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INSTRUMENTS AND TECHNIQUES

Viruses and Tumors

The electron microscope is proving to be a powerful tool for study of viruses and virus-induced tumors.

Leon Dmochowski

There exists a striking similarity between the progress made during the last 10 years in the studies on viruses as causative agents of infectious diseases in man and the advances in the studies on viruses implicated in the origin of tumors in animals. During this time more than 150 new infectious viruses have been described, and a number of viruses have been found responsible for various types of tumors in animals. The progress made in the discovery of these new viruses has largely been due to the use of newborn animals, improvements in tissue culture methods, and the introduction of the electron microscope as a tool for study of the submicroscopic structure, first of normal and then of cancerous cells, and later of viruses themselves.

Viruses and Cells: Present-Day Definition and Relationship

There is an intimate association between viruses and cells. Both cells and viruses have the ability to reproduce themselves, but the latter can only reproduce within cells. Viruses are nucleoprotein entities with one type of nucleic acid (ribo- or deoxyribonucleic acid); they are infectious (that is, capable of entering suitable susceptible cells); they reproduce from their own genetic material within the cells they infect; as a by-product of their reproduction within cells they may, but need not, induce a disease; they are unable to grow and divide; and they contain no enzymes (*1*).

As is discussed below, this definition of viruses has been confirmed by electron-miscroscope and biochemical studies. When a virus enters a cell, part or all of the metabolism of the cell is used for the manufacture of the virus. During this manufacturing process the cell may not show any visible signs of the entry and reproduction of the virus, but it may frequently be impaired in its various functions or even destroyed. This latter symptom of viral entry into the cell, described as cytopathic effect, has been successfully utilized in modern tissue-culture methods as a means of isolating and identifying many newly discovered infectious, and also tumor-inducing, viruses. It should be mentioned that the classification of viruses into "infectious" and "tumor-inducing" is misleading. It has lead to an artificial division of viruses into two seemingly unrelated types: "ordinary," or "infectious," and "tumor-inducing" viruses. It is now known that tumor viruses have all the properties of ordinary viruses (*2*) and that the latter may be implicated in the origin of cancer (*3*). There is no doubt now that certain viruses, following entry into suitable susceptible cells, lead to continuous, unrestricted proliferation of the cells and therefore to malignant or cancerous behavior in the organism of the host. Similar activity of the Rous sarcoma (*4*) and the SE polyoma virus (*5*) has recently been demonstrated in suitable susceptible cells grown in tissue culture. Thus, a virus may transform a normal cell into a cancer cell both in an animal and when maintained outside the body of the animal. It is now known that the same virus may lead to inflammatory and destructive changes in certain cells and to malignant proliferation of other cells, as shown in the case of the SE polyoma virus (*6*).

This manifold ability of a virus to enter a cell and destroy it or change it into a cancer cell may lead to the question, Is virus "alive," and if so, is it the smallest unit of life? This philosophical question is as difficult to answer as the question, What is life? Life is a process, and it is therefore hard to define a virus, a cell, or any cell constituent as a unit of life, no matter how small the virus or cell constituent may be. Recent advances in biochemistry have shown that nucleic acid, which is a virus constituent, is capable of inducing changes characteristic of the particular virus (*7*) and is therefore a carrier of viral activity. Furthermore, nucleic acid of a virus is capable of entering cells not susceptible to the virus in which the nucleic acid originated and of reproducing the virus within such cells (*8*). It would, however, be a mistake to relegate the complete virus to a purely secondary role or to ascribe to a cell only a secondary importance in favor of its nucleus. A cell with its nucleus and a virus with its nucleic acid represent an entity in each case, as in turn a cell infected with a virus may represent an entity (*8*).

Viruses and Cancer in Animals and Man

It is well known today that many types of cancer in animals are induced by viruses. There is, as yet, no experimental proof available that any one type of human cancer is induced by a virus. It would, however, be strange if nature were to impose such limits between the animal kingdom and man, or

The author is chief of the virology and electron microscopy section of the University of Texas M. D. Anderson Hospital and Tumor Institute and clinical professor of microbiology, Baylor University College of Medicine, Houston, Tex. This article is adapted from a lecture delivered 29 December 1959 at the annual meeting of the AAAS, in Chicago.

to divide so sharply the origin of cancer in animals from that of cancer in man. Studies of tumor-inducing viruses do indicate the possibility that at least some human cancers may be of viral origin.

Human cancer, like animal cancer, develops from a wide range of types of cells in any part of the body. It is also known that diverse factors may be responsible for the many types of cancer in different parts of the animal body. But an analysis of the different cancerogenic factors reveals a certain repetition of pattern, with emphasis on one or more factors, in any type of cancer. Thus, genetic, hormonal, and metabolic factors, and environmental factors such as various chemical carcinogens, radiation energy, and viruses, form a chain of events which leads to the formation of cancer. It is already known that in a number of virus-induced cancers in animals the genetic constitution and hormonal factors prepare a suitable background for the action of the virus, leading to the induction of cancer. Without these factors, the virus itself is almost powerless. However, frequently large amounts of virus may overcome low genetic susceptibility, which in turn leads to a less favorable hormonal environment.

In the case of some animal tumors, the same virus may induce as many as 23 different types of cancer in different parts of the body of the animal (mouse) and also different types of cancer in animals of several other species (*9*). The list of viruses responsible for animal cancers is constantly growing, especially since the discovery of the viral origin of a certain (lymphatic) type of leukemia in mice by Gross (*10*). The induction of tumors in animals which had been inoculated, when newborn, with extracts of tumor tissues filtered through cell- and bacteria-retaining filters, or with tumor extracts which had been passaged repeatedly in tissue culture, has led to various interpretations of the observed and confirmed experimental facts. The concept of a process similar to transduction or transformation in bacteria was introduced as a possible basis for the induction of tumors (*11*). In another interpretation, the concept of an antigen-bearing particle specifically interfering with the immunity-producing system of mice was put forward (*12*). Electron microscopy of ultrathin sections of tumor tissues and of various preparations obtained from tumors induced by cell-free preparations has, however, demonstrated that tumor-inducing agents have a morphological basis. It appears that electron microscopy may help us to understand the chemical basis of viral activity and contribute to our knowledge of the structure of tumor viruses and of their mode of activity within cells.

Biological experiments recently carried out have revealed the existence of yet another relationship of tumor viruses to cells. Extracts of organs from normal mice have been shown to induce tumors in other mice (*13*). Treatment of apparently normal mice with cortisone has led to the development of tumors, known to be of viral origin (*14*). X-irradiation of mice with a low incidence of leukemia has induced leukemia in these animals, which could then be transmitted by cell-free extracts to other animals of the same strain (*15*). Morphological studies carried out by means of the electron microscope have shown the presence of virus particles in cells of animals in which leukemia was induced by the cell-free extracts from tissues of mice that became leukemic after x-irradiation (*16*).

These experiments have demonstrated that some tumor viruses may exist in animals in a latent form without inducing cancer or leukemia. These viruses may be transmitted from generation to generation without any symptoms and thus appear noninfectious in the ordinary sense. Tumor viruses, however, may also spread, like any other virus, through contact between animals or in animal secretions and excreta (see *17*).

Ultrastructure of Normal and Virus-Infected Cells

Electron-microscope studies have supplied much detail on cell components known to exist from studies in the light microscope. They have led to the discovery of new constituents in cells and have clarified our knowledge of other controversial constituents. The appearance of cells processed for electron microscopy compares favorably with that of living cells seen by phase contrast microscopy.

There exists an amazing similarity, as revealed in electron-microscope studies, between the submicroscopic structure of plant, animal, and human cells. At least some plant cells have the same cellular constituents as animal cells and are of similar structure (*18*). Similarly, in type and structure of submicroscopic constituents, no differences between animal and human cells have been observed (*19*).

After normal cells had been studied through electron microscopy, the study of cells during various diseases, especially viral infections, was undertaken. This has led to the visualization of viruses in the infected tissues and has revealed the complicated structure of these agents and the behavior of various submicroscopic cell elements during the different stages of infection (*20–22*). Electron microscopy has revealed a basic similarity in the appearance and internal structure of bacterial, plant, animal, and human viruses, as seen in the infected cells. Although these viruses may vary in size and shape and in some details of internal structure, they show a common basic structure—a protein envelope or envelopes and an internal dense center, now known to be the nucleic acid (*22, 23*). Constant improvements in staining techniques have recently led to the visualization of structural organization of the different components of plant, animal, and human viruses, isolated from the infected cells (*24*).

Electron microscopy of ultrathin sections of cells infected with viruses has given us a picture of these subcellular particles in their natural surroundings. The complicated structure of the virus particles helps in differentiating these particles from normal cell components. It appears that we are gradually acquiring an understanding of the structure of virus particles both within and outside the infected cells.

Ultrastructure of Cancer Cells

Soon after the application of electron microscopy to cytology of normal and infected cells, electron-microscope studies of malignant cells were started. Particular attention was directed toward tumors of known viral origin (*22, 23*). The comparative ease with which viral agents could be detected in the diseased tissues and in some tumors of known viral origin also led to electron-microscope studies of tumors suspected to be or viral origin.

Electron-microcsope studies of cancer cells have, so far, failed to reveal any essential differences between the submicroscopic structure of cancer cells and that of normal cells (*22, 23, 25*).

Cancer cells from tumors of viral origin have, however, shown various degenerative changes in their ultrastructural components and have shown structural components now known to be virus particles. The changes in the various cellular components, such as Golgi apparatus, mitochondria, endoplasmic reticulum, and nuclear and cell membranes, if not directly related to the development of virus particles, could be described as nonspecific, as these changes have also been observed in cells subjected to various unfavorable environmental influences. An analysis of the changes which can be specifically related to the development of virus particles within cancer cells reveals a striking similarity to the changes observed in cells infected with various "ordinary" or "infectious" viruses, whether plant (*26*), animal, or human (*22*, *23*). It represents a morphological confirmation of the similarity in biological behavior of infectious and tumor-inducing viruses. Electron microscopy of tumor cells of viral origin has also revealed a basic similarity between virus-induced tumor cells in amphibia, birds, and mammals and similarity between the virus particles observed within these cells.

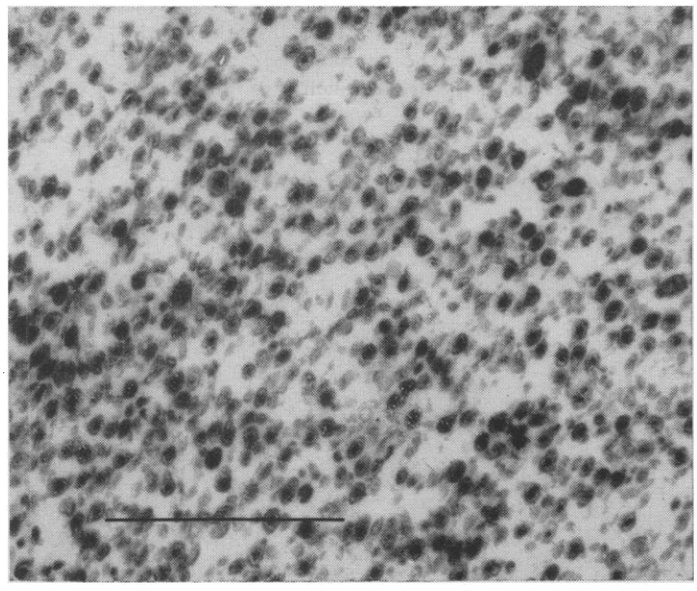
Detection of Virus Particles in Cancer Cells

Any description of the various changes observed in cancer cells would be incomplete without mention of the difficulties encountered in the search for virus particles in electron-microscope studies of tumors of known viral origin. This point is of extreme importance to any future studies of human cancer of unknown or suspected viral etiology.

In some virus-induced tumors, the large size of the etiological agent alone, or this factor combined with the high infectivity of the tumor extracts, has helped considerably in the detection of virus particles in the tumor cells. The Shope fibroma in rabbits (*27*) and molluscum contagiosum in man (*28*) are tumors caused by viruses of the pox group. Further electron-microscope studies on these viruses may perhaps demonstrate a connection between pox infections and some types of cancer. It should not be forgotten that the hypothesis of the viral origin of cancer was first put forward by Borrel and Bosc in 1903 (*29*), who observed the proliferative effect of pox viruses on tissues. This hypothesis was advanced before the discovery of the first tumor of viral origin—that is, chicken leukosis—by Ellermann and Bang in 1908 (*30*).

In cells of organs of chickens suffering from different forms of the so-called chicken leukosis complex, such as visceral lymphomatosis, erythroblastosis, and myeloblastosis, the presence of viral particles was detected with comparative ease, as one cell in 50 or one in 100 revealed the particles (*22*). However, the search for virus particles in the circulating blood cells of chickens with fowl leukosis was found to be extremely difficult and mostly unsuccessful (*31*). This may be an indication that in cancer affecting blood, the circulating blood cells, which would appear to be the natural target of a search for virus particles by means of the electron microscope (*32*), are not the choice material for morphological proof of viral etiology. This apparently is the case at least in certain types of cancer of the blood, not only in chickens but also in mice. This may also indicate an approach in electron-microscope studies of cancer of the blood (leukemia) in man.

Other chicken tumors of known viral origin may serve as an example of the difficulties encountered in the search for viral particles. It was many years



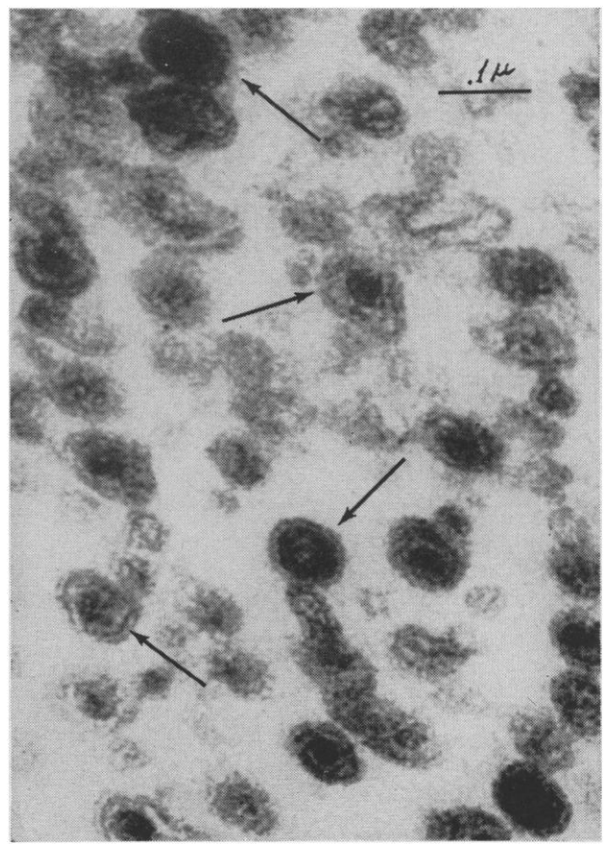


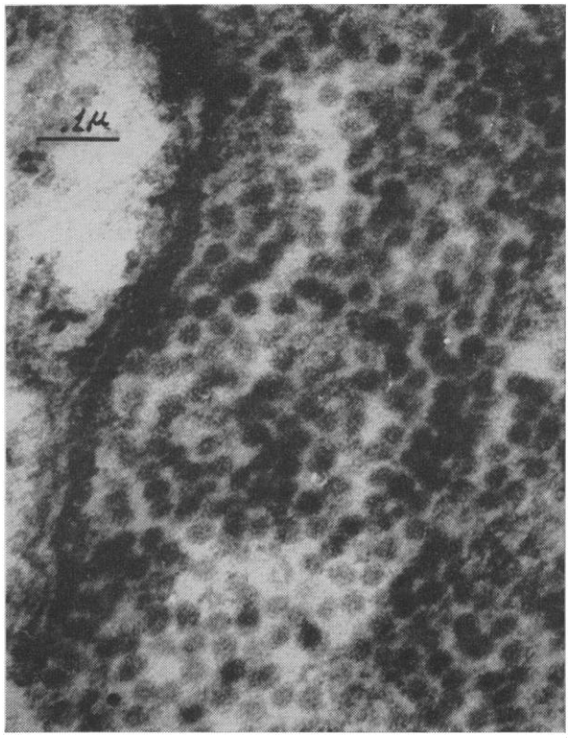
Fig. 1 (above). General appearance of virus particles in a pellet obtained by differential low- and high-speed centrifugation of milk from mice of virus-carrying strains, following defatting, decaseination, and fluorocarbon treatment (about × 32,000). Fig. 2 (right). Part of Fig. 1 at higher magnification (about × 134,000). The arrows point out virus particles, showing some details of internal structure. [L. Dmochowski, C. E. Grey, L. O. Pearson, R. G. Hughes]

before characteristic virus particles were discovered in the chicken tumor called Rous sarcoma (after the discoverer of the viral origin of this tumor). In spite of improved techniques, the search for virus particles in this and other chicken tumors of connective and endothelial tissues, although successful, has been difficult and time-consuming because of the small number of virus particles found, although biological proof of the viral origin of these tumors has been available for some time (*33*).

Although the viral origin of breast cancer in mice was conclusively shown by Bittner in 1936 (*34*), characteristic virus particles were not found in cells of these tumors until 1954 (*35*). Since then, the search for the morphologically characteristic agent in the cells of at least some breast cancers in certain strains of mice has presented comparatively little difficulty (*36*). It was soon found that this was not by any means the rule. Cells of breast cancers in mice from different strains showed considerable variation in the number of virus particles observed. In some breast tumors virus particles could not be found, although occasionally the agent could be demonstrated biologically (*37*). It was later shown that by certain chemical and biophysical procedures the agent can be concentrated and demonstrated both biologically and in the electron microscope (*38*) (Figs. 1 and 2). The part played by electron microscopy in the isolation and purification of tumor virus is discussed below.

After the discovery of cell-free transmission of lymphatic leukemia in mice (*10*), the presence of characteristic virus particles both within and outside the cells was demonstrated in 1956 (*39*). These particles could not be demonstrated in every case of leukemia in mice, and only after considerable search could they be found within cells (*40*). In spite of ample experimental evidence of the viral origin of this type of leukemia in mice (*17*), the demonstration of virus particles in this type of cancer is far from an easy one, as they can only be found in approximately one-third of the examined cases. This morphological finding coincides roughly with the results of bioassays for the presence of leukemia-inducing virus in leukemic tissues of mice with lymphatic leukemia (*41*).

During attempts at confirmation of the concept of cell-free transmission of mouse lymphatic leukemia, tissue-cul-



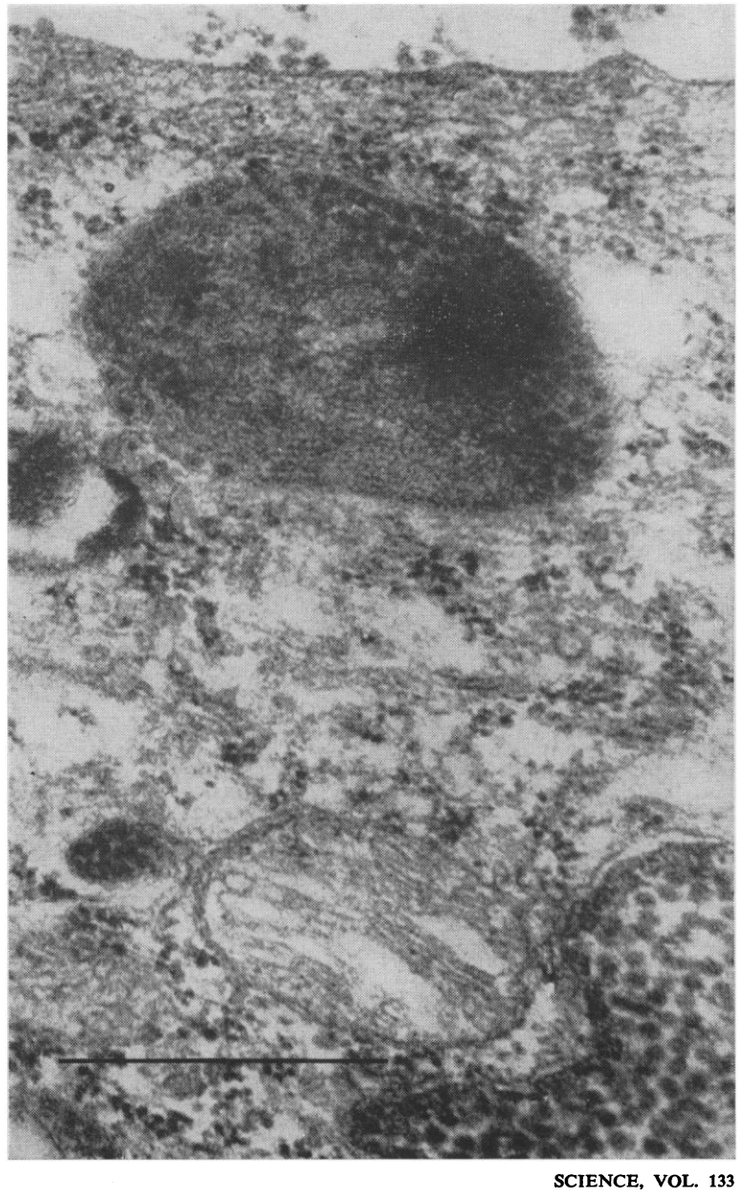


Fig. 3 (above). Part of the nucleus of a cell from a kidney tumor induced in a hamster by the polyoma virus. Virus particles within the nucleus and nuclear membrane are shown (about × 116,500). Fig. 4 (right). Part of the cytoplasm of a cell from a kidney tumor induced in a hamster by the polyoma virus. Shown are virus particles in an inclusion body (bottom right-hand corner), mitochondrion (above the inclusion body), and another inclusion body with some virus particles (about × 89,000; measure, 0.5μ). [L. Dmochowski, C. E. Grey, E. Bereczky, J. Blicharski]

ture studies resulted in the discovery of another tumor-inducing virus, the so-called polyoma, a virus inducing multiple tumors in mice, rats, and hamsters (*9*, *42*). This discovery immediately raised the question of whether one or more viruses are involved in the origin of these tumors. Evidence was eventually obtained, in suitable biological experiments, that one virus is the etiological agents of all these cancers (*9*). This observation required morphological confirmation which at first could not be readily obtained. The destructive changes induced by polyoma virus are easily observed in cells grown in tissue culture. Electron-microscope examination of cells showing these changes demonstrated with comparative ease the presence of characteristic virus particles, mostly in the nucleus and occasionally in the cytoplasm of the infected cells (*43*). Similar results were obtained with different types of cells grown in tissue culture (*44*). Thus, there was no difficulty in demonstrating characteristic virus particles—apparently the etiological agent—in the destructive lesions. Moreover, these particles could be shown with a remarkable consistency.

An entirely different problem, however, arose in the examination of cancers induced in animals by the polyoma virus. The virus particles were found only after an intensive and prolonged search in polyoma-induced cancer of the breast (*43*) and in salivary glands of mice (*43*, *45*) (Figs. 3–5). This in itself was not surprising in view of the already known difficulty in obtaining biologically active extracts of these tumors (*9*). The presence of the virus in polyoma-induced tumors could be demonstrated in biological tests such as passage of extracts of the tumor cells on embryo cells grown in tissue culture (*9*) or by growing the tumor cells in vitro (*46*). Thus, again, a certain correlation has been observed between the presence of characteristic virus particles in tumor cells and the presence of tumor-inducing activity in these cells.

In an extensive electron-microscope study of kidneys from polyoma-infected mice, rats, and hamsters, virus particles, similar to those observed in polyoma-infected cells in tissue culture, have been found in the nuclei and occasionally in the cytoplasm of the cells of proximal and distal convoluted tubules and in the cells of the collecting tubules of kidneys from animals of the three species (*47*). These particles have been found with decreasing frequency in the cells of proliferative lesions, and only with great difficulty in the neoplastic changes in the kidneys of these animals (*47*, *48*). Further studies of polyoma-induced tumors in the kidneys of mice, rats, and hamsters have revealed virus particles in the nuclei of cells of the tumors (*49*) similar to those reported in the cells of kidneys with inflammatory, destructive, and proliferative lesions. Again, electron-microscope studies amply indicate the difficulties encountered in the search for virus particles, even in tumors of known viral origin. In view of the morphological evidence of the presence in tumor cells of virus particles, which undoubtedly are the causative agent, as will be shown later, it may be concluded that the virus is both the initiating and the continuing cause of malignancy. This may perhaps serve as an example of the usefulness of electron-microscope studies of cancer, especially in combination with appropriate biological investigations.

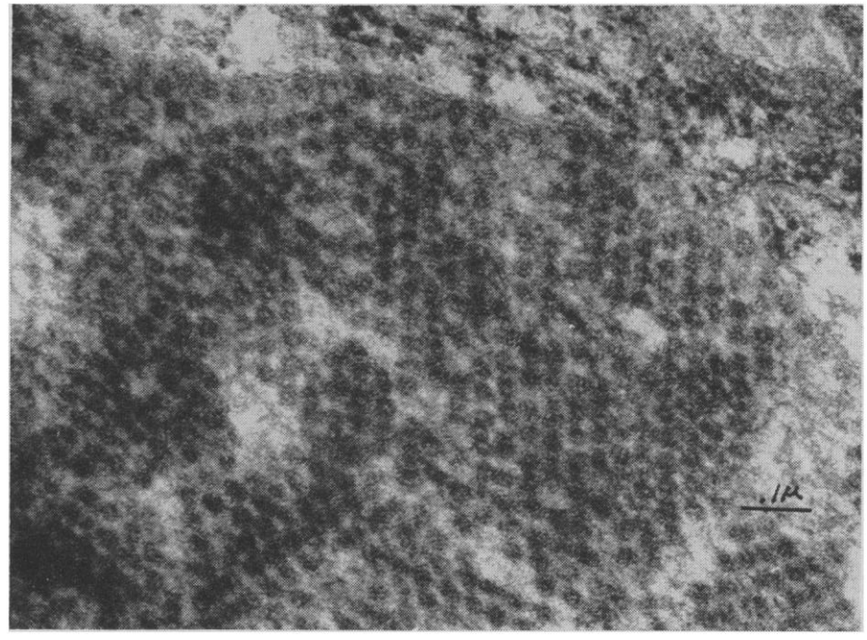
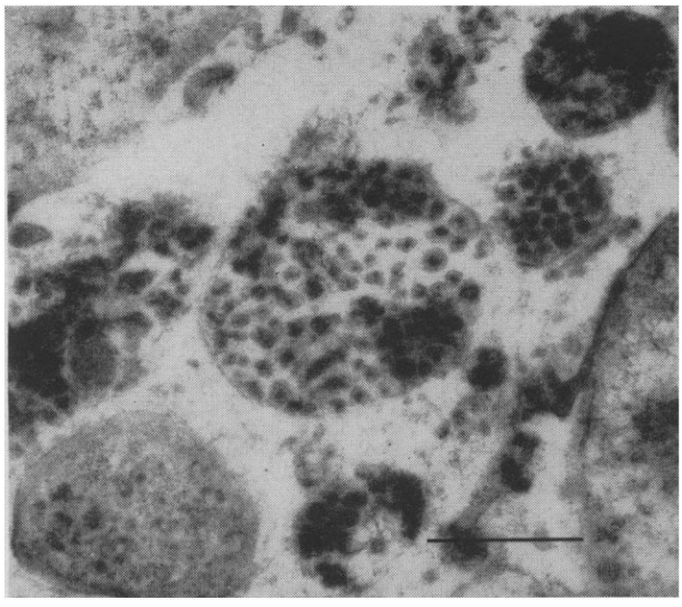


Fig. 5. Part of an inclusion body within the cytoplasm of a cell from a kidney tumor induced in a hamster by the polyoma virus. Virus particles may be seen in an orderly array (× 105,000). [L. Dmochowski, C. E. Grey, E. Bereczky, J. Blicharski]

A striking example of the continuous presence of the virus in tumors which it produces is shown by the adenocarcinoma of the kidneys of chickens. Burmester and his associates (*50*) have recently shown that cell-free preparation of material containing myeloblastosis virus induce in chickens not only myeloblastosis but also other types of chicken leukosis, such as visceral lymphomatosis, osteopetrosis, and cancer (adenocarcinoma) of the kidneys. A study of the submicroscopic morphology of the adenocarcinoma of the kidneys in chickens, both virus-induced and after repeated transplantations, has revealed similar changes in the cells of both types of tumors. Virus particles similar in appearance to those seen in the affected organs of chickens with myeloblastosis have been observed (*47*) (Figs. 6–9). Cell-free preparations of the transplanted adenocarcinoma induced in other chickens mostly the same type of cancer (*50*). Thus, again, electron microscopy appears to indicate the possibility of a virus being the initiating and continuing cause of yet another type of cancer—that is, of a tumor of chickens.

In view of the difficulties encountered in electron-microscope studies of animal tumors of known viral origin, it is not surprising to find as yet comparatively few studies of human cancer. Nevertheless, the progress made in the electron-microscope studies of animal tumors presented a challenge for similar studies of human tumors. The rapidly accumulating knowledge of submicroscopic morphology of animal tumors and the progress in the discovery of the viral origin of various animal cancers were sufficiently compelling to cause investigators to undertake electron-microscope studies of human tumors which, from comparison with animal tumors of known viral etiology,



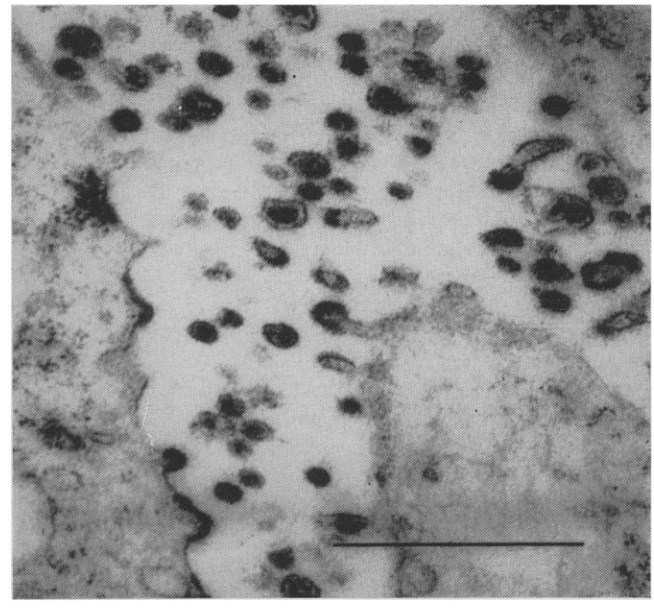


Fig. 6 (left). Part of the cytoplasm of a cell from a kidney tumor induced in a chicken by leukemia virus (myeloblastosis). Inclusion bodies with virus particles may be seen (about × 21,000). Fig. 7 (right). Kidney tumor induced in a chicken by leukemia (myeloblastosis) virus. Virus particles in the intercellular space and budding processes of cellular membranes may be seen (about × 36,000). [L. Dmochowski, C. E. Grey, B. R. Burmester, W. G. Walter]

were suspected of being also of viral origin. As far as is known, extensive studies of two types of human cancer have so far been carried out. These two types are leukemia in its various forms and breast cancer.

In an extensive study (*51*) of lymph nodes obtained by surgical procedures from patients with leukemia, changes were observed in the submicroscopic constituents of the cells surprisingly similar to those already found in the cells of leukemic organs from mice and chickens. As in murine and chicken leukemia, these changes alone could not be described as specific for cancer cells. In addition, however, virus particles were found both within and outside the cells of leukemic lymph nodes from human subjects (Figs. 10 and 11). There were no apparent differences in the size or internal structure of virus particles in the lymph nodes from human subjects with different types of leukemia. These particles were found in half of the cases examined but were not observed in cells from lymph nodes of patients apparently free of leukemia.

It is important that the difficulties encountered in the search for virus particles in human leukemic tissues be emphasized. Frequently, up to ten specimen blocks of tissue had to be cut in sequential sections before virus particles were found. (However, when they were observed in a certain block of tissue, they were present in considerable numbers in many sections of the block.) It is therefore hardly surprising that virus particles have not yet been observed in the circulating blood cells from leukemic patients (*32*) and that they have been observed in only one case of human leukemia in a surgical biopsy specimen (*52*). At this point it should be stressed that the observation of virus particles in human leukemic tissues and their absence in lymphoid tissues of patients apparently free of leukemia does not indicate in any way that these particles are the etiological agent of leukemia. Nevertheless, such studies constitute a preliminary and necessary step in the studies of viral etiology of human leukemia. These virus particles have also been observed in cells grown in tissue culture, derived from surgical biopsy specimens of leukemic patients (*51*). Such studies, combined with immunological and biochemical studies, may perhaps lead to characterization of the particles and may help in establishing the origin of leukemia in man.

Cells from 91 cases of human breast cancer studies in the electron microscope failed to reveal distinct virus particles (*53*). Virus particles have been observed in tumor cells in another study of human breast cancer recently reported (*54*). Since, as was mentioned above, there are no qualitative differences in the ultrastructure of normal and cancer cells, the observation of virus particles in a tumor is of great interest. Nevertheless, enthusiasm must be tempered with caution because of the realization that tumors may carry many viruses unrelated to their origin; they may also carry viruses capable of inducing tumors unrelated to the tumor in which they have been found and isolated. This has been amply demonstrated in the case of some animal cancers (*17*), such as leukemic tissues from which the polyoma virus was recovered.

The observation of virus particles in the cells of tumors raises the question of the relationship of these particles to various cell constituents and of the changes in these constituents which could conceivably be associated with the presence of virus particles. The scope of this discussion does not allow for a description of the submicroscopic structure of the various cell constituents, but there are excellent reviews on this subject available (*55*). The different cell constituents can also be isolated by physiochemical procedures, and their appearance can be studied by means of electron microscopy, which has contributed significantly to the study of the various cell fractions (*56*). Much remains to be done in electron microscopy of various cell fractions from tumors of viral origin, especially those which contain virus particles.

An observation of virus particles within different cell constituents can-

not in itself be interpreted as indicating the site of origin. It is often tempting to speculate about the mode of development of virus particles within various cell constituents because of their striking location. Pictures of the tumor cells taken by the electron microscope represent at best a series of stills. Tissue culture of cells infected with tumor viruses offers a better approach for study of the mode of development of tumor virus particles, especially in combination with electron microscopy.

Nucleolus and Nucleus

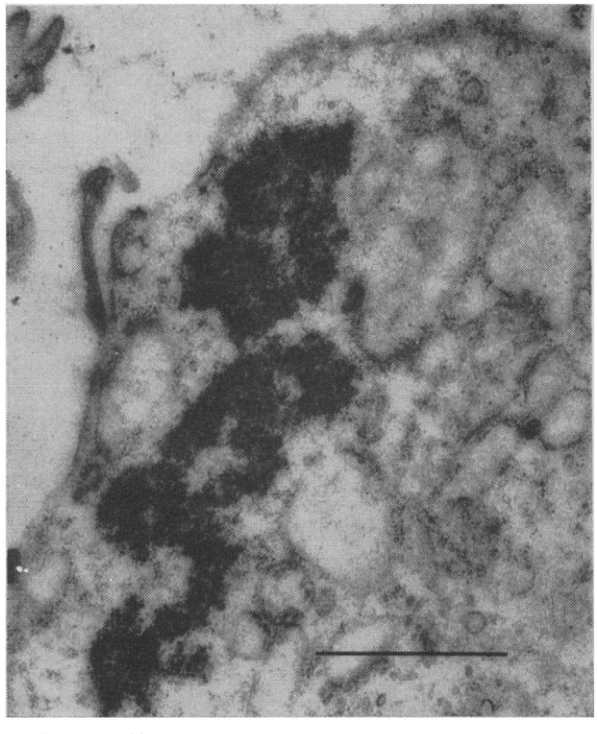
Enlargement of the nucleolus with condensation of some of its constituents has been observed in Rous sarcoma tumor of chickens (*57*) and in Shope papilloma tumor of rabbits (*58*). In the latter, virus particles have been observed in the network of the nucleolus (*58*). The nucleolus is frequently enlarged in cancer cells of the squamous cell carcinoma of the eye in cattle (*22*), in polyoma virus-induced tumors (*43*), and in human breast cancer (*53, 57*). Polyoma virus particles have occasionally been observed to be continuous with the denser filamentous material of the nucleolus (*59*). As in other virus infections, "dense bodies" have been observed scattered in the nucleolus and the nucleoplasm of the adenocarcinoma of the leopard frog (*60*), in Rous sarcoma of chickens (*61*), in polyoma-infected tissue-culture cells (*44*), in molluscum contagiosum, a benign human skin tumor (*28*), and in human breast cancer (*53, 57*). These aggregations within the nucleolus and nucleoplasm are strikingly similar to those noted in the cytoplasm of chicken adenocarcinoma of the kidney (*62*).

An intimate association of virus particles with threads of chromatin in the nucleus has been observed in rabbit papilloma cells (*58*), in polyoma-infected mouse embryo cells grown in vitro (*63*), and in polyoma-induced tumors of the kidneys in mice, rats, and hamsters (*49*). The distribution of chromatin, frequently considerably enlarged, along the nuclear membrane is a characteristic feature of cells infected with polyoma in vitro and in vivo (*49, 63*). As in other viral infections, inclusion bodies within the nucleus containing virus particles, frequently in regular "crystalline" arrays, have been found in polyoma virus-infected cells (*48, 49, 63*) and in polyoma-induced tumor cells (*49*). The virus particles have also been observed scattered at random in the nucleus of cells of Shope papilloma (*58*) and of polyoma-induced tumors (*43, 45, 49, 59*). A thickening and occasional duplication of nuclear membrane with polyoma virus particles between and outside the nuclear membranes have been occasionally observed (*63*) in polyoma-infected cells in vitro. All changes in the nucleus and nucleolus of virus-induced tumor cells are strikingly similar to those observed in cells infected with "ordinary" viruses (*22, 57*).

Cytoplasm

The changes observed in the different submicroscopic constituents of the cytoplasm are strikingly similar in virus-induced tumors in animals and in some human cancers in which virus particles have been observed or in which the particles could not be found (*22, 57*).

Mitochondria, one of the ultrastructural constitutents of the cytoplasm, may show only changes which can be described as degenerative, or they may show the presence of virus particles in what appear to be various stages of formation. Virus particles within mitochondria have been observed in visceral lymphomatosis, erythroblastosis, myeloblastosis, and renal adenocarcinoma of chickens (*22, 62, 64*) and in myeloblastosis cells in vivo and in vitro (*65*). They have also been found in regular arrays in mitochondria of polyoma-induced tumors of the salivary gland of mice (*59*) and of polyoma-induced tumors of the kidneys of mice, rats, and hamsters (*49*). In view of the known importance of mitochrondia in the biochemistry of cells, this "power plant" of tumor cells once again presents a



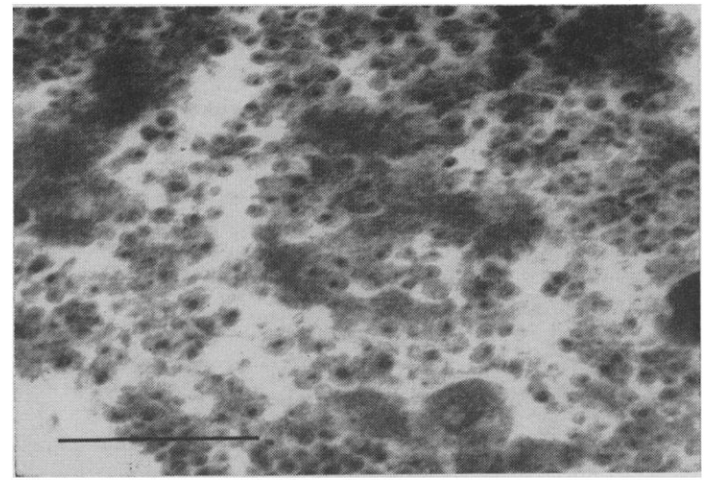
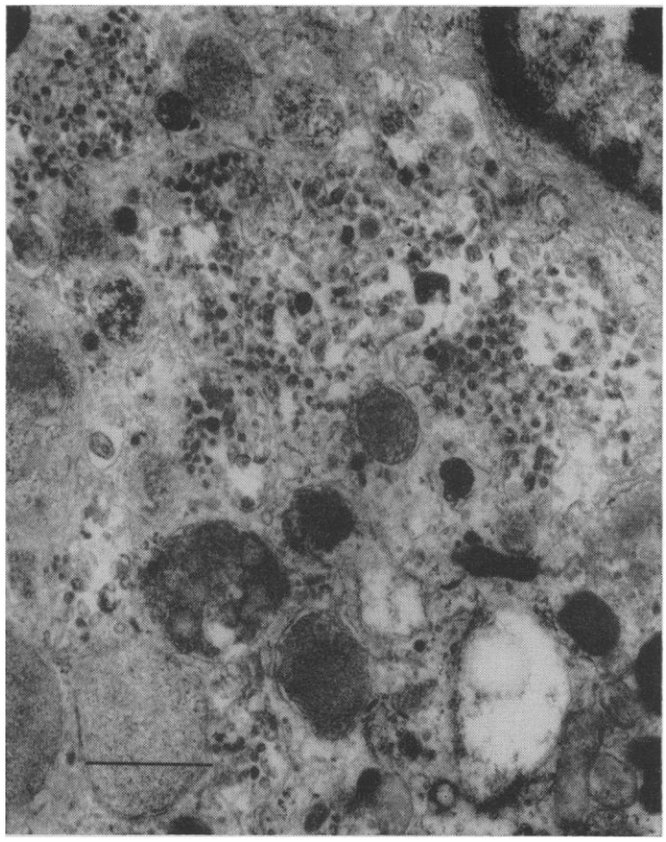


Fig. 8 (left). Part of the cytoplasm of a cell from a kidney tumor induced in a chicken by leukemia (myeloblastosis) virus. Characteristic aggregates, which precede the appearance of virus particles, may be seen in the cytoplasm (about × 26,000). Fig. 9 (above). Virus particles which appear within characteristic osmiophilic aggregates in the cytoplasm of some cells of chicken kidney tumor induced by leukemia (myeloblastosis) virus (about × 26,500). [L. Dmochowski, C. E. Grey, B. R. Burmester, W. G. Walter]



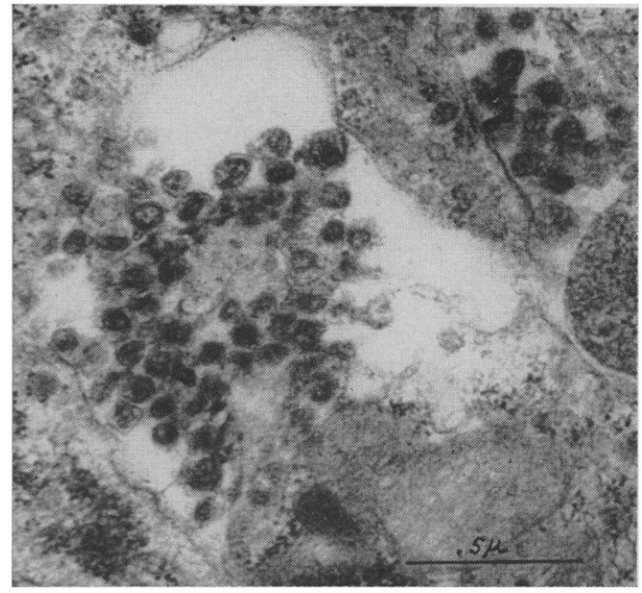


Fig. 10 (left). General appearance of a section of lymph node from a patient with acute lymphatic leukemia. Profound changes may be seen in the cytoplasm of cells, with inclusions and virus particles (about × 17,000). Fig. 11 (above). Virus particles in the intercellular space, in a section of lymph node from a patient with acute lymphatic leukemia (about × 50,000). [L. Dmochowski, C. E. Grey, J. A. Sykes, C. C. Shullenberger, C. D. Howe]

challenge to biochemists interested in oncology. The size of mitochondria may be increased to that of an inclusion body, as seen in the light microscope. Virus particles are occasionally found arranged in orderly arrays within the body, as seen in the electron microscope (*49, 59*). These morphological observations are indicative of profound changes in the biochemical economy of tumor cells, which appear at the same time to have their genetic apparatus affected by the intimate association of virus particles with chromatin in the nucleus.

An increase in the size of the Golgi apparatus in some virus-induced tumors has been encountered in breast cancer of mice (*23*), Rous sarcoma of chickens (*57*), bovine ocular squamous cell carcinoma cells grown in vitro (*66*), and human leukemic cells grown in tissue culture (*51*). However, no association with virus particles, except in breast cancer in mice (*23*), has been observed.

The ribonucleoprotein particles (*67*), another submicroscopic component of the cytoplasm, are known to increase in number in cancer cells. Characteristic aggregations of these particles have been observed in the cytoplasm of Rous sarcoma cells (*57*) and in chicken kidney adenocarcinoma cells (*62*), with virus particles present within these aggregates in cells of the kidney carcinoma of chickens (*62*).

Electron-microscope studies of normal and cancer cells have revealed an extensive system of membranes, interconnected and extending from nuclear membranes, through the membranes of endoplasmic reticulum or ergastoplasm, to the cell membrane (*22*). The latter appears to be intimately associated with virus particles in the cells of a number of virus-induced tumors, such as leukemia of mice (*68*), breast cancer of mice (*69*), erythroleukosis of chickens (*23*), and chicken renal adenocarcinoma (*62*). It is premature to speculate on the formation of virus particles from cell membranes, in view of observations of what appear to be progressive stages in virus development.

Tissue-Culture Studies

Susceptible cells grown in tissue culture and infected with tumor viruses have now been extensively studied in the electron microscope at different intervals of time after infection. The following tumor viruses have, so far, been studied in tissue culture: Rous sarcoma (*61, 70*), chicken myeloblastosis (*65*), Shope rabbit fibroma (*71*), and mouse breast cancer (*69, 72*) and polyoma (*43, 44, 63*). These studies gave a more detailed picture of changes which could be interpreted as the gradual development of particles of the various tumor viruses. Thus, combination of the method of tissue culture and electron microscopy gave support to the original observations on the ultrastructure of cells from virus-induced tumors, and to interpretation of the different ultrastructural forms as developmental stages of tumor virus particles. Bioassays of virus-infected cells in vitro, carried out at different intervals of time, lent further support to the conclusion that the particles are the various tumor viruses.

One of the important considerations in preparing tissue specimens for electron-microscope examination is the suitability of such specimens for phase and fluorescent microscope studies. In a study combining phase, fluorescent, and electron microscopy of cells grown in vitro and infected with polyoma virus (*63*), it has been possible to correlate morphological changes observed by these means with tumor-inducing activity of the tissue-culture material. The gross structure of these inclusions and their gradual formation could be ob-

served in the phase contrast microscope, their viral nature could be determined by observation of virus particles in the electron microscope, and their chemical composition could be assessed by pretreatment with nucleases, followed by staining with a fluorescent dye, acridine-orange. As there are as yet no staining procedures available which could differentiate viral from cellular ribonucleic or deoxyribonucleic acid, such integrated studies, in which fluorescent microscopy is combined with electron microscopy, are of extreme importance as they are helpful in indicating the type of nucleic acid carried by a virus.

Identification and Ultrastructure of Tumor Virus Particles

The increasing frequency with which characteristic particles have been encountered in the cells of various tumors of known and suspected viral origin has led to increasingly stringent criteria for the identification of certain characteristic structures, differing from normal ultrastructural components of cells, as virus particles and etiological agents of the disease under study. Again, electron microscopy, with its improved techniques of fixation and staining of specimens, combined with various biophysical and biochemical procedures, has greatly contributed to the morphological identification and biochemical characterization of different "ordinary" and tumor viruses (*22*).

An integrated electron-microscope and cytochemical study of chemically purified preparations of Rous sarcoma virus, combined with biological tests of such preparations, served as the means of identifying particles observed in Rous sarcoma tumor cells as Rous virus. This study showed, further, that the "nucleoid" or dense center of the virus particles is composed of ribonucleic acid (*73*).

Biophysical procedures, such as ultracentrifugation, combined with electron microscopy of biologically active pellets obtained by ultracentrifugation of plasma of chickens with myeloblastosis and erythroblastosis have provided evidence that particles present in such pellets are the causative agents of these forms of chicken leukosis (*74*). The ultrastructure of virus particles observed in ultracentrifugal pellets was found to be similar to that of particles seen in ultrathin sections of cells from these types of chicken leukosis.

Similar procedures have led to the identification of Shope papilloma virus particles and have clarified their ultrastructural appearance (*75*). Again, the structure of papilloma particles was found to be similar to the structure shown in sections of rabbit papilloma cells (*58*). These studies extended previously reported results on the size, shape, and density of papilloma particles (*76*).

Biophysical and biochemical procedures, combined with electron microscopy of ultracentrifugal pellets obtained from suitably treated milk of mice carrying the virus which induces breast cancer in mice (Bittner virus), have helped in identification of the characteristic particles as the Bittner virus (*38*). These particles are similar in size and appearance to those previously observed in breast cancer cells of mice (*35*, *36*). They do show greater details of ultrastructure (*77*), when an improved staining procedure is used (*78*). There appears to be agreement as to the size and appearance of Bittner virus particles, as similar results have also been obtained through the use of various biophysical procedures carried out on Bittner virus-carrying material obtained from different sources (*69*, *72*, *79*).

Thus, electron-microscope studies of tumor-inducing viruses have reached the second stage of their development, where it is now possible to devise experiments leading toward the identification of tumor virus particles. However, the requirements of quantitative electron microscopy (*21*) for identification of virus particles as causative agents of a disease can only be satisfied if a strict correlation is established between the number of characteristic particles and the titer of tumor-inducing activity of preparations containing the virus particles. Recently, new methods of staining for electron microscopy have become available which may meet the criteria of quantitative electron microscopy and, in addition, allow for a study of the ultrastructure of viruses on a molecular level (*24*).

As mentioned above, biological studies revealed a great similarity between "ordinary" viruses and tumor viruses. Electron-microscope studies of ultrathin sections of virus-infected cells and tumor cells induced by viruses revealed

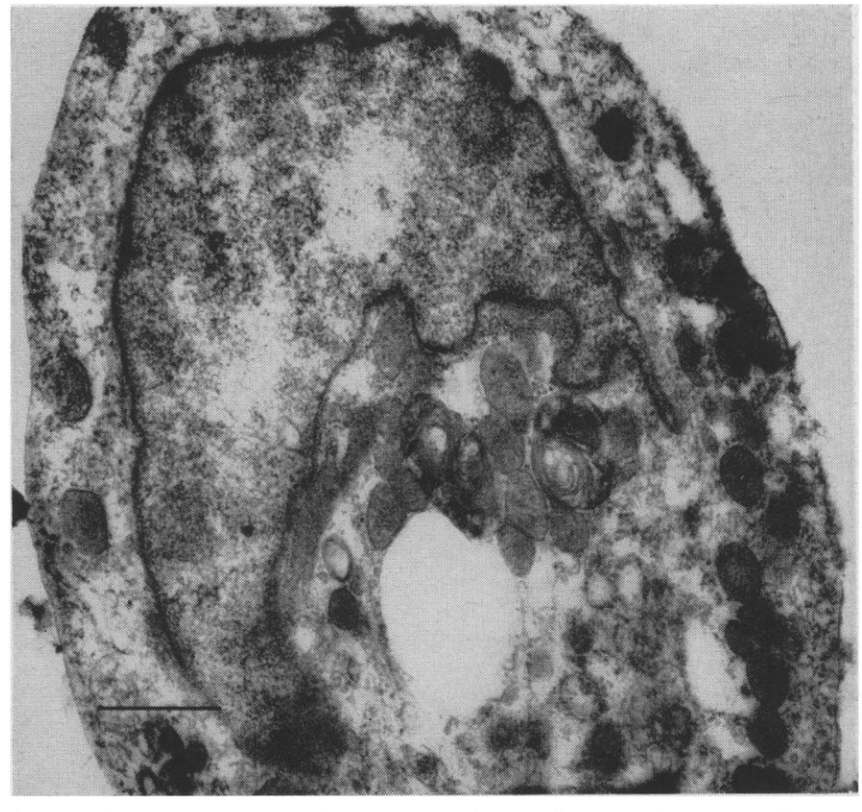


Fig. 12. General appearance of a mouse embryo cell grown in tissue culture and treated with nucleic acid preparation from mouse lymphatic leukemia. The altered mitochondria and characteristic onion-like structures that precede the appearance of virus particles may be seen (× 16,000). [L. Dmochowski, C. E. Grey, L. O. Pearson, J. A. Sykes, R. G. Hughes]

that "ordinary" viruses and tumor viruses are similar in their relationship to the various submicroscopic constituents of cells. These studies have also shown a general similarity in size, shape, and structure of plant, insect, animal, and human viruses. As mentioned above, most virus particles are composed of a dense center (now known to be one of the nucleic acids) surrounded by a single, double, or multiple membrane. High-resolution electron microscopy of sections of tumor cells stained with heavy metals has already led to the visualization of an inner reticular or filamentous structure of the dense center, or the so-called "nucleoid," of some of the tumor viruses (*22*, *23*, *49*, *59*).

The recent application of staining technique with potassium phosphotungstate (*24*) to purified or even partly purified virus preparations has revealed previously unseen details of the structure of virus particles in the electron microscope. It is of extreme importance that such details agree with data on virus structure obtained by x-ray diffraction and other physiochemical methods. This method has demonstrated the structure of the protein shell, which is composed of subunits varying in number in different viruses. It has also been possible to observe the inner structure of the nucleoid, which is composed of a number of subunits arranged in the form of a flexible helical array in some of the animal viruses (*24*). Thus electron microscopy appears to indicate the existence within viruses of a structure which fulfills the essential property of viruses, the construction of the particle by the use of multiple, similar, protein subunits (*24*). This technique appears to retain the three-dimensional structure at the molecular level and at the same time preserves the activity of virus particles.

The application of this staining technique has shown the structure of the shell or coat of virus particles (*80*) already identified as the polyoma virus by other studies (*81*). It has also shown the structure of the coat of Shope papilloma virus particles (*82*). These in general are similar to structures observed in the coat of other viruses. The presence of particles with a hollow center has also been observed. These may be noninfectious particles or stages in the development of the infective particles. Such particles have been described in sections of virus-induced tumor cells (*22*, *23*).

Viral Nucleic Acid and Cancer

It is now known that viral nucleic acids from plant, animal, and human viruses transmit viral infectivity and induce changes characteristic of the particular virus (*7*). The nucleic acid is therefore the basis of viral activity. Nucleic acid from a virus can also lead to virus production in cells from nonsusceptible hosts without signs of disease or morphological changes (*8*). Nucleic acid from tissues infected with polyoma virus has been found to behave in a manner similar to that of nucleic acid from other viruses. It will induce changes characteristic of the virus in susceptible cells grown in tissue culture (*83*, *84*) and will induce tumors in susceptible animals (*83*). As yet there are no morphological studies of the submicroscopic appearance of cells treated with nucleic acid from tissues infected with tumor viruses to which the cells are known not to be susceptible. Such studies may reveal the behavior of submicroscopic constituents of cells, and whether formation of virus particles takes place. Electron-microscope studies of susceptible cells treated with nucleic acid preparations from leukemic and polyoma virus-infected tissues have shown changes similar to those observed during polyoma infection and the presence of characteristic virus particles (*22*) (Fig. 12). These morphological observations are supported by the results of biological and serological tests. Thus, a morphological confirmation has been obtained of the formation of polyoma virus particles within susceptible cells treated with the nucleic acid preparations from tissues infected with this virus. Electron-microscope studies of cells treated in such a manner, carried out at various intervals, may permit observation of the gradual development of virus particles and indicate the involvement of the various ultrastructural components in the formation of the particles.

Electron microscopy has shown that deoxyribonucleic acid in purified preparations consists of macromolecules 20 angstroms in length (*85*), and that ribonucleic acid consists of two types of molecules differing in length (*86*). It may be too much to hope that electron microscopy may in the near future help to distinguish the ribonucleic and deoxyribonucleic acid of the host from the nucleic acid of a virus. Nevertheless, it has already shown details of structure which appear to be rapidly closing the gap between morphology and molecular biology.

The problems of molecular biology are now increasingly important; hence attempts are being made to detect viral antigens by means of electron dense antibody conjugants (*87*). This approach offers hope of visualizing sites of antigen-antibody interaction on the molecular level. It appears that specific identification of antigenically distinct viral particles can be made by electron microscopy. This offers considerable hope for future studies of human cancer and the characterization of virus particles encountered in the cells of some human cancers.

Recent progress in electron-microscope studies of virus-infected cells, of cells of tumors induced by viruses, and of viruses themselves has provided a common meeting ground for morphologists, virologists, chemists, and physicists. It has led, through mutual interest, to considerable progress in our knowledge of viruses and animal tumors. Although electron microscopy of human cancer is still in the pioneering stage and a virgin territory, the ground is bound to be cleared through the cooperation of specialists in the different disciplines of science (*88*).

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