VIRUSES, NUCLEIC ACIDS, AND CANCER

A Collection of Papers Presented at the Seventeenth Annual Symposium on Fundamental Cancer Research, 1963

Published for
The University of Texas M. D. Anderson Hospital and Tumor Institute

Baltimore
The Williams and Wilkins Company
1963

Library of Congress Catalog Card No. 63-21957

Manufactured in the United States of America
by the Printing Division of The University of Texas

Dedicated to the Memory of
Francisco Duran-Reynals, M.D.
(1899–1958)

Acknowledgments

Grateful acknowledgment is made of the work of Dr. Leon Dmochowski, chairman of the 1963 symposium committee, and of the following committee members who worked with him: Mr. Joe E. Boyd, Jr., Miss Frances E. Goff, and Drs. Arthur Cole, Murray M. Copeland, Russell W. Cumley, Felix L. Haas, Clifton D. Howe, T. C. Hsu, Saul Kit, Robert J. Shalek, John A. Sykes, and H. Grant Taylor. Drs. W. Ray Bryan, Werner Henle, Albert B. Sabin, and Wendell M. Stanley have graciously served as members of the advisory committee for this symposium.

Co-sponsor of this symposium was The University of Texas Postgraduate School of Medicine. We gratefully acknowledge the assistance of the National Cancer Institute and of the American Cancer Society, Texas Division.

This symposium volume was edited and arranged for publication by the following members of the Publications Department: Russell W. Cumley, Joan McCay, Dorothy Aldridge, Sally Connelly, Judith Haroz, Julie Sorrell, and Wendelyn White.

The book was produced by Joan McCay.

The staff members of the Publications Department acknowledge with thanks the kind assistance on references given by Miss Elizabeth Runge, Medical Librarian at The University of Texas Medical Branch in Galveston, and Miss Virginia Parker, Librarian for the Texas Medical Center Library in Houston.

Invited Discussants

In addition to the speakers invited to present formal papers at the Symposium and to take part in the discussions, the following individuals were invited as discussants.

H. J. Bendixen, Statens Veterinaere Serumlaboratorium, Copenhagen, Denmark

A. J. Dalton, Division of Viral Oncology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

V. Defendi, The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania

M. L. Duran-Reynals, Laboratory of Cellular Physiology, Department of Surgery, Albert Einstein College of Medicine, Yeshiva University, New York, New York

B. E. Eddy, Section of Experimental Virology, Division of Biologic Standards, National Institutes of Health, Bethesda, Maryland

H. Febvre, Department of Cytobiology, Institut de Recherches Scientifiques sur le Cancer, Villejuif (Seine), France

C. Friend, Viral Oncology Section, Sloan-Kettering Institute for Cancer Research, New York, New York

S. Gard, Department of Virus Research, Karolinska Institutet, Stockholm, Sweden

J. T. Grace, Jr., Roswell Park Memorial Institute, Buffalo, New York

J. Huppert, Institut Gustave-Roussy, Villejuif (Seine), France, and Institut Pasteur, Paris, France

L. M. Kozloff, Department of Biochemistry, The University of Chicago, Chicago, Illinois

R. A. Liebelt, Department of Anatomy, Baylor University College of Medicine, Houston, Texas

J. B. Moloney, Division of Viral Oncology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

A. E. Moore, Division of Virology and Immunology, Sloan-Kettering Institute for Cancer Research, New York, New York

M. Pollard, Lobund Laboratory, Department of Biology, University of Notre Dame, Notre Dame, Indiana

R. Rask-Nielsen, Department of Biochemistry, University of Copenhagen, Copenhagen, Denmark

C. Sirtori, Istituto Nazionale per lo Studio e la Cura dei Tumori, Divisione di Anatomia e Istologia Patologica, Milano, Italy

S. E. Stewart, Division of Viral Oncology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

L. Zilber, Department of Immunology and Oncology, Gamaleya Institute, Moscow, U.S.S.R.

Table of Contents

VIRAL NUCLEIC ACID: PROPERTIES AND MODE OF REPLICATION

AVIAN VIRUSES AND NEOPLASIA

BERTNER FOUNDATION LECTURE

VIRUSES AND INTERFERON

BIOLOGICAL ASPECTS OF TUMOR INDUCTION BY VIRUSES

INTERRELATIONSHIP OF VIRUSES: INTRACELLULAR AND EXTRACELLULAR FACTORS IN NEOPLASIA

Session Chairmen

W. R. BRYAN, Division of Viral Oncology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

J. S. COLTER, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada

H. L. FRAENKEL-CONRAT, Virus Laboratory, University of California, Berkeley, California

W. HENLE, Research Department, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

R. W. HORNE, Institute of Animal Physiology, Agricultural Research Council, Babraham Hall, Babraham, Cambridgeshire, England

R. J. HUEBNER, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

J. L. MELNICK, Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas

A. B. SABIN, Children's Hospital Research Foundation, University of Cincinnati, Cincinnati, Ohio

R. E. SHOPE, The Rockefeller Institute, New York, New York

W. M. STANLEY, Virus Laboratory, University of California, Berkeley, California

R. C. WILLIAMS, Virus Laboratory, University of California, Berkeley, California

and possible nature of such an inhibitor in the tissues and in the milk of low-cancer-strain mice are now being investigated. The genetic factors may be responsible for the substrate on which virus particles act and for chemical differences in the virus itself which reflect in the different mammary tumor incidence encountered.

The Mouse Leukemia Virus (Gross)

After the successful demonstration of the transmission of mouse leukemia by cell-free extracts from leukemic organs of high-leukemia-strain (Ak and C58) mice, and the induction of mouse leukemia by cell-free preparations of tissues from apparently healthy high-leukemia-strain mice by Gross (1951, 1952), he postulated that mouse leukemia is an "egg-borne" disease which is transmitted vertically (Gross, 1953, 1955). Recently, Gross (1961, 1962), Law and Moloney (1961), Law (1962), and Krischke and Graffi (1962) demonstrated the presence of leukemia-inducing activity in the milk of mice which had been inoculated with either the Gross, Moloney, or Graffi leukemia virus. Thus, there appears to be no doubt that leukemia-inducing virus, isolated from a number of sources and passaged through successive generations of suitable mice, is transmitted, like the Bittner virus, through the mother's milk. Stable high- and low-leukemia strains of mice were established through foster-nursing experiments, similar to those which led to the discovery of the Bittner virus (Bittner, 1936a). Reciprocal foster-nursing experiments have revealed that transmission of the leukemia virus of mice from mother to offspring may occur through the placenta during the prenatal period, although in an apparently less efficient manner than through the mother's milk (Law, 1962).

Data obtained from reciprocal foster-nursing experiments with mice of strains with a high and a low incidence of spontaneous leukemia, presented by MacDowell and Richter (1935), Fekete and Otis (1954), Furth, Cole, and Boon (1942), Law (1954), and Kirschbaum (1957) do not support an extrachromosomal or milk transfer of the leukemia-inducing property, at least in certain strains of mice. Law (1962) has recently shown that foster nursings and thymectomies in successive generations of mice of a high-leukemia strain (AKR) do not alter the tendency of these mice to develop a high incidence of leukemia. As the Gross leukemia virus was originally derived from leukemic or normal organs of high-leukemia strain (AKR or C58) mice, the data from reciprocal foster-nursing experiments with mice of strains with high and low incidence of spontaneous leukemia apparently contradict the extrachromosomal transmission of the leukemia virus of Gross or Moloney.

This apparent contradiction between the transmission of the naturally occurring leukemia virus in mice of the so-called high-leukemia strains and that of a leukemia virus of increased potency through planned (Gross) or apparently accidental laboratory manipulation (Moloney) in no way de-

tracts from the importance of these investigators' observations. This contradiction may only be based on the difference in potency of the respective viruses and on what is of no less importance, the difference in the genetic constitution between the so-called high- and low-leukemia-strain mice. It is conceivable that because of this, the foster-nursing experiments may be successful in the case of the "artificial" and not in the case of the "naturally occurring" leukemia virus. There appears to be little doubt that the mouse leukemia virus may be transmitted not only through the milk but also through the placenta, as shown by Gross (1951) in experiments with high-leukemia (AKR) strain embryos and, more recently, by Moloney (1962) in experiments with suitable mice inoculated with Gross or Moloney leukemia virus.

In the present experiments, a study was made of sections of high-speed (105,000 × g) centrifugal pellets of decaseinated and defatted milk obtained from mice of high-leukemia strains (AKR and C58) and from mice (C3H/f) inoculated with the passage A leukemia virus of Gross (1957). In the sections of high-speed centrifugal pellets of milk fixed in osmic acid, virus particles have been observed (Figures 24 and 25) which resemble in size and appearance virus particles found in sections of leukemic organs of mice with spontaneous leukemia (Dmochowski, Grey, and Law, 1956; Dmochowski and Grey, 1957, 1958; Bernhard and Guérin, 1958b) and with induced leukemia (Dmochowski and Grey, 1957, 1958; Bernhard and Gross, 1959). These particles are morphologically indistinguishable from virus particles present in sections of leukemic organs of rats with leukemia induced by passage A virus (Dmochowski, Gross, and Padgett, 1962).

In negatively stained preparations from high-speed centrifugal pellets of defatted and decaseinated milk of AKR high-leukemia-strain mice (Figures 26, 27, and 28) and of C58 high-leukemia-strain mice (Figure 29), the virus particles (900 Å to 1,400 Å) resemble myxovirus particles such as influenza virus (Figure 23), and the virus particles present in the milk of high- and low-mammary-cancer-strain mice. Similar virus particles have been found in potassium phosphotungstate-stained preparations of high-speed centrifugal pellets of defatted and decaseinated milk obtained from C3H/f strain mice inoculated with passage A Gross leukemia virus (Figure 30). It should be pointed out that milk of C3H/f mice inoculated with Gross passage A virus has a high-leukemia-inducing activity, as shown by Gross (1962). It appears, therefore, that these particles are leukemia virus. The activity of milk from high-leukemia-strain (AKR) and of passage A inoculated C3H/f mice is now being tested.

A study of preparations stained with potassium phosphotungstate and obtained from leukemic organs of AKR high-leukemia-strain mice and from leukemic organs of C3H/f strain mice which had been inoculated with passage A Gross leukemia virus has revealed characteristic virus particles, some of them with "tails" (Figures 31 and 32). These particles resemble those found in sections of high-speed centrifugal pellets from milk of AKR

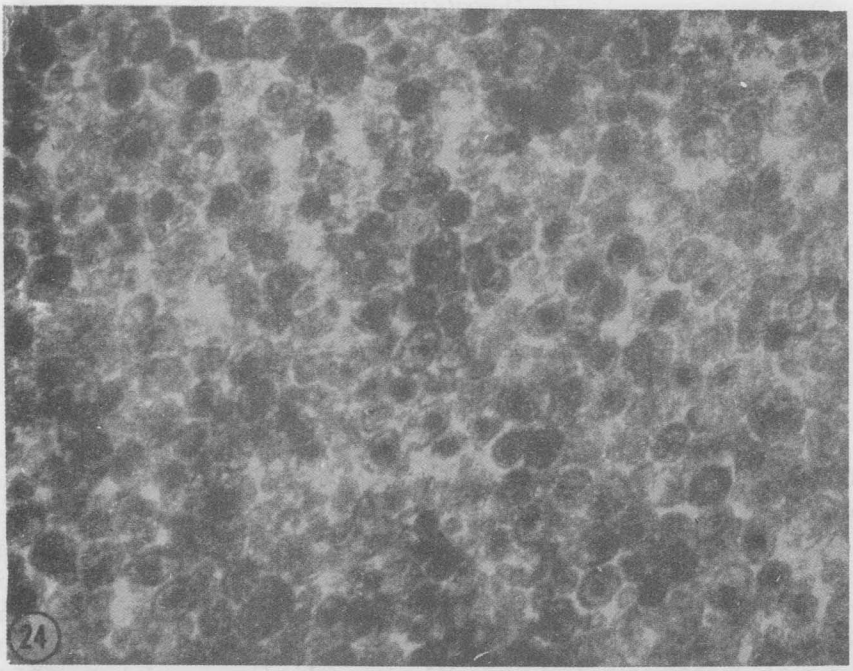

FIGURE 24. Characteristic virus particles present in section of osmic acid-fixed pellet obtained by high-speed centrifugation of defatted and decaseinated AKR high-leukemia-strain milk. × 60,000.

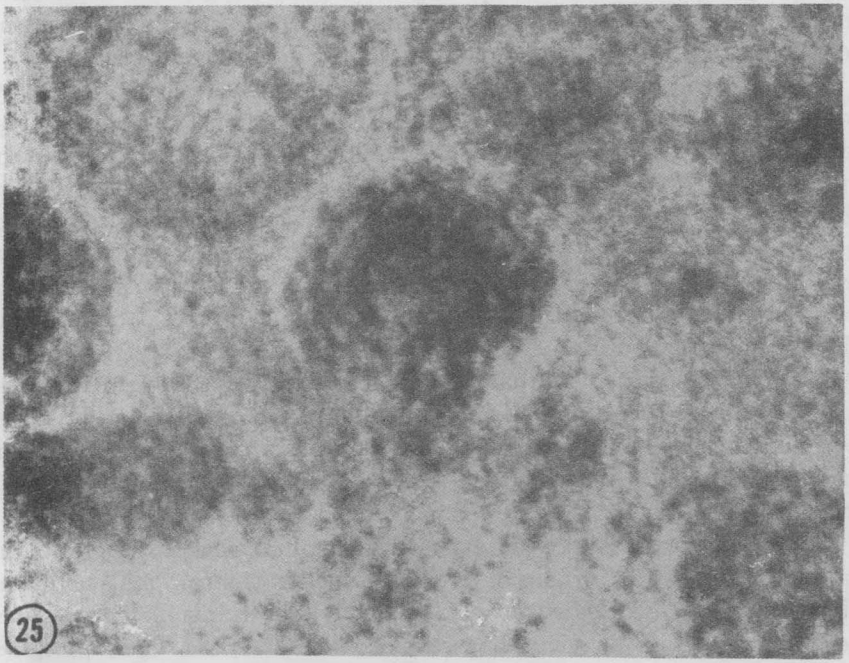

FIGURE 25. High-magnification view of a virus particle from osmic acid-fixed preparation of a pellet from AKR high-leukemia-strain milk. × 320,000.

strain mice (Figure 24). Similar virus particles were demonstrated in high-speed centrifugal pellets from plasma of mice and rats with leukemia induced by Moloney (Dalton, Haguenau, and Moloney, 1962) or by Rauscher leukemia virus (Zeigel and Rauscher, 1963). As already mentioned, the "tail-like" appearance of some of the virus particles in our preparations and in those obtained by others may be the result of the molarity of the resuspending medium, as originally observed by Bang (1946, 1947, 1948) and Cunha *et al.* (1947).

In view of the reported transmission of mouse leukemia virus through the placenta of high-leukemia-strain (AKR, C58) mice (Gross, 1951) and of mice inoculated with Gross or Moloney leukemia virus (Moloney, 1962), studies were carried out on sections of osmic acid-fixed organs of embryos from high-leukemia-strain (AKR) mice. It should be pointed out that "fully formed" virus particles have been found in the follicular cells of ovaries of AKR high-leukemia-strain mice long before the development of symptoms of leukemia (Figures 33 and 34). It is of interest that in sections of osmic acid-fixed organs of embryos from AKR high-leukemia-strain mice, only "immature" or doughnut-type virus particles have so far been found (Figure 35), in spite of a prolonged and careful search. This may have been because of the age of the embryos and the age of the pregnant AKR strain females. These possibilities are now being explored.

Similar "immature" or doughnut-type particles have been found in thin sections of osmic acid-fixed organs of 14-hour-, two-day-, and four-day-old mice of AKR high-leukemia-strain mice (Figures 36, 37, and 38). "Mature" or fully formed virus particles have been observed in sections of similarly prepared organs of six-day, eight-day, 14-day, or older AKR strain mice (Figures 39, 40, and 41). Some of the organs of two- to 10-week-old mice reveal a picture similar to that observed in leukemic organs of AKR

(text continued on page 116)

Type and Occurrence of Virus Particles in Different Tissues from AKR Strain Mice of Different Ages

Age	Tissues Examined*					
	Thymus		Spleen		Bone Marrow	
	Immature	Mature	Immature	Mature	Immature	Mature
Embryo	+	—	NE	NE	NE	NE
14 hours	++	—	NE	NE	NE	NE
2 days	+	—	—	—	NE	NE
4 days	+	—	—	—	NE	NE
6 days	++	+	—	—	NE	NE
8 days	+	+	—	—	NE	NE
10 days	+	—	++	+	NE	NE
14 days	—	—	++	+	++	+
28 days	NE	NE	++	++	++	++
42 days	NE	NE	++	++	++	++
56 days	NE	NE	++	++	++	++

* Not less than 25 sections of each tissue were examined.

Abbreviations: NE, not examined; —, no virus particles seen; +, small number of virus particles seen; ++, relatively large number of virus particles seen.

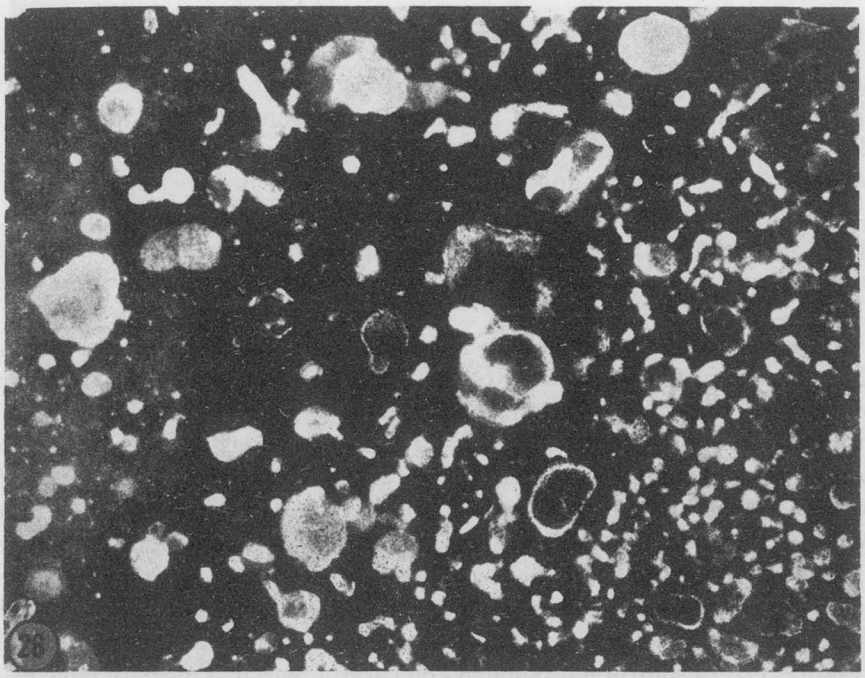

FIGURE 26. Low-magnification view of a negatively stained preparation from AKR high-leukemia-strain milk, showing a few characteristic virus particles as well as large and small cytoplasmic components. × 60,000.

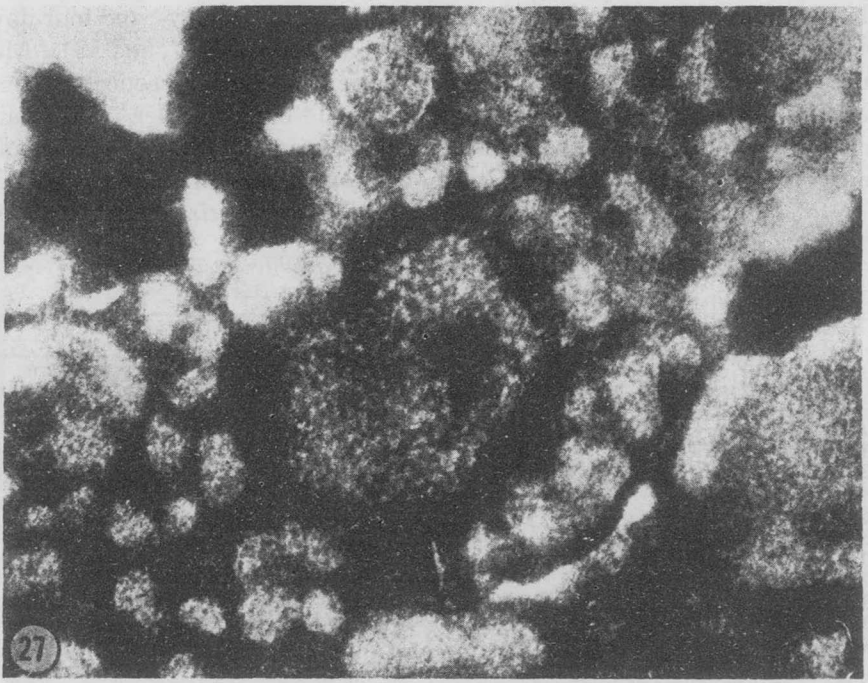

FIGURE 27. A negatively stained virus particle in AKR high-leukemia-strain milk, surrounded by cytoplasmic components. × 240,000.

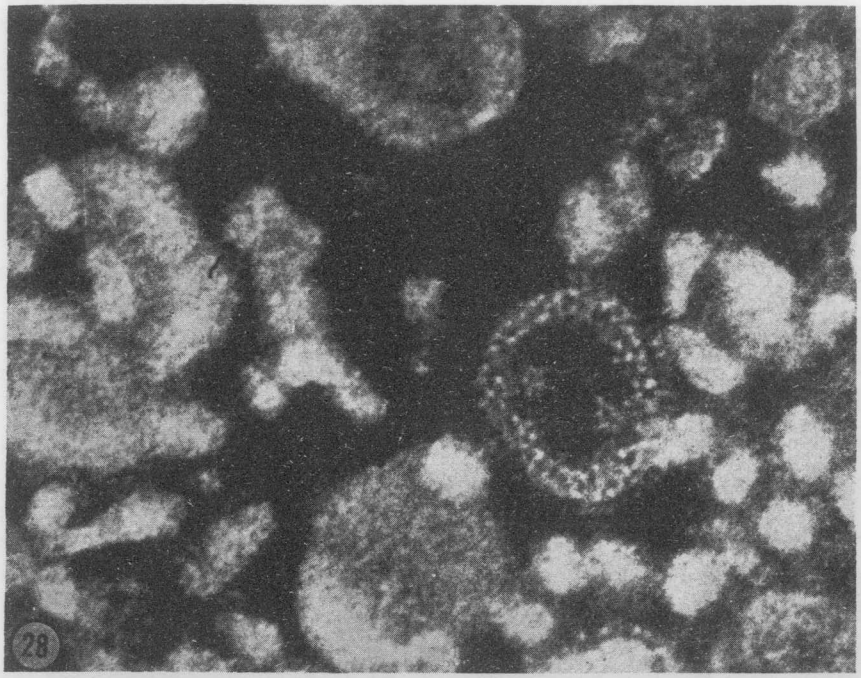

FIGURE 28. A partly disrupted virus particle, surrounded by cytoplasmic components in a negatively stained preparation from AKR strain milk. × 240,000.

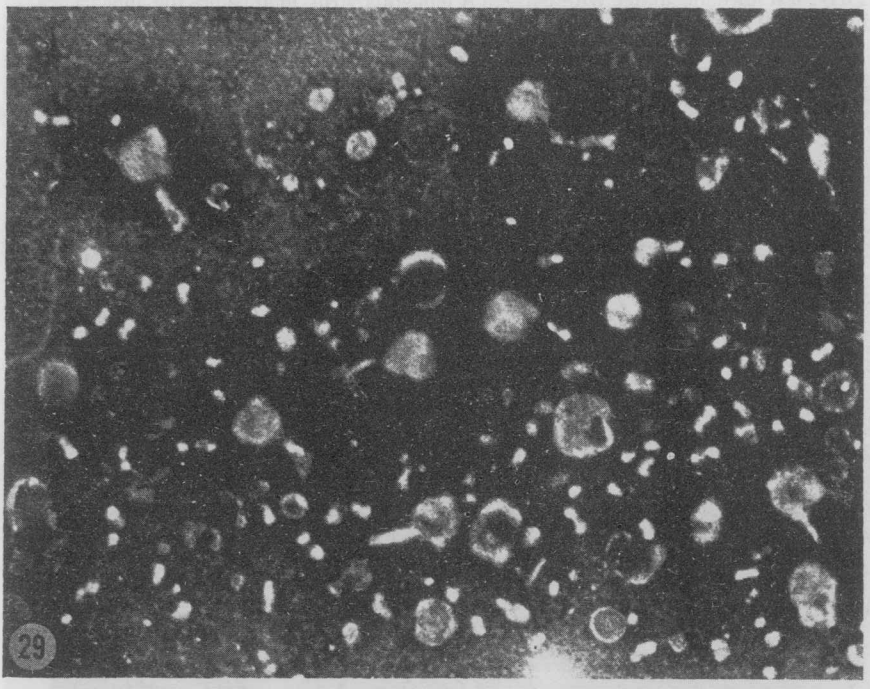

FIGURE 29. A low-magnification view of a negatively stained preparation from C58 high-leukemia-strain milk. Virus particles, some with "tails", and many small cytoplasmic components are present. × 60,000.

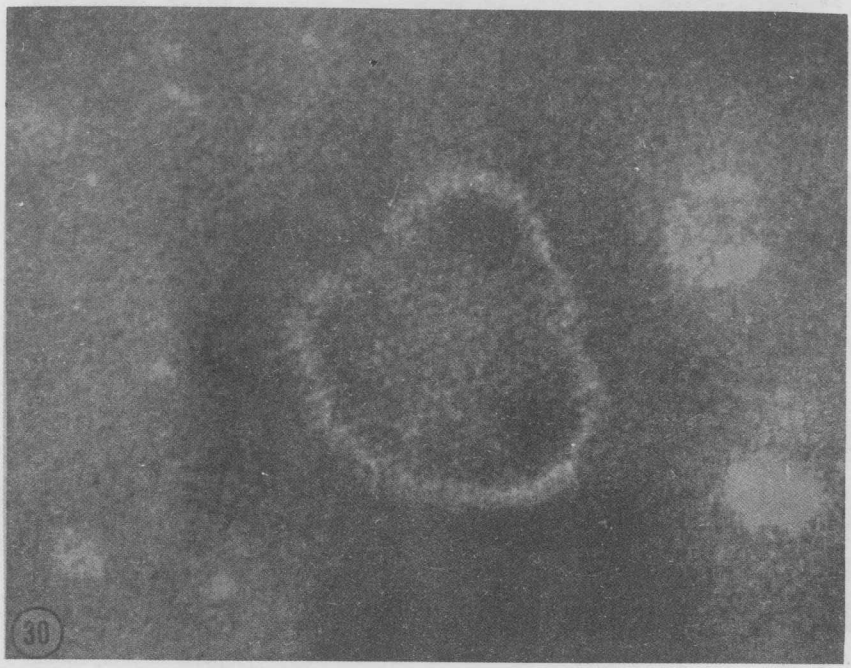

FIGURE 30. Appearance of virus particle in a negatively stained preparation from milk of C3H/f test mice inoculated with passage A leukemia virus, long before the appearance of disease. × 240,000.

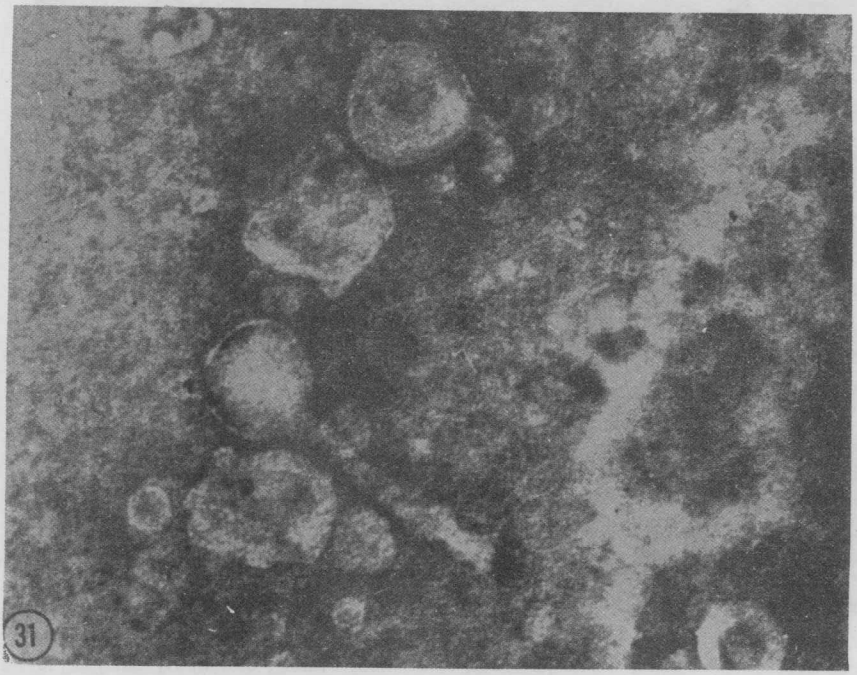

FIGURE 31. Appearance of virus particles in negatively stained preparation from AKR leukemia tissues. One particle has a well-defined "tail." × 120,000.

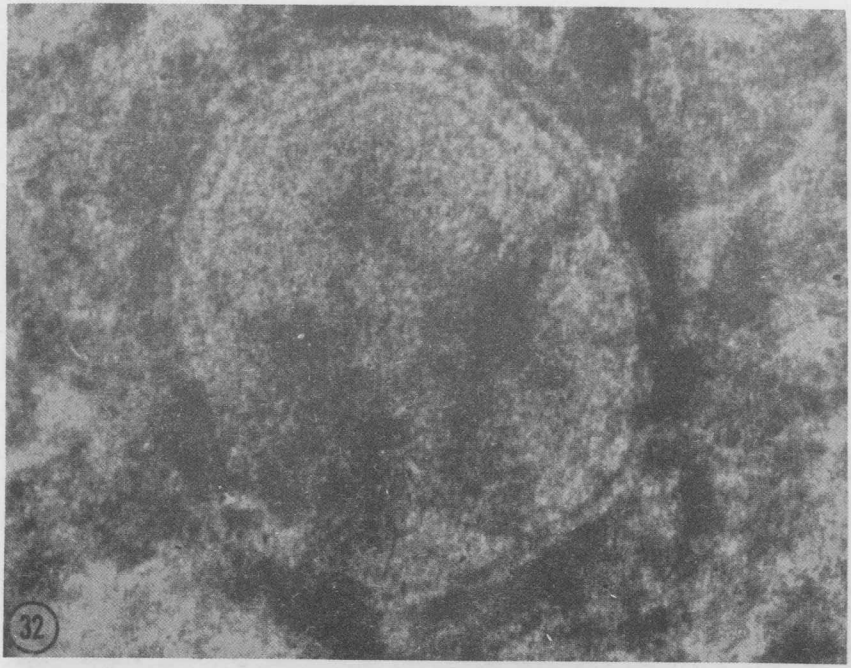

FIGURE 32. Appearance of a smaller and partially distorted particle seen in a negatively stained preparation of AKR leukemia tissues. × 240,000.

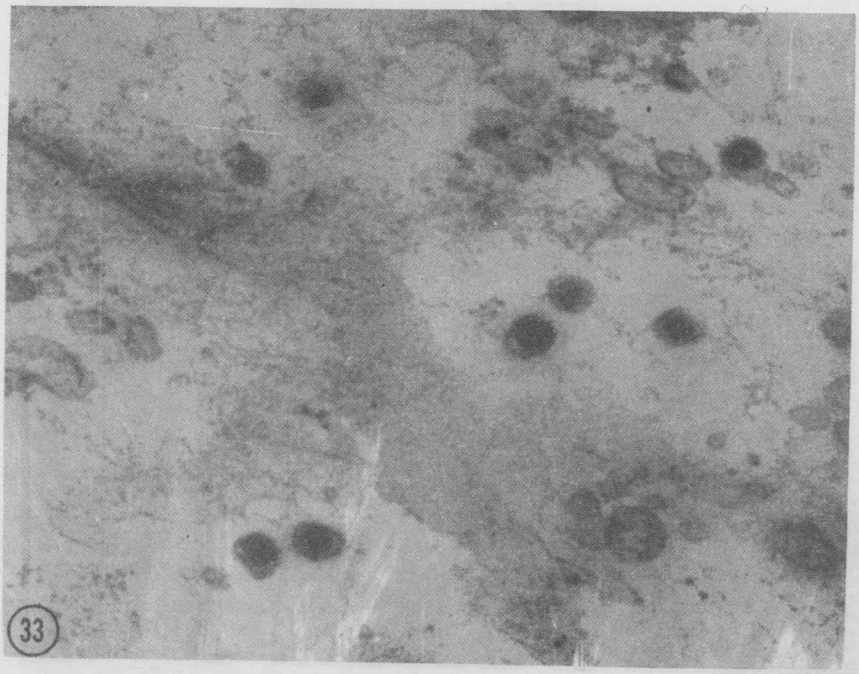

FIGURE 33. Appearance of virus particles in section of AKR adult mouse ovary. × 60,000.