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Electron Microscopic Studies of Rat Leukemia Induced with Mouse Leukemia Virus.* (27564)

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The mouse leukemia virus, initially isolated from spontaneous Ak mouse leukemia(1), and subsequently passed serially through newborn mice(2), proved to be pathogenic not only for mice but also for rats(3). Electron microscopic studies of the mouse leukemia virus were carried out at first using Ak or C58 mice which developed spontaneous leukemia(4,5). Ultrathin sections prepared from leukemic organs of such mice revealed in the cytoplasm or intercellular spaces of leukemic cells the presence of spherical particles varying in size from 750 to 1200 Å in diameter. In subsequent studies similar particles were found in ultrathin sections of leukemic organs of C3H mice with virus-induced leukemia(4,6). Essentially, therefore, there was no difference between particles found in animals with "spontaneous" leukemia and those in which leukemia developed following inoculation of the virus.

Experiments here reported were carried out with the purpose of studying in the electron microscope ultrathin sections of organs from leukemic rats, in which the disease was induced with the mouse leukemia virus.

Materials and methods. Suckling, less than 5 days old, Sprague-Dawley or Osborne-Mendel rats, were inoculated i.p. (0.5 ml each) with the passage A(3) mouse leukemia virus (Gross). The filtrates used for inoculation were prepared from either organs of C3H mice with virus-induced leukemia or from organs of leukemic rats in which the disease was induced with the mouse leukemia virus.

Eleven leukemic rats (3 Sprague-Dawley, and 8 Osborne-Mendel) about 2½ to 4 months old, and 12 normal, healthy control rats (8 Sprague-Dawley and 4 Osborne-Mendel) about 2 to 9 months old, were used as donors for the preparation of ultrathin sections. The rats were sacrificed by chloroform inhalation. Fragments of bone marrow, spleen, mesenteric, peripheral lymph nodes and mediastinal tumor were removed promptly and sliced into small pieces in cold phosphate buffered 2% osmic acid. After fixation for 2 hours in the cold (10°C), the tissues were brought through a series of graded

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alcohols at 15-minute intervals. After absolute alcohol the tissues were brought through a 1:1 mixture of absolute alcohol and n-butyl methacrylate (1 hour), then through 2 changes of the methacrylate (1 hour each), put into prepolymerized monomer, and polymerized overnight at 56°C in the monomer with 0.5% benzoyl peroxide. Sections were cut with a glass knife on a Porter-Blum ultramicrotome, stained with a saturated solution of uranyl acetate(7), coated with a thin film of carbon, and examined in an RCA EMU 3E electron microscope. Original magnifications of the electron micrographs varied from 2,700 to 30,000 diameters, which then were further enlarged photographically.

Results. In ultrathin sections of all 11 leukemic rats, characteristic spherical particles were found in leukemic lymph nodes, in mediastinal tumor, spleen, and also in the bone marrow. These particles could be divided into 2 main groups: those with an electron-dense nucleoid (average diameter value: 630 Å) centrally or excentrically placed, surrounded by one or 2 membranes with an average diameter value of 990 Å for the outer membrane; and those with an electron-lucent center (with an average diameter value of 490 Å), surrounded by 2 or 3 membranes (with an average diameter value of 840 Å for the outer membrane). The particles with an electron-dense center are described as virus particles, and those with an electron-lucent center are described as vesicular or doughnut-type particles. This nomenclature is for the purpose of convenience only; the doughnut-type particles may represent a stage in development of the virus. In addition to these 2 types of particles, structures were found in the megakaryocytes of both spleen and bone marrow which could be described as cylindrical or tubular. The diameter of the outer, intermediate, and inner membranes, surrounding these structures, corresponds with the diameter of the various membranes forming the vesicular or doughnut-type particles.

In sections of *bone marrow* of leukemic rats, both types of particles were found in the megakaryocytes. The proportion of the two types of particles may vary from one megakaryocyte to another. In megakaryocytes the virus particles may predominate, while in other cells the doughnut-type particles may be more numerous. Both types of particles were found in the system of channels, the outer membrane of doughnut-type particles frequently forming a continuity with the membranes lining the channels. The two types of particles as well as the cylindrical forms were frequently found within the specific granules of megakaryocytes (Fig. 1, 2). In addition, a phenomenon frequently observed was what could be described as invagination or budding of the membrane surrounding the specific granules (Fig. 1). In some cases, 3 membranes, similar to those found in vesicular particles, could be observed in the budding membrane of the specific granules (Fig. 1a). Occasionally the outer membrane of the cylindrical structures appeared to form a continuity with the membrane surrounding the specific granule or vacuoles in which they were found (Fig. 2). Frequently, the cylindrical forms were observed within the cytoplasm of megakaryocytes, apparently unconnected with either the specific granules or membranes of the channels. Occasionally, doughnut-type particles, found within the channels, specific granules, or the cytoplasm itself, showed a continuity of their outer membranes (Fig. 2). Infrequently, doughnut-type particles, of smaller diameter than that of similar type particles encountered elsewhere in megakaryocytes were found lying free in the cytoplasm of these cells. No other changes were found in the megakaryocytes of the bone marrow of leukemic rats, except for changes in density and distribution of osmiophilic material within the specific granules. Occasionally it was difficult to differentiate these granules from structures which could be interpreted as altered small mitochondria. No changes could be observed in the Golgi apparatus, but the occasional strands of ergastoplasm appeared to differ by the number of ribonucleoprotein particles aggregated along the membranes of ergastoplasm.

In sections of *spleen* of leukemic rats, megakaryocytes presented changes identical to those observed in megakaryocytes of the bone marrow. The characteristic virus particles were found in the intercellular spaces,

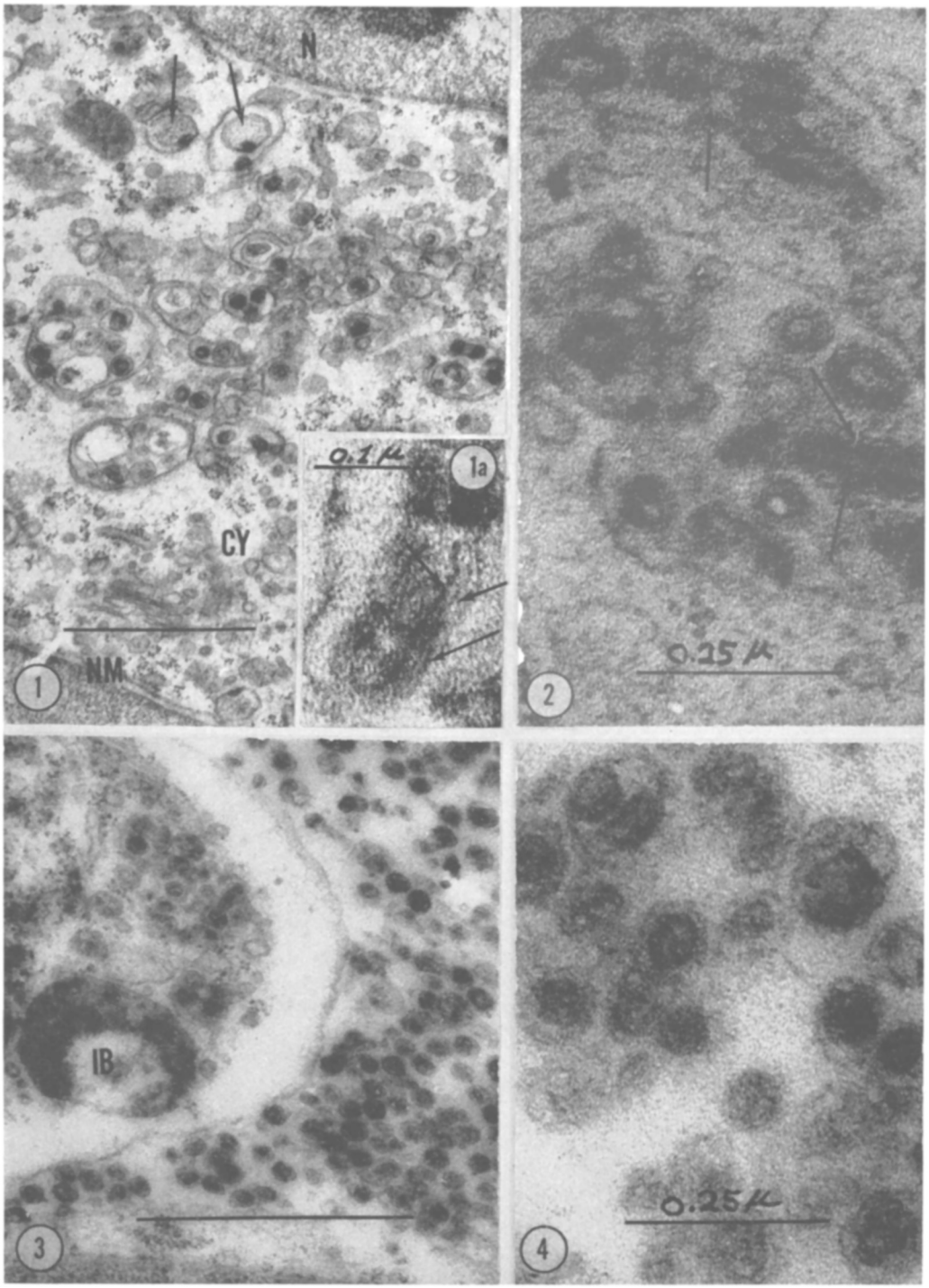

FIG. 1. Electron micrograph of section of bone marrow from rat with leukemia induced by passage-A-mouse-lymphoid leukemia virus. In the cytoplasm (CY) virus particles present within specific granules and vacuoles. Some specific granules show budding of membranes (arrows). Nucleus (N) and nuclear membrane (NM). Magnification 28,000 ×.

FIG. 1a. Example of invagination or budding of membrane in a specific granule of megakaryocyte. Arrows indicate 3 membranes. Magnification 195,000 ×.

FIG. 2. Part of cytoplasm of megakaryocyte from bone marrow of a leukemic rat. Vesicular particles, some showing continuity of outer membranes (arrow) and a cylindrical form (arrows) present. Magnification 120,000 ×.

FIG. 3. Part of the cytoplasm of degenerating cells from thymus gland of a leukemic rat. Virus particles and cytoplasmic inclusion (IB) present. Magnification 40,000 ×.

FIG. 4. Appearance of extracellular particles in mesenteric lymph node of a leukemic rat. Magnification 120,000 ×.

with occasional vesicular type particles present. The virus particles were found in close proximity to the plasma membrane of cells which could be described as lymphoblasts. Very occasionally, the plasma membrane of these cells was found to form protrusions with changes which could be described as the formation of doughnut-type particles. Among the mitochondria in comparatively small number, dense osmiophilic inclusions were found varying in size, shape, and density. In these inclusions were structures which could be interpreted as various stages of virus particle formation.

In sections of the affected peripheral and mesenteric *lymph nodes* and of *mediastinal tumors,* the cells, presumably lymphoblasts, revealed a picture the same as that described for similar cells in the spleen of leukemic rats. Both extracellular virus particles (Fig. 3, 4) and osmiophilic inclusions (Fig. 3) were frequently encountered.

Measurements of the size of virus particles and the vesicular or doughnut-type particles in the various organs of leukemic rats are recorded in Table I; average diameter of the virus particles was about 990 Å.

Twelve normal healthy rats varying in age from 2-9 months served as *control animals.* Ultrathin sections of bone marrow, spleen, peripheral and mesenteric lymph nodes, and thymus of these rats were examined. Over 30 sections of each of the organs were examined, but none revealed the presence of either virus particles or doughnut-type particles or other changes which were observed in sections of leukemic animals.

Discussion. It is of interest that virus particles could be found without difficulty on electron microscopic examination in leukemic organs of all 11 leukemic rats examined. These particles are essentially similar to those found in mice with virus-induced leukemia (4,5,6,8). Rather unexpected was the observation of frequent presence of such particles in megakaryocytes, that is, in cells which do not appear to participate directly in the leukemic process. Dalton and his coworkers have recently also observed mouse leukemia virus particles in megakaryocytes in spleen and bone marrow of affected animals(8). The possible significance of this observation requires further study.

The presence of cylindrical structures in megakaryocytes of bone marrow and spleen of leukemic rats may be of interest. The possible relationship of the cylindrical or tubular structures to the virus particles is now being investigated. Dalton and his associates(8) did not observe cylindrical forms in ultrathin sections of organs of mice with leukemia induced by passage A virus (Gross). However, they noticed similar cylindrical forms in organs of leukemic mice inoculated with the

TABLE I. Particle Measurement in Rat Leukemia Size in Angstroms.

Organs	Particle type	Outer diameter		Intermediate membrane		Nucleoid or inner membrane		No. particles measured
		Range	Avg	Range	Avg	Range	Avg	
Bone marrow	Virus	880–1160	990	—	—	500–740	650	83
	Vesicular	700–1000	810	500–600	580	390–570	490	33
Lymph nodes	Virus	950–1100	1040	—	—	510–660	640	80
	Vesicular	—	—	—	—	—	—	—
Thymus	Virus	750–1110	990	—	—	530–670	630	79
	Vesicular	780– 800	790	—	—	340–500	430	4
Spleen	Virus	900–1000	940	—	—	540–710	610	16
	Vesicular	880– 910	900	—	—	490–540	530	4

leukemia virus isolated by Moloney(9). We have observed cylindrical structures in megakaryocytes of bone marrow and spleen of both rats and mice with passage A virus-induced leukemia.

The leukemic virus isolated by Moloney (9) may not be different from that originally (1) recovered from spontaneous mouse leukemia by Gross(10). In our study, the size of passage A (Gross) virus particles, based on measurement of 270 particles, was 990 Å, which is about the same as that reported by Dalton(8) for the leukemic virus isolated by Moloney.

Summary. 1. Ultrathin sections of bone marrow, spleen, lymph nodes, and mediastinal tumors from 11 rats with leukemia induced by mouse leukemia virus (Gross), were examined in the electron microscope. 2. In organs of all 11 leukemic rats, 2 types of particles could be observed. One type, described as virus particles (990 Å average diameter), with an electron-dense center (630 Å average diameter), surrounded by a less dense zone, and limited by another membrane, not always clearly defined. 3. The other type, described as vesicular or doughnut-type particles, with an electron-lucent center, surrounded by an inner membrane or ring staining strongly with uranyl acetate (490 Å average diameter) and an outer ring or membrane (840 Å) reacting less with the uranyl acetate stain. Frequently, but not always, another membrane (580 Å diameter) could be seen between the inner and outer membranes. 4. Both types of particles were found in megakaryocytes of bone marrow and spleen. 5. Virus particles were also observed in intercellular spaces of lymph nodes and mediastinal tumors, and occasionally within cytoplasmic inclusions of cells present in these tissues. 6. Characteristic cylindrical structures were observed in megakaryocytes of bone marrow and spleen of leukemic rats. 7. Neither virus nor doughnut-type particles could be found in either bone marrow, spleen, thymus or lymph nodes of 12 normal rats.

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Effect of Ethionine in Castrated Male Rats, with and without Testosterone.* (27565)

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The methionine analogue, ethionine, has been shown to induce fatty livers in female but not in male rats(1,2). It has been demonstrated that ethionine inhibits incorporation of methionine and glycine into liver protein(3) and inhibits synthesis of liver enzymes(4-9) in mature female or immature male rats. Farber and Corban(10) reported that ethionine inhibited protein synthesis in the liver of female but not of male rats and suggested that the sex difference in the effects of ethionine was mediated by androgens. We have observed that ethionine is a more effective tumor-growth inhibitor, and is more toxic, in female than in male rats(11), and

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