

Persistent Infection of a Rat Kidney Cell Line with Rauscher Murine Leukemia Virus

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Received for publication 27 June 1966

ABSTRACT

DUC-NGUYEN, HUU (National Cancer Institute, Bethesda, Md.), EDITH N. ROSENBLUM, AND ROBERT F. ZEIGEL. Persistent infection of a rat kidney cell line with Rauscher murine leukemia virus. *J. Bacteriol.* **92**:1133-1140. 1966.—The propagation of a murine leukemia virus (Rauscher) in a kidney cell line, derived from a rat with lymphoid leukemia, was studied. A complement-fixing (CF) antigen reacting with Rauscher immune sera was detected at various passage levels, which correlated with the visualization by use of electron microscopy of viral buds and viral particles in different stages of maturation in all passages. Five-month-old monolayers continued to shed virus and to yield high CF antigen titers. The cell-free supernatant fluid from cultures of the 14th passage was shown to be infectious for a normal rat kidney cell line, as evidenced by the appearance of the CF antigen in this line. Interferon production was not demonstrated in infected cultures. The overall data indicated that rat kidney cells could be used to propagate Rauscher virus in a carrier state.

The long-term propagation of Rauscher murine leukemia virus in established cultures derived from mouse spleen cells (16), from a mixture of BALB/c mouse spleen and thymus cells (18), and from BALB/c mouse embryo cells (8) has been well documented. The virus has been shown to multiply in cultures of mouse kidney cells when mice were inoculated 7 days prior to the initiation of the cultures (12). Heretofore, all *in vitro* studies of Rauscher virus were concerned with mouse cells as the host system. Although rats are highly susceptible to leukemia induced by Rauscher virus (14), these animals have not been considered as the natural host of murine leukemia viruses (13). Agents apparently related to infrequent spontaneous leukemias in rats (6, 15) have not been well defined (17). It was of interest to study the response of cultured rat cells to Rauscher virus infection. The present study was undertaken to determine whether rat kidney cells are capable of supporting Rauscher virus replication *in vitro*, and whether carrier cultures can be established.

MATERIALS AND METHODS

Establishment of long-term cell cultures; NCI-RR cell line. These cells were derived from an adult, noninbred Osborne-Mendel (OM) rat, which was kindly provided by F. J. Rauscher. The rat had been inoculated at birth with 3 or 4 log infective units of Rauscher virus at its 17th passage in rats of the same

strain. At time of sacrifice, the animal presented a large lymphocytic neoplastic thymus, enlarged lymph nodes, and splenomegaly. The two kidneys were decapsulated, and the tissue was minced, washed with balanced salt solution (BSS), and treated with 0.25% trypsin solution containing penicillin and streptomycin. The resulting cell suspension was washed again with BSS, and resuspended in modified McCoy medium containing 30% fetal calf serum and antibiotics (Grand Island Biological Co., Grand Island, N.Y.). The cells were seeded into T-60 flasks. After 3 days, the growth medium was renewed for the first time, and, thereafter, the medium was renewed at 3- to 4-day intervals. On the 7th day after the initial planting, a complete monolayer was obtained. The cultures were split by trypsin treatment every 4 to 5 days, grown in modified McCoy medium with 15% fetal calf serum, and maintained in the same medium but with only 5% serum. This line is currently (June, 1966) in its 35th passage.

NCI-NR cell lines. The technique employed to establish two normal cultures was identical to that described for the NCI-RR line. They were derived from healthy young adult OM rats, and are currently (June, 1966) in their 27th and 29th passages. They were used throughout the present study as control cultures.

Antigen and virus pools. To prepare virus or antigen grown in tissue culture, 7- to 14-day-old NCI-RR cultures were harvested by scraping the cells from the surface of the flask. The cells of one T-60 or one 250-ml plastic flask (Falcon Plastic, Div. of B-D Laboratories, Inc., Los Angeles, Calif.) were resuspended in 0.9 ml of supernatant fluid; the suspen-

sion was then treated in a disintegrator (Ultrasonic Industries, Inc., Plainview, N.Y.) for 2 min at 1.8 amp. The resulting homogenized material was tested for complement-fixation activity or used as a source of virus pools after clarification by low-speed centrifugation. Culture media (7 or more days old) from NCI-RR cultures were also used as a source of seed virus.

The Indiana strain of vesicular stomatitis virus (VSV) provided by K. Paucker (The Children's Hospital of Philadelphia, Philadelphia, Pa.) was grown in chick embryo fibroblasts and used for interferon assay.

Complement-fixation (CF) test. The microtechnique described by Huebner et al. (9) was employed. The CF tests were performed in R. J. Huebner's laboratory (Laboratory of Infectious Diseases, National Institutes of Health, Bethesda, Md.). Rauscher immune serum was made in OM rats which had been implanted subcutaneously with a minced rat lymphosarcoma. The tumor used to prepare the immune serum was received from F. J. Rauscher, and was in its 83rd transplant passage in newborn OM rats. Immune serum, also prepared according to the method described for anti-Friend and anti-Moloney sera (7), was kindly provided by J. W. Hartley. Weanling OM rats were inoculated with supernatant fluids of secondary mouse embryo cells infected with Rauscher virus (1.0 ml intraperitoneally and 0.5 ml intravenously), and were bled out 5 to 6 weeks after inoculation. Antigen titrations were carried out by use of 1:20 dilution of anti-Rauscher virus rat serum containing 4 units of antibody as determined by titration against Rauscher virus antigen prepared in mouse embryo tissue culture (8). The serum pool used did not react when tested at a 1:10 dilution against a variety of normal mouse and rat tissue extracts and tissue culture cell antigens.

Assay of interferon. Interferon production and assay were essentially the same as previously described (5). The culture fluids to be tested for interferon activity were centrifuged at $200 \times g$ at 4°C for 15 min. The clarified supernatant fluids were concentrated 10-fold with Carbowax polyethylene glycol compound 20-M (Union Carbide Corp., Chemicals Div., New York, N.Y.). The resulting materials were then acidified for 24 hr and realkalinized for 24 hr with pH 2.0 KCl buffer and pH 7.0 phosphate buffer, respectively, at 4°C. Finally, the materials were dialyzed against BSS and stored at -20°C. In a few experiments, dialysis against buffers was replaced by high-speed centrifugation ($80,000 \times g$) for 90 min. Interferon was assayed in primary tube cultures of rat embryonic (RE) cells (Microbiological Associates, Inc., Bethesda, Md.). Serial twofold dilutions of test materials were preincubated with cells at 37°C. After 18 hr, following the removal of culture supernatant fluids, the cells in each tube were challenged with 10^8 TCID₅₀ of VSV. In control cultures, complete destruction of cells occurred regularly within 48 hr.

Preparation of cells and culture fluids for electron microscopy. The phosphotungstic acid (PTA) staining was carried out as follows. Culture medium was first

clarified by centrifugation at $200 \times g$ for 15 min; the viral particles in the supernatant fluids were then centrifuged at $54,000 \times g$ for 2 hr. Three to four drops of filtered 2% aqueous PTA solution, adjusted to pH 7.2 with 0.1 N KOH, was added to the viral pellet, and thoroughly mixed with it for 1 min; one small drop of the mixture was then placed on carbon-coated collodion-layered grids. For the preparation of thin sections, cell monolayers were gently scraped from the flask surface, sedimented by low-speed centrifugation, fixed successively in chrome-osmium mixture and 0.5% aqueous uranyl acetate solution, dehydrated with graded alcohol, and embedded in an Epon-araldite mixture. Thin sections were double-stained with uranyl acetate and lead citrate for 20 and 15 min, respectively.

RESULTS

Growth characteristics of Rauscher virus-infected rat kidney cells. The original cultures of NCI-RR cells consisted of a mixed population of epithelial, mesenchymal, and fibroblastic cells typical of primary kidney cultures. However, by the end of the third passage, only fibroblast-like cells were present in monolayers (Fig. 2 to 4). As expected, the refractile cells, described by Sinkovics et al. (16), were not observed in unstained cultures. Trypan blue staining failed to reveal the presence of live floating cells in the culture medium. No cytopathic effect (CPE) and no sign of morphological transformation was observed in monolayers maintained in modified McCoy medium or medium 199 for over a 5-month period. The two cell lines derived from uninfected normal rats showed the growth pattern described for NCI-RR cells. The cell population of these cultures was composed mainly of fibroblast-like cells, indistinguishable from the infected culture (Fig. 1). The time required to disperse the monolayer by treatment with trypsin was slightly, but consistently, shorter for NCI-RR cultures than for the controls. The supernatant fluids of cultures infected with Rauscher virus became highly viscous within 48 to 72 hr after subculture, or after medium changes. The change in viscosity was consistently observed whether modified McCoy, 199, or 109 culture medium was employed, and was independent of the concentration of fetal calf serum used.

Presence of a complement-fixing (CF) antigen reacting with Rauscher immune sera in the NCI-RR cell line. When cell extracts prepared by sonic treatment were tested by CF against rat Rauscher immune sera, an antigen was consistently demonstrated in each passage level in the NCI-RR cells, but not in the two normal control lines (Table 1). The CF antigen titers fluctuated between 1:8 and 1:32, the highest titers being obtained 9 to 10 days after subculture. To

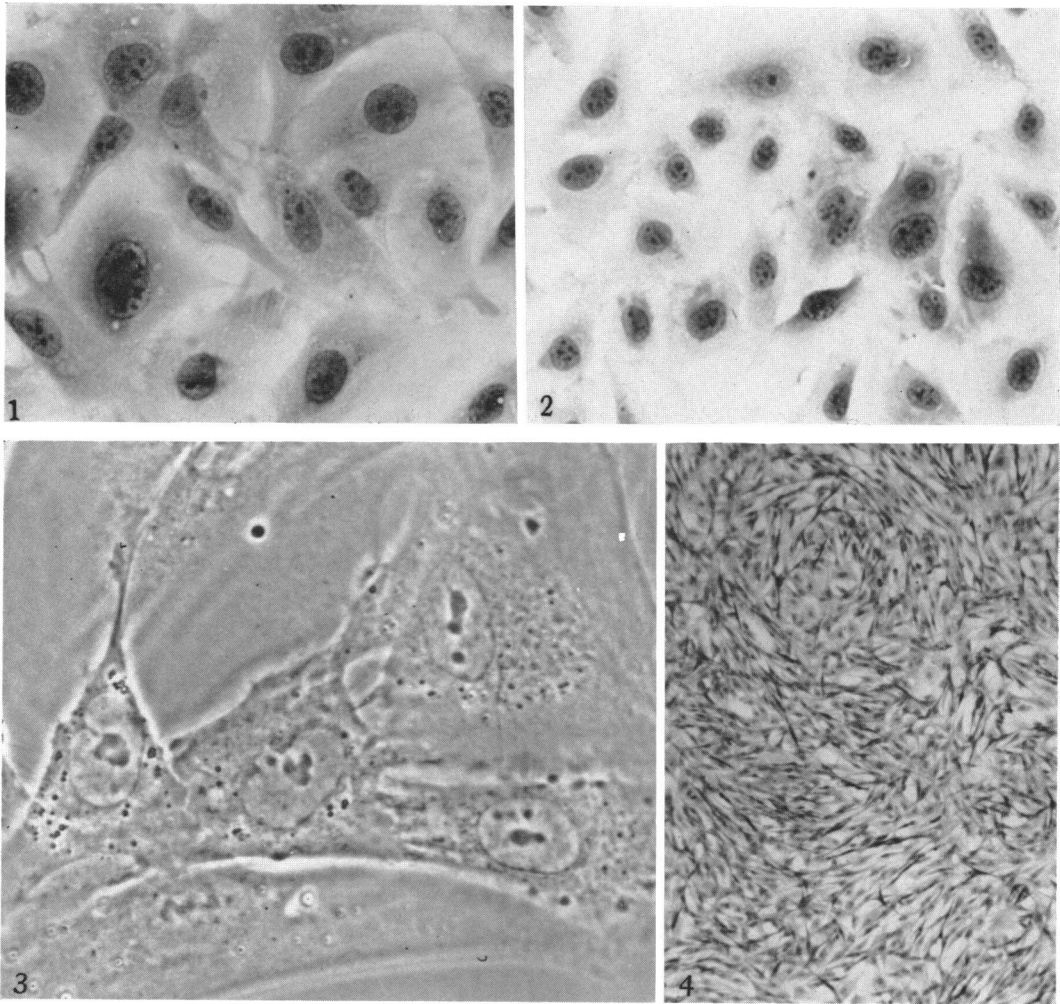


FIG. 1. Normal rat kidney tissue culture in its 12th passage. Fibroblast-like cells; Wright stain. $\times 400$.

FIG. 2. The 13th subculture of kidney cells derived from a rat infected with Rauscher virus. Fibroblast-like cells; Wright stain. $\times 160$.

FIG. 3. Same culture as in Fig. 2. Phase-contrast. $\times 1,000$.

FIG. 4. Appearance of the monolayer shown in Fig. 2, 24 hr after subculture. Fibrocyte-fibroblast cells; Wright stain. $\times 60$.

determine whether an old monolayer of the 4th passage (prepared on 17 November 1965), which was retained without subculture for the 5 following months, was still producing antigen, 20 ml of the cell-free culture medium was sedimented at $30,000 \times g$ for 2 hr, and the pellet was suspended in 1.0 ml of supernatant fluid. The resulting suspension yielded a titer of 1:32 when tested for CF antigen. These findings indicated that the cell line which was derived from the Rauscher lymphoid leukemia rat was actively producing antigen. The question was raised whether the

antigen was directly related to the new viral progeny, or whether it was merely the expression of a cellular reaction resulting from the incorporation of viral genome into the cell without viral replication.

In vitro transmission of the antigen-inducing agent to a normal rat kidney cell line. Supernatant fluids (5 days old) from the NCI-RR cells in their 14th passage were clarified by low-speed centrifugation at $30,000 \times g$ for 2 hr. The pellets were suspended in culture media to obtain a 10-fold concentrated suspension. The material

TABLE 1. *CF antigen titers of the three established cell lines as tested against rat anti-Rauscher serum^a*

Passage	CF antigen titers ^b		
	NCI-NR(1) ^c	NCI-NR(2) ^c	NCI-RR
4		<2	(32) ^d
11	<2	<2	
12			8
13		<2	8
14	<2	<2	16
16	<2	<2	16
17	<2	<2	8
18		<2	
20			32
21			32
22	<2		
24		<2	
29			16

^a Antiserum was used at a 1:20 dilution.

^b The titers are expressed as the reciprocals of the highest positive dilutions.

^c Normal cell lines.

^d Figure in parentheses indicates the titer of 20 times concentrated culture medium.

was inoculated into three sets of 48-hour-old monolayers prepared from one of the normal cell lines in its 12th passage. The first set of cells was subcultured on the 7th; the second, on the 10th; and the third, on the 13th day after inoculation. Each of the three sets of monolayers was accompanied by an uninoculated control. Subsequent splitting of cells and CF testing for antigen reacting with Rauscher immune sera were carried out weekly. As can be seen in Table 2, detectable antigen was observed approximately 3 weeks after inoculation of the virus, and significant titers were obtained by the end of the 4th week. No evidence of antigen was noted in the first two passages. The control cultures remained free from this antigen at each passage level. In the third subculture, antigen in set C reached a significant titer, whereas set A barely showed the presence of antigen at dilution 1:2, and set B had a low titer. A linear correlation seemed to exist between the number of days elapsed before the first subculture was performed and the titer of CF-reactive antigen at the third passage. The results indicated that a CF antigen reacting with Rauscher immune sera was induced in a normal rat cell line exposed to the supernatant fluids of cultures which were derived from the kidneys of a leukemic rat.

Electron microscopy. Supernatant fluids and cells of NCI-RR line and those of the control lines were examined at various passage levels.

Viral buds were observed in cultures of NCI-RR cells in all passages examined (Fig. 5, 6, 9). Viral particles in different stages of maturation were seen scattered or bridged in clusters (Fig. 11) around the cell. Figure 5 shows two interconnecting particles with electron-lucent nucleoids. Each particle has a dense inner ring, an intermediate shell, and a thin outer coat. Occasionally, budding virus with a long pedicle was observed (Fig. 5). Figure 7 illustrates at least eight particles sectioned at various distances from their centers. One of them shows an angular head with a long double-membraned tail. Three mature particles with electron-dense nucleoids and irregular outer coats are shown in Fig. 8. The upper one is engulfed in the invagination of a portion of the plasma membrane, beneath which deposits of electron-dense material are evident (arrow). The viral surface is separated from the cell membrane by thin filamentous materials. Whether this picture represents the early stage of a pinocytotic process cannot be ascertained. It is conceivable that such activity might occur in cultures in which viral replication is taking place. Incomplete particles with discontinuous coats (Fig. 12) were more frequently observed in early passages than in the later ones. Observations by thin section of

TABLE 2. *Detection of CF antigen in NCI-NR(2) cells inoculated with supernatant fluids from antigen-producing NCI-RR cells*

Culture ^a	Passage	Age of culture when subcultured and CF-tested ^b	CF reaction ^c when cell extract was diluted				
			1:2	1:4	1:8	1:16	1:32
A	1	7	0				
	2	14	0				
	3	21	2	0	0	0	0
	4	28	4	4	3	1	0
	5	35	4	3	1	0	0
B	1	10	0				
	2	17	0				
	3	24	4	4	1	0	0
	4	31	4	3	1	0	0
	5	38	4	3	1	0	0
C	1	13	0				
	2	20	1				
	3	27	4	4	4	2	0
	4	34	4	4	3	1	0
	5	41	4	4	3	1	0

^a For the sake of simplicity, the negative CF reaction of the controls is not tabulated.

^b Cumulative number of days from initiation of experiment.

^c Degree of CF reactivity; 4+ = complete fixation of complement.

the fifth subcultures, which were derived from the normal rat kidney cells inoculated with supernatant fluid of NCI-RR cultures, as described above, revealed the presence of virus in small number. This seemed to correlate with the low CF antigen titers (Table 2). Negative-staining techniques applied to the 5-day-old culture medium of a culture passaged 14 times revealed tailed particles (Fig. 10). Viral particles with tails were also observed in the supernatant fluids of the culture dated 17 November 1965, which had, as shown above, a CF antigen titer of 1:32.

Induction of leukemogenic diseases in animals by NCI-RR cells. When cell extracts from the 24th passage of the NCI-RR line were inoculated into newborn OM rats and BALB/c mice, these animals developed leukemogenic diseases indistinguishable from the original diseases described by Rauscher (14). Control animals, uninoculated or inoculated with NCI-NR cells, were free from the diseases. The results of further studies on the potential antigenicity and pathogenicity of tissue culture-grown Rauscher virus in rats and mice will be the subject of a later report.

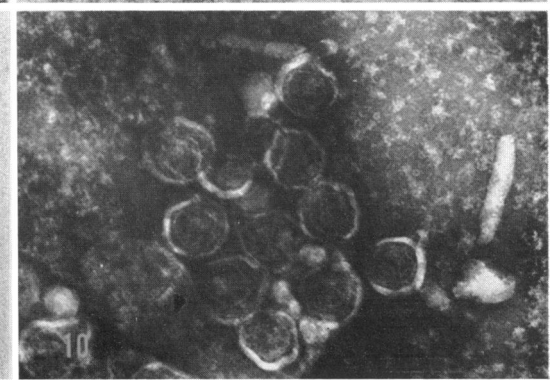
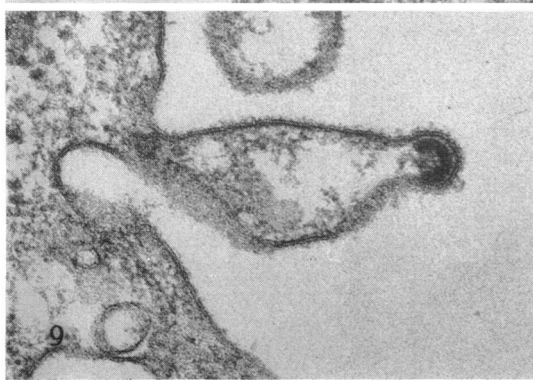
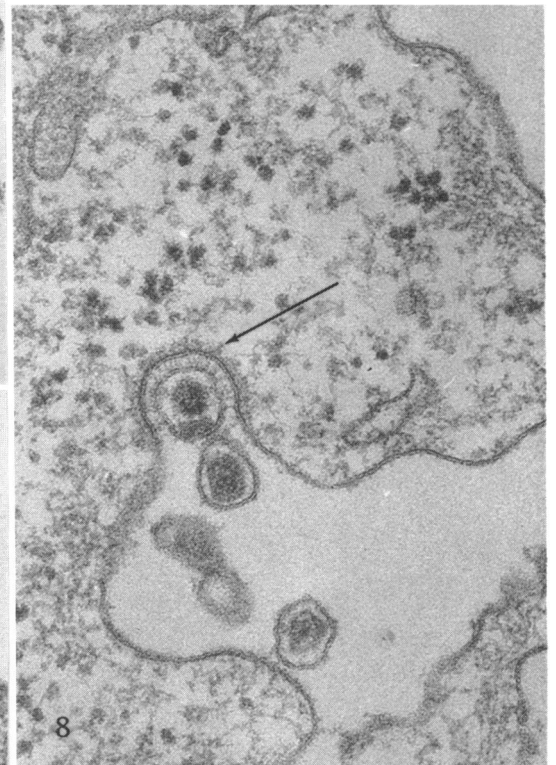
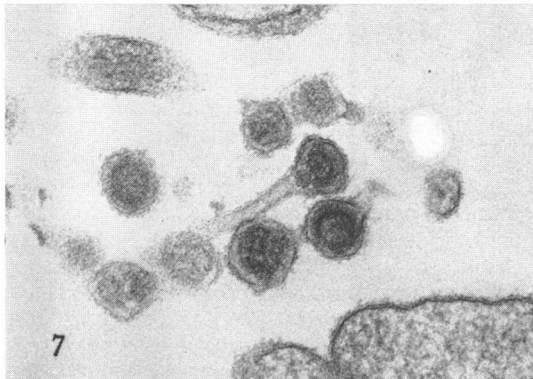
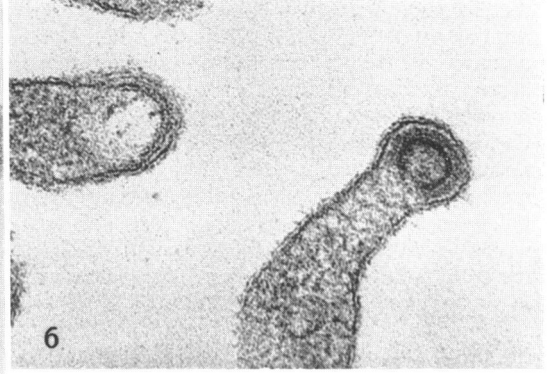
Storage of cell lines. Attempts were made to determine the viability of control cells and NCI-RR cells with 8% dimethylsulfoxide and 30% fetal calf serum at -70°C . After thawing, viable-cell counts were performed, by use of 0.1% trypan blue as diluent. After 9 weeks of storage, 85 to 90% of viable cells were recovered. When seeded into flasks, these cells grew out luxuriantly after two passages. Electron microscopic observations revealed large numbers of viral particles and occasional viral buds in the second and seventh subcultures of frozen cells.

Interferon production. The supernatant fluids from three cultures of NCI-RR cells at the 12th, 17th, and 28th passages were tested for the presence of interferon. A slight delay in the CPE of VSV in tubes pretreated with these supernatant fluids was noted on the 1st day, as compared with the tubes inoculated with culture fluids from the control lines. Complete protection of cells was not obtained in tubes pretreated with undiluted tissue culture supernatant fluids. To test for direct viral interference, subcultures from the 19th, 20th, and 30th passages of NCI-RR line and from the 17th, 19th, and 21st passages of NCI-NR line were challenged with VSV in serial 10-fold dilutions. With 10^2 TCID₅₀ of virus per tube, 4+ CPE was observed in both NCI-RR and NCI-NR monolayers within 48 hr. The data suggested that, under the present testing conditions, detectable interferon was not produced in NCI-RR cells.

Physical properties. The CF antigen derived from culture media or sonic-treated extracts of NCI-RR cells was sedimented at $20,000 \times g$ for 60 to 90 min. When culture fluids, showing the high viscosity noted above, were centrifuged at $80,000 \times g$ for 2 hr, a thick viscous material was sedimented into the pellet. Further studies are in progress to determine its nature. The data indicated that CF antigen was directly related to the presence of viral particles, and that the virus can be separated from the viscous material by high-speed centrifugation.

DISCUSSION

The persistent infection in vitro of a rat kidney cell line with Rauscher virus was indicated by: (i) the use of a host system in which no leukemia viruses had been demonstrated; (ii) the typical pathological findings of the leukemic animal from which the infected line NCI-RR was derived; (iii) the demonstration of a CF antigen reacting with specific Rauscher immune sera at various passage levels; (iv) the electron microscopic observations of typical murine leukemia viral buds at different stages of maturation in several subcultures; (v) the recovery of viral particles from the culture media of 5-month-old NCI-RR monolayers; and (vi) the observation of the leukemogenic effect of Rauscher virus in rats and mice inoculated with NCI-RR cells. A successful transmission of a CF antigen-inducing agent to a normal rat cell line was indicated by the gradual rise of CF titers, and the electron microscopic observation of Rauscher virus particles in the subsequent passages of inoculated cultures, but not in uninoculated ones. Persistence of residual Rauscher virus in passaged cultures was very unlikely, since 14 passages and 28 medium changes were performed before the supernatant fluid was used for inoculation into normal cells. The absence of detectable CF antigen in the first two subcultures could be due to one, or a combination, of the following factors: (i) the low input multiplicity employed, (ii) the age of the culture when infected, (iii) the small number of susceptible cells, (iv) the long incubation period in these cells. Relevant data presented by Manaker et al. (10) suggested that serial passage of Moloney virus in mouse-spleen cells was successful with a 14-day interval between subcultures but not with a 7-day interval. Working with Rauscher virus grown in secondary mouse embryo tissue cultures, Hartley et al. (8) carried out routine serial passages, between 14 and 20 days. They noted that, in general, definite CF antigen was produced in first-passage cultures, and that only rarely were blind passages needed



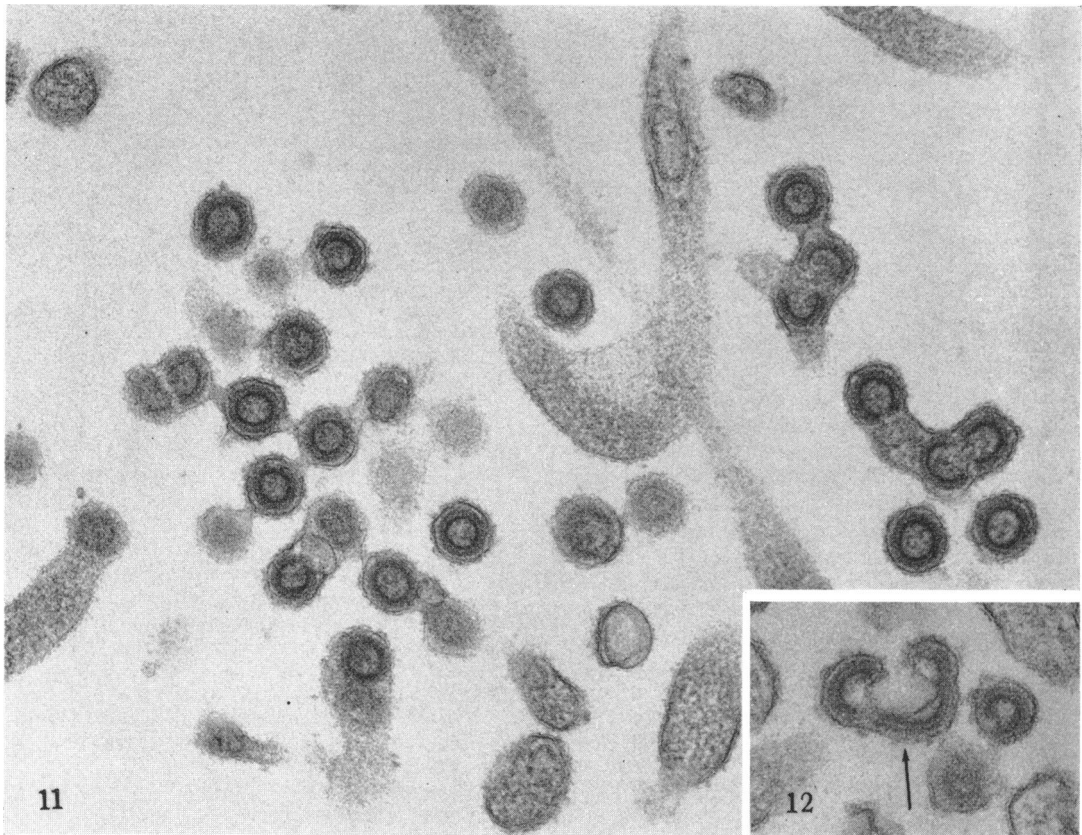


FIG. 11. Several viral particles lying at various distances from the plane of section. They are bridged together, giving a "dumbbell or chainlet" appearance (left half of picture). Partially completed particles with wide cytoplasmic bridges are shown on the right. Approximately $\times 75,000$.

FIG. 12. An abnormally incomplete particle (arrow) lying near two normal ones. Approximately $\times 75,000$.

before the CF antigen became positive. These authors also pointed out that cultures infected 18 to 24 hr after seeding gave higher antigen titers than did cultures infected 5 to 6 days after seeding. Titration of input and recoverable virus in animals, as well as kinetic studies of inoculated cultures by specific fluorescent antibody, might provide further information on this point.

Since the classical method of production and assay of interferon was used, the failure of concentrated culture media of NCI-RR cells to protect test cultures indicated that Rauscher virus did not induce the production of interferon in these cells. This deduction is further supported by the absence of cellular resistance to VSV infection in NCI-RR and NCI-NR cultures.

FIG. 5. Portion of cytoplasm of a rat kidney cell from the second subculture. The two Rauscher virus particles in the upper right corner are interconnected by a bridge and have an outer coat, a dark inner ring, an intermediate shell, and an electron-lucent nucleoid. The viral bud in the lower left corner is sectioned on a plane parallel to its diameter. Note the long pedicle. Approximately $\times 52,000$.

FIG. 6. Detail of viral bud. Note the incomplete intermediate membrane. Approximately $\times 100,000$.

FIG. 7. Viral particles lying at various distances from the plane of section. One particle has an angular head and a long tail. Approximately $\times 75,000$.

FIG. 8. Portion of the cytoplasm of a rat kidney cell. The plasma membrane shows an invagination in which one viral particle with an electron-dense nucleoid is lodged. Note the deposit of electron-dense material beneath the invaginated plasma membrane, and the filamentous material which separates the viral surface from the cytoplasmic membrane. All three particles have a "baggy" appearance and a single outer coat. Approximately $\times 75,000$.

FIG. 9. Another viral bud in tissue culture of the 10th passage of rat kidney cells. Approximately $\times 75,000$.

FIG. 10. Negatively stained high-speed centrifugal pellet of supernatant fluids from rat kidney cultures in their 14th passage. Some particles show tails. Approximately $\times 75,000$.

Sinkovics et al. (16) described an interferon-like substance in leukemic mouse spleen cultures. On the other hand, Peries et al. (11), using the classical method for demonstrating interferon, were not able to detect interferon in a mouse cell line infected with Rauscher virus (18).

The spherical forms of Rauscher virus propagated in rat kidney culture are morphologically very similar to the agents which cause Gross leukemia (1), Friend leukemia (3), and Moloney leukemia (2). Dmochowski et al. (4) and Dalton et al. (2) described cylindrical forms found in cytoplasmic vacuoles of megakaryocytes of bone marrows of rats infected with Gross and Moloney virus, respectively. Unlike megakaryocytes, the fibroblast-like cells examined in the present study only occasionally showed cytoplasmic vacuoles. In no instances were there tubular forms of virus in these vacuoles. Morphologically, the Rauscher virus present in these rat kidney cultures more closely resembles the spherical particles described in mouse spleen and thymus cultures (Zeigel et al., National Cancer Institute Monograph 22, *in press*).

ACKNOWLEDGMENTS

We are indebted to J. W. Hartley for supervising the complement-fixation tests. The excellent assistance of Marian V. A. Smith, Barbara V. Allen, and Priscilla B. Hildebrand is fully acknowledged.

LITERATURE CITED

1. BERNHARD, W., AND L. GROSS. 1959. Presence de particules d'aspect viral dans les tissus tumoraux de souris atteintes de leucémies induites. *Compt. Rend.* **248**:160-162.
2. DALTON, A. J., F. HAGUENAU, AND J. B. MOLONEY. 1964. Further electron microscopic studies on the morphology of the Moloney agent. *J. Natl. Cancer Inst.* **33**:225-275.
3. DE HARVEN, E., AND C. FRIEND. 1960. Further electron microscope studies on a mouse leukemia induced by cell-free filtrates. *J. Biophys. Biochem. Cytol.* **7**:747-752.
4. DMOCHOWSKI, L., C. E. GREY, F. PADGETT, AND J. A. SYKES. 1963. Studies on the structure of the mammary tumor-inducing virus (Bittner) and of leukemia virus (Gross), p. 85-121. *In* Viruses, nucleic acids, and cancer. The Williams & Wilkins Co., Baltimore.
5. DUC-NGUYEN, H., AND W. HENLE. 1966. Replication of mumps virus in human leukocyte cultures. *J. Bacteriol.* **92**:258-265.
6. DUNNING, W. F., AND M. R. CURTIS. 1957. A transplantable acute leukemia in an inbred line of rats. *J. Natl. Cancer Inst.* **19**:845-853.
7. HARTLEY, J. W., AND W. P. ROWE. 1966. Production of altered cell foci in tissue culture by defective Moloney sarcoma virus particles. *Proc. Natl. Acad. Sci. U.S.* **55**:780-786.
8. HARTLEY, J. W., W. P. ROWE, W. I. CAPPS, AND R. J. HUEBNER. 1965. Complement-fixation and tissue culture assays for mouse leukemia viruses. *Proc. Natl. Acad. Sci. U.S.* **53**:931-938.
9. HUEBNER, R. J., W. P. ROWE, H. C. TURNER, AND W. T. LANE. 1963. Specific adenovirus complement-fixing antigens in virus-free hamster and rat tumors. *Proc. Natl. Acad. Sci. U.S.* **50**:379-389.
10. MANAKER, R. A., P. C. STROTHER, A. A. MILLER, AND C. V. PICZAK. 1960. Behavior *in vitro* of a mouse lymphoid leukemia virus. *J. Natl. Cancer Inst.* **25**:1411-1419.
11. PERIES, J., M. BOIRON, AND M. CANIVET. 1965. Recherche d'une production d'interféron et d'une interférence virale hétérologue dans une lignée cellulaire chroniquement infectée par le virus de Rauscher. *Ann. Inst. Pasteur* **109**:595-600.
12. PERIES, J., J. P. LEVY, M. BOIRON, AND J. BERNARD. 1964. Multiplication of Rauscher virus in cultures of mouse kidney cells. *Nature* **203**:672-673.
13. POLLARD, M., AND M. KAJIMA. 1966. Leukemia in germfree rats. *Proc. Soc. Exptl. Biol. Med.* **121**:585-589.
14. RAUSCHER, F. J. 1962. A virus-induced disease of mice characterized by erythrocytopenia and lymphoid leukemia. *J. Natl. Cancer Inst.* **29**:515-543.
15. SCHREINER, A. W., AND J. J. WILL. 1962. A transplantable spontaneous chloroleukemia in Wistar rat. *Cancer Res.* **22**:757-760.
16. SINKOVICS, J. G., AND C. D. HOWE. 1964. Growth characteristics, virus yield, and interferon assay of leukemic mouse spleen tissue cultures. *J. Infect. Diseases* **114**:359-372.
17. WEINSTEIN, R. S., AND W. C. MOLONEY. 1965. Virus-like particles associated with chloroleukemia in the rat. *Proc. Soc. Exptl. Biol. Med.* **118**:459-461.
18. 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.