JOURNAL OF VIROLOGY, July 1973, p. 177-187
Vol. 12, No. 1

Printed in U.S.A.

Induction of Type C Viruses in Cultured Guinea Pig Cells

D. P. NAYAK AND PATRICK R. MURRAY

Department of Medical Microbiology and Immunology, School of Medicine, University of California, Los Angeles, California 90024

Received for publication 24 October 1972

Particles morphologically resembling type C viruses were activated by bromodeoxyuridine (BUdR; 10^{-4} M) treatment of cultured guinea pig cells. Virus particles were isolated from the cells of normal and leukemic strain 2 and random-bred guinea pigs (adult and embryonic). Immature virus particles with electron-lucent cores were found in the cytoplasmic matrix. The mature particles with electron-dense cores were found outside the cells and some appeared in the process of budding from the plasma membrane. The peak of virus production was observed within 4 days of BUdR treatment. When compared to the amount of virus produced in darkness, visible light enhanced virus production, whereas exposure of BUdR-treated cells to UV light either had no effect or inhibited virus production. Virus particles had a density of 1.16 gm/ml, possessed an oncornavirus-specific reverse transcriptase, and contained a large-molecular-weight RNA (65–70*S*) which dissociated into 36*S* subunits after heat denaturation. The BUdR-activated virus particles, therefore, possessed the morphological, biophysical, and biochemical characteristics of type C oncornaviruses.

There is a considerable amount of evidence for the presence of type C RNA virus genetic information in the cells of many vertebrates (25, 33, 35). In guinea pigs, virus-like particles have been observed in normal and leukemic lymphoid cells (9, 17, 18, 22) but there have been no studies demonstrating that these particles possess the biochemical, biophysical, or biological properties characteristic of a type C oncornavirus. In this report we present evidence that a type C guinea pig virus (GPV) can be activated after 5-bromo,2'-deoxyuridine (BUdR) or 5-iodo,2'deoxyuridine (IUdR) treatment of cells cultured from leukemic and normal adult guinea pigs, and from guinea pig embryos (leukemia-susceptible strain 2 and leukemia-resistant random-bred guinea pigs). These particles have the morphology of type C viruses, possess a density of 1.16 gm/ml, contain an oncornavirus-specific RNA-dependent DNA polymerase (reverse transcriptase) and a large-molecular-weight RNA (65-70*S*) which dissociates to 36*S* and smaller RNA components when denatured. Recently, Hsiung reported that virus particles morphologically similar to type C particles could be activated by BUdR treatment of spleen cells cultured from leukemic guinea pigs (12).

MATERIALS AND METHODS

Animals. Strain 2 guinea pigs and leukemic cells were originally obtained from S. Opler of Stanford University (22). Leukemia in strain 2 animals is maintained as transplantation leukemia by inoculating leukemia cells into healthy animals which develop leukemia in 2 to 4 weeks. Chromosome analysis of leukemia cells has shown that they are always of female karyotype irrespective of their source in male or female leukemic animals (36; Nayak, unpublished observations). Random-bred guinea pigs were purchased from a local farm (Curd's Caviary, Los Angeles).

Cell culture. Cells from the spleen, kidney, or muscle of nonleukemic adult guinea pigs and from 1-month-old guinea pig embryos were dispersed by trypsin and cultured in growth medium (Eagle minimal essential medium, Earle salt) supplemented with 15 to 20% inactivated fetal calf serum, glutamine (0.0292%) and tryptose phosphate broth (10%). When the organs of the leukemic animals were cultured after trypsin dispersion, an endogenous guinea pig herpesvirus (GPHV) became activated within 1 to 2 weeks in 100% of the cultures, and the entire cell population was destroyed by 3 weeks. To avoid this, 1-mm pieces of leukemic spleen, lymph nodes, or tumors were placed on stainless steel organ culture grids (Falcon Co.) and maintained in vitro for 2 to 3 weeks (7) as follows: the grid containing the pieces of tissue was transferred every day or every

other day to new organ culture dishes containing fresh medium. This avoided the leukocytes, especially macrophages and leukemic lymphoblasts, which were shed onto the bottom of the dish. Within 2 to 3 weeks, the shedding of leukocytes ceased, and a layer of fibroblastic cells grew on the grid. At this time the tissues and cells on the grid were dispersed by trypsin and cultured on plastic dishes in growth medium containing 20% inactivated fetal calf serum. By the second subculture, a relatively uniform cell population of fibroblasts became predominent. These cells, if inoculated into strain 2 guinea pig (10^6 to 10×10^6 cells/animal), failed to produce leukemia; whereas as few as 1,000 leukemic lymphoblast cells cause leukemia in all inoculated strain 2 guinea pigs (26; Nayak, unpublished data). By using this organ culture technique, we obtained six cell cultures from eight leukemic animals. These cells have undergone 10 to 40 subcultures in 3 to 8 months without any apparent GPHV activation. In two other cultures, GPHV became activated at the second and fifth passage (SP 392, Table 1). The tissues (spleen) of these 2 animals were maintained for 1 week only on the organ culture grid instead of 3 weeks. Apparently, the leukemic lymphoblasts and other mononuclear cells either maintained GPHV as a chronic infection or contained GPHV genome in cryptic form which became activated in tissue culture (20). Thus, by the organ culture technique, it was possible to establish cultures of stromal cells free from infectious GPHV by the preferential exclusion of leukemic lymphoblasts and other mononuclear cells.

Treatment of cells with BUdR. Nonconfluent cell cultures (usually 24 or 48 h after subculture) were treated with BUdR (10^{-4} M, Schwarz BioResearch, Inc.) in minimal essential medium containing dialyzed fetal calf serum (15%) and glutamine (14). After 48 h of treatment, the BUdR medium was replaced with fresh growth medium.

Assay of reverse transcriptase activity. The supernatant media from control (mock exposed) or BUdR-treated cultures were harvested and freed from subcellular debris in a Sorvall centrifuge at 10,000 rpm for 10 min. The media then was either processed immediately or after storage at 4 C for 2 to 3 days. The virus particles were pelleted at 25,000 rpm for 60 min in a SW 27 rotor. Pellets were resuspended in the DNA polymerase reaction buffer (23) consisting of 50 mM Tris-hydrochloride, pH 8.0; 30 mM KCl; 10 mM $MgCl_2$; 5 mM dithiothreitol; and 0.1% NP-40. After 10 min at 4 C, 10 nmol of dATP, 560 pmol of ^{3}H-TTP (8,700 counts per min per pmol), and 5 μg of the indicated template or an equal volume of water were added to make the final reaction mixture (100 μliters). This was incubated for 60 min at 37 C. Samples (25 μliters) were taken at zero and sixty minutes, and trichloroacetic acid precipitable counts were determined. Each assay was done in duplicate.

Purification of GPV. Cell culture supernatant was freed from subcellular debris (10,000 rpm for 10 min in a Sorvall centrifuge) and used for the purification of type C virus by two procedures reported previously (3, 24). (i) All of the proteins, including virus, were precipitated by adding $(NH_4)_2SO_4$ to 50% saturation. The precipitate was redissolved in TE buffer (0.01 M Tris, pH 7.4, 0.001 M EDTA), concentrated at the interface of 20 and 60% sucrose, and then purified by density equilibrium centrifugation in a 15 to 60% sucrose gradient. (ii) In the second method, culture supernatant was layered on the top of a 60 and 20% discontinuous sucrose gradient, and the virus was collected at the interface by centrifugation for 1 h at 27,000 rpm. This step was repeated to further concentrate the virus, and the cushioned virus band was then collected, diluted, and analyzed by sucrose density equilibrium centrifugation. Procedures used for isolation and characterization of the virus RNA have been previously described (3, 24).

Electron microscopy. The procedure used for tissue and cell fixation, thin sectioning, and negative staining have been reported earlier (19).

RESULTS

The effect of BUdR on cultured guinea pig cells. BUdR treatment (10^{-4} M) for 48 h had little or no cytopathic effect on cultured guinea pig cells. However, the multiplication of BUdR treated cells was slower than that of untreated cells. By day 4 of BUdR treatment (i.e., day 2 after replacing the BUdR medium with growth medium), there were half as many cells in the BUdR-treated plates as in the control (mock exposed) plates. A few cells became round and detached from the monolayer. Whereas in the electron micrographs, the membrane of the cells without BUdR treatment (not shown) was distinctly trilaminar (19), the membranes of BUdR-treated cells (endoplasmic reticulum, nuclear membrane, mitochondrial membrane, plasma membrane) lost the typical trilaminar structure and appeared blurred (Fig. 1). The toxic effect of BUdR on the synthesis of cellular macromolecules is not well understood. BUdR has been reported to inhibit cellular RNA synthesis in normal and Rous associated virus-infected chicken cells, but not in Rous sarcoma virus-transformed chicken cells (15). The effect of BUdR on the synthesis of specific macromolecules in cultured guinea pig cells has not yet been determined.

Morphology of BUdR-activated particles. Virus-like particles are not found in cells cultured from embryonic or adult normal and leukemic guinea pigs (12, 19). A few virus-like particles budding from endoplasmic reticulum have been consistently found in leukemic lymphoblasts (9, 18, 22). These particles have electron-lucent cores and are always associated with cell cysternae, and rarely are found in the extracellular space. There is a great deal of controversy whether these particles represent type A or type C viruses (9, 11, 22).

Subsequent to the BUdR treatment of cul-

TABLE 1. *Activation of oncornavirus reverse transcriptase in guinea pig cells*[a]

Group	Cells[b]	Passage	BUdR treatment[c]		No BUdR treatment[c]	
			Poly rA: oligo dT	Poly dA: oligo dT	Poly rA: oligo dT	Poly dA: oligo dT
(A) Leukemic strain 2	LN 173	(13)	1,864	112	85	119
	LN 173	(14)	1,785	72	530	300
	LN 173	(18)	1,920	20	72	84
	SP 325	(1)	668	0	242	328
	LN 392	(4)	1,307	25	146	209
	SP 392	(4)	1,903	0	72	87
	SP 392/GPHV	(5)	102	124	205	460
	LN 394	(21)	2,394	170	285	209
(B) Non-leukemic strain 2	GPEF-1	(2)	6,025	1,195	284	430
	GPF-4	(2)	2,090	145	NT[d]	NT
	GPEF-6	(2)	3,961	387	0	0
(C) Random-bred guinea pig	GPEK-1	(2)	2,462	188	146	72
	GPEF-3	(2)	1,800	156	NT	NT
	GPK-2	(2)	1,060	202	NT	NT
	GPEF-4	(2)	1,972	176	187	243
	GPEF-5	(2)	2,391	209	109	163
	GPEF-2	(2)	2,520	210	18	36
	GPEF-2/GPHV	(4)	104	160	180	148
D) Mouse leukemia virus[e]	G-MuLV R-MuLV		1,490	272	NT	NT
E) Cellular DNA polymerase[f]	Guinea pig Leukemic Lymphoblasts		105	1,251	NT	NT

[a] Cultures were treated with BUdR (10^{-4} M) for 48 h and then fed with fresh medium. Forty-eight hours later the supernatant medium was removed and assayed for virus-specific reverse transcriptase activity as described in Materials and Methods. Four dishes were used in each experiment.

[b] Trichloroacetic acid precipitable counts of 25-μliter samples of the reaction mixture after 60 min of incubation at 37 C. Trichloroacetic acid precipitable counts at zero time of the reaction (average, 60–100 counts/min) were deducted.

[c] LN 173 refers to the cells prepared from the lymph node (axillary) of leukemic guinea pig no. 173. LN 392 and SP 392 cells were cultured from the axillary lymph node and spleen of leukemic guinea pig no. 392. SP 392/GPHV cells spontaneously underwent guinea pig herpesvirus activation at passage 5, GPEF-1 (guinea pig embryo fibroblast), GPEF-2, GPEF-3, GPEF-4, GPEF-5 and GPEF-6 cultures were derived from 6 separate guinea pig embryos. GPF-4 (muscle fibroblasts from adult normal guinea pigs), GPEK-2 (guinea pig embryo kidney), and GPK-2 (adult guinea pig kidney) cultures were initiated as described above. GPEF-2/GPHV cells were infected with guinea pig herpesvirus before BUdR treatment.

[d] NT = not tested.

[e] Gross murine leukemia virus (G-MuLV)-producing mouse lymphoblasts and Rauscher murine leukemia virus (R-MuLV)-producing rat fibroblasts were the gifts of D. Imagawa and R. Saxton, respectively.

[f] Cellular DNA polymerase was isolated from the cytoplasm of leukemic guinea pig lymphoblasts suspended and disrupted in RSB, pH 8.5 (0.01 M Tris, pH 8.5; 0.01 M KCl; 0.0015 M $MgCl_2$; and 0.001 M B-mercaptoethanol). The homogenate was clarified of nuclei, whole cells, mitochondria and was then centrifuged at 105,000 $\times$ *g* for 1 h. The resulting supernatant fluid was subjected to ammonium sulfate fractionation and G-200 filtration. Fractions containing the DNA polymerase were stored at −70 C. (M. Shoyab and D. P. Nayak, manuscript in press).

tured cells, virus particles (GPV) were activated from all of the cell cultures established from normal and leukemic animals. These viruses resembled both mature and immature type C viruses and had a diameter of 100 nm. The majority of the immature GPV were found inside the cell, whereas mature particles were found outside the cells (Fig. 1). Intracellular particles were found in the cytoplasmic matrix but did not appear budding from endoplasmic reticulum as found in leukemic lymphoblasts in vivo. Some viruses were also associated with

plasma membrane and appeared in the process of budding (Fig. 1B and C). Relatively larger numbers of GPV were found associated with the round cells floating in the medium after BUdR treatment (Fig. 1D). There was no morphological difference between the particles activated from cultured cells of normal embryos and normal or leukemic adult guinea pigs.

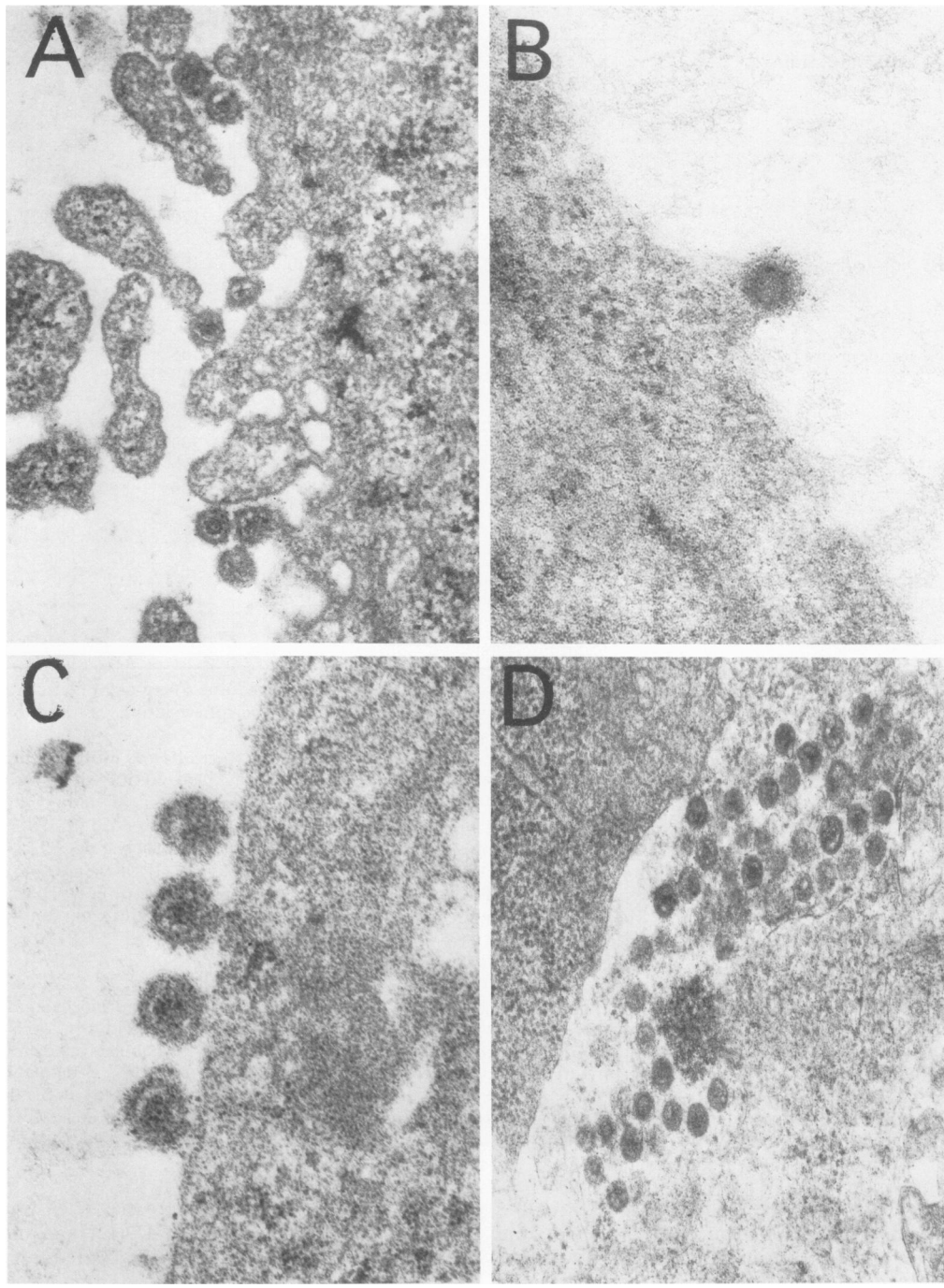

FIG. 1. *(A) Morphology of the extracellular BUdR activated virus particles in LN 394 cell cultures, ×45,750. (B) Budding virus particles, ×83,800. (C) Mature virus particles, ×112,750. (D) Extracellular virus particles associated with cell floating in the culture medium, ×34,800.*

Activation of oncornavirus reverse transcriptase. BUdR treatment resulted in the production of particles containing oncornavirus specific reverse transcriptase. This enzyme had the same template specificity as Gross murine leukemia virus (G-MuLV) and Rauscher murine leukemia virus (R-MuLV), and was distinct from the cellular DNA polymerase isolated from leukemic guinea pig lymphoblasts (Shoyab and Nayak, manuscript in press). The results are presented in Table 1. In brief, both the BUdR-activated GPV reverse transcriptase (Table 1A, B, C) and the MuLV transcriptase (Table 1D) have a higher activity with poly rA:oligo dT than with poly dA:oligo dT as the template. The reverse was true for guinea pig cellular DNA polymerase (Table 1E) (22). All cell cultures obtained from guinea pigs (leukemic strain 2, nonleukemic adults and embryos, random bred and strain 2) were induced by BUdR treatment to release particles containing the oncornavirus-specific reverse transcriptase (Table 1). In addition, the results were the same whether primary, secondary, or long term (e.g., 30 passages) cultures were used. Occasionally, the supernatant of untreated cells appeared to contain increased enzyme activity (Table 1A, LN 173, passage 14). However, this did not represent a spontaneous activation of GPV, but rather resulted from the insufficient washing of the sample during trichloroacetic acid precipitation. (i) A high count was present also in the poly dA:oligo dT reaction; (ii) no activity was found in the supernatant of the same culture in other passages (Table 1A, LN 173, passage 13 and 18); (iii) particles containing the virus-specific reverse transcriptase could not be detected in density gradients; and (iv) GPV could not be found by electron microscopy. In no case did we observe the spontaneous activation of virus in cultured guinea pig cells.

Particles containing virus-specific reverse transcriptase were not found in any leukemic guinea pig plasma. Although leukemic lymphoblasts contained virus-like particles budding from the endoplasmic reticulum in vivo (9, 18, 22), we failed to detect the release of any type C particles by lymphoblasts cultured in vitro. BUdR treatment did not activate detectable quantities of GPV from these lymphoblasts since they could not be grown and maintained in vitro for a sufficient length of time (20). These lymphoblasts contained a guinea pig herpesvirus (GPHV) genome which was not expressed in vivo but became partially activated in vitro. Consequently, all of the lymphoblasts in tissue culture died within 96 h. Cultures of guinea pig cells exhibiting the cytopathic effect of GPHV, due to spontaneous activation (Table 1A, SP 392/GPHV, passage 5) or exogenous infection (Table 1C, GPEF-3/GPHV, passage 4), did not release any viral reverse transcriptase or type C particles after exposure to BUdR. However, in the absence of GPHV, both of these cell cultures released particles containing virus-specific reverse transcriptase. Even the partial expression of GPHV genome killed the cells, and, therefore, interfered with the production of type C virus which required continuous cellular function especially transcription and translation (32, Nayak, unpublished data). Limited experiments with IUdR indicated that it was equally effective as the activating agent in this system.

Effect of visible and UV light on GPV production. Because of the previous reports of BUdR incorporated DNA sensitivity of light (28), the initial experiments of GPV activation with BUdR were carried out in the dark. Since this was somewhat inconvenient, we decided to determine the effect of visible laboratory light (General Electric F40CW) and UV light on the BUdR activation of virus particles. The results (Fig. 2A) show that visible laboratory light and short exposure to UV light (5 s) did not have an inhibitory effect on virus activation with BUdR. Rather, visible light enhanced the BUdR activation of virus by more than twofold. However, the longer exposure to UV light (30 s or more) significantly reduced the virus production in BUdR-treated cells. In another system involving the induction of polyoma virus from polyoma transformed hamster cells, UV exposure of BUdR-treated cells increased the virus production over that produced by non-UV-exposed BUdR-treated cells (10). Inhibition of virus production in guinea pig cells may be due to differences in cellular sensitivity and variations in exposure to UV. UV light or visible light alone did not activate any GPV in non-BUdR-treated guinea pig cells as determined by the polymerase assay (Fig. 2B). All of the subsequent experiments involving BUdR treatment, therefore, were carried out under normal laboratory lighting.

Kinetics of GPV production. The kinetics of the production of type C virus particles after BUdR treatment were determined by assaying for reverse transcriptase activity in the supernatant. Cultured cells from a random-bred guinea embryo (GPEF-3) and a leukemic adult guinea pig (LN 394) were studied. The results of a representative experiment performed with a guinea pig embryo fibroblast culture (GPEF-3) are shown in Fig. 3. As in the murine system (29), the induction of type C viruses were in-

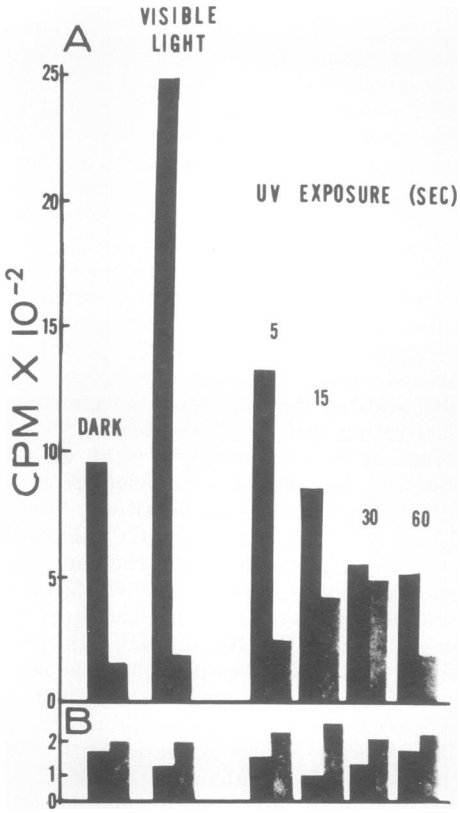

FIG. 2. *Effect of visible light and UV light on GPV production. Cultures were exposed to BUdR (10^{-4} M) (A) or mock exposed (B). The cultures were handled and maintained in complete darkness or handled and maintained in the presence of normal laboratory light. The UV-treated cultures were handled and maintained in complete darkness. They were exposed to UV irradiation (4 ergs per mm^2 per s) for variable periods of time immediately after BUdR treatment. The old media in all cultures was replaced with fresh growth medium after 48 h of BUdR treatment. Forty-eight hours later the culture media were harvested and assayed for virus DNA polymerase activity with poly rA:oligo dT (heavy shade) and poly dA:oligo dT (light shade) in duplicate.*

duced for a brief period of time after exposure to BUdR. The peak of virus production was observed on the day 3 or 4 of BUdR treatment (i.e., the day 1 or 2 after removal of BUdR medium). By day 6 the induction of GPV containing virus-specific reverse transcriptase ceased. No enzyme activity was detected in the supernatant of uninduced cultures. The kinetics of virus activation were similar in cells cultured from leukemic and nonleukemic animals (adult or embryonic). The mechanism of BUdR activation and the subsequent suppression of type C virus particles in murine or cavian cells is not presently understood. The continuous incorporation of BUdR into cellular DNA may be required for the virus activation, whereas the toxicity of BUdR may subsequently suppress the production of type-C viruses. As mentioned earlier, BUdR treated cells grew more slowly, and the membranes of BUdR-treated cells did not have the distinct trilaminar appearance of the untreated cells (Fig. 1).

Biophysical properties of GPV. To determine the physical nature of the particles containing the oncornavirus-specific reverse transcriptase, the culture fluid from 40 BUdR-treated dishes was harvested on day 4. GPV

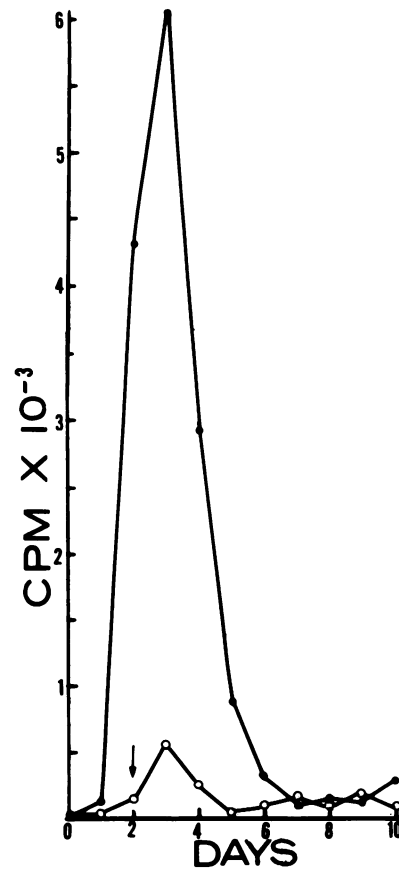

FIG. 3. *Kinetics of GPV activation after BUdR treatment. Groups of GPEF cultures were treated with BUdR (10^{-4} M) medium which was replaced on day 2 as shown by the arrow. Each point represents the virus-specific polymerase activity present in a 24-h harvest of culture medium. On day 1 and 2, GPV released in the BUdR medium was assayed. On day 3 and onwards GPV released in the growth medium was determined. Poly rA:oligo dT (●), Poly dA:oligo dT (○).*

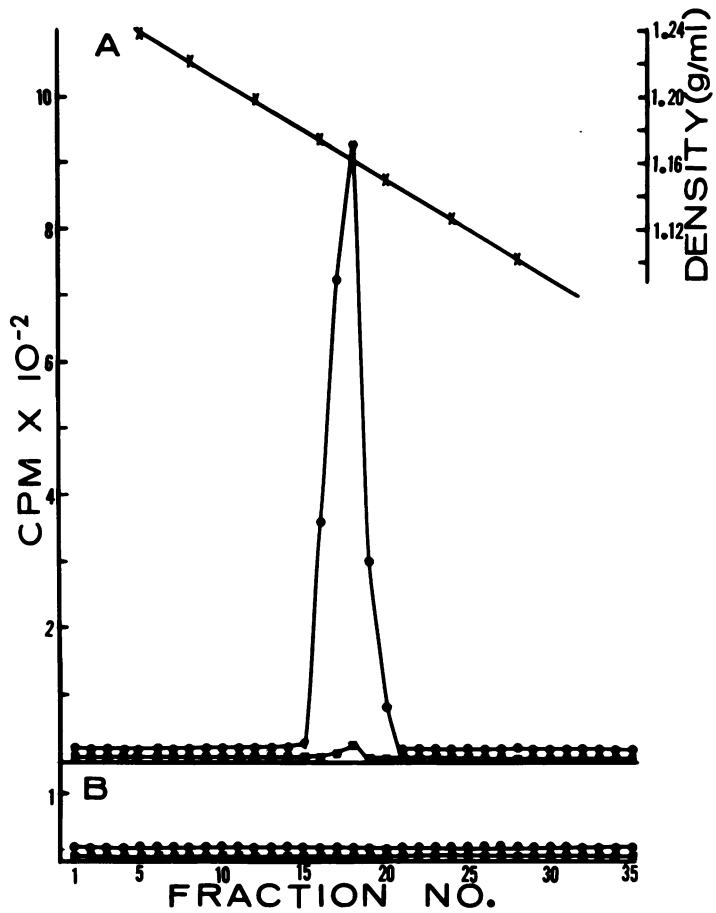

FIG. 4. *Sucrose gradient analysis of virus particles containing DNA polymerase. Groups of cultures were exposed to BUdR (A), and others kept as control were mock exposed (B). At the end of 72-h post-BUdR treatment the culture media from both groups were collected twice consecutively at 8-h intervals. The medium was freed of cellular debris and concentrated at the interface of 70 and 20% sucrose by centrifuging at 25,000 rpm for 1 h at 2 C in a SW27 rotor. The virus band was diluted with NT pH 7.4 (0.1 M NaCl; 0.01 M Tris-hydrochloride, pH 7.4) and centrifuged over a preformed linear sucrose gradient (70 to 20% wt/vol in NT, pH 7.4; 16 h at 38,000 rpm in a SW40 rotor at 4 C). Fractions were collected from the bottom of the gradient and analyzed for refractive index. Samples of each fraction were diluted, pelleted at 30,000 rpm for 1 h in a SW50 rotor, and analyzed for DNA polymerase activity by using poly rA : oligo dT (●) and poly dA : oligo dT (■). Acid precipitable counts from duplicate assays of the same fraction are presented.*

present in the medium was freed from cellular debris, concentrated by the second method at the interface of 60 and 20% sucrose (see Materials and Methods), and analyzed by density equilibrium centrifugation in a sucrose gradient (3, 24). Fractions of the gradient were collected, measured for density, and assayed for enzyme activity. When poly rA : oligo dT was used as the template, a peak of polymerase activity was found at the density of 1.16 gm/ml (Fig. 4A). No appreciable activity was found with poly dA : oligo dT in any fraction. The same amount of supernatant from mock BUdR-treated cultures failed to exhibit activity with either synthetic template (Fig. 4B). In a similar experiment using ^{3}H-uridine, a radioactive peak of virus particles was found at the density of 1.16 gm/ml (Fig. 5). In control cultures without BUdR treatment no such peak was observed. When samples were examined by negative staining, electron micrographs confirmed that virus-like particles were confined to peak fractions. When GPV was concentrated by $(NH_4)_2SO_4$, the profile of GPV in a density equilibrium gradient was broad, and the peak appeared at 1.18 to 1.20 gm/ml. Ammonium sulfate apparently disrupted the labile virus envelope and released partial cores.

Nature of GPV-RNA. ^{3}H-uridine-labeled virus particles were isolated from peak fractions. The virus RNA was extracted by the phenol–sodium dodecyl sulfate method (21), precipitated with ethanol overnight, and analyzed in sucrose velocity gradient (Fig. 6A). A high-molecular-weight RNA (65-70*S*) was found, along with varying amounts of 28, 18, and 4*S* RNA components. The amount of the small RNA components ($<28S$) in virus preparations varied from batch to batch and increased invariably with the longer interval between the harvest of the culture medium. GPV-RNA molecules often formed aggregates in sucrose gradients containing 0.1 M NaCl. Addition of unlabeled carrier RNA during phenol extraction and ethanol precipitation might have facilitated such aggregation. Such nonspecific aggregates have been reported in RNAs of tumor viruses and also in influenza viruses (3, 4, 6, 8). The profile of the RNA did not change when analyzed in sucrose gradients containing sodium dodecyl sulfate (0.5%) which reduces the association of any protein in forming RNA aggregates. GPV-RNA sedimented slowly in a low-salt (0.001 M) sucrose gradient (Fig. 6B) as would be expected of single-stranded RNA (8). The profile of GPV-RNA was similar to that of the RNA of Gross-MuLV when analyzed simultaneously in low-salt (Fig. 6B, C) as well as in high-salt (0.1 M) sucrose gradients. Finally, when the GPV-RNA was denatured by heat at 75 C for 3 min, we found a major peak around 36*S* with minor smaller components (Fig. 6D). The relative proportion of RNA components smaller than 36*S* increased after denaturation

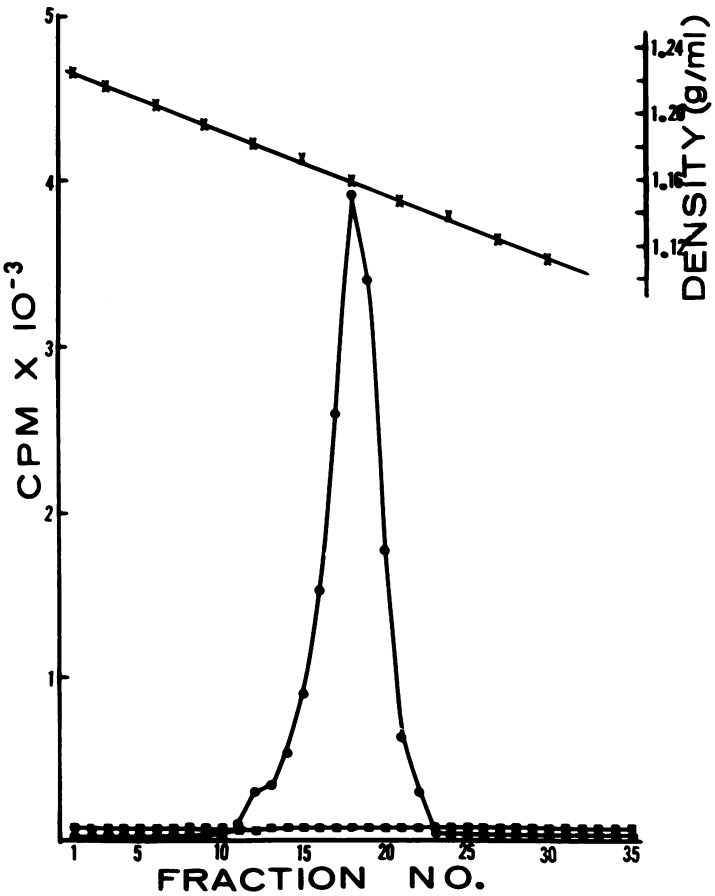

FIG. 5. *Sucrose gradient analysis of ^{3}H-uridine-labeled virus particles. Seventy-two hours after BUdR treatment, the cultures were labeled with ^{3}H-uridine (10 μCi/ml; specific activity, 29.5 Ci/mmol, Schwarz/Mann) for 2 consecutive 8-h periods. The medium was analyzed for ^{3}H-uridine-labeled virus in a sucrose density gradient. A sample of each fraction was used to determine radioactivity and density. Symbols:* (O) *BUdR treated;* (■) *control.*

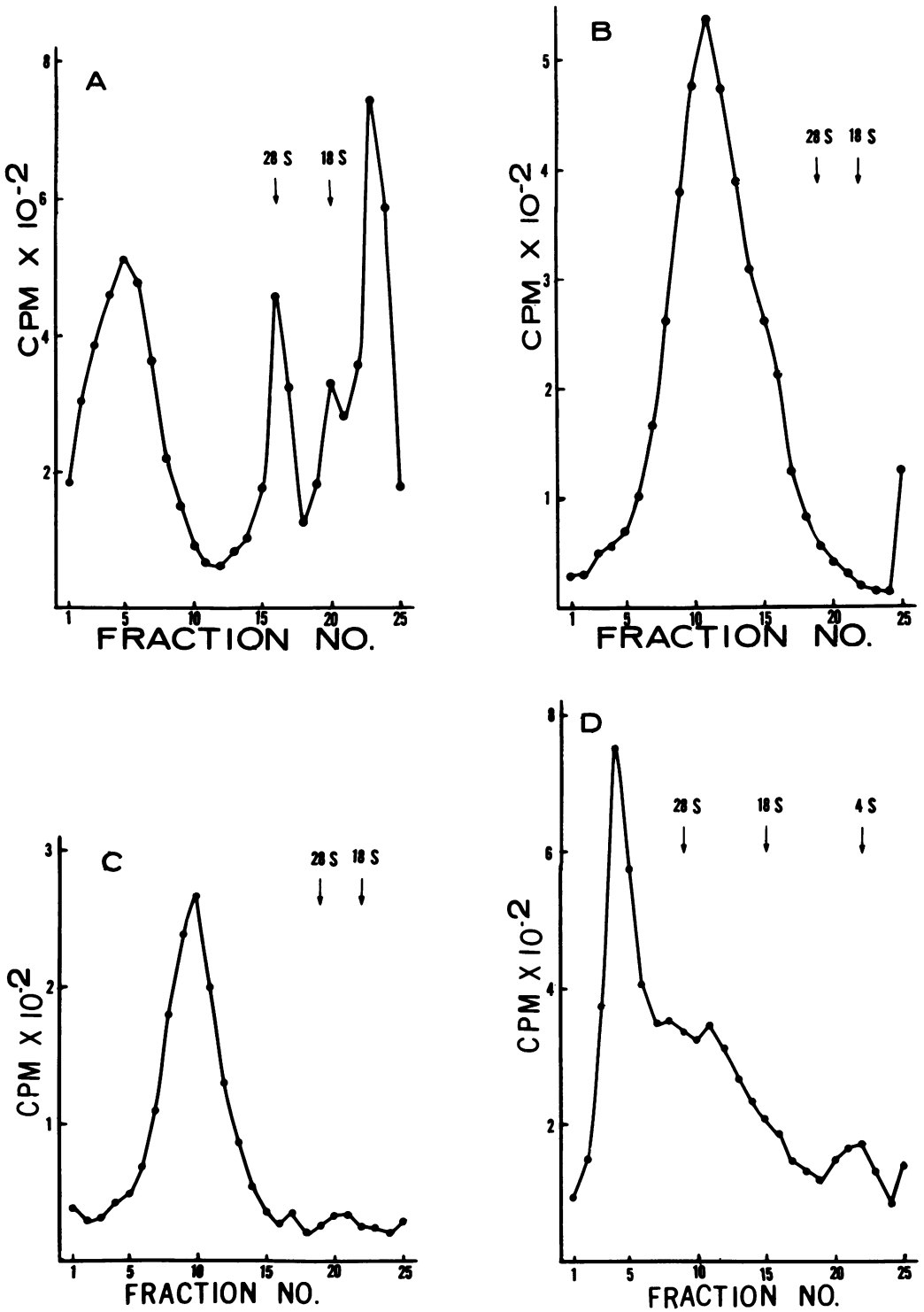

FIG. 6. *Velocity sedimentation of ^{3}H-RNA of GPV in sucrose gradients. (A) ^{3}H-uridine-labeled virus particles were harvested at 2-h intervals for 4 consecutive changes. The virus was concentrated and purified by equilibrium centrifugation. The virus fractions were pooled and diluted with NTE, pH 7.4 (0.1 M NaCl; 0.01 M Tris-hydrochloride, pH 7.4; 0.001 M EDTA). RNA was extracted by phenol-SDS method and analyzed in a sucrose velocity gradient (20–5%) containing NTE (pH 7.4) with a 60% sucrose cushion (0.3 ml) in a SW50.1 rotor (49,000 rpm for 50 min at 4 C). A sample of each fraction was counted for acid precipitable radioactivity. Fractions 2 to 7 were pooled. The RNA was precipitated by adding two volumes of alcohol and used for further analysis in B and D.*

of 70S RNA when the guinea pig virus preparation was harvested every 16 to 18 h instead of every 2 to 3 h.

DISCUSSION

This report extends the previous observations that various chemical inducers, particularly BUdR and IUdR, can activate type C virus particles from mouse (2, 16), chicken (35), human (30, 31), rat (14) and, recently, from guinea pig cells (12). Type C viruses are quite prevalent in mouse and chicken tissues, and their spontaneous induction in the laboratory is also common (1, 34). Therefore, one cannot disregard the possibility of a chronic infection in these cells releasing virus particles at a nondetectable level prior to chemical induction. However, experimental evidence supports that IUdR or BUdR may, at least in some cases, activate an unexpressed or partially expressed cellular gene coding for virus-specific information (13). In human cells the only evidence of the BUdR-induced virus is based on morphology (30, 31).

Guinea pig cells may provide an experimental system to study the activation of type C viruses with less concern about the possibility of exogenous infection. For example, as opposed to the murine and avian systems, we are unaware of any evidence that guinea pigs contain infectious type C viruses. In fact, to our knowledge there are no guinea pig tumors actively releasing infectious type C viruses. Furthermore, virus-like particles in guinea pigs are rare; i.e., they are only observed in leukemic lymphoblasts (9, 18, 22) and occasionally in chemically-induced sarcomas (11) and the germinal centers of normal guinea pigs (17). These particles are noninfectious, but from endoplasmic reticulum, lack electron-dense cores, are rarely found outside the cell, and cannot be grown in tissue culture. The morphological classification and the biological identity of these particles have been disputed (9, 11, 22). Our preliminary data suggest that the virus-like particles found in leukemic guinea pig cells is due to the expression of endogenous virus genes (Nayak, unpublished data). There is no evidence of any spontaneous induction of type C viruses from cultured guinea pig cells either in this laboratory or, to our knowledge, in other laboratories.

BUdR caused activation of type C virus particles in all of the cultures of normal and leukemic guinea pig cells tested to date. Cells of strain 2 and cross-bred guinea pigs were equally susceptible to BUdR induction. Thus, GPV, potentially inducible from any guinea pig cell, appears to be a truly endogenous virus.

Mature GPV with electron-dense cores were found outside the cell. Some were in close association with the plasma membrane, while others were in the process of budding (Fig. 1). Numerous virus particles were also found in the cytoplasmic matrix. Both extracellular and intracellular particles have been observed in BUdR-treated human cells (30, 31). The morphology of GPV appeared to be similar to that of type C viruses. However, we are unable to determine the details of the morphological characteristics of GPV with certainty since the viral envelope and cellular membrane have lost their trilaminar structure after BUdR treatment (Fig. 1).

GPV has a density of 1.16 gm/ml, contains an endogenous oncornavirus-type reverse transcriptase, and has a high-molecular-weight RNA (65-70S) which dissociates after denaturation into smaller RNA components (36S). Based on these morphological, biophysical, and biochemical observations, GPV induced by BUdR appears to have properties common to that of type C oncornaviruses (37).

The observations reported here support the postulation of Huebner and Todaro (13) that a type C RNA virus genome may be present in normal cells. However, the role that the endogenous virus genome may play in tumor induction in guinea pigs or in other animal systems remains uncertain. Attempts to infect uninduced cells in vitro or induce leukemia in vivo by BUdR-induced GPV or by the cells releasing virus particles after BUdR treatment have not yet proven successful.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of Della Goldblatt, Zane Price's help with the electron microscopy, and the assistance of Maureen Murray.

This investigation was supported by grants from the American Cancer Society, by a Dernham Fellowship (D-152) of the American Cancer Society, California Division, and a United States Public Health Service Training Grant.

LITERATURE CITED

1. Aaronson, S. A., J. W. Hartley, and G. J. Todaro. 1969. Mouse leukemia virus: "spontaneous" release by mouse embryo cells after long term *in vitro* cultivation. Proc. Nat. Acad. Sci. U.S.A. **64**:87-94.
2. Aaronson, S. A., G. J. Todaro, and E. M. Scolnick. 1971. Induction of murine C-type viruses from clonal lines of virus-free BALB/3T3 cells. Science **174**:157-159.
3. Baluda, M. A. 1967. Characterization of some properties from leukemia viruses, p. 19-35. *In* Subviral carcinogenesis, Monograph First International Symposium on Tumor Viruses, Nagoya, Japan.
4. Barry, R. D., and P. Davies. 1968. The sedimentation of influenza virus and its RNA in sucrose density gradients. J. Gen. Virology **2**:59-69.

5. Dalton, A. J., 1972. RNA tumor viruses—terminology and ultrastructural aspects of virion morphology and replication. J. Nat. Cancer Inst. **49**:323-327.
6. Duesberg, P. H., and W. S. Robinson. 1967. On the structure and replication of influenza virus. J. Mol. Biol. **25**:383-405.
7. Eisinger, M., S. M. Fox, E. de Harven, J. L. Biedler, and F. K. Sanders. 1971. Virus-like agents from patients with Hodgkin's disease. Nature (London) **233**:104-108.
8. Erickson, R. L. 1969. Studies on the RNA from avian myeloblastosis virus. Virology **37**:124-131.
9. Feldman, D. G., and L. Gross. 1970. Electron microscopic study of guinea pig leukemia virus. Cancer Res. **30**:2702-2711.
10. Fogel, M. 1972. Induction of virus synthesis in polyoma-transformed cells by DNA antimetabolites and by irradiation after pretreatment with 5-BUdR. Virology **49**:12-22.
11. Holmes, E. C., D. L. Morton, G. Schidlovsky, and E. Trahan. 1971. Cross reacting tumor-specific transplantation antigens in methylcholanthrene-induced guinea pig sarcomas. J. Nat. Cancer Inst. **46**:693-700.
12. Hsiung, G. D. 1972. Activation of guinea pig C-type virus in cultured spleen cells by 5-bromodeoxyuridine. J. Nat. Cancer Inst. **49**:567-570.
13. Huebner, R. J., and G. J. Todaro. 1969. Oncogenes of RNA tumor viruses as determinants of cancer. Proc. Nat. Acad. Soc. U.S.A. **64**:1087-1094.
14. Klement, V., and M. O. Nicolson. 1971. Rescue of the genome of focus-forming virus from rat non-productive lines by 5-bromodeoxyuridine. Nature N. Biol. **234**:12-14.
15. Levinson, W. E., J. M. Bishop, N. Quintrell, J. Jackson, and L. Fanshier. 1970. Synthesis of RNA in normal and Rous sarcoma virus infected cells: effect of bromodeoxyuridine. Virology **42**:221-224.
16. Lowy, D. R., W. P. Rowe, N. Teich, and J. W. Hartley. 1971. Murine leukemia virus: high frequency activation *in vitro* by 5-iododeoxyuridine and 5-bromodeoxyuridine. Science **174**:155-156.
17. Ma, B. I., D. C. Swartzendruber, and W. H. Murphy. 1969. Detection of virus-like particles in germinal centers of normal guinea pigs. Proc. Soc. Exp. Biol. Med. **130**:586-590.
18. Nadel, E., W. Banfield, S. Burstein, and A. J. Tousimis. 1967. Virus particles associated with strain-2 guinea pig leukemia (L2C/N-B). J. Nat. Cancer Inst. **38**:979-982.
19. Nayak, D. P. 1971. Isolation and characterization of a herpesvirus from leukemic guinea pigs. J. Virol. **8**:579-588.
20. Nayak, D. P. 1972. Activation of guinea pig herpesvirus antigen in leukemic lymphoblasts of guinea pigs. J. Virol. **10**:933-942.
21. Nayak, D. P., and M. A. Baluda. 1968. Ribonucleic acid synthesis in cells infected with influenza virus. J. Virol. **2**:99-109.
22. Opler, S. R. 1967. Observation of a new virus associated with guinea pig leukemia: preliminary note. J. Nat. Cancer Inst. **38**:797-800.
23. Robert, M. S., R. G. Smith, R. C. Gallo. P. S. Sarin, and J. W. Abrell. 1972. Viral and cellular DNA polymerase: comparison of activities with synthetic and natural RNA templates. Science **176**:798-800.
24. Robinson, W. S., and M. A. Baluda. 1965. The nucleic acid from avian myeloblastosis virus compared with the RNA from the Bryan strain of Rous sarcoma virus. Proc. Nat. Acad. Sci. U.S.A. **54**:1686-1692.
25. Rowe, W. P., J. W. Hartley, M. R. Lander, W. E. Pugh, and N. Teich. 1971. Noninfectious AKR mouse embryo cell lines in which each cell has the capacity to be activated to produce infectious murine leukemia virus. Virology **46**:866-976.
26. Sarma, P. S., P. J. Uterhorst, U. Zebe, H. T. Wepsic, J. Whang-Peng, B. Zabar, and R. Huebner. 1970. L2C/N-B guinea pig leukemia: failure to demonstrate transmissible leukemogenic virus. *In* Proceedings of 4th International Symposium on Comparative Leukemia Research, Cherry Hill, N. J., Karger, Basel, New York.
27. Silagi, S., D. Beju, J. Wrathall, and E. de Harven. 1972. Tumorigenicity, immunogenicity, and virus production in mouse melanoma cells treated with 5-BUdR. Proc. Nat. Acad. Sci. U.S.A. **69**:3443-3447.
28. Stahl, F. W., J. M. Crasemann, L. Okun, W. Fox, and C. Laird. 1961. Radiation-sensitivity of bacteriophage containing 5-bromodeoxyuridine. Virology **13**:98-104.
29. Stephenson, J. R., and S. A. Aaronson. 1972. Genetic factors influencing C-type RNA virus induction. J. Exp. Med. **136**:175-183.
30. Stewart, S. E., G. Kasnic, Jr., C. Draycott, and Theresa Ben. 1972. Activation of viruses in human tumors by 5-iododeoxyuridine and dimethyl sulfoxide. Science **175**:198-199.
31. Stewart S. E., G. Kasnic, Jr., C. Draycott, W. Feller, A. Golden, E. Mitchell, and T. Ben. 1972. Activation *in vitro* by 5-IUdR or a latent virus resembling C-type virus in a human sarcoma cell line. J. Nat. Cancer Inst. **48**:273-277.
32. Temin, H. M. 1963. The effects of actinomycin D on growth of Rous sarcoma virus *in vitro*. Virology **20**:577-582.
33. Todaro, G. J., and R. J. Huebner. 1972. The viral oncogene theory: new evidence. Proc. Nat. Acad. Sci. U.S.A. **69**:1009-1015.
34. Vogt, P., and R. R. Friis, 1971. An avian leukosis virus related to RSV(O): properties and evidence for helper activity. Virology **43**:223-234.
35. Weiss, R. A., R. R. Friis, E. Katz, and P. K. Vogt. 1971. Induction of avian tumor viruses in normal cells by physical and chemical carcinogens. Virology **49**:920-938.
36. Wepsic, H. T., B. Zbar, J. Whang-Peng, T. Borsos, and H. J. Rapp. 1970. Guinea pig leukemia L2C: characterization of chromosomes and attempts to demonstrate antigenicity. J. Nat. Cancer Inst. **45**:99-105.
37. Wilson, S. H., and E. L. Kuff. 1972. A novel DNA polymerase activity found in association with intracisternal A-type particles. Proc. Nat. Acad. Sci. U.S.A. **69**:1531-1536.