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Structure and Leukemogenic Activity of a Murine Leukemia Virus

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Purified Friend viruses obtained from chronically infected tissue cultures were studied under the electron microscope in an effort to correlate the fine structure of the particles to their leukemogenic activity under varied experimental conditions, i.e., temperature treatments and exposure to Tween 80, amyl acetate, or ether. It was observed that an intact viral envelope was a prerequisite to leukemogenic activity as tested by intraperitoneal inoculation of newborn mice. It was also noted that the percentage of C particles was not increased after heating for 1 hr at 45 C (treatment which, however, completely inactivated the viruses). Digestion with ribonuclease indicated the presence of ribonucleic acid within the nucleoids of "enveloped A particles," which shows that these are not immature particles. The significance of the simultaneous presence of "enveloped A" and C particles is discussed.

The viruses which induce murine leukemia (15, 17, 18, 22, 26) are morphologically similar. Under an electron microscope, they appear as spherical particles (about 90 to 100 mμ in diameter) present in the extracellular spaces or within cytoplasmic vacuoles (3, 7). They are formed by a budding process during which an envelope is acquired from the plasma membrane of the infected cell (8).

A leukemia, serially transmissible to adult Swiss or DBA/2 mice by cell-free filtrates, was originally described in 1957 (15). In these leukemic animals, viremia appears on the 4th day after inoculation (10). The viruses found in the plasma of leukemic mice are not of a uniform morphological type. As previously shown (6), the plasma particles are predominantly type C particles [according to Bernhard's nomenclature (3)] with a very low percentage of "enveloped type A" particles. Conversely, the majority of the virus found in the supernatant fluid of a line of cultured leukemic cells (16) are "enveloped type A" particles (10; E. de Harven, *in press*). [The "enveloped type A" particles are referred to as type A particles in this study. This terminology is in complete agreement with the definition of type A particles originally proposed by Bernhard et al. (3). The definition of type A particles proposed by these authors clearly stated that these particles "se trouvent le plus souvent attachés à la surface cellulaire ou dispersés dans les espaces *INTERCELLULAIRES*" (which is "dispersed in the *EXTRACELLULAR* spaces"). This definition was illustrated by Fig. 3 and 5, which leave no doubt of the fact that type A particles can be extracellular in murine leukemias according to Bernhard's original classification.]

Although there is no direct evidence that A particles transform into C particles, the consensus is that A and C particles represent sequential evolutionary stages of the same agent.

Our present studies were designed to correlate the leukemogenic activity and the fine structure of the Friend virus. Although the relationship of A and C particles remains unclear (E. de Harven, *in press*), it was found that the integrity of the viral envelope is a prerequisite for leukemogenic activity of preparations inoculated by the intraperitoneal route and that the viruses can be completely heat-inactivated without a recognizable change in their fine structure.

MATERIALS AND METHODS

Virus. In most of our experiments, the virus was obtained from a tissue culture line of leukemic cells designated C-1A (16). The cells were grown in suspension in Eagle's basal medium and Earle's balanced salt solution supplemented with 15% fetal calf serum. Electron microscopy of these cells revealed a continuous production of viruses (10).

Purification of the virus was performed with a slight modification of a previously described method (6, 10). Tissue culture supernatant fluid was filtered by aspiration through a 0.45-μ silver Flotronics membrane (Selas Flotronics, Spring House, Pa) and was then centrifuged at 30,000 × *g* for 1 hr in a Spinco model L ultracentrifuge rotor 30. The pellet resulting from each

30-ml sample was homogenized in Ringer's solution for injection.

In those instances where virus was obtained directly from DBA/2 mice, filtrates of 20% homogenates of leukemic spleens were prepared as previously described (15), and 0.2 ml was inoculated intraperitoneally into each mouse. Seven days after inoculation, plasma from the infected mice was filtered sequentially through 0.45- and 0.2-μ silver Flotronics membranes and centrifuged at 35,000 $\times$ g for 2 hr. The resulting pellets were submitted to electron microscope analysis by the thin-sectioning method.

Mice. Swiss mice from Taconic Farms (Germantown, N.Y.) were bred in our laboratory, and mice from litters 1 to 5 days old were injected intraperitoneally with 0.2 ml of purified viral material (filtrates, or the resuspended pellets prepared from them). DBA/2 mice from Jackson Memorial Laboratory (Bar Harbor, Me.) were occasionally used, as noted in the text.

Temperature treatment. Filtrates of supernatant fluid were exposed to room temperature for 24 hr, 37 C for 6 hr, 37 C for 24 hr, 45 C for 1 hr, and 60 C for 30 min. They were then submitted to bioassay as such. Parts of the heated filtrates were centrifuged to obtain pellets for electron microscope study.

Solvent treatment with Tween 80. Filtrates of the supernatant fluid were mixed with 0.5 mg/ml of Tween 80 (polyethyleneoxide-sorbitanmonooleate, Amend Drug and Chemical Co., New York, N.Y.), stored in ice for 30 min, and shaken in a Cyclomixer every 5 min, throughout this period. The suspension was centrifuged at 44,000 $\times$ g for 1 hr in a type 30 rotor of a Spinco model L ultracentrifuge. Tween 80-treated filtrate was injected into newborn Swiss mice, and pellets were studied under the electron microscope.

Solvent treatment with amyl acetate. Amyl Acetate (Mallinkrodt Chemical Works, New York, N.Y.) was added (10 to 20%) to filtrates of the tissue culture supernatant fluid. The emulsion was stored at 4 C for 1 hr and was shaken vigorously every 10 min in a Cyclomixer. The aqueous phase was then collected, and nitrogen was blown near its surface for about 40 min. The fluid was centrifuged at 44,000 $\times$ g as described above. Filtrates were used for bioassay and pellets were used for electron microscope study.

Solvent treatment with ether. Ether (Merck, anesthesia grade), 10 to 20%, was added to the filtrate of tissue culture supernatant fluid at 4 C for 30 min and was vigorously shaken every 5 min. The resulting emulsion was centrifuged at low speed (500 $\times$ g), and the aqueous phase was collected. The residual ether was evaporated by blowing nitrogen near the surface for about 40 min. Samples of the aqueous phase were bioassayed and centrifuged for electron microscopy.

To obtain a better evaluation of the results obtained with tissue culture material, identical experiments with the plasma of leukemic DBA/2 mice were also performed. Both the ether-treated and control filtrates were injected intraperitoneally into DBA/2 mice. Pellets were studied under an electron microscope.

Treatment with ribonuclease. The supernatant fluid was filtered through a 0.45-μ membrane and treated with ether prior to ribonuclease exposure (12). After evaporation of the ether, 1 mg of ribonuclease per ml (Worthington Biochemical Corp., Freehold, N.J.) was added, and the mixture was kept overnight at 37 C (pH 7). After this treatment, the fluid was centrifuged at 54,500 $\times$ g for 1 hr, and the pellets were studied under the electron microscope.

Electron microscopy. To determine a suitable method for fixation of the pelleted viruses, the preservation of the particles was compared after the following treatments: (i) fixation with OsO_4 1% (24), in pH 7.2 phosphate buffer (21) for 90 min, followed by ethyl alcohol dehydration and Epon embedding (20); (ii) fixation with 1% OsO_4 in buffer for 60 min, followed by rinsing in Ringer's solution, post-treatment with 0.5% uranyl acetate (14), dehydration, and embedding in Epon; (iii) fixation with 1% glutaraldehyde (28) in phosphate buffer for 15 min, washing in buffer, and postfixation in 1% OsO_4 for 30 min, post-treatment in 0.5% uranyl acetate for 30 min, and then dehydration with ethyl alcohol and embedding in Epon.

The procedure which was adopted as routine was the double fixation with glutaraldehyde and osmium tetroxide and post-treatment with uranyl acetate. Sections were prepared with a Porter-Blum or LKB microtome, stained in 3% uranyl acetate (30) or lead citrate (27), carbon-coated (5), and viewed under a Siemens Elmiskop I electron microscope. Counting procedures to estimate the ratio of A particles to C particles (A/C ratio) were performed on an average of 200 virus particles per experiment through this study.

RESULTS

Ultrastructure of the virus. A good preservation of the viruses was obtained after fixation with glutaraldehyde followed by osmium tetroxide and uranyl acetate. The unit membrane structure of the viral envelope and the structure of the viral capsid were clearly seen; the contrast of the whole particles was satisfactory (Fig. 1). After fixation with OsO_4 alone, the fine structure of the envelope was not discernible (Fig. 2), except when the osmium fixation was followed by uranyl acetate treatment (Fig. 3).

These studies were started with the 67th serial passage of tissue culture line C-1A, which contained a high proportion of A particles. As the passage number increased, the number of A particles decreased (Table 1). Since there did not appear to be a correlation between infectivity and the A/C ratio, the significance of this observation remains unexplained.

Bioassay. To demonstrate the leukemogenic activity of tissue-cultured virus, newborn mice, 1 to 5 days old, which are more sensitive to small amounts of virus, were inoculated with filtrates or resuspended pellets prepared from filtrates.

The results compared in Table 2 show that activity, although variable, was present in both preparations. Since this activity was too low to

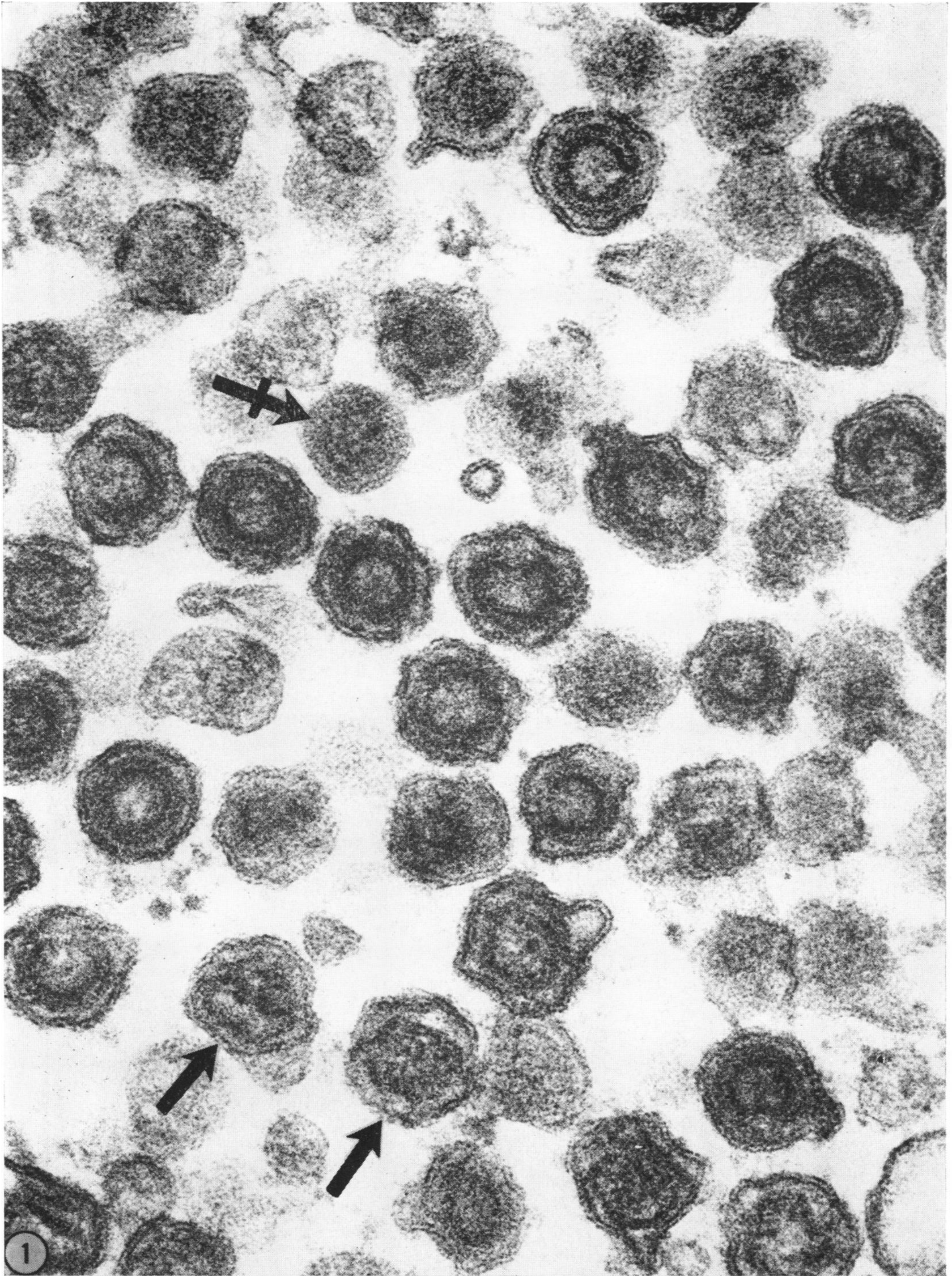


FIG. 1. *Thin section of a pellet of purified Friend virus from the supernatant fluid of C-1A cells. The pellet was fixed in 1% glutaraldehyde followed by osmium tetroxide and uranyl acetate. Most of the particles are "enveloped" type A; some (arrows) are of type C, and some are smaller and less contrasted because they are too far from the plane of sectioning. These last particles (indicated by crossed arrow) should not be regarded as transition forms between type A and C. The viral envelopes have a well-preserved unit membrane structure. The nucleocapsids of the enveloped type A particles have the typical doughnut shape with electron-lucent center. The section was stained with urynal acetate and with lead citrate.* × *188,000.*

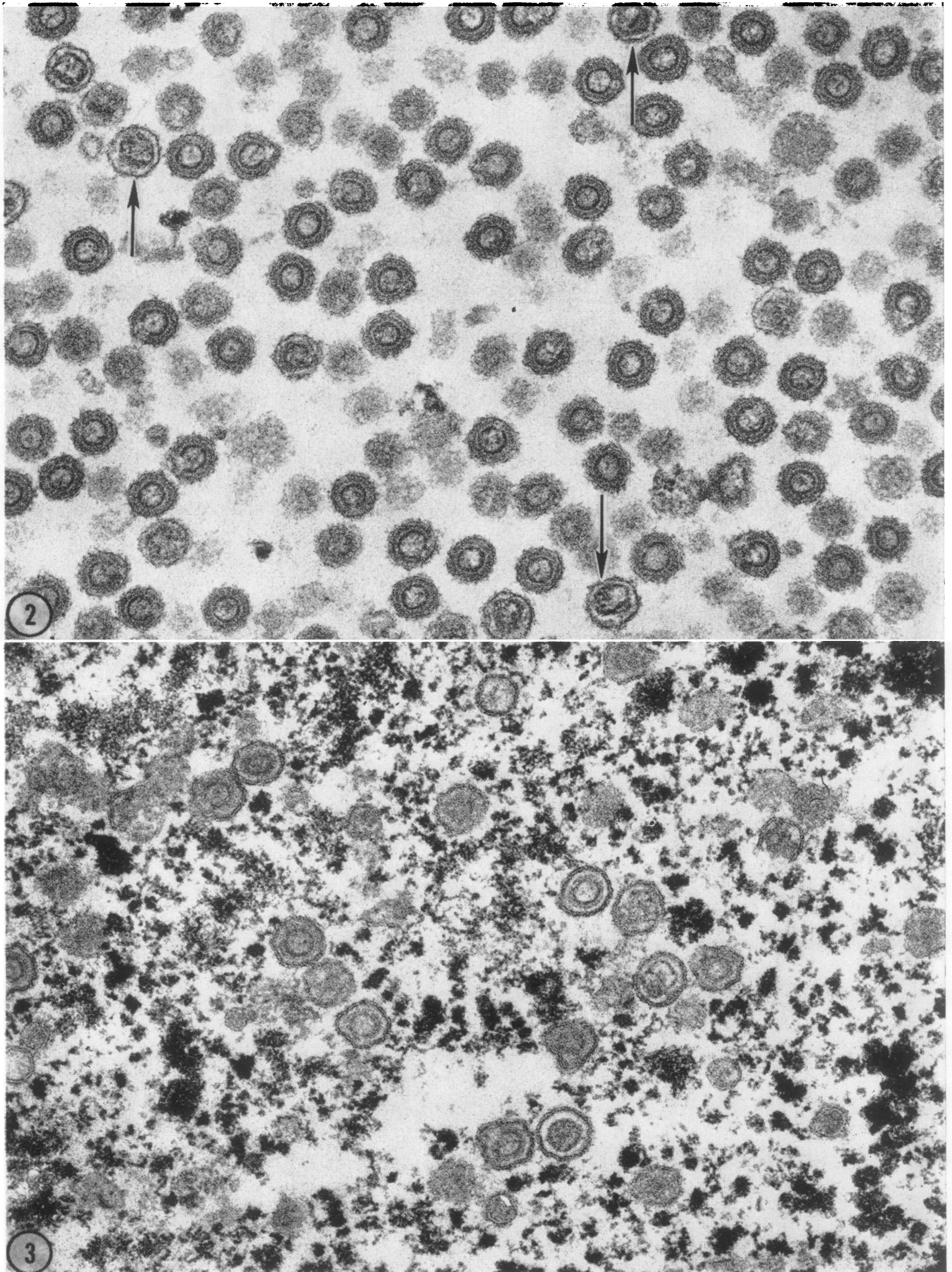


FIG. 2. *Same material as in Fig. 1. The virus pellet was fixed only in osmium tetroxide. The preservation of the viral envelopes is very poor. The majority of the particles are of type A; five are of type C (arrows). Staining with uranyl acetate and lead hydroxide.* × *70,000.*

FIG. 3. *Similar material as in Fig. 1 and 2. The viral pellet has been fixed in osmium tetroxide followed by uranyl acetate. The majority of the particles are of type A; their envelopes are as well preserved as in Fig. 1, indicating that the crucial step in the stabilization of the viral envelopes is the uranyl acetate treatment before starting the ethyl alcohol dehydration of the sample. Staining with uranyl acetate and lead hydroxide.* × *70,000.*

TABLE 1. *Comparison of the number of serial tissue culture cell passages, the ratio of A particles to C particles, and the leukemogenic activity*

Cell passage no.	Particle ratio (% A/%C)	Leukemogenic activity (%)[a]
65	99/1	—[b]
70	92/8	—[b]
78	99/1	60
83	83/17	58
93	35/65	80
128	39/61	—[b]
148	49/51	50
159	22/78	33

[a] A minimum of 1 litter (~10 animals) was used for each bioassay.
[b] Not done.

TABLE 2. *Leukemogenic activity of viruses purified from the murine C-1A tissue culture line in newborn Swiss mice*

Age of mice (days)	Percentage of leukemia in mice injected intraperitoneally with viruses from			
	Pellet	Particle ratio (% A/%C)	Filtrate	Particle ratio (%A/% C)
1	22.2	—[a]	28.5	—[a]
2	80	40/60	44	98/2
3	58	83/17	89	97/3
4	22	98/2	55.5	96/4
5	46.5	99/1	20	—[a]

[a] Not done.

permit estimation of the titers, the percentage of the inoculated animals which developed leukemia is given.

Temperature treatment. To further correlate the fine structure of the viruses with their leukemogenic activity, the effect of temperature was determined.

When heated at 45 C for 1 hr, particles were completely inactivated but still retained an unaltered morphology; the A/C ratio remained the same as in the control unheated samples. Only at higher temperatures, such as 60 C for 30 min, was a visible alteration in the morphology of the particles obtained (Fig. 4). The results of heat treatment are summarized in Table 3.

Solvent treatment. Amyl acetate, as well as ether, completely abolished the leukemogenic activity (Table 4) and uncoated the particles by solubilizing the viral envelopes, as shown in Fig. 5 where "naked" nucleocapsids can be seen.

After exposure to Tween 80, the leukemogenic activity was also lost; however, the viral envelopes, although obviously damaged, were still recognizable and appeared as a slight halo around the particles (Fig. 6).

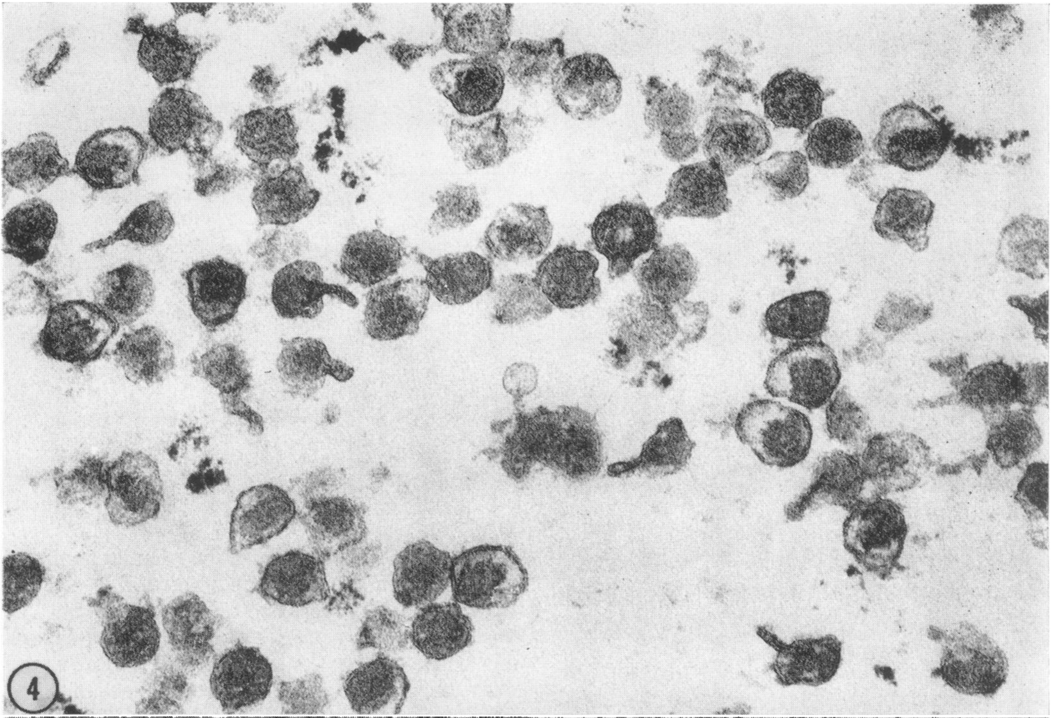
There was always a decrease in the number of particles recovered after solvent treatment. This may be due to the fact that the solvents completely destroy the majority of viruses, leaving only a few particles as uncoated nucleocapsids. The possibility that the treated viruses have altered properties and hence do not sediment under the same centrifugation conditions should also be considered.

To confirm these results, a similar experiment was carried out on viruses harvested from the plasma of leukemic mice, where the number of viral particles present appeared to be greater than in tissue culture preparations (Fig. 7). In plasma filtrates treated with ether, the number of uncoated virus particles was higher than in similarly treated tissue culture preparations (Fig. 8), but no leukemogenic activity was evident in DBA/2 mice inoculated with this material. These experiments suggest that the loss of activity is due to the destruction of the viral envelope rather than to the decrease in the number of viral particles after solvent treatment (Table 5). However, it is hoped that a reliable counting technique will soon be available to confirm this point.

Ribonuclease treatment. The purified tissue culture virus, treated with ether to remove the viral envelope and exposed to ribonuclease, showed a significant decrease in the electron density of the nucleoids of type A particles (Fig. 9), a finding which confirms observations previously made by Padget et al. (23) and de Thé et al. (12). Therefore, it appears that type A particles contain ribonucleic acid within their nucleoids and can hardly be regarded as immature particles as was suggested previously (1, 4, 12). [The term "immature" is, however, used by several authors (1) "in reference only to time after formation and is not meant to imply anything in regard to activity."]

DISCUSSION

The presence of two morphologically distinct types of particles in the plasma of leukemic mice remains a perplexing problem. It has been proposed that type A particles are immature and type C particles are complete infective particles (4,12). It was previously thought that the high degree of purity demonstrated by morphological studies, and the relatively low infectious titer of leukemic mice plasma pellets, might be due to the large percentage (98%) of C particles (6). Indeed, it had been remarked that the decrease in titer observed in tissue culture preparations of Rauscher leukemia virus may be due to heat inactivation (25). Therefore, the possibility that C particles are heat-inactivated virions had to be



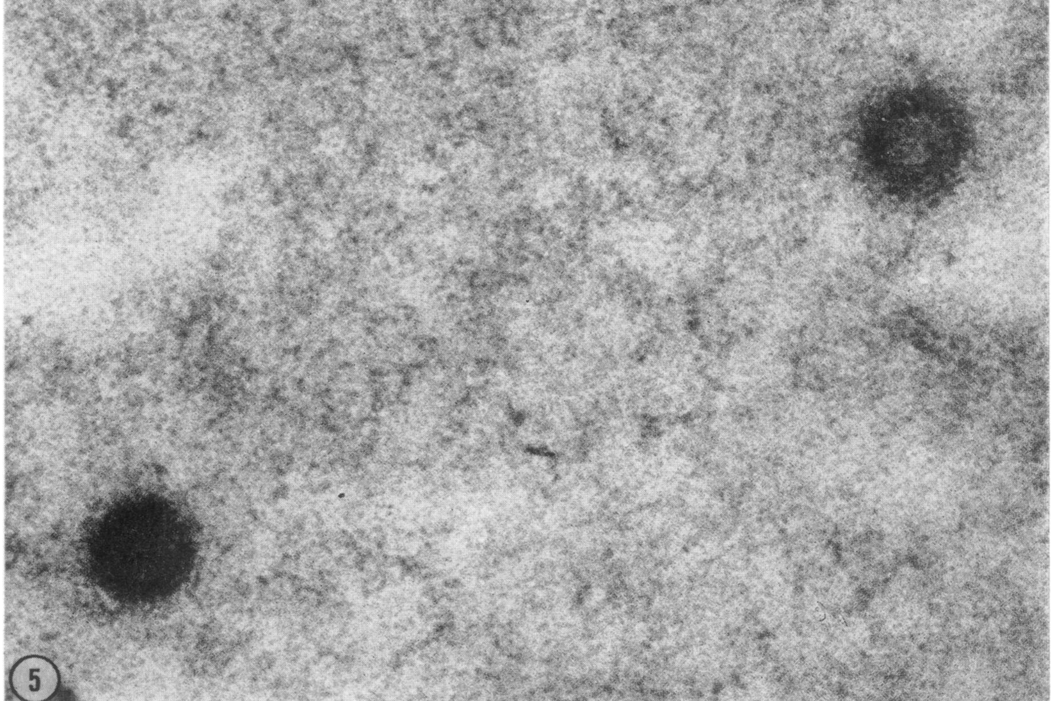


FIG. 4. *Friend virus purified from the supernatant fluid of C-1A cell cultures. The fixation was identical to that illustrated in Fig. 1. However, the material was heated at 60 C for 30 min before fixation. The viral envelopes are well preserved but all the nucleocapsids appear condensed and homogenized. Staining with uranyl acetate and lead hydroxide.* × *70,000.*

FIG. 5. *Purified Friend viruses from the fluid of C-1A cultures treated with ether prior to fixation. Rare "naked" nucleocapsids were found in the pellet. Two are illustrated in this picture. Note that viral envelopes have completely disappeared and the nucleocapsids are recognizable as those of type A particles. Staining with uranyl acetate and lead citrate.* × *188,000.*

considered. In this hypothesis, the low biological activity of the viruses obtained from the plasma could be attributed to the low percentage of A particles present.

However, it has not been possible to retain this hypothesis because the A/C ratio of our preparations was not shifted in the direction of C particles by heat inactivation. Moreover, the leukemogenic activity of the virus does not correlate with high or low A/C ratio.

Conversely, we might hypothesize that the low leukemogenic activity of our tissue culture-grown virus is due to the large percentage of A particles. This hypothesis should also be rejected, because in tissue culture preparations of Rauscher virus where type C particles predominated, the leukemogenic activity has also been found to be very low (2, 31). It seems, therefore, that very low leukemogenic activity is related to the fact that the virus is grown in vitro, and that the precise rela-

TABLE 3. *Correlation between structure and leukemogenic activity of viruses purified from the murine C-1A tissue culture: effects of various temperatures*

No. of experiments	Temp (C)	Time at indicated temp (hr)	Leukemogenic activity (%)	Presence of viral envelope	Particle ratio (% A/% C)
4	24[a]	24	50	+	60/40
1	37	6	60	+	50/50
2	37	24	50	+	30/70
2	45	0.5	30	+	40/60
2	45	1	0	+	30/70
4	60	1	0	+	Damaged particles

[a] Room temperature.

TABLE 4. *Correlation between structure and leukemogenic activity of viruses purified from the murine C-1A tissue culture: effects of various solvents*

No. of experiments	Solvent	Leukemogenic activity (%)	Presence of viral envelope	Particle ratio (%A/%C)
5	Amyl acetate	0	−	50/50
4	Ether	0	−	60/40
3	Tween 80	0	−	30/70

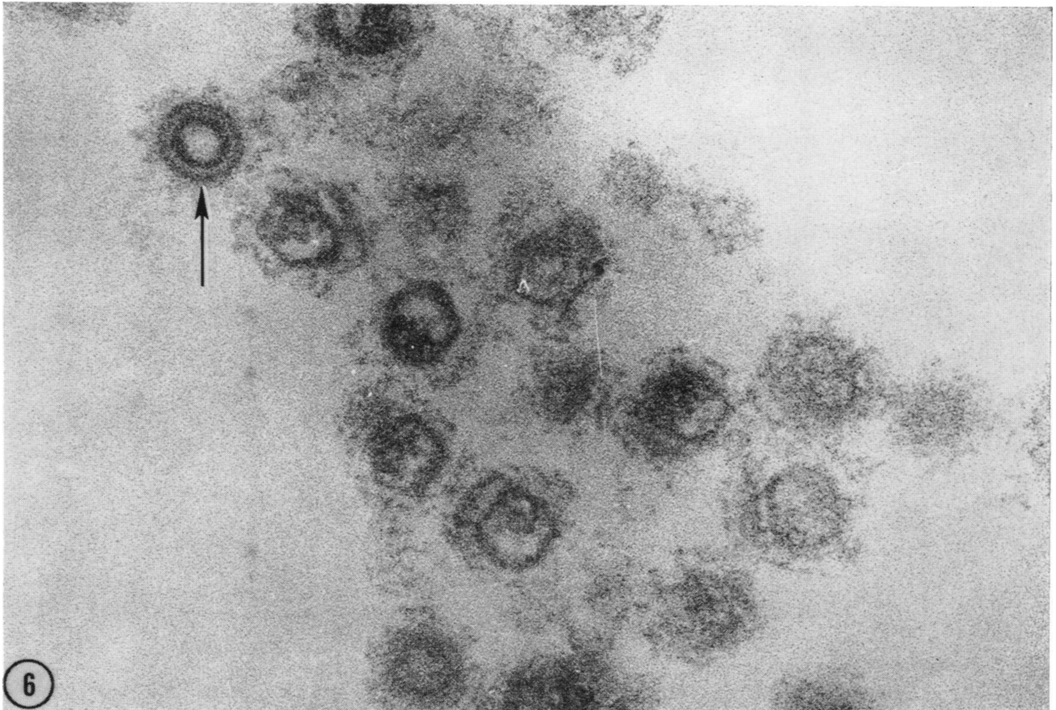


FIG. 6. *Same material as in Fig. 5 treated with Tween 80 prior to fixation. Remnants of the viral envelopes can be seen around the nucleocapsids, which in this case resemble those of type C particles. Only one type A nucleocapsid is seen in this field (arrow). Staining with uranyl acetate and lead hydroxide.* × *140,000.*

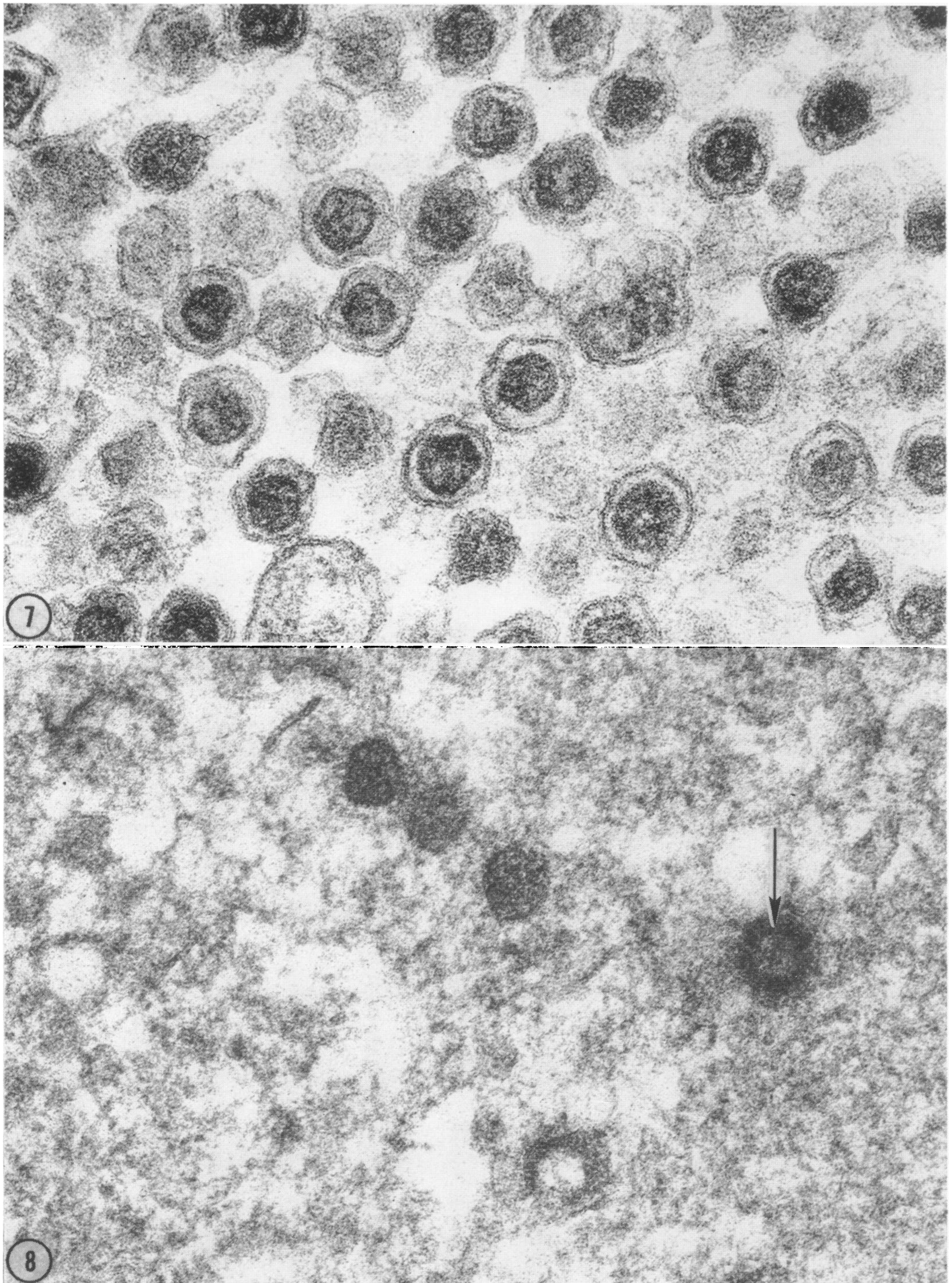


FIG. 7. *Friend virus purified from the plasma of leukemic DBA/2 mice 7 days after inoculation. Most of the particles are of type C. Staining with uranyl acetate and lead citrate.* × *105,000.*

FIG. 8. *Same material as in Fig. 7. The purified viruses have been, however, shaken in presence of 20% ether for 30 min prior to fixation. Rare nucleocapsids are found in the pellet. Most of them correspond to C particles. However, one type A nucleocapsid is seen in this field (arrow). Staining with uranyl acetate and lead citrate.* × *140,000.*

TABLE 5. *Correlation between structure and leukemogenic activity of virus purified from the plasma of DBA/2 leukemic mice*[a]

Plasma	Virus dilution					Viral envelope	Particle ratio (%A/%C)
	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}		
Ether-treated.....	0/5[b]	0/5	0/5	0/5	0/5	Absent	38/62
Control, untreated..	5/5	5/5	5/5	5/5	5/5	Present	5/95

[a] Determined 7 days after inoculation of virus.
[b] Number dead/number injected.

tionship between morphology and leukemogenic activity of the particles is yet to be defined.

Procedures which lead to the destruction of the viral envelopes bring about a complete loss in leukemogenic activity. It appears, therefore, that the presence of the viral envelope is a prerequisite for biological activity as tested by the intraperitoneal route, possibly because the envelope protects the viral nucleic acid from the action of ribonuclease present in the environment.

This interpretation receives some support from the fact that ether-treated Rauscher virus, with no visible envelope, has a demonstrable leukemogenic activity when inoculated intracranially but not intraperitoneally (29). Inactivation of the Rauscher leukemia virus by various physical and chemical agents was recently studied by Levy et al. (19). Their results of assays with heat-inactivated material are in general agreement with our findings. De Thé (11) recently obtained excellent preparations of "naked nucleocapsids" of Rauscher virus treated with digitonin, in an electron microscope study not correlated with biological observations.

Only type A particles bud from the surface of leukemic cells. Type C particles are never seen in the process of budding. Both A and C particles, however, are found in the extracellular spaces. This raises the question of the origin of the C particles. Two possibilities should be considered: (i) A and C particles are completely independent agents, and (ii) A particles are the precursors of C particles.

Against the first hypothesis is the fact that both particles are always observed in association (in vivo and in vitro). Moreover, type C particles are never intimately associated with the cells. They are sometimes seen within cytoplasmic vacuoles which can be regarded as the equivalent of extracellular spaces, or within phagocytic vacuoles, but they are never seen in intimate association with the infected cells, in a way which could suggest their independent origin.

In favor of the second possibility, i.e., that C particles represent transformed A particles, a basic ultrastructural resemblance between them should be stressed. Both types of particles are of approximately the same size, and they both have a well-defined envelope and a central nucleocapsid. However, in the case of type A particles, the nucleocapsid appears as a well-defined double shell with an electron-lucent center, whereas only an indistinct dense material forms the nucleoid of the type C particles. When observing the particles under an electron microscope, one might have the impression that transition forms can be observed between type A and type C particles. This is, however, not the case. As illustrated in Fig. 1, type A

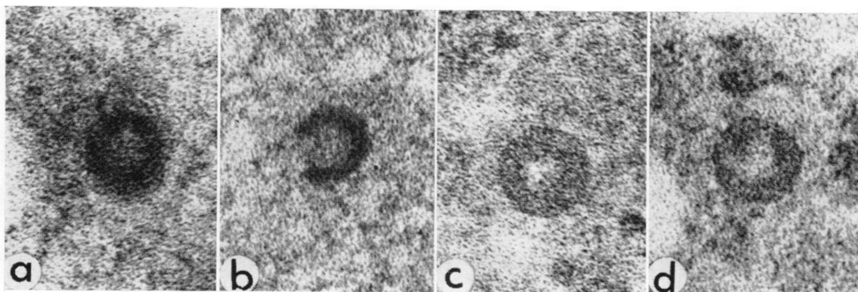


FIG. 9. *Friend viruses purified from the supernatant fluid of C-1A cell cultures. The viruses were first treated with ether to remove the viral envelopes and then incubated overnight at 37 C with 1 mg of ribonuclease per ml at pH 7. A and B are controls kept overnight at 4 C with no enzyme; C and D were incubated in the presence of ribonuclease. The four nucleocapsids seen in Fig. 9 correspond to type A particles, as indicated by their electron-lucent centers. The enzyme-treated particles have a significantly decreased electron density. Maximal attention in the staining as well as in the photographic procedures has been given to make certain that differences in contrast observed here actually reflect a specific enzymatic digestion. Therefore, the micrographs strongly suggest that ribonucleic acid is associated with the nucleocapsids of type A particles. Staining with uranyl acetate and lead citrate.* × *147,000.*

particles show their typical electron-lucent center only if the plane of sectioning is "equatorial," i.e., showing the maximal diameter of the particles and showing the unit membrane structure of the viral envelope. If particles are tangentially cut, their electron-lucent center is no longer clearly seen and the diameter of the particles appears somewhat smaller. These tangential sections of the particles should not be regarded as transition forms between type A and type C particles.

A parallel situation exists in mouse mammary carcinoma, where transformation from A into B particles was recently observed by Dolowy (13). The author showed a series of transformations inside the cytoplasm, although the complete B particle with a dense excentric nucleoid only appears in the extracellular space.

Type A particles probably transform into C particles. However, the cause of the transformation is still unclear. It is not a simple matter of aging, because our experiment on heat treatment can also be regarded as an in vitro aging experiment, and it appears from Table 3 that after 24 hr in vitro the A/C ratio was still close to the normal values given in Table 1. Is the transformation from type A to type C a last step in maturation or the beginning of some ultrastructural degeneration?

It is most unlikely that a major step in virus maturation takes place outside the cell. Enveloped type A particles are infective, as suggested by bioassay (Table 1), and they contain ribonucleic acid, as demonstrated by ribonuclease digestion; therefore, they can hardly be regarded as "immature." Type C particles might be considered as morphologically degenerated viruses still carrying leukemogenic activity. None of the presently available experimental data permits one to discard this hypothesis.

Finally, it appears that the in vivo transformation from type A to type C does not result from the immune response of the host because in chronically infected tissue culture systems (31) type C particles predominate (2).

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LITERATURE CITED

1. ANONYMOUS. 1966. Suggestions for the classification of oncogenic RNA viruses. J. Natl. Cancer Inst. **37**:355–397.
2. BARSKI, G., AND K. J. YOUN. 1966. Protective effect of specific immunization in Rauscher leukemia. Conference on Murine Leukemia. Natl. Cancer Inst. Monograph **22**:659–669.
3. BERNHARD, W., AND M. GUÉRIN. 1958. Présence de particules d'aspect virusal dans les tissus tumoraux de souris atteintes de leucémie spontanée. Compt. Rend. **247**:1802–1805.
4. DALTON, A. J., L. W. LAW, J. B. MOLONEY, AND R. A. MANAKER. 1961. An electron microscopic study of a series of murine lymphoid neoplasms. J. Natl. Cancer Inst. **27**:747–791.
5. DE HARVEN, E. 1958. A new technique for carbon films. J. Biophys. Biochem. Cytol. **4**:133–134.
6. DE HARVEN, E. 1965. Viremia in Friend murine leukemia: the electron microscope approach to the problem. Pathol. Biol. Semaine Hop. **13**: 125–134.
7. DE HARVEN, E., AND C. FRIEND. 1958. Electron microscope study of cell-free induced leukemia of the mouse: a preliminary report. J. Biophys. Biochem. Cytol. **4**:151–156.
8. DE HARVEN, E., AND C. FRIEND. 1960. Further electron microscope studies of a mouse leukemia induced by cell-free filtrates. J. Biophys. Biochem. Cytol. **7**:747–752.
9. DE HARVEN, E., AND C. FRIEND. 1964. Structure of virus particles partially purified from the blood of leukemic mice. Virology **23**:119–124.
10. DE HARVEN, E., AND C. FRIEND. 1965. Origin of the viremia in murine leukemia. Natl. Cancer Inst. Monograph **22**:125–134.
11. DE THÉ, G. 1967. Action of digitonin on murine leukemogenic virions. Compt. Rend. **D264**: 2347–2349.
12. DE THÉ, G., AND T. E. O'CONNOR. 1966. Structure of a murine leukemia virus after disruption with Tween-ether and comparison with two myxoviruses. Virology **28**:713–728.
13. DOLOWY, C. W. 1965. Morphology of the development of type B virus particles in a transplantable mammary tumor of ICR/Ha mice. J. Natl. Cancer Inst. **34**:815–825, 1965.
14. FARQUHAR, M. G., AND G. E. PALADE. 1965. Cell junctions on amphibian skin. J. Cell Biol. **26**: 263–291.
15. FRIEND, C. 1957. Cell free transmission in adult Swiss mice of a disease having the character of leukemia. J. Exptl. Med. **105**:307–318.
16. FRIEND, C., M. C. PATULEIA, AND E. DE HARVEN. 1965. Erythrocytic maturation in vitro of murine (Friend) virus induced leukemic cells. Natl. Cancer Inst. Monograph **22**:505–522.
17. GRAFFI, A. 1957. Chloroleukemia of mice. Ann. N.Y. Acad. Sci. **68**:540–558.
18. GROSS, L. 1954. Is leukemia caused by a trans-

missible virus? A working hypothesis. Blood **9:** 557–575.

19. LEVY, J. P., S. OPPENHEIM, P. CHENAILLE, D. SILVESTRE, A. FAVITIAN, AND M. BOIRON. 1967. Quantitative study of Rauscher virus inactivation by various physical and chemical agents J. Natl. Cancer Inst. **38:**553–565.
20. LUFT, J. H. 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. **9:**409–414.
21. MILLONIG, G. 1962. Further observations on a phosphate buffer for osmium solutions in fixation. Intern. Congr. Electron Microscopy, 5th, Philadelphia, p. 8.
22. MOLONEY, J. B. 1960. Properties of a leukemia virus. Natl. Cancer Inst. Monograph **4:**7–37.
23. PADGETT, F., V. KEARNS-PRESTON, H. VOELZ, AND A. S. LEVINE. 1966. Effects of ribonuclease and deoxyribonuclease on a murine leukemia virus (Rauscher). J. Natl. Cancer Inst. **36:**465–476.
24. PALADE, G. E. 1952. A study of fixation for electron microscopy. J. Exptl. Med. **95:**285–298.
25. PLUZNIK, D. H., AND L. SACHS, 1964. Quantitation of a murine leukemia virus with a spleen colony assay. J. Natl. Cancer Inst. **33:**535–546.
26. RAUSCHER, F. J. 1962. A virus induced disease of mice characterized by erythrocytopoiesis and lymphoid leukemia. J. Natl. Cancer Inst. **29:** 515–543.
27. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. **17:**208–212.
28. SABATINI, D. D., K. BENSCH, AND R. J. BARNETT. 1963. Cytochemistry and electron microscopy. J. Cell Biol. **17:**19–58.
29. SHIBLEY, G. P., F. E. DURR, G. SCHIDLOVSKY, B. S. WRIGHT, AND R. SCHMITTER. 1967. Leukemogenic activity of ether-extracted Rauscher leukemia virus. Science **156:**1610–1613.
30. WATSON, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. J. Biophys. Biochem. Cytol. **4:**475–478.
31. WRIGHT, B. S., AND J. C. LASFARGUES, 1965. Long term propagation of the Rauscher murine leukemia virus in tissue culture. J. Natl. Cancer Inst. **35:**319–327.