INTERACTION AMONG AVIAN TUMOR VIRUSES GIVING ENHANCED INFECTIVITY

By Teruko Hanafusa and Hidesaburo Hanafusa

THE PUBLIC HEALTH RESEARCH INSTITUTE OF THE CITY OF NEW YORK, INC.

Interactions between Rous sarcoma virus (RSV) and avian leukosis viruses take place on two different levels, at one of which avian leukosis virus exerts a determining influence on the properties of RSV during its development in the infected cell. Certain strains of RSV such as the Bryan high-titer strain are unable to produce infectious progeny without the presence in the same cells of multiplying avian leukosis virus which is therefore called helper virus.[1] It is now generally assumed that the defectiveness of RSV lies in its inability to make a viral envelope, and RSV depends on the helper virus to supply a stable outer structure.[2] This concept is supported by the fact that a number of RSV properties are controlled by the superinfecting helper virus. These include antigenic specificity,[2] host range,[3] sensitivity to interference,[3, 4] certain growth characteristics,[5] and the capacity to induce tumors in mammals.[6] All these properties are determined by the character of the viral envelope; RSV acquires this envelope from other viruses by a mechanism that was first discovered with bacteriophages and is called phenotypic mixing.[7]

A second type of interaction between RSV and helper virus involves an interference reaction at a cellular level. Cells infected with avian leukosis virus become resistant to superinfection with RSV, if there has been time for multiplication of the first agent.[8] The resistance appears to be specific for RSV and does not extend to Newcastle disease, vesicular stomatitis, Western equine encephalomyelitis, or vaccinia viruses.[8, 9] Resistance does not, in fact, extend equally to all RSV strains.[3, 4] As a rule, a strain of avian leukosis virus induces resistance to RSV particles which are coated with material of the same or closely related antigenic type. When the RSV coat is unrelated to that of the interfering virus, RSV can infect control or infected cells equally well.[3, 10] Table 1 illustrates these relationships. These findings suggest that resistance to RSV occurs at an early stage of RSV infection because viral envelope presumably plays important roles in the early steps of cell-virus interaction. More recently, Steck and Rubin[12] showed that RSV can be adsorbed equally well on normal and resistant cells but further penetration does not occur with the latter.

This paper will describe a new type of interaction between RSV and avian leukosis virus which is direct and can occur *in vitro*. As shown in Table 1, certain types of RSV produce about 20 times more foci in cultures infected with a helper virus (Rous-associated virus-1, or RAV-1) than in cultures of normal cells.[3, 6, 13] The studies on the mechanism for this enhanced infectivity show that certain strains of avian leukosis virus and the cognate RSV particles such as RSV(RAV-2) have a poor capacity for adsorption to normal chick embryo cells. However, RSV(RAV-2) may be modified by contact with extracts of RAV-1-infected cells so that adsorption to normal cells is greatly enhanced.

Materials and Methods.—Viruses: Stocks of avian leukosis viruses, RAV-1 and RAV-2, were obtained from culture media of heavily infected monolayers of chick embryo cells. The Bryan high-titer strain of RSV was prepared by infecting "nonvirus-producing transformed cells" (NP

cells)[1] [originally induced by RSV(RAV-1)] with various avian leukosis viruses. Every stock of the defective RSV contained helper virus in about 10 times higher amount than RSV.

Cell culture: Chick embryo cells were prepared from individual embryos derived from the eggs of SPAFAS, Inc., Connecticut. The genetically determined susceptibility of cultures to RSV variants was tested before use. In most experiments, K type or C/O type of embryos which are sensitive to both RAV-1 and RAV-2 were used. The methods for cell culture have been described.[5, 8]

Infection of cells with RSV: Two methods were employed. One is the method used for routine assay of RSV[8] in which virus in 0.1 ml was added to suspension of trypsinized cells in 5 ml of medium and cells were overlaid with agar 16–20 hr after infection. In the other method, cell monolayers were exposed to 0.2–0.5 ml of virus for 1 hr, then overlaid with agar.

Preparation of labeled virus: Virus-infected cells in plates of 10-cm diameter were incubated with 5 ml of medium containing 10 μc of H^3-uridine (Nuclear-Chicago, 30 c per mmole) per ml for 24 hr. Virus was purified essentially by the method described by Duesberg and Robinson.[14] The buffer used for sucrose solution contained 0.1 *M* NaCl, 0.001 *M* ethylenediaminetetraacetate (EDTA), 0.01 *M* Tris-HCl, pH 7.4. The same buffer plus 0.01% bovine serum albumin was used for a Sephadex column. All procedures were carried out at 4°C or in ice water. Harvested culture fluid was kept in ice water and 20-ml aliquots were layered over the two layers of 5 ml of 65% sucrose (w/v) in D_2O and 5 ml of 16% sucrose in 20% D_2O, and centrifuged in the Spinco SW25.1 rotor at 24,000 rpm for 2 hr. Virus banded over 65% sucrose solution was collected. The concentrated virus was then diluted with 3 vol of Tris-buffer and laid on a preformed linear gradient of sucrose in Tris-buffer (65% sucrose in D_2O–16% sucrose in 20% D_2O). It was centrifuged in the SW25.1 rotor at 24,000 rpm for 2.5 hr. One-ml fractions were collected in drops from the bottom of punctured tubes. Fractions of labeled virus were pooled and run through a column (20 × 2.5 cm) of Sephadex G-200 equilibrated with Tris-buffer. The labeled virus was pooled, its ionic strength was adjusted with concentrated Scherer's solution, and calf serum was added to a final concentration of 2%. The labeled virus thus purified was kept as long as a week.

Measurement of adsorption of labeled virus: About 20 million trypsinized chick embryo cells were resuspended in 1 ml of the labeled virus which had been diluted with medium to a concentration of about 1–2 × 10^4 cpm labeled virus per ml. The cell-virus suspension was incubated at 37°C for 30 min, being dispersed by a mixer every 10 min. Then the mixture was chilled in ice water, centrifuged, and the supernatant fraction removed. The cells were washed twice with 1 ml of medium in the cold, and then dissolved in 1 ml of 0.1 *N* NaOH and diluted with 1 ml of water. One-tenth-ml aliquots from each fraction were added to vials containing 0.4 ml of water, and 10 ml of scintillation solution (5 gm PPO, 0.2 gm of dimethyl POPOP, and 100 gm of naphthalene in 1 liter of dioxane) was added. Radioactivity was measured by a liquid scintillation counter (Nuclear-Chicago) with an efficiency of about 16%.

Results.—Conditions for enhanced RSV infection: As shown in Table 1, enhanced focus formation was observed when RAV-1 was the interfering virus and RSV(RAV-2), RSV(RAV-50), and SR-RSV were the challenge viruses. In the present study, RAV-1 and RSV(RAV-2) were used as representative agents for this type of interaction.

TABLE 1

INTERFERING RELATIONSHIP BETWEEN RSV AND HELPER VIRUSES*

Challenge virus	None	Efficiency of RSV Infection on Cells Previously Infected with: RAV-1	RAV-2	RAV-50
RSV(RAV-1)	1.0	0.00012	1.0	1.0
RSV(RAV-2)	1.0	~20†	0.00017	0.02
RSV(RAV-50)	1.0	~20	0.058	0.0024
SR-RSV	1.0	~20	0.055	0.0020

Chick embryo cells (C/O type) were infected with about 1 × 10^6 infectious units of helper viruses. After two cell transfers at 3-day intervals for RAV-1 or RAV-2 and after four transfers for RAV-50, the cultures were challenged with various RSV's. The number of Rous sarcoma foci in the helper-infected cultures was compared with that in control cultures uninfected with helper viruses.

* Data was taken from ref. 11.

† 10- to 30-fold increase.

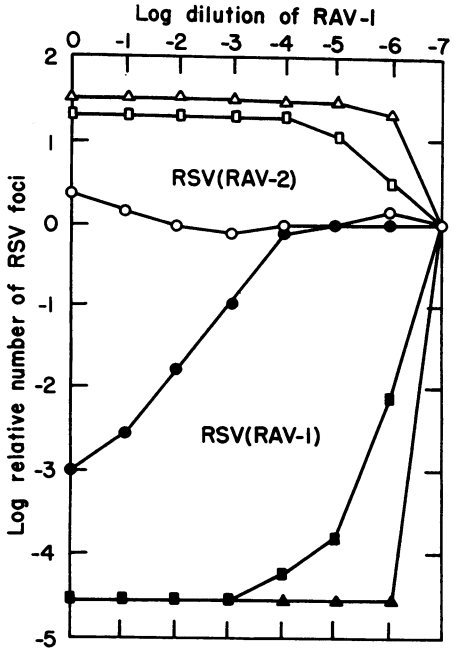

FIG. 1.—Typical development of interference and enhancement in RAV-1-infected cells. About 1×10^6 chick embryo cells were infected with 0.1 ml of serial dilutions of RAV-1 (10^7 infectious units per ml) and transferred at 3-day intervals. The cultures were challenged at each transfer with RSV(RAV-1) and RSV(RAV-2). The number of Rous sarcoma foci in RAV-1-infected cultures was compared with that in control cultures uninfected with RAV-1. Open symbols represent results with RSV(RAV-2) and closed ones with RSV-(RAV-1). *Circles:* 1st transfer; *squares:* 2nd transfer; *triangles:* 3rd transfer.

In the experiment shown in Figure 1, the degree of enhancement was measured as a function of the time after primary infection and the size of the primary infecting dose. Cells were first infected with various amounts of RAV-1 and the cells were transferred at three-day intervals, the cultures being challenged at each transfer by RSV(RAV-1) and RSV(RAV-2). The results were plotted as the ratio of the number of foci obtained in infected cells over the number of foci in normal cells and show that (1) with RSV(RAV-2) there was almost no enhancement in the cells of the first transfer, (2) there was a 20- to 30-fold enhancement of titer in the cells of the second and third passage, and (3) there was an interference effect against RSV(RAV-1) infection which became more marked with passage. The delay in the enhancement effect suggests that the production of rather large amounts of RAV-1 may be necessary for this phenomenon. On the basis of these results further enhancement experiments were carried out on secondary chick embryo cells infected with about 10^5 infectious units of RAV-1 and transferred twice before challenge.

Effect of trypsin on enhancement: It has been shown that RSV(RAV-2) is not enhanced in infectivity when added to RAV-1-infected monolayers one day after subculture, and this led to the finding that treatment of cells with trypsin just before RSV infection is essential for induction of enhancement.

Focus formation by RSV(RAV-2) was 20 times enhanced in RAV-1-infected cells when they were subcultured with trypsin four hours before their exposure to RSV (0.5 ml virus for 1 hr). However, the enhancement of RSV(RAV-2) in RAV-1-infected cells declined with time following the trypsin treatment. Twenty-four hours after trypsinization, virtually no enhancement was observed. The effects of other enzymes were examined and are summarized in Table 2. Enhancement in RAV-1-infected cells was found only when they had been subcultured with the use of proteolytic enzymes.

Adsorption of RSV(RAV-2): The requirement of proteolytic enzyme treatment for enhanced focus formation suggests that some early step in the virus cycle is involved such as adsorption or penetration. These two steps can be examined separately through use of the fact that (1) the former proceeds at low temperature

TABLE 2

EFFECT OF SEVERAL ENZYMES ON ENHANCEMENT OF RSV(RAV-2) INFECTION IN RAV-1-INFECTED CELLS

Reagents	Ratio of number of RSV foci on RAV-1-infected cells to that on uninfected cells
EDTA (0.02%)	1.0
EDTA + hyaluronidase (0.05%)	1.5
Pronase (0.01%)	11.3
Collagenase (0.1%)	5.0
Trypsin (0.05% crystalline)	21.0

Both uninfected and RAV-1-infected cells were subcultured by the aid of the reagents listed. RSV(RAV-2) in 0.1 ml was added to suspensions of the subcultured cells and the cultures were overlaid next day. The number of RSV foci was compared between RAV-1 infected cells and control cells uninfected with RAV-1. Focus formation by RSV(RAV-2) in normal cells was not significantly altered with the type of reagents used for subculture.

but the latter only at high temperature,[12] and (2) viruses adsorbed to the cells remain sensitive to inactivation by antibody at 10°C but become insensitive to antibody after penetrating the cells, which occurs rapidly at 37°C.[12, 15]

The rate of adsorption of RSV(RAV-2) was examined through the use of radioactively labeled virus. The results show mainly the adsorption of RAV-2, since the majority of the virus in RSV(RAV-2) preparation is RAV-2 and both viruses were probably equally labeled. Trypsinized normal and RAV-1-infected cells were resuspended in a small volume of labeled virus and incubated at 10°C for one hour, and the radioactivity remaining in the solution and attached to the cells was measured. Representative results are shown in Table 3. Large differences were observed between the capacity of normal and of RAV-1-infected cells to adsorb RSV(RAV-2) and RAV-2, whereas a labeled virus mixture of RSV(RAV-1) and RAV-1 adsorbed only slightly better to RAV-1-infected cells. A substantial amount of radioactivity adsorbed to cells at 10°C is released from cells by washing with medium, whereas virus adsorbed at 37°C was firmly attached to the cells, suggesting that the stage of adsorption found at 10°C is largely reversible. These results are compatible with the idea that RSV(RAV-2) is adsorbed to trypsinized RAV-1-infected cells at an increased rate which is the basis of the enhanced focus formation found with this combination. The results with labeled RSV also indicate that enhanced adsorption occurred not only with RSV(RAV-2) but with RAV-2. This was confirmed directly by the enhanced adsorption of labeled RAV-2 to RAV-1-infected cells (see Table 4). This fact also supports the above-men-

TABLE 3

ADSORPTION OF LABELED RSV AT 10°C

	Cells	Radioactivity (cpm) Total	Adsorbed	Absorbed (%)
H^3-RSV(RAV-2) + RAV-2				
Lot 1	Normal	2539	127	5.0
	RAV-1-inf.	2974	2121	71.5
Lot 2	Normal	6860	600	8.8
	RAV-1-inf.	7165	3770	52.7
H^3-RSV(RAV-1) + RAV-1				
Lot 1	Normal	4840	1140	23.6
	RAV-1-inf.	5830	3460	59.4
Lot 2	Normal	5580	2840	51.0
	RAV-1-inf.	5480	3920	71.7

About 2×10^7 uninfected or RAV-1-infected cells were trypsinized and resuspended in 1 ml of medium containing H^3-labeled RSV. The mixtures were incubated at 10°C for 60 min and the radioactivity in both fluid and cell fractions was measured. Two different preparations of labeled viruses were used for each RSV(RAV-1) and RSV(RAV-2).

TABLE 4

EFFECT OF AN EXTRACT OF RAV-1-INFECTED CELLS ON THE ADSORPTION OF RAV-2 TO NORMAL CELLS

Adsorption of H^3-RAV-2 together with:	Cells	Radioactivity (cpm) Total	Radioactivity (cpm) Adsorbed	Adsorbed (%)
Complete medium	Normal	10400	252	2.4
Complete medium	RAV-1-inf.	12530	7845	62.5
Extract from normal cells	Normal	11050	240	2.2
Extract from RAV-1 infected cells	Normal	11980	5368	44.8

About 2×10^7 of normal and RAV-1-infected cells were incubated with 1 ml of medium at 10°C for 1 hr. The supernatants of the mixtures were used as extracts. H^3-labeled RAV-2 (0.2 ml) was added to trypsinized cells together with 0.8 ml of complete medium or the extract. The cell suspensions were incubated at 37°C for 30 min, and radioactivity bound to cells was measured.

tioned mechanism for enhancement, because both RAV-2 and RSV(RAV-2) have the same viral envelope and must have equal efficiency in adsorption.

The effect of antiserum to RSV(RAV-2) incubated with cells for various times was examined. At one hour after infection (end of adsorption period at 37°C), focus formation by RSV(RAV-2) was already insensitive to antibody in both normal and RAV-1-preinfected cells, indicating the rate of penetration is not significantly altered in the process of enhancement.

The requirement of trypsin treatment of RAV-1-infected cells was also shown for the enhanced adsorption of labeled RSV(RAV-2). When cells were dispersed with EDTA and hyaluronidase, there was no significant difference between normal and RAV-1-infected cells in adsorption of labeled RSV(RAV-2).

Nature of interaction between RAV-1-infected cells and RAV-2 or RSV(RAV-2): In order to analyze further the nature of enhanced adsorption of RAV-2 or RSV-(RAV-2), the effect of the supernatant of trypsinized RAV-1-infected cells on adsorption of labeled RAV-2 was studied. An extract was prepared by incubating about 2×10^7 freshly trypsinized RAV-1-infected cells suspended in 1 ml of complete medium at 10°C for one hour. The extract was added to normal cells together with labeled virus, and adsorption of the virus was measured. As shown in Table 4, adsorption of RAV-2 to normal cells was enhanced by addition of the extract from RAV-1-infected cells. Effect of the extract on RSV(RAV-2) was also demonstrated in enhanced focus formation in normal cells by simultaneous inoculation with RSV.

The following experiments were performed to see whether the extract acts on the surface of normal cells or on RSV(RAV-2) particles. RSV(RAV-2) was incubated with RAV-1-infected cells or with the extract from them, and focus-forming activity of the incubated RSV was assayed on normal cells after diluting the mixtures. As shown in Table 5, in both instances RSV titer was increased by incubation with cell extracts and the number of foci was proportional to dilution. Since the effect of the extract on RSV(RAV-2) on simultaneous inoculation could be markedly diminished by dilution (no enhancement at 10- and 100-fold dilutions), the increased number of RSV foci at high dilutions of the mixture shown in Table 5 is a strong indication that RSV(RAV-2) was altered *in vitro* by product(s) of RAV-1 infected cells.

Characterization of the active principle in the extract of RAV-1-infected cells remains for further studies. It has been inseparable from RAV-1 by centrifugation in sucrose density gradients. It is sensitive to inactivation by RAV-1 antiserum

TABLE 5

INFECTIVITY OF RSV(RAV-2) FOLLOWING INCUBATION WITH RAV-1-INFECTED CELLS OR WITH EXTRACTS FROM RAV-1-INFECTED CELLS

	RSV(RAV-2) incubated with:	Number of RSV Foci Produced in Cultures of Normal Cells at Dilutions: 10^{-0}	10^{-1}	10^{-2}	10^{-3}
Expt. 1*	None	2000	206	16	3
	Normal cells	2100	156	9	0
	RAV-1-infected cells	Confl.‡	>3000	414	29
Expt. 2†	Medium	204	19	3	0
	Extracts from normal cells	180	12	1	0
	Extracts from RAV-1-infected cells	1090	150	14	6

* RSV(RAV-2) in 1 ml of medium was added to 2 × 10^7 trypsinized cells and incubated at 10°C for 1 hr. The mixture was centrifuged, and serial dilutions were made from the supernatant and inoculated to normal cells.

† Extracts were made as described in Table 5. RSV(RAV-2) in 0.1 ml was mixed with 0.9 ml of the extracts or medium, and incubated at 10°C for another hour. Then the mixtures were diluted and inoculated into normal cells.

‡ Confluent.

and resistant to sonic oscillation at 10 kc for 3 minutes. However, the enhancing activity was resistant to heat at 56°C for 30 minutes, which reduces infectivity by a factor greater than 10^3. The results suggest that RAV-1 itself or RAV-1-induced-specific antigen released from cells following treatment with trypsin is responsible for the enhancing activity.

Effect of trypsin on RAV-2 or RSV(RAV-2): Table 6 shows the effect of trypsin on focus-forming activity of RSV(RAV-2) and on adsorption of RAV-2 to host cells. It can be seen that the infectivity of RSV(RAV-2) in normal cells was not markedly affected, but enhancement in RAV-1-infected cells was almost totally abolished by treatment of RSV(RAV-2) with trypsin. The table also shows no enhanced adsorption of trypsin-treated RAV-2 to RAV-1-infected cells. Apparently, trypsin destroys the reaction sites on RAV-2 or RSV(RAV-2) particles which otherwise could interact with the extract from RAV-1-infected cells.

Discussion.—The present study shows that RAV-2 or RSV(RAV-2) is enhanced in adsorption to the RAV-1-infected cells and the critical reaction seems to occur *in vitro*. There was a complete correlation between enhancement of focus formation and enhancement of adsorption after various conditions of infection, including different treatments of viruses and cells. This correlation was also observed with SR-RSV; cells infected with RAV-1 absorb ten times more SR-RSV focus-forming units than do normal cells.

The mechanism of blocking of RSV infection in interference has been suggested also to be at some step in early cell-virus interaction.[3] Recent studies by Steck and Rubin[12] showed that penetration is blocked for RSV(RAV-1) in resistant cells infected with RAV-1. The similar rate of adsorption of RSV(RAV-1) to normal and RAV-1-infected cells was confirmed in the present study. In a separate experiment, we found that adsorption of RSV to the genetically nonpermissive chick embryo cells,[3, 10] occurred at the same rate as to the genetically permissive cells.[17] With cells that are nonpermissive, either on a genetic basis or as the result of viral interference, the block must be in the steps following adsorption, and this is in contrast to the enhancement reaction where all the available evidence suggests that adsorption itself is the crucial step.

There are several reports describing enhancement with other viruses.[18–24] The mechanism for these enhanced interactions has been studied in some systems where

TABLE 6

LOSS OF ENHANCEMENT EFFECT BY TREATMENT WITH TRYPSIN

Expt. 1 Treatment of RSV(RAV-2)	Number of RSV Foci on: Normal cells 10^{-1}	Normal cells 10^{-2}	RAV-1-infected cells 10^{-1}	RAV-1-infected cells 10^{-2}
Tris-saline	356	33	Confl.*	450
Trypsin	116	12	109	7

Expt. 2 Treatment of H^3-RSV(RAV-2) (total 9300 cpm)	Radioactivity Bound to: Normal cells	RAV-1-infected cells
Tris-saline	348 (cpm)	6494
Trypsin	272	400

RSV(RAV-2) was mixed with trypsin in final concentration of 0.05% and incubated at 37°C for 1 hr. The mixture was diluted and inoculated into both normal and RAV-1-infected cells (expt. 1) or diluted 5 times and incubated with suspended cells and the radioactivity bound to cells was measured (expt. 2).
* Confluent.

the induction in infected cells of substances which counteract with antiviral action of interferon was observed.[23–25] No intervention of interferon, however, has been demonstrated in the resistance induced by avian leukosis virus to RSV infection.[8, 9, 16] Interferon has no inhibitory action on adsorption of virus to cells,[26, 27] but blocks translation of virus-specific RNA.[28, 29] Therefore, interferon or its counterparts are probably not involved in the enhancement of RSV.

A unique feature of this enhancement is the modification of virus *in vitro*. The nature of the interaction between RAV-2 or RSV(RAV-2) with the extract of RAV-1-infected cells is not entirely clear. Preliminary studies show that RSV(RAV-2) modified by incubation with the extract becomes sensitive to neutralization by antiserum for either RAV-1 or RAV-2. It seems likely that RAV-2 or RSV(RAV-2) is activated by attachment of a host-adsorbing site of RAV-1 from the extract, rather than by unmasking of inactive particles by enzymes in the extract. The adsorbing site may be present either as the RAV-1 particle itself or as a viral subunit released from cells by trypsin. Using concentrated RAV-1 obtained from culture medium (containing about 6×10^8 infectious units per ml), we have thus far been unable to enhance adsorption of RAV-2. There was no enhancement of RAV-2 by RAV-1 obtained by sonication of infected cells in a small volume of medium. However, these results do not exclude the possibility of formation of aggregates of the two viruses. The titer or RAV-1 is generally higher in the extract from the trypsin-treated cells than in culture medium, and RAV-1 released by trypsin treatment may be different from the virus normally released into medium in its adhesiveness to other viruses.

Summary.—Production of Rous sarcoma foci by certain strains of RSV such as RSV(RAV-2) in chick embryo cells was enhanced about 20-fold if the cells were previously infected with RAV-1, and the enhanced infectivity was correlated with enhanced adsorption of RSV(RAV-2). RSV(RAV-2) was modified by contact with a trypsin digest of RAV-1-infected cells, with the result that its adsorbing capacity and infectivity for normal cells was increased by a factor of 20.

* This work was supported by U.S. Public Health Service research grant no. CA-08747 from the National Cancer Institute.

[1] Hanafusa, H., T. Hanafusa, and H. Rubin, these PROCEEDINGS, **49**, 572 (1963).
[2] *Ibid.*, **51**, 41 (1964).
[3] Hanafusa, H., *Virology*, **25**, 248 (1965).

[4] Hanafusa, T., H. Hanafusa, and H. Rubin, *Virology*, **22**, 643 (1964).
[5] Hanafusa, H., and T. Hanafusa, *Virology*, **28**, 369 (1966).
[6] Hanafusa, H., and T. Hanafusa, these PROCEEDINGS, **55**, 532 (1966).
[7] Novick, A., and L. Szilard, *Science*, **113**, 34 (1951); Hirst, G. K., and T. Gotlieb, *J. Exptl. Med.*, **98**, 41 (1953).
[8] Rubin, H., these PROCEEDINGS, **46**, 1105 (1960).
[9] Hanafusa, H., T. Hanafusa, and H. Rubin, *Virology*, **22**, 591 (1964).
[10] Vogt, P. K., *Virology*, **25**, 237 (1965).
[11] Hanafusa, H., and T. Hanafusa, in *Subviral Carcinogenesis*, ed. Y. Ito (International Symposium on Tumor Viruses, Nagoya, Japan, 1966), in press.
[12] Steck, F. T., and H. Rubin, *Virology*, **29**, 628, 642 (1966).
[13] Hanafusa, H., *Natl. Cancer Inst. Monograph*, **17**, 543 (1964).
[14] Duesberg, P. H., and W. S. Robinson, these PROCEEDINGS, **55**, 219 (1966).
[15] Mandel, B., in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 27 (1962), p. 123.
[16] Rubin, H., *Virology*, **13**, 200 (1961).
[17] Hanafusa, T., and H. Hanafusa, unpublished results.
[18] Kumagai, T., T. Shimizu, S. Ikeda, and M. Matsumoto, *J. Immunol.*, **87**, 245 (1961).
[19] Frothingham, T. E., *Virology*, **19**, 583 (1963); Frothingham, T. E., *J. Immunol.*, **94**, 521 (1965).
[20] Hermodsson, S., *Virology*, **20**, 333 (1963).
[21] Tisdale, W. A., *Proc. Soc. Exptl. Biol. Med.*, **114**, 774 (1963).
[22] Rabson, A. S., G. T. O'Conor, I. K. Berezesky, and F. J. Paul, *Proc. Soc. Exptl. Biol. Med.*, **116**, 187 (1964).
[23] Chany, C., and C. Brailovsky, *Compt. Rend.*, **261**, 4282 (1965); Chany, C., and C. Brailovsky, these PROCEEDINGS, **57**, 87 (1967).
[24] Kato, N., A. Okada, and F. Ota, *Virology*, **26**, 630 (1965).
[25] Isaacs, A., and Z. Rotem, *Virology*, **29**, 248 (1966).
[26] Wagner, R. R., *Virology*, **13**, 323 (1961).
[27] Isaacs, A., *Advan. Virus Res.*, **10**, 1 (1963).
[28] Marcus, P., *Virology*, **30**, 502 (1966).
[29] Joklik, W. K., and T. C. Merigan, these PROCEEDINGS, **56**, 558 (1966).

---

*TUMOR VIRUS RNA'S**

BY WILLIAM S. ROBINSON, HARRIET L. ROBINSON, AND PETER H. DUESBERG

DEPARTMENT OF MOLECULAR BIOLOGY AND VIRUS LABORATORY,
UNIVERSITY OF CALIFORNIA, BERKELEY

There are three known groups of RNA tumor viruses: the avian leukosis and sarcoma viruses, the murine leukemia viruses, and the murine mammary tumor virus. Besides having RNA as their nucleic acid, these viruses have other features in common. Electron micrographs show that all have similarities in ultrastructure such as flexible or compressible outer viral envelopes and electron dense inner cores. All contain a significant fraction of lipid most or all of which they acquire from cellular membranes as virus assembly is completed by a mechanism similar to that described for the myxoviruses.

Experiments to characterize the RNA's from representative members of each group of RNA tumor viruses will be described first. The results show that all