VIRUSES AND TUMORS

An Old Problem in the Light of Recent Advances[1]

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I. INTRODUCTION

A. Definition of a Virus

A discussion on viruses and their relationship to tumors must of necessity include an attempt at the definition of what is a virus and what is a tumor.

It is apparent that the definition of a virus is not an easy matter. It often leads to a discussion of the notion of organism and even of life itself, both, to say the least, rather difficult subjects (32). Is a virus a microorganism or an organism, is it a molecule, or is it a replicating agent of cellular origin? The definition is almost invariably reflected by one's training and even emotional make-up. Recently a penetrating analysis of the various definitions of a virus was given by Lwoff (145). He has proposed the following definition: "Viruses are infectious, potentially pathogenic nucleoprotein entities, with only one type of nucleic acid, which reproduce from their genetic material, are unable to grow and divide, and are devoid of enzymes." This last property may still be debatable, as some tumor-inducing viruses (84, 148) and infectious viruses (138) appear to have enzymes which have not been separated from these agents.

Like any definition, this one may be subject to lengthy discussion or argument, but for the time being it is acceptable as perhaps the least controversial and the most realistic.

B. Relationship of "Infectious" and "Tumor" Viruses

The next problem is one of possible doubt in some quarters whether the definition of viruses causing infections is acceptable for viruses causing tumors. According to some investigators (41, 42), these two types of agents differ from one another.

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Doubt has even been cast whether the tumor-inducing agents are viruses and not agents in a class by themselves. Reasons will be given as a basis for the contention that this differentiation between infectious and tumor-inducing viruses (46) is not supported by facts. The differentiation of viruses into "infectious" and "tumor-inducing" is a purely artificial one and refers to the clinical disease produced by a virus, either infection or tumor or both. All viruses by their very nature infect cells. However, according to Lwoff (145): "The essence of infection is not the disease, but the introduction into an organism of a foreign entity able to multiply, to produce a disease, and to reproduce infectious entities." This definition eliminates from infectiousness normal structures which can penetrate into another cell, such as the transforming principles and the lethal genes (145). Attempts at describing tumor viruses as bits of chromosomes, plasmagenes, cellular constituents, or enzymes are speculations lacking any factual basis.

Infectious viruses share with tumor viruses their requirement for living cells, their cell and host species specificity which may change or vary, their ability to remain latent for long periods of time, and their ability to produce different lesions according to the age of the host, to mention some of the common properties (57). Morphological, immunological, and biochemical studies have failed to reveal any essential differences between infectious and tumor-inducing viruses. Difficulties encountered in isolation and purification of these viruses are similar, if not greater, than those faced in similar procedures applied to infectious viruses. Only quite recently have the virus of poliomyelitis and Coxsackie virus been purified. Yet the purification of the papilloma virus of rabbits was accomplished many years previously (34).

Infectious viruses cause lesions ranging from suppurative to necrotizing, and also proliferative lesions. So far, only the tumor-inducing viruses have been found to induce all the lesions mentioned and also continuous, unrestricted, proliferative change, that is, malignancy. No known infectious virus has been found as yet to be responsible for malignancy. Experimental evidence may become available on the part played by infectious viruses in hosts with tumors and the possible difference in their behavior following sojourn in such hosts. In other words, is it possible that such viruses may carry material, genetic or nucleic, from hosts with cancer to other normal hosts, and thus act as initiators of malignancy (171)?

C. Definition of a Tumor

This brings us to the definition of what is a tumor. The term is frequently used of "benign" and "malignant" tumors. It is now known that the distinction between these two types is at best arbitrary (197), as it is closely associated with the development of tumors. There exists a new outlook on the development of tumors (95) which is outside the present discussion. It has been stressed, however, that the concept of a sharp distinction between normal and cancer cells is no longer tenable (179). It has been replaced by a gradual, stepwise, progressive change from well organized cellular patterns to the unrestricted behavior of cells in malignancy, combined with invasion of surrounding and then distant normal tissues and their ultimate destruction. It would appear, therefore, that the more we know about viruses and about tumors, the more facts become available, the more difficult is the definition of what tumors really are. Views formerly held and accepted are no longer tenable.

A proper assessment of the part played by viruses in the origin of tumors appears to have been seriously hindered by the simplified question, are viruses the cause of cancer? A great deal of time and also temper might have been saved and more rapid progress achieved by simply phrasing the question in a somewhat different way: are viruses one of the causes of cancer? Time and progress in many disciplines of cancer research were required before it was fully realized that cancer, no matter of what type and origin, is the result of many factors of the most diverse nature (166). Viruses may or may not be one of these factors, besides genetic, hormonal, and many environmental factors. Their mode of action, as yet, is almost entirely unknown. The proper approach therefore would be a question: are viruses involved in the origin of some or of many tumors, while acting in combination with other known factors? This combined action is also important in tumors already known to be induced by viruses, because the action of a virus is conditioned by the host and his properties, such as susceptibility, that is, genetic factors, hormonal factors, metabolism, and age. The dose and

route of inoculation of the virus also play an important part; their importance was largely elucidated by the work on chicken tumor viruses (5, 6, 35) and on rabbit papilloma (10).

This brings us to the relationship of viruses and tumors. It cannot be properly understood without looking into the past, discussing the present, and appraising the future. It would be impossible to discuss the vast amount of literature of experimental work in the field of cancer within the framework of the present review. Reference should be made to an excellent book by Oberling (152) and to other reviews (57, 156). An attempt will be made to present only some fundamental facts and their bearing on the part played by viruses in tumors.

II. PAST ACHIEVEMENTS

A. Decade 1908–1918

The first suggestion that some tumors may be caused by viruses came from French investigators (28–31). They based their theory on the similarity of fowl pox lesions to those of human molluscum contagiosum, and of proliferative lesions of vaccina to a neoplastic process. Since the discovery of viruses by Iwanowski in 1892 (130), and its confirmation by Beijerinck in 1899 (12), the first report of a tumor-inducing virus was made by Ellermann and Bang in 1908 (88), who discovered the virus of chicken leukosis. The report on cell-free transmission of chicken leukosis passed at first almost unnoticed. The following decade had one highlight—the discovery of the virus of chicken sarcoma by Rous in 1911 (165), and of the virus causing chicken osteochondrosarcoma (168). The Rous sarcoma, like any other virus-induced tumor, exhibits all the properties of a tumor (167). The heated arguments which followed the discovery of Rous need not be described in detail. They were a foretaste of arguments which followed almost every discovery of a tumor-inducing virus. Even quite recently, the old argument was put forward that metastases in virus-induced tumors are not true metastases, as they are only infections of distant parts of the body, and are not due to transported tumor cells (110, 111). It has been known, however, that they are true metastases since 1911 (165). Another argument was that the filterable tumors are a "disease *sui generis*," distinct from "true" tumors of birds (2). This argument has also failed to withstand the test of time.

Single cells, getting through defective filter candles, were also put forward as the real cause of Rous sarcoma. Then a transmissible mutagenic factor was suggested as the real cause of the tumor (151). Finally, the argument was brought forward that if it was a virus, this was of little significance, as it was a chicken tumor virus, far removed from animal not to say human tumors. The understanding that many tumors of animals are of viral origin came only gradually and very slowly. To recollect the famous and now forgotten argument: "Viruses, worms, tar—all can produce cancer." Why not maintain that all tumors are caused by worms? It is as reasonable as postulating a virus for every new growth (3).

B. Decade 1918–1928

The next decade, from 1918 until 1928, had comparatively little to add, except for continuous argumentative discussion, and the discovery of the viral etiology of human warts by Wile and Kingery in 1919 (196). The confirmation of the discovery of Rous and the discovery of other chicken tumor viruses by Fujinami and Inamoto in 1910, Lange in 1914, Pentimalli in 1916, and Teutschlaender in 1921 (see (46)) should be mentioned. Alas, because of untimely attempts at generalization that all tumors are caused by viruses (123, 124), they rather added fuel to the fire of criticism to which viruses as causative agents of cancer were subjected. The idea of a universal virus being the cause of the multitude of tumors in animals and man appeared unfounded and somewhat ridiculous. In the words of the British pathologist, Dr. Boycott (33), "If a virus present in normal cells and ordinarily non-pathogenic were responsible for all tumors differing so much in the histological structure and course of development, then one had better call it something other than a virus."

C. Decade 1928–1938

The next decade, from 1928 to 1938, still carried on the arguments and the sharp and greatly justified criticism of the possibility of viruses being implicated in the origin of "real" tumors. At the beginning of this decade unequivocal evidence of a relationship between condyloma acuminatum and cancer was established (44). During the first half of this period, the number of viral avian sarcomas reached eighteen (46). They were: spindle cell sarcomas, myxomas,

bone and cartilage tumors, and endotheliomas. The virus of each of these tumors was observed to induce only the same type of tumor. However, the possible complexity of avian tumor viruses had become apparent already (154, 155) in the reported induction of malignant tumors by chicken leukemia viruses. The problem as to whether one or many viruses cause leukemia and other tumors of birds came to be hotly disputed. Experiments during the following decades have contributed greatly towards the elucidation of this problem, though it is as yet not entirely solved.

In the second half of this decade, the writer became interested in the then rather unpopular problem of tumor viruses. Fortunately, perhaps, for him and others a number of discoveries during this decade laid solid foundations for things to come. Hardly twenty years have passed since the time when the writer in collaboration with Gye was engaged in unsuccessful attempts at cell-free transmission of mammary tumors of mice (*unpublished*). The technique of preparation of cell-free extracts was correct but genetically suitable test mice were not available and the importance of newborn mice was not realized. The same technique with suitably bred test mice now gives positive results in almost every case and is almost taken for granted. In the writer's mind there were frequent doubts whether the line of interest taken up was right after all, as there was no "real" mammalian carcinoma in which a virus could be shown to be implicated. Meanwhile, however, evidence was accumulating of viruses playing a part in other tumors.

Dogs were found to suffer from an infectious oral papillomatosis caused by a virus (149, 158). Also in 1932, Shope discovered that a fibroma of rabbits is caused by a virus (180) and in the following year he demonstrated the viral etiology of infectious papilloma of rabbits (181). Considerable effort was spent on studies of the newly discovered rabbit tumors, which were crowned by the description of the rabbit papilloma-carcinoma sequence (167), with, alas, the eventual "disappearance" of the virus from the carcinoma. This, at first sight, disappointing finding may be a clue to the behavior of at least some viruses in malignant disease. During the next decade, this sequence was subjected to further, critical examination (190), but this phenomenon is far from having been completely explored. The fascinating biological phenomenon of the fibroma-myxoma transformation was also described during this time (23). The heat-inactivated myxoma virus transforms the live fibroma virus into a myxoma virus. This is now known to be due to deoxyribonucleic acid of the myxoma virus (183).

In 1936, Bittner made his fundamental discovery of the mammary-tumor-inducing virus (24). The discovery proved to be of far-reaching importance, as there was for the first time a "true" carcinoma of a mammal found to be caused by a virus. The discovery of this virus is all the more important because it led gradually to the realization of the importance of the host in the final action of the virus. It is all the more to the credit of Bittner that he pointed out the equal importance of the genetic background and of the hormonal make-up of the host in the final result of virus activity, that is, the formation of a mammary tumor (25). The age of the host, as with many infectious viruses, also plays an important part in excluding the tumor-formation but not viral persistence and multiplication. The discovery of this virus came after more than thirty years of intensive studies on mammary tumors of mice, and was brought about by the introduction and development of inbred strains of mice, originated by C. C. Little in 1909. Soon after the discovery of Bittner another carcinoma, that of the kidney of a frog, was also found to be caused by a virus (144). In the true fashion of the period, both the virus of mammary carcinoma of mice and that of the carcinoma of the kidney of the frog were considered by many either as "factors," whatever these may mean, or frankly as anything else but a virus. It is characteristic of the attitude prevailing at that time that pressure was brought on the discoverer to call the mammary tumor virus a "milk factor" and not an agent, as "an agent" implied a virus.

During this decade, the leukemic forms of chicken leukosis were classified as erythroblastosis and myeloblastosis (102–104) and a proper morphological understanding of the fowl leukemias was reached. Both the erythroblastosis and myeloblastosis (102) frequently associated with sarcoma (89, 103) were found to be transmissible and virus-induced. The possible variation of viruses causing leukosis and sarcoma in chickens was reported, as already mentioned, by French investigators (154, 155) and also by Danish workers (89). This decade's contributions may be

concluded by the mention of the induction of lesions in the ectodermal layer of chick embryos by the Rous sarcoma virus (133).

D. Decade 1938–1948

During the next decade, 1938–1948, several forms of chicken leukosis, classified as lymphomatosis, were examined extensively by Burmester and his associates and the part played by a virus in visceral lymphomatosis was shown (37, 131).

This decade provided a great deal of new information on some of the known tumor-viruses, their relationship to the specific host, and their variation and adaptation. It is of interest and of importance to note that the naturally occurring tumors in chickens have provided so much basic information on the viral origin and pathogenesis of virus-induced tumors. The discovery of hemorrhagic disease induced in chickens by Rous sarcoma virus (76), the induction by this virus of a similar disease in ducklings and of a multitude of other tumors in older ducks, turkeys, and guinea fowls (77, 79–81), for which credit is due to Duran-Reynals, seemed at first incredible. The observations were interpreted by some as a simple mix-up of viruses and hailed by others as a proof of a single virus being the cause of many types of tumors. Thus, it was shown that chicken-tumor viruses can be adapted to foreign species of birds, the adaptation being the more successful, the younger the foreign host (80, 81). The range of tumors induced was indeed very broad. It seemed incredible that Rous sarcoma virus may induce: hemorrhagic disease, myxosarcoma, spindle-cell sarcomas of periosteum and endosteum, bone sarcomas, lymphoid tumors, leukemias, angiomas, and endotheliomas. The change in species and tissue specificity of Rous sarcoma virus may also occur following the transplantation of the Rous tumor in the anterior chamber of the eye of an animal of foreign species (182), storage in glycerol, and prolonged cultivation in tissue culture (for literature, see (57)). Although the problem whether one or more viruses are involved has not yet been entirely solved, there appears to be no doubt that avian viruses like those of some mammals can under certain conditions behave as destructive or necrotizing agents.

The phenomenon of virus variation is not limited to avian tumor viruses, but was shown to occur in some mammalian viruses, *e.g.*, the fibroma virus of rabbits was shown to induce malignant tumors in rabbits which had some degree of resistance to the virus, and a lethal inflammatory disease in newborn susceptible rabbits (4, 78). During this decade, the first isolation and purification of a tumor-inducing virus, the rabbit papilloma virus, was achieved by Bryan and Beard (34). During the same period the epidemiology of avian lymphomatosis was subjected to careful study (37). The visceral lymphomatosis is probably the most important disease in chickens. It is a contagious malignant disease caused by an agent which has many properties common with viruses (37). It is spread by direct contact and other means which were elucidated during the next decade. The decade can be closed by mentioning the discovery of virus-like agents causing tumors in plants (26, 27). Thus, the kingdom of plants is also affected by viruses causing the formation of tumors.

III. RECENT ACHIEVEMENTS: DECADE 1948–1958

A. General Description of Present-Day Methods

The next decade, from 1948 through 1958, started rather uneventfully and seemingly inauspiciously. When the cell-free transmission of mouse lymphatic leukemia into newborn mice of certain strains was described by Gross (113–115), the report was treated with lack of enthusiasm, if not great reserve and even disbelief. In spite of some reports of investigations which failed to repeat this fundamental discovery (140), it was later to be amply confirmed, although in some cases with somewhat different interpretation. Before a more detailed discussion of this discovery and of the subsequent findings to which it led, some attention should be devoted to the methodology employed in the study of tumor-inducing viruses during this decade.

The methods used for the study of tumor-inducing viruses do not differ from those used in the study of infectious viruses. However, as in the study of the latter, so in the study of the former long known techniques received new attention and appraisal. All biological techniques for study of infectious viruses were available by 1914: all immunological techniques except for hemagglutination of Hirst (129), tissue culture, avian embryos, and living adult animals. As in the study of infectious viruses, the potentialities of old techniques were not fully utilized until fairly recently. The use of newborn mice was not fully exploited until the work of Gross and the subse-

quent studies of Stewart *et al.* (184–189). It might be a disservice to these investigators to state that the importance of newborn mice was indicated by the discovery of the mouse mammary tumor virus and subsequent work of Bittner and others. All the more credit goes to these investigators for having been first in the field with the use of newborn mice in mouse leukemia studies. The observation of cytopathogenic changes induced *in vitro* by the Rous sarcoma virus (143) was not fully exploited until the cultivation of the mouse leukemia-inducing virus on monkey kidney cells grown in tissue culture (189). More recently, the observation was made of a cytopathogenic effect of this virus on mouse embryo cells grown in tissue culture (86).

The cultivation of the leukemia-inducing virus in cells grown *in vitro* has led to a perplexing and most fascinating discovery of the potentialities of this agent (or these agents), as revealed by the induction in newborn mice of a multitude of tumors of different types and in various sites. The cytopathogenic effect exerted by this virus may prove to be a rapid and convenient method of determining the presence of this agent, better than the experimental animal. It may also prove to be a convenient method for assaying the infectivity of this virus and for the identification of specific antibodies. It may also lead to the isolation of new tumor-inducing viruses. The method of hemagglutination could be used successfully in assaying the presence of this virus, which induces multiple tumors in mice and hamsters and has a cytopathogenic effect in mouse embryo cells grown *in vitro*, as it has been shown that it will agglutinate guinea pig, hamster, and human erythrocytes (85). In addition, hemagglutination-inhibiting antibodies have been observed to develop in mice, hamsters, and rabbits inoculated with infected tissue culture fluids (85).

There is no intention to review here the extensive literature on studies of the antigenic properties of tumor-inducing viruses. They have extended over all decades, beginning soon after the discovery of Rous in 1911. A great deal of effort was spent during the last three decades to analyze the antigenic properties of preparations of tumor-inducing viruses which contained very little, if any, of purified viral antigen. Claims were put forward, either unfounded or only partly justified, about immunological properties of such preparations. Cross-reactions with normal tissue components and with immune sera against such components have been obtained. These have served as a basis for some interpretations of the existence of essential differences between infectious and tumor-inducing viruses (41). There exist extensive reviews on the immunological properties of the mouse mammary-tumor-inducing virus (53), the viruses of chicken leukosis (7), Rous sarcoma virus (128), and tumor-inducing viruses in general (57). Even reasonably purified preparations of some of the viruses still show considerable antigenic similarity to normal tissue components. This in itself should not be surprising and should not serve as a basis of seemingly firm conclusions about the antigenic structure of the tumor-inducing viruses. Since the 1920's a great deal of study has been devoted to the immunological properties of Rous sarcoma, with many contradictory results based upon the differences in the amount of impurities present in the preparations and on the techniques employed (2, 6, 7, 52, 132, 169). It is quite possible that conclusions about antigenic interrelationship of different avian tumor viruses may have to be revised, when more purified preparations are achieved and used in immunological studies.

As long as viruses responsible for different neoplastic conditions in the same species, such as erythro- and myelo-blastosis viruses, can be differentiated, and if various immune reactions can be utilized for the diagnosis of the presence of such viruses, the task of an immunologist will have been, at least for the time being, completed. It appears, however, that tumor immunologists have taken up a task more ambitious than the immunologists who study ordinary infectious viruses. Standard techniques of neutralization can now be used to detect antibodies against some viruses of fowl leukosis (83), or hemagglutination-inhibition tests for antibodies against the so-called Stewart-Eddy polyoma virus of mice (85). Neutralizing antibodies to the virus of Friend's leukemia of mice have been described and an effective formalinized vaccine against the virus has been reported (100, 101). The induction of passive immunity against lymphomatosis virus has been reported by Burmester (38). Passive immunity, following treatment with rabbit antisera, to the induction of parotid gland and other tumors of mice has also been reported (187). Thus, at least in certain tumor-inducing viruses, effects similar to those in infectious viruses can be

obtained. It should not be forgotten that even highly purified preparations of influenza virus contain host proteins (137), and the preparation of vaccines against infectious viruses was a long-drawn effort, at least against some of them. Diseases caused by other infectious viruses still elude effective and lasting vaccination.

There is no doubt, however, that the difficulties encountered in immunologic studies of tumor-inducing viruses are greater than those with infectious viruses (for review of literature, see (57)). But it is now established that the tumor-inducing viruses are antigenic in foreign species and that many, but not all, induce various types of antibodies in the homologous host species.

The comparatively recent method of electron microscopy of ultrathin sections of tissues infected by ordinary and tumor-inducing viruses presents an additional method in the study of tumor viruses. It has contributed greatly to our knowledge of the structure of viruses and to the structure of normal cells and of those infected by viruses (57). It presents a convenient method of search for structures resembling virus particles in tumors of known or suspected viral origin (58, 59). The usefulness of this method in studies of tumor-inducing viruses appears now amply justified (8, 9).

B. Reappraisal of Old Methods and Results of New Methods

The discovery by the use of newborn mice of the virus of lymphatic leukemia in mice by Gross (113–115) is one of the landmarks of this decade. He succeeded, where others failed by not using newborn mice. The discovery of the parotid gland carcinoma virus in mice soon followed (116, 117, 184–186). The virus of lymphatic leukemia apparently induces also subcutaneous sarcomas (118), and tumors of the adrenal glands (186). Some or all of these results have been confirmed by a number of investigators (74, 140, 170, 198). The induction of leukemia and other tumors in mice by cell-free filtrates of mouse leukemic or other tumor tissues has also been reported by others (172, 173). It appears that under certain conditions the leukemia-inducing agent may have other potentialities, unless it is assumed that more than one virus is present in the original cell-free extract of leukemic tissues of mice. This actually appears to be the case, as will be discussed later. However, the presence of two or more viruses does not preclude their variation. We will return to this point later. It should be mentioned that the use of newborn mice does not appear to be a strict condition, for the induction of leukemia in 14-day-old mice is possible following repeated passages of the agent (121). There are indications that the parotid carcinoma virus of mice may spread by cross infection from inoculated newborn mice to untreated litter mates (107). Similar results have been obtained in the case of the Stewart-Eddy polyoma virus by ourselves (71). In addition, some of the mice, inoculated with infected tissue culture fluids, show a curious involvement of the lungs (71), which histologically can be diagnosed as lobar pneumonia. It is of interest that some newborn mice of the same stock inoculated with tissue culture fluid from uninfected cells have shown similar symptoms (71).

In view of the many different types of neoplasms of the reticular system of mice, the question arises whether viruses are involved in other types of mouse leukemia. The virus of chloroleukemia in mice was discovered by Graffi in 1956 (112), and the virus of reticular cell-type leukemia of mice by Friend in 1957 (99, 100). The relationship of these two viruses to that of lymphatic leukemia of mice as described by Gross and that of the parotid gland tumor virus described by Stewart is not yet known.

The virus of parotid gland tumors in mice following cultivation in monkey kidney or mouse embryo cells grown *in vitro*, has been found to have a much wider range of activity, inducing a variety of cancers at a relatively early age. This appears to have been entirely unexpected and it is most fascinating. The virus can induce as many as 23 different types of tumors (189), affecting, to mention a few, the following organs: lung, pleura, thyroid, kidney, adrenals, skin, stomach, thymus, mucous glands, and mammary glands in both female and male mice (189). This virus, as already mentioned, has been found to induce cytopathogenic changes in mouse embryo cell cultures and to induce a variety of tumors in hamsters, animals of a species foreign to its original host species (86, 87). The size of the virus present in tissue culture fluids which induce cytopathogenic changes was found to be in the region between 1000 and 600 A (86). It is therefore similar in size to virus particles observed in the affected organs of mice with leukemia induced by cell-free filtrates of organs of mice with sponta-

neous leukemia (69), and also similar in size to virus particles found in ultrathin sections of parotid gland tumors of mice by means of the electron microscope (70). This point will be discussed later.

Before going into greater detail about these findings, the discovery should be mentioned of the transformation of the Lucké tumor virus in amphibia (162). The virus is tissue- and race-specific, but after growth in young salamanders, the virus shows affinity for the iris and skeletal tissues of the frog. It will also induce anaplastic lesions which lead to lysis and complete destruction of tissues, following transplantation of the renal tumor in young newts (191). The same will happen following adaptation of the virus to other resistant races of frogs. Thus, again an example was obtained showing how the specificity of a tumor-inducing virus can be broken down either by the injection of the virus into the adult host of a resistant race or into a young host of a foreign species. It leads to virus variation and to infectivity for tissues different from the original organ, and to the appearance of a new property of the virus, that of inducing necrotizing lesions.

Transformation of both rabbit and squirrel fibroma virus into live myxoma virus, when mixed with heat-inactivated myxoma virus, has been shown to take place in tissue culture of rabbit kidney cells (135). Thus, again tissue culture presents a convenient tool for study of virus recombination with nucleic acid or other constituents of cells of the host. Tumor-inducing viruses of widely separate species, under certain conditions, appear to exhibit similar behavior. The Rous sarcoma virus of chickens and other birds and the virus of other tumors of birds will induce in the embryos of newborn birds a non-neoplastic, hemorrhagic disease (76–81, 147, 157, 192, 193). The virus of rabbit fibroma behaves in a similar fashion (78, 79). The virus of parotid gland tumors of mice may exhibit affinity for different tissues and different species (85, 87, 187–189). It has been found recently by Dmochowski and his associates (71) that the Stewart-Eddy polyoma virus adapted to hamsters in addition to described tumors of liver, lungs, and subcutaneous tumors may also induce hemorrhagic disease, following inoculation into newborn hamsters. The virus of the hemorrhagic disease induces cytopathogenic changes in monkey kidney cells, rat embryo cells, and human amnion cells grown *in vitro* (71). The titer of the virus inducing these changes may be as high as 10^{-7} (71, 86). The virus of Lucké renal adenocarcinoma of the frog will induce sarcomas and necrotizing disease, following the passage in foreign or resistant environment (162, 191). Thus, tumor-inducing viruses of amphibia, birds, and mammals may behave in a similar fashion, and under certain circumstances exhibit similar variation. This is a great step forward, since the days of the discovery of the hemorrhagic disease in birds by Duran-Reynals (76).

During the present decade, a great deal of useful information has been obtained on neoplasms of chickens, classified as chicken leukosis complex, which were the first tumors shown to be induced by a virus. There are several known forms of neoplasia in this complex of diseases: lymphomatosis, which can be visceral, ocular, or neural (range paralysis), erythroblastosis, and myeloblastosis (57). Visceral lymphomatosis is as yet the only known neoplastic disease of frankly contagious character. It can be transmitted through the egg, in the saliva, feces, and through drinking water (39, 40). The virus of lymphomatosis has been found highly mutable, and under certain conditions may induce the following neoplasms: osteopetrosis, hemangio-endotheliomas, myxosarcomas, fibrosarcomas, osteochondrosarcomas, and erythroblastosis, from the so-called RPL-12 strain (Burmester, *personal communication*). The virus of myeloblastosis will, under certain conditions, induce renal carcinoma (Burmester, *personal communication*). A similar neoplasm is also induced by the virus of erythroleukosis (45). Thus, we have yet another example of virus variation or mutation, which has been found to depend upon the age of the chickens, the dose of virus, and route of inoculation (37).

Extensive studies on viruses of chicken leukosis during this decade led to the isolation and characterization of the viruses of erythroblastosis and myeloblastosis by Beard and his associates (6, 7, 9, 11). Electron microscope studies of ultrathin sections of ultracentrifugal pellets of preparations of these viruses have undoubtedly been very helpful in differentiating virus particles, with their characteristic structure, from normal cell components (20). They will be discussed later.

The induction of cytopathogenic changes in chicken embryo liver tissue cultures by the virus of visceral lymphomatosis has recently been re-

ported (93, 178). A great deal of work still remains to be done along these lines. However, the virus of visceral lymphomatosis appears to behave in cells grown in tissue culture in a fashion similar to that of the Rous sarcoma virus.

During the present decade, the squirrel fibroma has been found, like Shope fibroma, to be contagious in nature and may be transmitted by insect vectors (51, 134, 136). Last, but not least, deer are also affected by a virus-induced fibroma (75, 181*a*).

The general survey of discoveries would not be complete without mentioning the induction of tumors in drosophila by a virus-like agent (43, 98, 127). Thus, plants, insects, amphibia, and mammals have viral agents responsible for the induction of neoplastic conditions or cancer. Only cancer of man still eludes determination of whether or not viruses are responsible for at least some of its types.

This chapter of studies on virus-induced tumors would also be incomplete without at least a brief discussion of the mouse mammary-tumor-inducing virus. Following the discovery of its transmission through mother's milk, a series of most extensive studies on this virus have been carried out during the present decade (53, 57). The virus has been found to be present in males, and the male infects the female during mating (94, 150). It is of great interest that the presence of the virus is not limited to highly inbred, "artificial" strains of mice, but has been found in wild house mice from which it can be transmitted to pure-bred mice and vice versa (1). The virus can gradually increase in concentration from an inapparent infection to the stage of overt infection (54). This may serve as one of the examples of the use of pure-bred mice in studies on tumor-inducing viruses. Their genetic make-up can be varied, almost at will, and applied most usefully in the study of virus-host relationship. A considerable effort has been spent on attempts at isolation and purification of this virus, which are unfortunately much complicated and delayed by the long latent period of tumor-induction (57). Some time may elapse before these studies will reach a satisfactory conclusion. This tumor-inducing virus, like that of chicken lymphomatosis (39), can pass from one host to another, even for many generations, without tumor formation (56). Application of tissue culture to the study of this virus has shown that it survives, if not multiplies, in chicken fibroblasts grown in tissue culture (Pikovski, see (57)). More extensive studies of this virus in tissue culture may lead to a number of important observations by those who are prepared to be patient and are not put off by its long latent period.

The mode of action of tumor-inducing viruses, like that of infectious viruses, still remains largely in the realm of mystery. Newly developed systems, like the chorioallantoic membrane of the chick (169) or chick embryo brain (122), the application of tissue culture to suitable selected tumors (161), or monolayer cultures of chick embryo fibroblasts combined with chick embryo chorioallantoic membrane cells (194), may in due course provide an understanding of the mechanism of tumor-induction by viruses. At this stage of investigation, it appears premature to attempt to solve the mode of action of tumor viruses by the application of the observations on bacteriophages, still less bacteria. That is not to say that in the future a comparison may be drawn between the course of events in these different systems.

C. Electron Microscopy

An attempt has been made to show the usefulness of methods, long known in virology, in the study of tumor-inducing viruses. During the last decade, electron microscopy of ultrathin sections of tumor cells has proved to be a method of great importance in the study of virus-tumor cell relationships. It appears to be the method of choice in the study of the development of viruses in tumor cells, if combined with other already known techniques, and it is most revealing in the study of the structure of virus particles. This method is of great importance in the search for viral agents in tumors of known or suspected origin of both animals and man, if as in any other method a critical judgment and knowledge of the whole background of the problem is applied. The method is also helpful in assessing the progress of isolation and purification procedures of tumor-inducing viruses, and in the establishing of the specific relationship between the isolated particles and tumors which result from the inoculation of suspensions containing such particles. Like any other method, no matter how good, it has its limitations, which will come to the forefront all the sooner, the more extensively it is applied.

1. Mouse neoplasms. It was natural that one of the first tumors of animals to be examined by this

method was mammary cancer in mice. The availability of suitable, genetically identical strains of mice, differing only by the presence or absence of the mammary-tumor-inducing virus, presented the best control material. Virus particles of a characteristic structure were first observed by Dmochowski (55) in intercellular spaces and in the cytoplasm of tumor cells of mice bearing the agent. The particles have an internal, dense center, outer, paler zone, surrounded by a membrane of a double wall (55–57, 59). This observation was soon confirmed and extended to other strains of mice by Bernhard and his associates (18). The usefulness of this method became immediately apparent on examination of mammary tumors from mice apparently free of the virus. It revealed the presence of the virus particles in variable numbers in some of the tumors originating in the so-called virus-free mice (21, 56, 57). Thus, it appears that the virus may be more widespread than originally expected. This observation has also been confirmed by biological tests (56, 59).

Electron microscopic studies of ultrathin sections of leukemic organs of mice of several strains with a high incidence of spontaneous leukemia, revealed a number of changes in the various submicroscopic constituents of these cells as well as virus particles present both intra- and extracellularly (68). A similar study of the affected organs of mice with leukemia induced by cell-free preparations of organs from mice with spontaneous leukemia revealed virus particles of similar structure and of similar size range (59). Organs of young mice of the same strains failed to reveal any of the changes seen in the cells of the same organs from mice with spontaneous and induced leukemia (59).

It is well known that a number of etiological factors are responsible for the induction of leukemia in mice (60, 139). Among the many types of radiation energy known to be leukemogenic, X-irradiation is effective in either single or repeated doses. In spite of intensive search, electron microscope examination of ultrathin sections of organs from mice with X-ray induced leukemia failed to show the presence of characteristic virus particles and inclusion-like bodies, although various kinds of damage in the submicroscopic cell elements were found similar to those seen in spontaneous leukemia (69). This observation is all the more interesting since the leukemia was induced in the same strain of mice, the organs of which, when leukemia was induced by cell-free material from mice with spontaneous leukemia, revealed the presence of virus particles and inclusion bodies in the cytoplasm of cells. It is even of greater interest that cell-free extracts of these organs of mice with X-ray-induced leukemia induced leukemia in mice of the same strain, although in comparatively low incidence. Electron microscope study of organs of the latter mice showed the presence of virus particles and inclusion-like bodies (70). The virus particles, although of similar structure, are smaller in size than those in spontaneous leukemia and in leukemia induced by cell-free material of spontaneous leukemia, although there is some overlap in size. Further studies are required to establish the relationship of the different types of leukemia in mice. Electron microscope studies, if combined with biological, biochemical, and immunological tests may help to solve the relationship of viruses to leukemia induced by various factors.

The induction of parotid gland tumors in mice by cell-free preparations of leukemic organs of mice with spontaneous leukemia, presents an interesting aspect of the studies on cell-free transmission of leukemia (116). It has now been amply confirmed in a number of studies (74, 185, 186, 198), some of which led to the conclusion that the parotid gland tumor-inducing virus is a separat agent, distinct from the leukemia-inducing virus (74, 119, 120). In ultrathin sections of parotid gland tumors, characteristic virus particles in the cytoplasm of cells have been observed by Dmochowski and his associates (70), falling in size within the lower range of virus particles observed in spontaneous leukemia. The size of these particles was much more uniform than that of particles in spontaneous leukemia, the latter showing a considerable range of size. Whether this is indicatory of the presence of several viruses in spontaneous leukemia or of variation of the same agent remains to be shown. It is of considerable importance that virus particles similar in size to those seen in parotid gland tumors, have been observed in ultracentrifugal pellets of cell-free leukemic extracts which showed parotid gland tumor-inducing activity (70). It appears that the virus particles may therefore be the agent itself.

Similar studies combined with other approaches may help to solve the puzzling problem, whether the Stewart-Eddy virus, the so-called "polyoma"

virus, responsible for the multiple tumors in mice and hamsters and for the hemorrhagic disease in hamsters (71), is one agent showing extremes of variability and mutation, or several agents, with similar, although more limited, ability to mutate or vary. The problem is one of a tumor-virus showing under certain conditions, extremes of what could be described as "cancer-pantropism," with the ability to induce tumors in different organs and tissues, or of a number of tumor-viruses, present in the original tumor and showing different types of "cancer-tropism." There is no doubt that electron microscopy of these tumors will help in the elucidation of the problem. There is indeed a prodigious amount of work in front of tumor virologists, hard and painstaking but nevertheless rewarding in the end.

2. Avian neoplasms. Electron microscopy of ultrathin sections of various avian tumors may serve as another example of the usefulness of this method in the search for causative agents of these tumors. Some time elapsed before the observations made on the ultrastructure of Rous sarcoma cells grown *in vitro* (47) could be confirmed (22, 108). The ultrastructure of this virus has been described later in greater detail (125, 126, 153). It offers a basis for future studies of the biochemical composition of this tumor-inducing virus. Quantitative studies combined with bioassays and biophysical and chemical procedures seem to have established the identity of these virus particles as the Rous sarcoma virus (90, 125). There seems to be little doubt, therefore, that the virus particles seen in ultrathin sections of tumor cells and of ultracentrifugal pellets of cell-free extracts of the tumors are the agent of Rous sarcoma. Thus, 47 years after the discovery of the cell-free transmission of chicken sarcoma tumors, the identity of the virus appears to have been established. It may now be possible to employ both biochemical and immunological studies, combined with morphological tests, for further characterization of the agent, provided the inherent difficulties of the purification problem are well understood and born in mind (36).

Virus particles of similar structure, but somewhat larger in size, have been observed in the cells of Murray-Begg endothelioma (164) and in Fujinami myxosarcoma (16). It could be mentioned here as at any other points of the discussion that the term "virus particles" and not "virus-like particles" has been used purposely. There is so far no known component of normal cells whose structure resembles that of particles proved to be viruses (57). The term "virus-like" implies caution about reaching a conclusion that the particles observed are the tumor-inducing virus and has been used by the writer himself and many other investigators. It is, however, cumbersome and there is no reason to avoid the term "virus particles," as in itself it does not imply any conclusion about the relationship between these particles and the origin of any particular tumor. Overcautious or supercritical people have the tendency to accuse an investigator of jumping at a conclusion about the origin of the investigated tumor as soon as the term "virus particles" is used by him. In view of what has been stated, more freedom in the use of the term virus particles should be allowed, and the writer has made use of this freedom.

Electron microscope studies on the submicroscopic structure of several tumors of the chicken leukosis complex, known to be transmitted by cell-free preparations, have either already been reported (61–67) or are in press (60). Virus particles have been observed in the affected organs of chickens with all forms of leukosis studied, except for neural lymphomatosis, where only occasional particles have been observed in the affected nerves. Particles with characteristic internal structure as well as inclusion-like bodies containing virus particles have been observed in the cytoplasm and in the intercellular spaces in the following forms of chicken leukosis complex: visceral lymphomatosis (so called extravascular lymphomatosis), both spontaneous and induced; erythroblastosis RPL-strain 12 (so-called intravascular lymphomatosis); erythroblastosis of Engelbreth-Holm (89); and in granuloblastosis (myeloblastosis) of Hall, Bean, and Pollard (126*a*). Although in all cases the virus particles failed to show any difference in their internal structure, they did show differences in size. Virus particles in visceral lymphomatosis and erythroblastosis RPL-strain 12 are similar in size to Rous virus particles, whereas those in both erythroblastosis and granuloblastosis are similar in size, but smaller than those in the two former types of chicken leukosis (64). Whether these observed differences in size are statistically significant and whether they indicate that different viruses may be involved in the origin of the various forms of chicken leukosis remains to be determined. There

appears to be a similarity in the developmental cycle of viruses in all forms of chicken leukosis. The virus particles develop within mitochondria, are released into the cytoplasm of the cells, and following breakdown of the cells appear in the intercellular spaces (65).

Virus particles of similar structure, but somewhat larger in size, have been observed in cells of the bone marrow of chickens with erythromyeloblastosis (17). They were also observed in the affected organs of a different breed of chickens with erythroblastosis (15). Similar virus particles have been found in the ultracentrifugal pellets of plasma from chickens with erythroblastosis and granuloblastosis (20), and in association with myeloblasts maintained in tissue culture (159), but not in myeloblasts of the circulating blood. They have, however, been frequently observed in cells other than myeloblasts in the affected organs (159). There seems to be little doubt that these particles are the causative agents of at least some of the tumors of chicken leukosis, but final proof is still lacking for others of the tumors. It is to be hoped it will be forthcoming in the near future.

The study of ultrastructure of chicken leukosis tumors offers another opportunity for gaining inside information of virus-host relationships and for the intimate exploration of virus variation or mutation. It will help to answer the question whether variation of a virus has a morphological expression, at least as seen in the electron microscope.

It should be mentioned that some studies revealed the presence of virus particles of similar size and structure in variable but small numbers in organs of some normal chickens and of chick embryos (13, 14, 17). This does not seem surprising in view of the widespread occurrence of lymphomatosis in chickens. Normal, young chickens of specially bred lines, such as the East Lansing-strain 15, maintained under controlled conditions do not appear to harbor these virus particles (66).

Electron microscope studies on tumors of chicken leukosis could be summed up by stating that such studies combined with other methods and bioassays may settle the problem of whether one or more viruses are involved in the origin of neoplasms described as chicken leukosis. The results so far obtained indicate, at least on the basis of differences in particle size, that there may be more than one virus involved (7, 11, 64).

3. Rabbit tumors. Benign Shope fibroma cells and those of its malignant form in newborn rabbits, have revealed in the electron microscope characteristic changes, virus particles, and what appear to be various stages of their development (19, 42). The gradual formation of virus particles of Shope fibroma and the various developmental stages have been described in cells of rabbit fibroblasts grown *in vitro* and infected with Shope fibroma virus (50, 92). This is an example of an approach most helpful in proving that the particles originally observed in tumor cells are the agent itself.

4. Tumors of amphibia. To complete the ultramicroscopic studies of animal tumors, the study of renal adenocarcinoma of the leopard frog has to be mentioned. Virus particles and what may be developmental forms of these particles have been observed (91). Although no parallel bioassay studies have been carried out, it seems highly probable that the virus particles are the causative agent.

5. Human tumors. A natural question now comes to mind, what have similar studies revealed in human tumors of known, suspected, or unknown viral origin? From among the human tumors of known viral etiology: molluscum contagiosum, laryngeal papilloma, common warts, and venereal warts, only the first two have so far been examined in ultrathin sections. In molluscum contagiosum, virus particles similar to those of pox group viruses and various developmental forms have been described (16, 109). Virus-like inclusions have also been described in two cases of laryngeal papilloma (146). Cytoplasmic inclusions of mixed fibrillar and granular ultrastructure have been described in a case of malignant human myxosarcoma (141). It would be almost superfluous to say that such studies should be continued with an intensified effort.

The study of submicroscopic structure of the affected lymph nodes from cases of human leukemia appears to be of interest. The original observation of virus particles in cells of a lymph node from a case of acute lymphatic leukemia (58) has been extended to two other patients, one with acute lymphatic leukemia and one with acute myeloid leukemia (72, 73). The virus particles have a characteristic internal structure, appear to develop in inclusion-like bodies, and the cytoplasm of cells of the lymph nodes shows changes observed in cells of mouse and chicken

leukemia. Similar virus particles have been observed in the bone marrow of a case of myelogenous leukemia (9). The relationship of these virus particles to the origin of leukemia requires further investigation. It should not be forgotten that human tissues, normal and malignant, may harbor viruses, latent or other, unconnected with the origin of leukemia. Nevertheless such studies should continue as they appear to be a convenient first step in the investigations of a possible viral origin of at least some types of human leukemia.

In connection with electron microscope studies of human leukemia, some recent biological experiments should be mentioned. Fresh but not heated, cell-free filtrates of the brains of leukemic mice (176), of the brains of patients who died from different types of leukemia, but not of apparently normal human brains (174, 177) have been found to accelerate or to induce leukemia in certain strains of mice within a short time. The filtrates of human leukemic brains have also been passed serially through mice without loss of the leukemia-stimulating activity (175). It is of extraordinary interest that filtrates of leukemic organs of mice with the induced leukemia have so far failed to yield the virus, which could only be recovered from brains of the same mice (174, 175). These are extremely interesting observations which will have to be repeated and correlated with morphological studies.

Electron microscope studies of animal tumors have contributed greatly to the knowledge of the tumor-inducing viruses. Their importance is even greater, if one considers that these studies constitute a convenient basis for similar studies of human tumors. Much has already been learned about virus-induced animal tumors but a great deal remains to be explored by means of the electron microscope and other techniques. Although the interpretation of electron microscope observations must of necessity be governed by the greatest caution, it cannot be passed without the expression of satisfaction and hope for better things to come. The hope, however, must be tempered with understanding of the limitations to which this, like any other method, no matter how rewarding, is ultimately subject. To hope that it will be possible to differentiate all the tumor-inducing viruses on the basis of their internal structure means to do injustice to electron microscope studies. The present stage of these studies compares somewhat with that of the investigations during the late 19th and the beginning of the 20th century, when various types of bacteria were being discovered. Not all of them, far from it, can be diagnosed on the basis of differential staining by means of the light microscope alone. It is certain that the same statement applies to infectious or tumor-inducing viruses studied in the electron microscope. It will, however, take some time before the electron microscope studies of viruses reach a stage similar to that already achieved by light microscope studies of bacteria.

D. Appraisal of the Results

It seems that the history of critical comments and appraisal repeats itself again in connection with the discoveries of Gross, Stewart, and Eddy. Some of the criticism was not entirely unjustified. Both the possibility of a comparatively high incidence of spontaneous leukemia in mice of the strains used in these experiments and the possibility of induction of leukemia in these mice by irradiation or inoculation of bone marrow of mice from certain other strains (140) have to be considered. The criticism was met by the repetition of the experiments with suitable litter-mate control mice. Additional criticism was based on the possibility of the presence of cells in the tumor-inducing preparations but this cannot be taken as an explanation of the first observations of Gross and Stewart. The later tissue culture experiments provide indeed a justified basis for criticism about the presence of cells, as Stewart and Eddy did not use what could be agreed by everybody to be a cell-free extract, and in some experiments they used what was a suspension of tumor cells. This in itself, however, need not necessarily serve as an argument against the presence of a tumor-inducing virus. After all, the varicella-herpes zoster viruses, true infectious agents, move from infected cells to contiguous normal cells which they infect, and only infected cells employed as passage material produce cytopathogenic changes in cells grown *in vitro* (197). Yet nobody doubts that varicella and herpes zoster are produced by a virus or viruses.

The criticism, that transplantation of tumor cells and not induction of tumors takes place may be dispensed with as an argument against the presence of a tumor-inducing virus. If some of the leukemias induced by cell-free preparations are not transplantable in the strain of origin (139),

it is not necessarily an argument against their neoplastic character. Not all recognized leukemias are easily transplantable; and virus-induced leukemias may belong to the so-called dependent neoplasms which will grow only in suitably conditioned hosts (105). The argument about ease of transplantation may be entirely misleading, as there are tumors induced, for example, in chickens by carcinogenic hydrocarbons which are not easily transplantable in other chickens, and there are virus-induced tumors in chickens which are easily transplantable (3).

Another interpretation of Gross's and Stewart's findings is that of induction or transduction. The leukemias induced in the experiments of Gross (119, 120) and of Woolley and Small (198) were characteristic of the recipient strain of mice and not of the donor strain. They believe that induction has taken place. In other experiments, suggestive evidence was obtained that the induced leukemias had the genetic character of the donor and of the recipient strain (106). This constituted the basis of another interpretation, namely, that transduction had taken place (195). The suggestion was put forward that the agent, being related to chromosomal nucleoproteins, enters the genetic apparatus of cells of the host and causes perpetuation of certain genetic characteristics (107). This process, similar to transduction or transformation in bacteria, had been advanced more than twenty years ago, but then it was related to mutation (151). It is obviously unnecessary to introduce this latter interpretation to explain the long latent period, the lack of infectivity under natural conditions, the necessity of using newborn mice, or the limited strain specificity of the agent. The biophysical and biochemical properties of the leukemia-inducing agents are different from those of the pneumococcal transforming factor (112). It seems equally unnecessary, to say the least, to introduce the concept of an antigen-bearing particle specifically interfering with the immunity-producing system of mice (128).

The criticism and arguments are mentioned in detail, as similar criticism was raised almost thirty years ago about Rous sarcoma virus. The facts and observations are correct, but arguments exist and the same ones persist. The real basis of these criticisms is our present lack of knowledge of how viruses act. Any arguments or criticism should be welcome, provided that, as in the past, they will not interfere with the active pursuit of studies on tumor-inducing viruses. Fortunately, the tools at present available may help shortly to settle the problem, whether the leukemia-inducing agent or agents have a morphological character or are biochemical factors. The future may reveal, perhaps sooner than we expect, the chemical basis of their activity.

Although the hypothesis of acquired immunological tolerance (128) may explain some of the aspects of leukemia-induction, it fails to be an argument against a leukemia-inducing virus, which may be at the start like the mammary-tumor-inducing virus, a "very low grade virus" (42), which through experimental interference has increased in potency.

There is no doubt that the last decade has led to many discoveries in the field of tumor-inducing viruses; even though their importance may be difficult to assess at the moment, it certainly should be realized.

IV. FUTURE PROSPECTS

How about the future decade? It may appear unnecessary to attempt to predict future developments. It may well take another ten years to work out all the details of the present-day observations. Ten years ago, it would have appeared incredible, if not ridiculous, to say that a leukemia-inducing agent of one species may induce leukemia and many other tumors in a foreign species. Yet, an example of similar events was already available in chicken tumors. Time is bound to take care of the arguments which may even be called arguments in semantics. Only a concerted attack, with the employment of as many techniques as possible, most of them already available, will help to characterize the viruses involved in tumor-induction. This, unfortunately, involves, more than ever, teamwork and cooperation of highly trained specialists, which more often than not is rather difficult to achieve. This is not to say that the "lone wolf" has lost his part, for he undoubtedly will have a great deal to say yet.

It has now been amply demonstrated that tumor-inducing viruses may behave like infectious viruses and also behave as carcinogens. This latter effect has been especially well documented by the classical experiments of Rous and his associates (57). Whether infectious viruses can act as carcinogens or co-carcinogens still remains

to be demonstrated. Vaccinia virus appears to behave in this manner as indicated in the studies of Duran-Reynals (82). Experiments along these lines should be continued and extended. The use of tissue culture, in spite of the difficulties when it is used for this purpose, should be brought into such studies.

Even at this stage to claim that viruses are involved in the origin of tumors induced by radiation or chemical carcinogens, or in the neoplastic transformation in tissue culture, may be a little premature, to say the least, and is also unnecessary. Such claims have so far resulted in more harm than good to the theory that viruses are one of the causative agents of cancer. There is no doubt that they are involved in the origin of some tumors, and the present-day question is not, whether they are involved in the origin of tumors, but in how many tumors they are involved. Only the future may show this through the application of the techniques which have already yielded results or through the use of entirely new methods. If an example of some recently discovered virus-induced tumors can be used for illustration, parotid gland tumors may be taken as one. They ordinarily either yield no virus or do so only with difficulty, but when cultured with mouse embryo cells they yield the virus with comparative ease (187–189). Therefore, caution should be exercised in statements regarding the origin of at least some tumors. The use of tissue culture is most promising and should be applied to other tumors in mice and other species as well as to those of man. It may lead to the discovery of "new" tumor-inducing viruses.

A similar approach with the help of electron microscope studies may help to explain the basis of the increase in the range of tumor-inducing activity. It may show whether it is a modification of a tumor-inducing virus or an increase in activity of the same virus, or is based upon the "unmasking" of additional viruses.

The foretaste of things to come may already be taking shape. It is now known that ribonucleic acid of the tobacco mosaic virus is infective, although at a lower titer than the original nucleoprotein (97). Even more surprising, the virus may be reconstituted by combining protein and nucleic acid moieties of the same or different strains of tobacco mosaic virus, the infectivity in the latter case following that of the virus supplying the nucleic acid (96).

There are indications that ribonucleic acid may be the carrier of infectivity of Mengo encephalitis, West Nile encephalitis, and poliomyelitis viruses (48, 49), or of Semliki forest virus (160). Although these experiments have opened more questions than they have answered, they indicate the way along which future experiments with tumor-inducing viruses should be conducted. The discovery that ribonucleic acid is infectious is a discovery of the first order. It is bound to influence the experiments in the field of infectious and tumor-inducing viruses.

It is a temptation to suggest, and the best way to deal with the temptation is to yield to it, that what electron microscopy has already revealed of the structure of infectious and tumor-inducing viruses may be the morphological expression of the biochemical constitution of viruses. The central dense core, or the so-called nucleoid, may be the ribo- or deoxyribo-nucleic acid, and the surrounding membrane the protein coat. The double membranes already seen frequently in a number of tumor-inducing viruses must certainly have their meaning, as yet uncomprehended. These viruses may shed one coat to adjust themselves to the environment, or both coats, depending upon where they find themselves.

Thus, probably in the near future, the morphological dissection of viruses will be followed by a biochemical analysis of these viruses, from which a great deal of useful information may be derived. This approach in the study of tumor-inducing viruses may lead to the understanding not only of these viruses, but perhaps to the comprehension of the tumor development itself, and in the long run to a more successful form of cancer treatment. "Tumor virologists" and "ordinary" virologists should watch each other's results and methods of approach closely. A great deal of useful information may thus be derived, especially by the former investigators.

There is no doubt that basic biological laws established in animals are applicable to man. The techniques used in the present decade of cancer research in general and in the study of tumor-inducing viruses in particular lend themselves to the study of human tumors, a number of which have for sometime been suspected of viral origin. The time is one of great challenge which must be met and taken up. Cancer research is at the doorstep of great discoveries. It would be surprising indeed if the present fundamental knowl-

edge of the behavior of virus-induced tumors and tumor-inducing viruses were not to lead to important observations on cancer of man.

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