimally small".

The existence of such infinitesimally small microorganisms, invisible to the human eye under the optical microscope and suspected by Pasteur already in 1881 (Roux, 1903), was substantiated some ten years later, in 1892, by Iwanowski.

* It is quite apparent that such limitations may cause almost insurmountable difficulties in attempts to transmit experimentally certain obscure, progressive and generally fatal human diseases, such as pemphigus, malignant neoplasms and leukemias.

† Dr. Bouley, vice-president of the French Academy of Sciences (Pasteur *et al.*, 1884).

The mosaic disease of tobacco plants causes mottling of leaves with a brown discoloration; the leaves become brittle and damaged. In 1892, Iwanowski, in St. Petersburg, Russia, reported (1894*, 1898) that the juice obtained from leaves of tobacco plants afflicted with this disease could be passed through porcelain filters, which retained all visible microbes, and that such filtrates were fully capable of reproducing mosaic disease when inoculated into healthy plants. A few drops of the infectious fluid placed on leaves of a new plant, or instilled into the ground on which such a plant was growing, transmitted the disease.

In 1898, Beijerinck, in Delft, the Netherlands, made a similar observation. Since he was able to transmit the mosaic disease in tobacco plants by filtrates which did not contain any visible microbes, he assumed that this disease is caused by a "contagium vivum fluidum" (Beijerinck, 1898). Although his experiments were reported four years later than those of Iwanowski (1894), Beijerinck claimed (1899) that he had no knowledge of Iwanowski's studies when he wrote his report.

At about the same time, similar observations were made with pathogenic agents causing certain diseases in animals and in man. In 1898, G. Sanarelli in Montevideo, Uruguay, described a contagious and fatal disease afflicting rabbits and causing multiple myxomatous swellings in the subcutaneous tissue around the nostrils, mouth, ears, anus, and vagina. The causative agent was found to be so small that it could not be detected under a microscope. Thus, curiously enough, a tumor was one of the first diseases recognized to be transmissible by a submicroscopic agent. Sanarelli, however, did not filter his agent. Filtration experiments were carried out later†.

That same year, in 1898, Løeffler and Frosch, in Berlin, Germany, reported that the "foot-and-mouth disease" of cattle, a highly contagious disease of cloven-footed animals, could be transmitted by filtrates. It was thus evident that this disease is caused by a submicroscopic, invisible agent.

Gradually, many other communicable diseases of animals and man, for which no specific causative microbes could be detected under the microscope, were found to be transmissible by filtrates. Blood, or pathologically altered tissues, removed from hosts suffering from such communicable diseases as smallpox, hydrophobia, infantile paralysis, herpes, encephalitis, etc., were found to contain invisible, and filterable pathogenic agents. These agents could be transferred indefinitely from one host to another; hence, it was evident that they reproduced themselves in their hosts.

It thus became apparent that among transmissible diseases, some were

* The paper was read at the Academy of Sciences in St. Petersburg on February 12, 1892, but was published (in German) in the Bulletin of the Academy in 1894 (Iwanowski, 1894).

† See Rabbit Myxomatosis on p. 17 in this monograph.

caused by agents so small that they could not be detected with an ordinary optical microscope. The term "viruses" which had been previously employed on a more general basis, and which at first included a broad group of pathogenic microorganisms, gradually became restricted to very small, submicroscopic pathogenic agents*.

Filtration

Most of the larger microbes are retained by bacteriological filters, whereas viruses generally pass through. There exists a large variety of filters employed in the laboratory. The filter pads, filter membranes, or filter candles, vary in porosity, and can be selected according to needs. Most of the filters employed, such as Chamberland, Berkefeld, or Selas filter candles, or Seitz filter pads, retain the larger microbes, but allow the smaller viruses to pass through. A certain quantity of virus particles is lost during filtration procedure, because of absorption of the particles by filter pads or filter candles. This is particularly true when Seitz asbestos filter pads, to a lesser extent when infusorial earth filter candles (Berkefeld) or unglazed porcelain filter candles (Chamberland or Selas), are employed. Sinter glass filter candles are also available, as well as graded collodion membranes; the latter allow most of the virus particles to pass with relatively minimal loss due to filtration procedure.

A filtrate passed under good technical conditions through bacteriological filters of proper porosity, such as Berkefeld N, Chamberland L 3, or Selas O2, or O3, may contain viruses, but is considered "bacteriologically sterile", i.e. free from the larger microbes. Minute defects may occur,

* Émile Roux, a close associate of Pasteur, reviewed the problem of "invisible" microbes in 1903. He noted that although all microbes are very small, as their name implies, and require a microscope to be seen, their size actually varies to a considerable extent. The Schaudin's *Bacillus Bütschlii* (a very large microbe found in the intestine of cockroaches) has a diameter of 3 to 6 μ. This is a real giant as compared with Pfeiffer's bacillus; the latter (*Hemophilus influenzae*) has a diameter of only 0.5 μ. Since, for all practical purposes, using transmitted light, the limit of visibility is about 0.2 μ, a microbe only four or five times smaller than Pfeiffer's bacillus could not be seen even under the best optical microscope.

What justification do we have, asked Roux, to assume that the world of microbes ceases to exist at the level of 0.1 or 0.2 μ? It is only logical to assume, Roux noted, that there exist microorganisms much smaller, and therefore invisible to the human eye.

Roux quoted Pasteur who suspected as early as 1881 that rabies is caused by a virus so small that it can not be seen under the microscope. At that time the invisible viruses were only "beings of reason" ("êtres de raison").

Since it was established, however, that tobacco mosaic disease in plants (1892) and foot-and-mouth disease in cattle (1898) could be transmitted by filtrates, the "beings of reason" became "beings of reality".

A short half of a century later, electrons, employed instead of light waves, opened the world of viruses to the human eye.

however, in the filter candles, and for that reason filtrates should always be tested for bacterial sterility.

* * * * *

Filtration thus became a very convenient tool in the study of virus diseases. Whenever an investigator was confronted with an obscure, and possibly infectious disease, one of the initial experiments consisted usually of an attempt to transmit such a disease from one host to another by means of a filtrate. If an extract prepared from diseased tissues could be filtered without losing its pathogenic potential, i.e. when the filtrate reproduced symptoms of the same disease following inoculation into a susceptible host, it was generally assumed that such a disease was caused by a virus.

The term "filterable viruses" was employed to designate very small pathogenic agents capable of passing through bacteria-tight filters and responsible for many transmissible diseases in humans, animals, and plants.

Some Fundamental Properties of Viruses

Viruses are generally smaller than ordinary microbes. As a rule, they are less than 400 mμ in longest diameter. Some viruses are very small, about 20 mμ or even less in particle diameter. Others exceed 200 mμ in diameter. Their shapes vary from round or spheroidal particles, to rods, filaments or brick-shape crystals.

Although our knowledge of the biochemical structure of viruses is still very fragmentary, it is quite probable that most viruses consist of a central core of nucleic acid, which is the genetic material responsible for infectivity, and of an outside protein shell.

Viruses are specific pathogenic agents. They cause symptoms of characteristic diseases. Some have a wide range of hosts. Other viruses have a narrow host range and infect only a single species. Some are transmitted directly from host to host, whereas others require intermediary hosts, such as insect vectors, for transmission. In certain instances man may be only an accidental, occasional host of a virus which usually may be maintained in nature primarily in other species.

Many viruses have a preference to invade and multiply in certain cells of particular organs. This selectivity of a virus for a particular cell type may be quite strict. Yet, most viruses may adapt themselves to various types of tissues and may thus be able to infect, under proper conditions, a broad spectrum of cells.

In a group of virus particles of the same kind produced in a multiplication cycle, a certain proportion may contain genetic material slightly different from the rest. This altered genetic constitution may then be

retained in successive virus generations. Such spontaneous mutations occur now and then among viruses.

Viruses may frequently remain latent, causing only occasionally symptoms of disease. In this respect they do not differ from many bacterial infections. Latent carriers of viruses and pathogenic microbes are by far more frequent than those with frank symptoms of disease.

Viruses are obligate cell parasites. After entering a susceptible cell, the virus loses its identity, and disappears within the cell. The virus may then become latent and completely incorporated in the genetic material of the host's cell. The cells may later multiply, and carry along the invisible virus through successive cell generations.

The virus may, on the other hand, induce fundamental changes in the metabolism and morphology of the cell, which may lead to degeneration and eventually to cell destruction, with a concurrent liberation of a large number of newly formed virus particles.

The reproduction of viruses differs fundamentally from that of the ordinary microbes. Some of the larger microbes, such as gonococci or brucella organisms, also grow intracellularly. The ordinary microbes multiply, however, by binary fission. From one microbe, two are formed; the two now split, and four are formed; under ideal conditions, the number increases in geometrical progression, to eight, sixteen, thirty-two, sixty-four, and so forth.

The reproduction of the viruses is quite different. Actually, what happens in the cell following the entry of an infective virus particle is poorly understood. It is apparent, however, that the virus particle disintegrates and ceases to be infective on bio-assay, or to be detectable in the electron microscope. After a latency period (eclipse phase) which varies for different viruses, a large number of newly formed, reconstituted virus particles, as many as two hundred or more, reappear in groups (inclusion bodies), or scattered, and at about the same time in the nucleus or in the cytoplasm.

The cell's genetic material serves as a ready source for the replication of the genetic cores of the newly formed virus particles. The cell's cytoplasm then serves to complete the formation of virus particles providing the outer protein shell for the virus. Degeneration and eventual destruction of the cell follows, with the liberation of a large number of newly formed virus particles.

The virus particles can now infect new cells and either remain latent, or proceed, after a brief eclipse phase, with their reproductive cycle, which ends again with the destruction of the cell and the liberation of newly formed virus particles.

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