

# VIRUSES, NUCLEIC ACIDS, AND CANCER

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Symposium on Fundamental Cancer Research, 1963*

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

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## 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.*

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## Table of Contents

Session Chairmen . . . . .	xii
Invited Discussants . . . . .	xiii
Introduction . . . . .	1
R. LEE CLARK	

### STRUCTURE OF VIRUSES

A Comparative Study of the Structures of a Variety of Bacteriophage Particles with Some Observations on the Mechanism of Nucleic Acid Injection . . . . .	7
DESMOND KAY	
Structure and Assembly of Regular Virus Particles . . . . .	27
D. L. D. CASPAR and A. KLUG	
Electron Microscope Studies on the Structure and Symmetry of Virus Particles . . . . .	40
R. W. HORNE	
Icosahedral Viruses—A Geometric Approach to Their Maturation . . . . .	63
HEATHER DONALD MAYOR	
Morphology of the RNA Phage FH5 . . . . .	68
J. HUPPERT, ANTOINETTE RYTER, and JEANNE FOUACE	
The Arthropod Viruses . . . . .	72
KENNETH M. SMITH	
Studies on the Structure of the Mammary Tumor-Inducing Virus (Bittner) and of Leukemia Virus (Gross) . . . . .	85
L. DMOCHOWSKI, C. E. GREY, F. PADGETT, and J. A. SYKES	
Structure and Genetic Properties of Bacterial Viruses . . . . .	122
THOMAS F. ANDERSON	
Virus Particles of Mouse Lymphomas and Mammary Carcinoma . . . . .	141
R. KINOSITA and T. KAKEFUDA	

### REPLICATION OF VIRUSES: BIOCHEMISTRY OF VIRUS-INFECTED CELLS

Influence of Phages on the Synthesis of Host Enzymes of Bacteria . . . . .	151
MICHAEL B. YARMOLINSKY	

The Functions of the RNA of Bacteriophage f2 . . . . .	173
NORTON D. ZINDER	
Substances Produced During Replication of Plant Viruses . . . . .	180
ROY MARKHAM	
Biosynthesis of Viral Ribonucleic Acids . . . . .	191
M. HOMMA, A. V. RAKE, W. PARANCHYCH, D. B. ELLIS, and A. F. GRAHAM	
The Programing of Herpes Virus Multiplication in Mammalian Cells . . . . .	205
BERNARD ROIZMAN	
Studies of Replication and Properties of the Bittner Virus . . . . .	224
DAN H. MOORE and MICHAEL J. LYONS	

### VIRAL NUCLEIC ACID: PROPERTIES AND MODE OF REPLICATION

Viral Nucleic Acid: Properties and Mode of Replication. Intro- duction . . . . .	245
W. M. STANLEY	
Replication of the Nucleic Acids of the Bacterial Viruses . . . . .	246
ROBERT L. SINSHEIMER	
Biosynthesis of Infectious Ribonucleic Acid of Tobacco Mosaic Virus by Extracts of Healthy Plants . . . . .	252
GEORGE W. COCHRAN	
Properties of the DNA of Polyoma Virus . . . . .	271
RENATO DULBECCO	
Early Events in the Reproduction Cycle of Animal Viruses . . . . .	282
P. HAUSEN, H. HAUSEN, R. ROTT, C. SCHOLTISSEK, and W. SCHÄFER	
Early Changes Following Virus Infection: Thymidine Kinase In- duction in Cells Infected with Vaccinia and Herpes Simplex Viruses . . . . .	296
SAUL KIT	
Changes in RNA and Protein Synthesis in Mammalian Cells In- fected with a Virulent Virus . . . . .	310
RICHARD M. FRANKLIN and DAVID BALTIMORE	

### AVIAN VIRUSES AND NEOPLASIA

Avian Viruses and Neoplasia: Introduction . . . . .	329
W. R. BRYAN	
Biological and Structural Properties of Rous Sarcoma Viruses . . . . .	331
R. J. C. HARRIS	
Studies on the Biological, Biochemical, and Biophysical Properties of Avian Tumor Viruses . . . . .	344
J. W. BEARD, R. A. BONAR, URSULA HEINE, GUY DE THÉ, and DOROTHY BEARD	

The Infection of Chicken Fibroblast Cultures by Avian Myeloblastosis Virus . . . . .	374
PETER K. VOGT	
Induction of Differentiation in Certain Target Cells by Avian Myeloblastosis Virus: An In Vitro Study . . . . .	387
M. A. BALUDA, I. E. GOETZ, and S. OHNO	

#### BERTNER FOUNDATION LECTURE

Properties of a Virus Isolated from Leukemic Mice, Inducing Various Forms of Leukemia and Lymphomas in Mice and Rats . . . . .	403
LUDWIK GROSS	

#### VIRUSES AND INTERFERON

Viruses and Interferon: Introduction . . . . .	429
W. HENLE	
Interference and Cell Division . . . . .	430
KURT PAUCKER	
The Effect of Interferon on the Synthesis of Viral Nucleic Acid . . . . .	447
ROYCE Z. LOCKART, JR., and T. SREEVALSAN	
Interference and Interferon in Relation to Tumor Viruses and Tumor Cells . . . . .	462
A. C. ALLISON	

#### BIOLOGICAL ASPECTS OF TUMOR INDUCTION BY VIRUSES

The Interaction of Polyoma Virus with Hamster Fibroblasts . . . . .	487
MICHAEL STOKER	
The Role of the Mammary Tumor Virus in Mouse Mammary Noduligenesis and Tumorigenesis . . . . .	498
K. B. DEOME	
Significance of the Absence of Infectious Virus in Virus-Induced Tumors . . . . .	508
H. RUBIN and H. HANAFUSA	

#### INTERRELATIONSHIP OF VIRUSES: INTRACELLULAR AND EXTRACELLULAR FACTORS IN NEOPLASIA

The Possible Role of a "Transmissible Factor" in Leukemia Induction by Radiation Plus Urethan . . . . .	529
I. BERENBLUM	
The Combined Action of Viruses and Other Carcinogens . . . . .	544
M. H. SALAMAN, K. E. K. ROWSON, F. J. C. ROE, J. K. BALL, J. J. HARVEY, and G. DE BENEDICTIS	



Studies on the Oncogenicity of Human Adenovirus . . . . .	559
JOHN J. TRENTIN, YOSHIRO YABE, and GRANT TAYLOR	
Observations on a Specific Adenovirus 12 Antigen in Virus-Free Adenovirus Tumor Transplants . . . . .	564
R. J. HUEBNER, W. P. ROWE, and L. D. BERMAN	
Lymphoma in ICR Mice Treated with 4-Nitroquinoline N-Oxide (4-NQO) . . . . .	571
R. KINOSITA and T. TANAKA	
"Newer" and "Older" Viruses in Mammalian Malignancy . . . . .	580
MAURICE R. HILLEMANN	
A Children's Cancer Dependent on Environment . . . . .	615
DENIS BURKITT	
The Role of Viruses in Relation to Cancer in Animals and Man . . . . .	630
FRANK L. HORSFALL, JR.	
Index . . . . .	643

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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 ( $105,000 \times g$ ) 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 ( $900 \text{ \AA}$  to  $1,400 \text{ \AA}$ ) 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.  $\times 60,000$ .

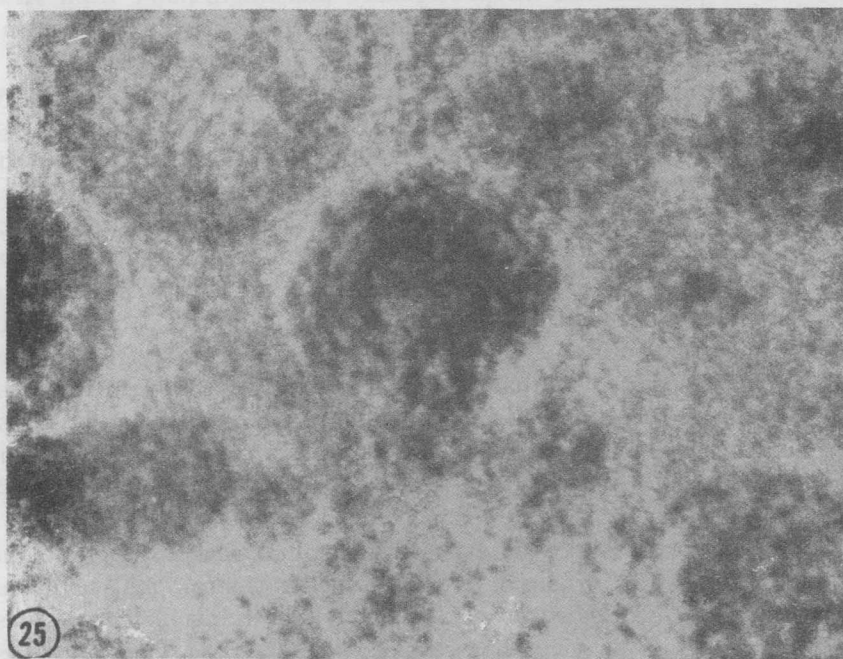


FIGURE 25. High-magnification view of a virus particle from osmic acid-fixed preparation of a pellet from AKR high-leukemia-strain milk.  $\times 320,000$ .

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, Haguénau, 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.



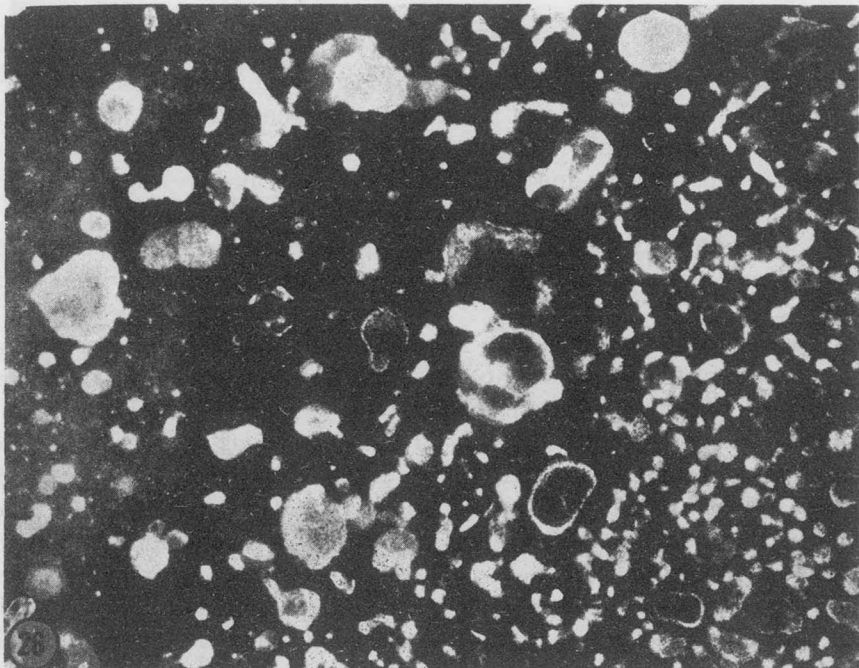


FIGURE 26. Low-magnification view of a negatively stained preparation from AKR high-leukemia-strain milk, showing a few characteristic virus particles as well as large and small cytoplasmic components.  $\times 60,000$ .

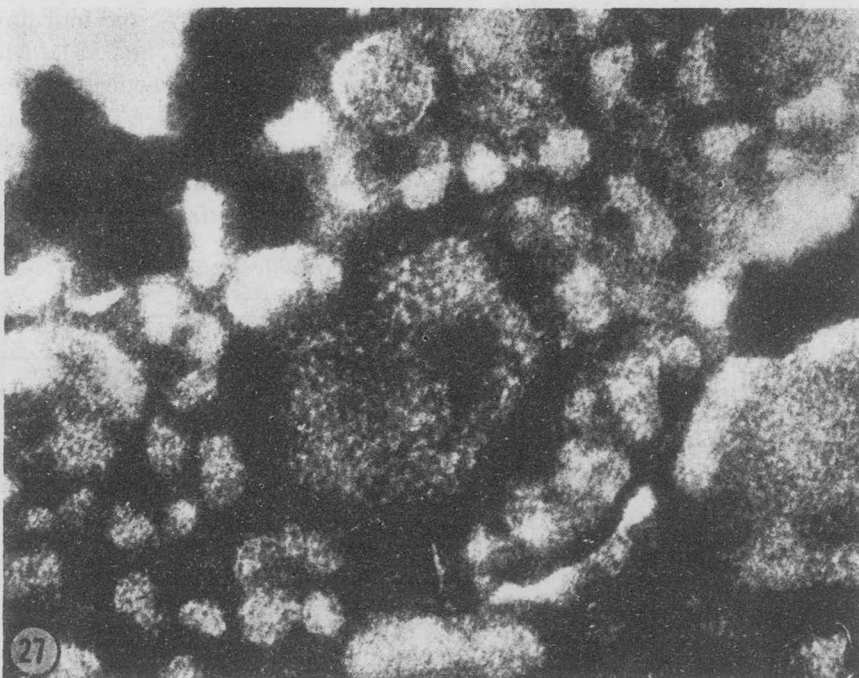


FIGURE 27. A negatively stained virus particle in AKR high-leukemia-strain milk, surrounded by cytoplasmic components.  $\times 240,000$ .

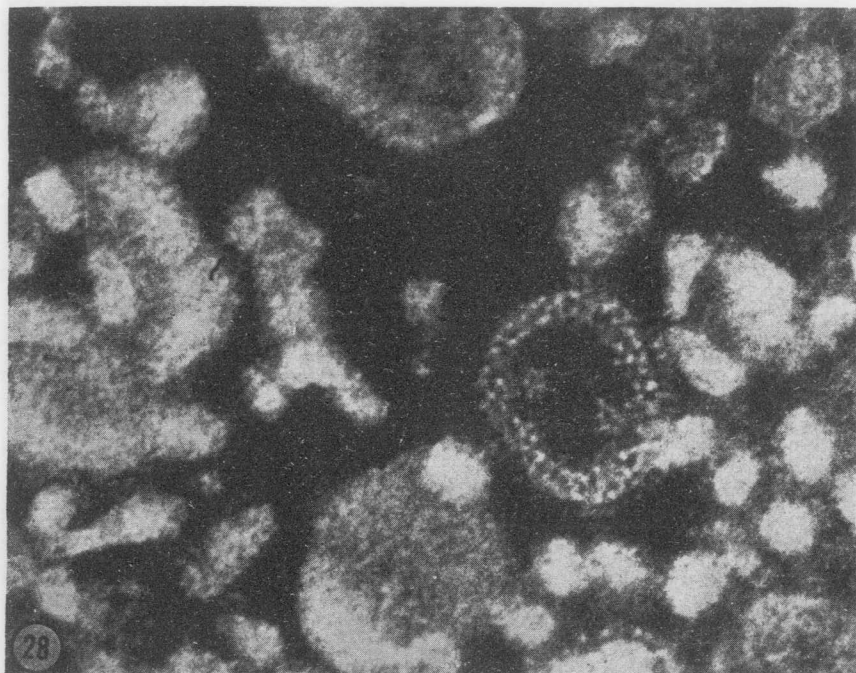


FIGURE 28. A partly disrupted virus particle, surrounded by cytoplasmic components in a negatively stained preparation from AKR strain milk.  $\times 240,000$ .

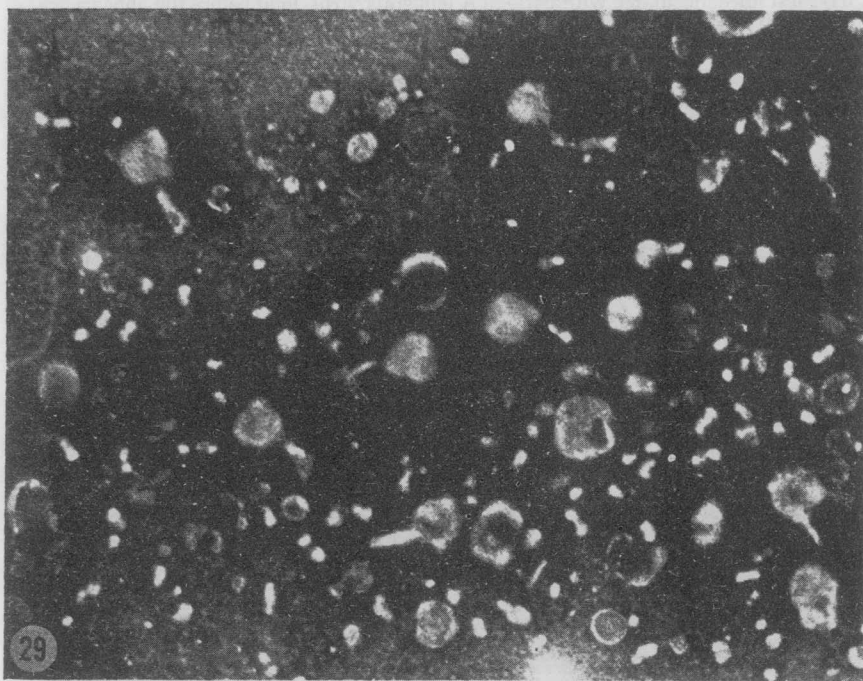


FIGURE 29. A low-magnification view of a negatively stained preparation from C58 high-leukemia-strain milk. Virus particles, some with "tails", and many small cytoplasmic components are present.  $\times 60,000$ .

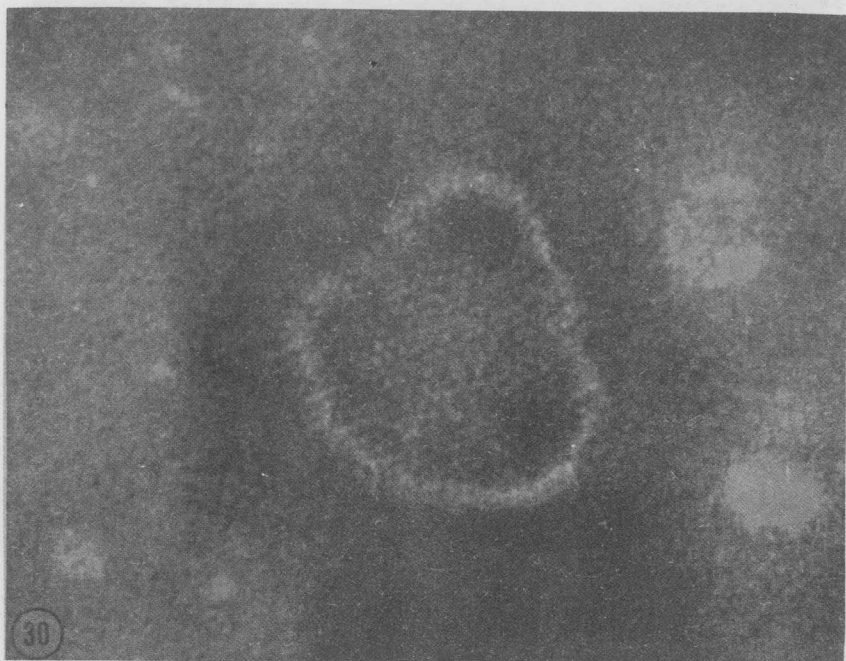


FIGURE 30. Appearance of virus particle in a negatively stained preparation from milk of C3H/f test mice inoculated with passage A leukemia virus, long before the appearance of disease.  $\times 240,000$ .



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



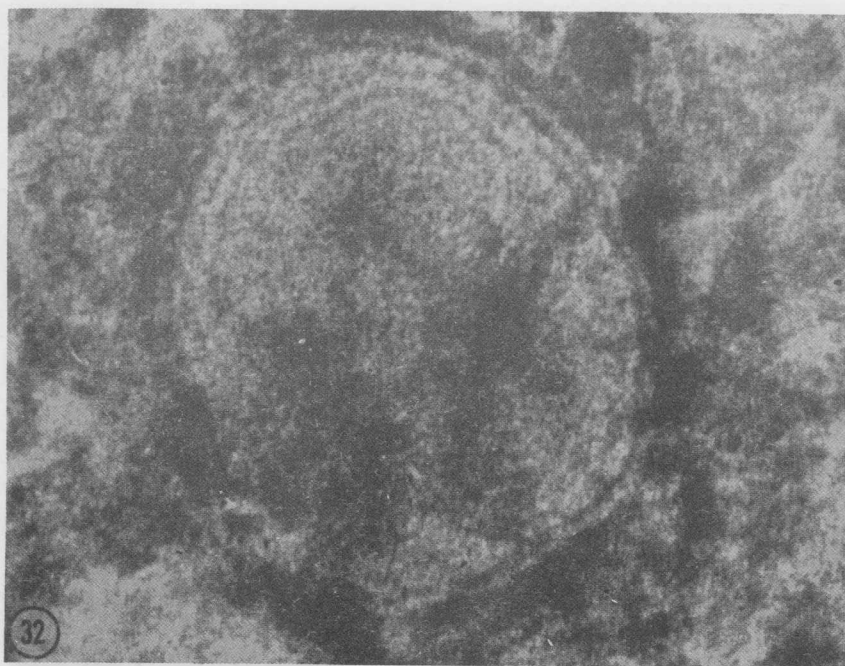


FIGURE 32. Appearance of a smaller and partially distorted particle seen in a negatively stained preparation of AKR leukemia tissues.  $\times 240,000$ .

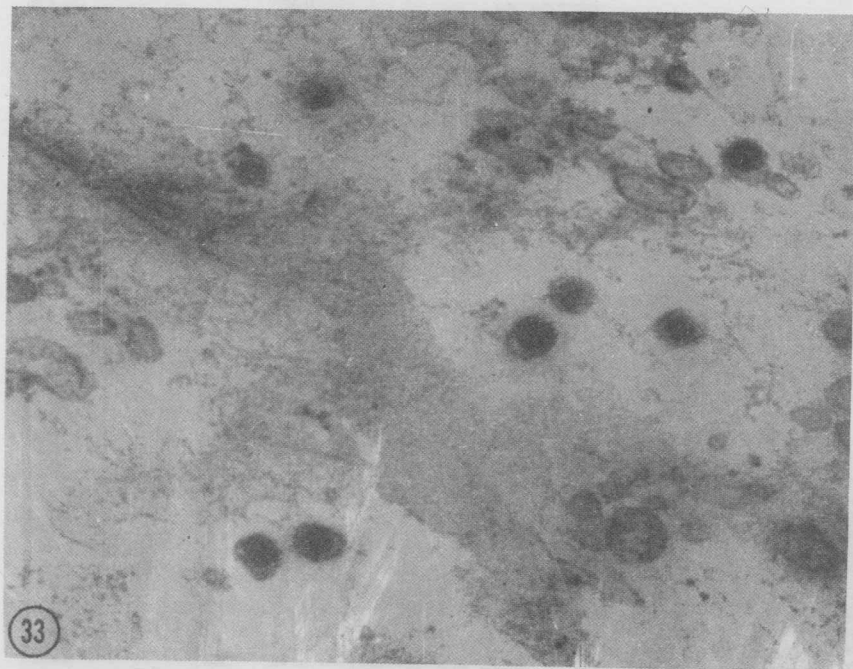


FIGURE 33. Appearance of virus particles in section of AKR adult mouse ovary.  $\times 60,000$ .