An Electron Microscope Study of Rat Leukemia Induced with Mouse Leukemia Virus (Gross)*

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SUMMARY

A comparative electron microscope study was carried out on ultrathin sections of bone marrow, spleen, lymph nodes, and mediastinal tumors from eighteen rats with leukemia induced by mouse leukemia virus (Gross).

In organs of all eighteen rats with leukemia, two types of particles were observed: one type, described as virus particles of 990 A average diameter, with an electron-dense center of 630 A average diameter, centrally or eccentrically located and surrounded by one or two membranes; the other type, described as vesicular or doughnut-type particles, with an electron-lucent center with an average diameter of 490 A, surrounded by an inner membrane and an outer membrane with an average diameter of 840 A. Occasionally, another membrane (580 A) could be seen between the inner and outer membranes of these particles. Both types of particles were found in the megakaryocytes of bone marrow and spleen, and the virus particles in the intercellular spaces in the lymph nodes, spleen, and in the mediastinal tumors. Occasionally, the particles were also found in cytoplasmic inclusions of cells present in the mediastinal tumors and in the lymph nodes. These particles were observed with equal frequency in these tissues, but apparently more frequently than in the spleen and bone marrow.

Budding of plasma membrane of blast cells leading to particle formation was occasionally observed in the cells present in all organs examined. Similar budding of the membranes of cytoplasmic channels and of specific granules in the megakaryocytes of bone marrow and of spleen was also observed.

Characteristic cylindrical or tubular structures were observed in the megakaryocytes of the bone marrow and spleen of leukemic rats. Segmentation of these structures into doughnut-type particles and budding into similar particles was also frequently seen.

No particles of any type or cylindrical structures could be found in either bone marrow, spleen, thymus, or lymph nodes of fifteen normal rats.

Following the successful cell-free transmission of mouse leukemia virus (17, 18), numerous electron microscope studies have been carried out on organs of mice with various types of spontaneous and induced leukemia. These studies have been described in a number of extensive reviews (2, 3, 9–11). The present study deals with the results of electron microscopic examination of ultra-thin sections of different organs of leukemic rats in which the disease had been induced with passage A leukemia virus (Gross). This virus, initially isolated from spontaneous Ak mouse leukemia (17), proved to be pathogenic not only for mice (18) but also for rats (19). It could be passed serially through newborn rats, inducing in the great majority of the inoculated animals either lymphatic or stem-cell leukemia, and in some instances also myelogenous leukemia (20).

In a previous report (16) the results of electron microscope studies of organs of rats with passage A virus-induced leukemia were described. The present report deals with a more detailed description of these studies and includes results based on comparative examination of material, prepared by improved methods, and obtained from different organs of leukemic and normal control rats.

MATERIALS AND METHODS

Rats with leukemia induced with passage A mouse leukemia virus (Gross) were used as a source of material. Altogether eighteen leukemic rats were used. Twelve of these animals were Sprague-Dawley, and six were Osborne-Mendel rats, varying in age from 2 ½ to 4 months. In
addition, eleven Sprague-Dawley and four Osborne-Mendel healthy rats, 2–9 months old, were used as a source of control material.

Leukemia was induced in the rats by the intraperitoneal inoculation of 0.5 ml. of passage A leukemia virus preparation into animals less than 5 days old. The virus preparation used for inoculation was made from organs of C3H mice with virus-induced leukemia, or from organs of rats with leukemia induced by the passage A virus. The virus preparation used for inoculation was made according to a method described previously (18).

Bone marrow, spleen, peripheral and mesenteric lymph nodes, and mediastinal tumors were removed from leukemic rats, sacrificed by chloroform inhalation. Bone marrow, spleen, thymus gland, and lymph nodes were obtained from the control healthy animals. The organs from leukemic and control rats were cut into small pieces and fixed in cold 2 per cent osmic acid buffered with sodium and potassium phosphate with the addition of glucose, according to the method of Millonig (25). The tissues were dehydrated in a series of graded alcohols, at 15-minute intervals, varying from 30 per cent to absolute. They were then brought through a mixture of absolute alcohol and n-butyl methacrylate (equal volumes) for 1 hour, followed by two changes of the methacrylate (1 hour each), pre-polymerized monomer overnight, and polymerized in the methacrylate with 0.5 per cent benzoyl peroxide at 56° C. for 24 hours. Sections were cut with glass knives on a Porter-Blum microtome, and stained with either a solution of uranyl acetate alone for periods varying from 30 minutes to 1 hour (37) or with uranyl acetate for 2 hours, followed after washing in triple-distilled water by a solution of lead tartrate (26) for 30 minutes, according to a method developed by one of us (32). After being stained the sections were coated with a thin film of carbon and examined in an RCA EMU 3E electron microscope, at 50 or 100 kv. Original magnifications of the electron micrographs varied from 2,000 to 60,000 diameters, which were then further magnified by photographic enlargement. On an average, 30 sections of each organ from the experimental and 50 sections of each organ from the control animals were examined.

RESULTS

In all the eighteen leukemic rats examined, characteristic particles were found. These particles could be divided into two categories: one with an electron-lucent center, varying in diameter from 340 A to 570 A and with an average diameter of 490 A surrounded by two or three membranes, with an average diameter of 840 A, and varying from 700 A to 1000 A for the outer membrane. These particles are described as vesicular or doughnut-type particles and are believed to be immature virus particles (Fig. 23). The second category of particles comprised particles with an electron-dense nucleoid with an average diameter of 630 A, varying from 500 A to 740 A, centrally or eccentrically placed, surrounded by one or occasionally two membranes, with an average diameter of 990 A for the outer membrane and varying from 750 A to 1160 A. The particles with an electron-dense center are described as mature virus particles (Fig. 22). This definition of the observed particles as "immature" and "mature" virus particles is for the purpose of simplification only. The vesicular particles may be a stage in the formation of particles with electron-dense centers, but so far no convincing evidence to this effect has been obtained, except for gradually increasing density of the electron-lucent center. It is possible that another type of structure observed and described as tubular or cylindrical structures may represent a stage in the formation of the vesicular or doughnut-type particles. The diameter of the outer, intermediate, and inner membranes of these structures (Figs. 4, 10–12) corresponds to the diameter of the different membranes constituting the vesicular or doughnut-type particles. The length of these structures so far observed may be as much as 0.7 μ. They may be straight, ending rounded up with what appears to be a half of the vesicular particle (Figs. 4, 8, 10, 11). These structures have also been found to curve gradually (Fig. 8) or abruptly (Fig. 11), and on occasions seem to branch off (Figs. 8, 10). They have also been found to show septa or segmentation (Figs. 4, 8, 10).

Bone marrow.—In the bone marrow of all leukemic rats both types of particles have been observed in the megakaryocytes. The number and proportion of the two types of particles may vary from one megakaryocyte to another in the same bone marrow and may also vary in similar cells of the bone marrow from one rat to another. Both types of particles have been found in the system of channels and vacuoles, some of which may have originated from specific granules of the megakaryocytes.

During examination at low magnification, it has been found difficult to observe any characteristic differences between the megakaryocytes in bone marrow of healthy young control rats (Fig. 1) and those present in the bone marrow of leukemic animals (Fig. 2). Vacuoles of varying size have been observed in the megakaryocytes of the bone marrow of leukemic rats (Fig. 2). These, however, could not be described as characteristic changes.

Examination of the bone marrow of fifteen control rats at higher power failed to reveal any of the changes observed in the megakaryocytes of the bone marrow from leukemic rats. As already mentioned, both immature and mature particles have been found in the vacuoles of the cytoplasm of megakaryocytes (Fig. 3), and in the channels of these cells as well as in the specific granules (Figs. 3 and 7). Occasionally, these particles have also been observed freely scattered in the cytoplasm of these cells. Frequently, invagination or budding of the membrane surrounding the specific granule has been noted (Figs. 3, 6). This budding phenomenon appears to be a stage in the formation of vesicular particles and may represent, at least in some cases, a cross-section of the tubular or cylindrical structures forming a continuity with membranes of a specific granule. Budding of the membrane of cytoplasmic channels leading to the formation of vesicular particles has also been observed.

The cylindrical or tubular structures have been found in the cytoplasm of megakaryocytes (Figs. 4, 8–12). They appear to originate from the membrane of cytoplasmic channels and of specific granules (Figs. 4, 10). They may appear to be straight in longitudinal sections.
(Figs. 9, 14), or they may appear to bend gradually (Fig. 8) or abruptly (Fig. 11). The cylindrical or tubular structures may on occasion appear to form a continuity with the membranes of the system of channels or vacuoles of megakaryocytes (Figs. 9, 10). Frequently, the cylindrical structures appear to be rounded off on either one or both ends (Figs. 4, 8, 10, 11). Occasionally they appear to segment (Figs. 4, 10), may show what appears to be branching (Fig. 8), and apparent formation of spherical or doughnut-type particles (Figs. 8, 10, 11).

In view of the frequency of observation of these tubular or cylindrical structures in the megakaryocytes of the bone marrow of leukemic rats infected with passage A leukemia virus, and their apparent absence in tissues of normal control animals, these structures seem to be a characteristic phenomenon of murine leukemia.

No other changes have been observed in the megakaryocytes of the marrow of leukemic rats. However, changes in the distribution and density of osmiophilic material within the specific granules have frequently been observed (Figs. 6–8). Occasionally, an impression was gained that at least some of the specific granules could be interpreted as altered mitochondria of small diameter. Infrequently, an apparent increase of ribonucleoprotein granules has been noted along the strands of ergastoplasm (Fig. 3), but no changes could be observed in the Golgi zone.

As far as other types of cells of the bone marrow of leukemic rats are concerned, the phenomenon of budding of the plasma membrane, occasionally observed in the megakaryocytes (Fig. 20), has also been found in the eosinophils (Figs. 13–16) and in the normoblasts (Figs. 17, 18). Very infrequently either mature or vesicular type particles have been observed within the cytoplasm of eosinophils (Fig. 16) or of normoblasts (Fig. 19).

Spleen.—In the megakaryocytes of the spleen of leukemic rats changes have been found identical to those observed in the megakaryocytes of the bone marrow of the same animals. Both vesicular and mature particles and cylindrical or tubular structures have been observed in the megakaryocytes of the spleen in the same relationship to the cytoplasmic components as in the megakaryocytes of the bone marrow (Fig. 21). However, the number of the observed mature particles (Fig. 22) and vesicular or doughnut-type particles (Fig. 23) appeared to be smaller than that encountered in the megakaryocytes of the bone marrow. Fully formed or mature virus particles have been observed in cytoplasmic inclusions in the proximity of mitochondria in various stages of alterations and of dense osmiophilic bodies of varying size within cells of undetermined origin (Fig. 24). Frequently virus particles have been found in the intercellular spaces in close proximity to the plasma membrane of cells of different types (Fig. 5). Very occasionally the budding phenomenon of the plasma membrane of lymphoblasts has been encountered and could be interpreted as the formation of vesicular or doughnut-type particles.

Lymph nodes and mediastinal tumors.—Numerous fully formed virus particles have been observed both in the affected peripheral lymph nodes (mesenteric, axillary, inguinal) and in the mediastinal tumors. From the results of the present study it appears that the virus particles have been found to be more numerous in these tissues than in the megakaryocytes of the bone marrow or spleen. However, only occasional signs of what could be interpreted as formation of virus particles could be observed in these tissues. Only occasional budding of the plasma membrane of lymphoblasts could be observed, and infrequently fully formed virus particles have been found in what appeared to be cytoplasmic inclusions of undetermined cells in the lymph nodes (Fig. 25) and in the mediastinal tumors (Fig. 28). Fully formed virus particles have most frequently been encountered in the intercellular spaces and in what could be interpreted as fragments of broken down cells (Figs. 25, 27). Occasionally, these particles have been observed in the proximity of blood vessels or within the lumen of the blood vessels. The characteristic cylindrical or tubular structures have not been observed in the lymph nodes or mediastinal tumors.

In a study of over 50 sections each of bone marrow, spleen, peripheral and mesenteric lymph nodes, and of the thymus gland of fifteen normal 2- to 9-month-old control rats (eleven Sprague-Dawley and four Osborne-Mendel) no virus particles could be found or any other changes comparable to those observed in the eighteen leukemic rats.

DISCUSSION

In the present study, virus particles have been found in the organs of all eighteen leukemic rats examined. These particles have most frequently been observed in the mediastinal tumors and in the peripheral and mesenteric lymph nodes. No difference could be found between the number of virus particles observed in the lymph nodes and the mediastinal tumors. The number of particles with electron-dense centers or mature particles (within the limits of the error of sampling imposed by the number of sections examined and only too frequently encountered in electron microscope studies) was apparently smaller in the bone marrow and spleen than in the lymph nodes or mediastinal tumors.

No difference could be observed in the present study between the number of structures interpreted as cytoplasmic inclusions, containing electron-dense spherical particles, and encountered in the cells of mediastinal tumors, and the number of similar structures present in the cytoplasm of cells of the peripheral or mesenteric lymph nodes. The part played by these structures in the possible mode of the development of the passage A leukemia virus remains to be determined.

The budding phenomenon of the plasma membrane of blast cells has been observed infrequently but with similar frequency in the cells of the leukemic lymph nodes and in those of the mediastinal tumors. It appears of interest that budding of plasma membranes leading to particle formation has occasionally been observed in the blast cells and also in eosinophils present in the bone marrow of leukemic rats.

Measurements of particle size may not be a sufficiently dependable basis for the classification of virus particles under certain conditions (7). The emphasis on particle size is probably not justifiable, since different embedding materials produce different degrees of shrinkage. The
particles would be larger, for example, if embedded in epoxy resin instead of methacrylate. The size of virus particles may also depend on their location, whether extracellular or intracellular (7, 11). This variation in the size of virus particles, depending on their location, has been encountered in mouse, rat, chicken, and human leukemia (11). In the present study the average size of passage A leukemia virus particles, based on the measurement of the size of 270 intracellular and extracellular particles, has been found to be 990 Å. This particle size is similar to that of virus particles present in the organs of mice with leukemia induced with the virus strain isolated by Moloney (7). The size of the leukemia virus strain isolated by Moloney (27, 28), if one considers the limitations of the criteria based on size measurements, appears to be similar to that of passage A leukemia virus (17, 20). The present study appears to have demonstrated also that the mode and sites of particle formation are similar in leukemia induced by either virus strain.

Although in the present study examination of sections of the various organs failed to reveal particles with tail-like structures originally reported by Dalton (5, 6), with the possible exception of Figure 25 in the bottom right-hand corner, it is probable that differences in the methods of embedding and staining were responsible for the failure to observe such structures. They may, of course, have simply been overlooked. Particles with "tails" have been observed in various organs of mice and rats with passage A virus-induced leukemia by Feldman et al. (1). Similar structures have recently been observed by Okano et al. (31) in the intercellular spaces of organs of rats with leukemia induced by the passage A virus. Particles with tail-like structures have been observed by us in preparations of milk, negatively stained with potassium phosphotungstate, and obtained from mice of AKR and C58 strains known to develop a high incidence of spontaneous leukemia (15), and occasionally in sections of organs of mice with passage A virus-induced leukemia (2).

Megakaryocytes, especially those present in the bone marrow of leukemic rats, have been found to show changes similar to those described by Dalton et al. (7) in the megakaryocytes of the spleen and bone marrow of leukemic mice. Budding of the membrane of cytoplasmic channels of megakaryocytes of the bone marrow and of spleen has been observed by Dalton and his co-workers (7) in mouse leukemia induced by the passage A virus, as well as that induced by leukemia virus strains isolated by Moloney, Manaker, and by the Friend virus, and also in spontaneous leukemias of AKR and C3H/Fj mice. De Harven and Friend (8) made similar observations on megakaryocytes of the bone marrow of mice with Friend leukemia. Furthermore, Dalton and his co-workers (7) observed particle formation within specific granules of megakaryocytes in mouse leukemia induced by the passage A virus, as well as in spontaneous leukemia in mice of strains AKR and C3H/Fj; they did not observe such particle formation in mice with leukemia induced by the virus strains isolated by Moloney or Manaker, or in Friend mouse leukemia. Similarly, characteristic cylindrical or tubular structures have been found (7) in the megakaryocytes of the bone marrow and spleen of mice with leukemia induced by the virus strain isolated by Moloney, but not in cells of mice with leukemia induced by the passage A virus or by the virus strain isolated by Manaker.

These discrepancies in the finding of either the formation of particles from specific granules or the cylindrical or tubular structures in different leukemias may be due to differences in technical methods of preparing tissue specimens and also in sampling and persistence in looking for certain structures in the course of electron microscopic examinations. Thus, contrary to previous negative reports of Dalton and his co-workers (7), the characteristic cylindrical or tubular structures have been found frequently in our present study in the megakaryocytes of the bone marrow and spleen but not in the lymph nodes of rats with leukemia induced by the passage A virus. Similar structures, apparently originating from the membranes of cytoplasmic vacuoles and leading to the formation of spherical translucent particles by segmentation and budding, have also been found by us in the mesenteric lymph nodes of AKR strain mice with spontaneous leukemia (15) and in the megakaryocytes of the bone marrow of mice with passage A (Gross) virus-induced leukemia (16). The failure to observe the cylindrical or tubular structures in the lymph nodes and mediastinal tumors of the examined rats may only indicate that these structures have been overlooked, since they may not appear as frequently in these tissues as in the megakaryocytes of the bone marrow and spleen of rats with leukemia virus of Gross. From the available morphological evidence it appears that these tubular or cylindrical structures are a stage in the development of the spherical particles with electron-lucent center.

The cylindrical or tubular structures have not been reported in a recent study of rat leukemia induced by passage A virus (31). A number of reasons for this may be advanced, but it should be mentioned that, in our original studies on spontaneous leukemia in AKR strain mice and on leukemia induced in C3H strain mice with passage A virus, these structures have also been overlooked (12, 13). Again, the error of sampling as well as the improvements in the methods of specimen preparation may be mentioned as a possible explanation of this discrepancy in the electron microscope findings.

The cylindrical or tubular structures do not appear to be a unique phenomenon characteristic of leukemia virus. Similar structures, described as filamentous, have been demonstrated in the nuclei of cells infected with polyoma virus of mice and have been interpreted as a stage in virus

Fig. 1.—Appearance of a megakaryocyte in the bone marrow of a control rat. Multilobed nucleus (N) and cytoplasm (CY) containing numerous specific granules may be seen. Section stained with uranyl acetate. Original magnification, ×2,000; final, ×4,500.
Fig. 2.—Appearance of a megakaryocyte in the bone marrow of a leukemic (Osborne-Mendel) rat. Part of the cytoplasm (CY) is more electronlucent than the rest of the cytoplasm, as in the control bone marrow. The rest of the cytoplasm appears more dense and contains vacuoles (V). No other changes can be seen at this magnification. Section stained with uranyl acetate. Original, ×3,500; final, ×8,000.
Fig. 3.—Part of the cytoplasm (C) and lobulated nucleus (N) of a megakaryocyte in the bone marrow of a leukemic rat. In the cytoplasm, specific granules (SG) may be seen, showing budding of their membrane (double arrows) and presence of mature and immature particles (arrows). Numerous vacuoles, showing the budding phenomenon and presence of immature and fully formed virus particles (arrows), are present. Part of Golgi zone (G) and endoplasmic reticulum (ER) may be seen. Uranyl acetate staining. Original, X8,000; final, X30,000,
Fig. 4.—Part of an enlarged channel in the cytoplasm of a megakaryocyte in the bone marrow of a leukemic rat (Osborne-Mendel). Within surrounding membrane (arrows), a number of cylindrical structures showing segmentation (double arrows). Uranyl acetate and lead tartrate stain. Original, ×30,000; final, ×150,000.

Fig. 5.—Virus particles present in the intercellular space in the spleen of a leukemic rat. Cytoplasm (CT) of some cells may be seen. Osmiophilic inclusion (INC) may also be seen. Uranyl acetate stain. Original, ×10,000; final, ×40,000.

Fig. 6.—Specific granules of a megakaryocyte of a leukemic rat, showing characteristic budding (arrows). Uranyl acetate staining. Original, ×10,000; final, ×120,000.

Fig. 7.—Appearance of an immature particle in a late stage of development, present in a specific granule, in the cytoplasm of megakaryocyte in the bone marrow of a leukemic rat. Uranyl acetate and lead tartrate staining. Original, ×60,000; final, ×300,000.
FIG. 8.—Part of the cytoplasm of a megakaryocyte from a leukemic rat (Osborne-Mendel), showing numerous cylindrical structures, some apparently branching off (double arrows) and some in cross-section. Specific granules (arrows), some apparently showing loss of densely osmiophilic granular material. Uranyl acetate and lead tartrate stain. Original, X30,000; final, X135,000.
Figs. 9-12.—Examples of variation in appearance of the observed cylindrical structures in megakaryocytes of the bone marrow of leukemic rats. Sections stained with uranyl acetate and lead tartrate.

Fig. 9.—Original, X30,000; final, X75,000.
Fig. 10.—Original, X30,000; final, X90,000.
Fig. 11.—Original, X30,000; final, X210,000.
Fig. 12.—Two immature particles apparently linked together (arrows). Original, X30,000; final, X150,000.
Fig. 13.—Budding of plasma membrane of an eosinophil in the bone marrow of a leukemic rat. Characteristic inversion of plasma membrane (arrow). Uranyl acetate and lead tartrate stain. Original, X30,000; final, X93,000.

Fig. 14.—Eosinophilic granule and almost completed budding in an eosinophil. Uranyl acetate and lead tartrate stain. Original, 30,000; final, X93,000.

Fig. 15.—Another example of budding of plasma membrane of an eosinophil. Characteristic membrane (arrow) frequently seen in connection with budding. Uranyl acetate and lead tartrate stain. Original, 30,000; final, X93,000.

Fig. 16.—Part of the cytoplasm of an eosinophil, showing a mature virus particle (arrow) and characteristic spherical membranes (double arrows). Uranyl acetate and lead tartrate stain. Original, 15,000; final, X60,000.
Fig. 17.—Budding of plasma membrane of a normoblast in the bone marrow of a leukemic rat (double arrows). Uranyl acetate and lead tartrate stain. Original, ×30,000; final, ×93,000.

Fig. 18.—Another example of budding of plasma membrane of a normoblast in the bone marrow of a leukemic rat (double arrows). Uranyl acetate and lead tartrate stain. Original, ×30,000; final, ×75,000.

Fig. 19.—Budding from within a cytoplasmic membrane in a normoblast (arrow) present in the bone marrow of a leukemic rat. Uranyl acetate and lead tartrate stain. Original, ×15,000; final, ×53,000.

Fig. 20.—Budding from a plasma membrane of a megakaryocyte in the bone marrow of a leukemic rat. Uranyl acetate and lead tartrate stain. Original, ×30,000; final, ×93,000.
Fig. 21.—Appearance of a megakaryocyte in the spleen of a leukemic rat (Osborne-Mendel). Both immature and fully formed virus particles (arrows) may be seen in the cytoplasmic channels. Budding of the membranes of the channels (double arrows) may also be seen. Uranyl acetate stain. Original, ×8,000; final, ×20,000.
Fig. 22.—Mature particles present in a megakaryocyte of the spleen of a leukemic rat. Uranyl acetate and lead tartrate stain. Original, X30,000; final, X120,000.

Fig. 23.—Immature particles present in a megakaryocyte of the spleen of a leukemic rat. Multiple membranes (arrow) may be seen. Uranyl acetate and lead tartrate stain. Original, X30,000; final, X180,000.
Fig. 24.—Cytoplasmic inclusions, in part surrounded by a double membrane (arrows) containing mature virus particles present in a cell of spleen from leukemic (Osborne-Mendel) rats. Dense osmiophilic inclusions (INC) and mitochondria (M) may be seen. Uranyl acetate stain. Original, X10,000; final, X65,000.
Fig. 25.—General appearance of a section of lymph node from an Osborne-Mendel leukemia rat, showing virus particles present among cell debris. Osmiophilic bodies (OB) and lipid bodies (LB) are seen. Uranyl acetate stain. Original, ×8,000; final, ×28,000.
FIG. 26.—Cytoplasmic inclusion, containing mature particles (double arrows) in the mesenteric lymph node of a leukemic Osborne-Mendel rat. Uranyl acetate stain. Original, $\times 15,000$; final, $\times 60,000$.

FIG. 27.—Numerous mature virus particles present in the intercellular spaces in the mesenteric lymph node of a leukemic (Osborne-Mendel) rat. Uranyl acetate stain. Original, $\times 20,000$; final, $\times 80,000$.

FIG. 28.—Cytoplasmic inclusions, containing mature virus particles (arrows) present in a blast cell of mediastinal tumor of a leukemic (Osborne-Mendel) rat. Uranyl acetate stain. Original, $\times 10,000$; final, $\times 75,000$. 
multiplication (4, 21, 22). They have also been demonstrated in tissues infected with vesicular stomatitis virus (23, 33-35), rabies virus (24), and with infectious laryngotracheitis virus (36). Filamentous forms have been demonstrated also at the surface of cells infected with influenza virus (29, 30). In these studies the characteristic spherical particles of influenza virus, however, have not been observed to develop by segmentation of the filamentous forms, although apparently they may form by budding at the end of these structures (29, 30). Recently Archetti and Bocciairelli (1) demonstrated filamentous forms in two influenza A virus strains, grown in embryonated chicken eggs. Sections of these filamentous structures revealed regular succession of septa or constrictions, giving rise to particles, appearing as "bean-like" forms. Apparent formation of spherical particles at the end of these structures (29, 30). Recently Archetti and Bocciairelli (1) demonstrated filamentous forms in tissues infected with vesicular stomatitis virus (29, 30). In these studies the characteristic spherical particles revealed regular succession of septa or constrictions, giving rise to particles, appearing as "bean-like" forms. Apparent formation of spherical particles at the end of these structures has also been demonstrated (1). A conclusion was reached that the spherical virus particles are formed from the filaments by a process of segmentation (1).

The presence of cylindrical or tubular structures in the megakaryocytes of leukemic mice and rats (5, 7, 15, 16) appears to indicate the possibility of a similar mode in the development of leukemia virus in the cells of spontaneous and induced leukemia of mice and in the cells of induced leukemia of rats. Our recent studies on lymph nodes of leukemic patients indicate that cylindrical or tubular structures, similar to those found in mouse and rat leukemia, are also present in the cells of these tissues (14). Further studies are required to ascertain the significance of these observations in leukemic tissues of man.

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