[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[17] Hanafusa, T., and H. Hanafusa, unpublished results.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).

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*TUMOR VIRUS RNA'S**

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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

the RNA's studied are single-stranded and have sedimentation constants $s_{20,w}$ between 69 and 74S in 0.1 M salt. The sedimentation behavior indicates that they are very similar to each other in size, with molecular weight values around 10^7.

The specific viruses that we have studied are (1) the mixture of the Bryan strain of Rous sarcoma virus and its helper Rous-associated virus (RSV + RAV),[1] (2) the BAI strain of avian myeloblastosis virus (AMV),[2] (3) the Rauscher mouse leukemia virus (MLV),[3] and (4) the mouse mammary tumor virus (MTV).[4]

Virus Purification.—The purification procedures that we use take advantage of the low buoyant density and relatively large size of the RNA tumor viruses which allows them to be rapidly centrifuged to density equilibrium in density gradients of sucrose. Each virus was purified by several steps of centrifugation in stepwise and linear gradients of buffered sucrose. Ethylenediaminetetraacetate (EDTA), which has been shown to stabilize the infectivity of RSV,[1] was included in all buffers and sucrose solutions. Such rapid and gentle purification allows recovery of all virus infectivity[1, 2] and intact viral RNA.[1] When virus is frozen or thawed several times during purification or is centrifuged for prolonged periods of time in concentrated salt solutions such as CsCl, much infectivity is lost and some or all of the intact viral RNA is degraded.[1]

The Viral RNA's.—The detergent sodium dodecyl sulfate (SDS) effectively disrupts the lipid-containing RNA viruses, following which the viral RNA's can be purified by phenol extraction and alcohol precipitation.[1] The RNA's of all four tumor viruses have been prepared in this way.

Figure 1 shows the results of fractionation of radioactively labeled RNA from RSV + RAV, AMV, MLV, and MTV by zone sedimentation in sucrose density gradients containing 0.11 M salt. Tobacco mosaic virus RNA which sediments at 31S under these conditions was used as carrier in each experiment and is represented by the A_{260} tracing (*dotted line*). Sedimentation is from right to left in each case. Figure 1*A* shows sedimentation of RNA extracted from RSV + RAV labeled with P^{32}. It can be seen that there are two components of labeled RNA, one sedimenting faster than TMV-RNA and one sedimenting more slowly. The faster-sedimenting component is thought to be the intact viral RNA. This is suggested by the fact that in highly purified virus preparations this RNA component represents as much as 95 per cent of the total RNA from virus. Furthermore, as mentioned above, when virus is repeatedly frozen and thawed, infectivity is lost and this RNA component progressively represents a smaller and smaller fraction of the total RNA isolated from virus. However, infectivity of the faster-sedimenting RNA component has not been demonstrated, and final proof that this RNA is the complete complement of viral RNA must await this demonstration. The purified preparation of Bryan high-titer virus used in this experiment was a mixture of RSV and RAV. The ratio of infectious RAV to infectious RSV is probably around 5 or 10.[5, 6] Thus most of the P^{32}-labeled RNA in Figure 1*A* is probably RAV-RNA.

The viral RNA component sedimenting more slowly than TMV-RNA is thought to contain degraded viral RNA resulting from breakdown of RNA in the virus before the time of RNA isolation. This is suggested by the observation that when virus is repeatedly frozen and thawed, the faster-sedimenting component is reduced in amount and the slower-sedimenting component increases in amount. The fact

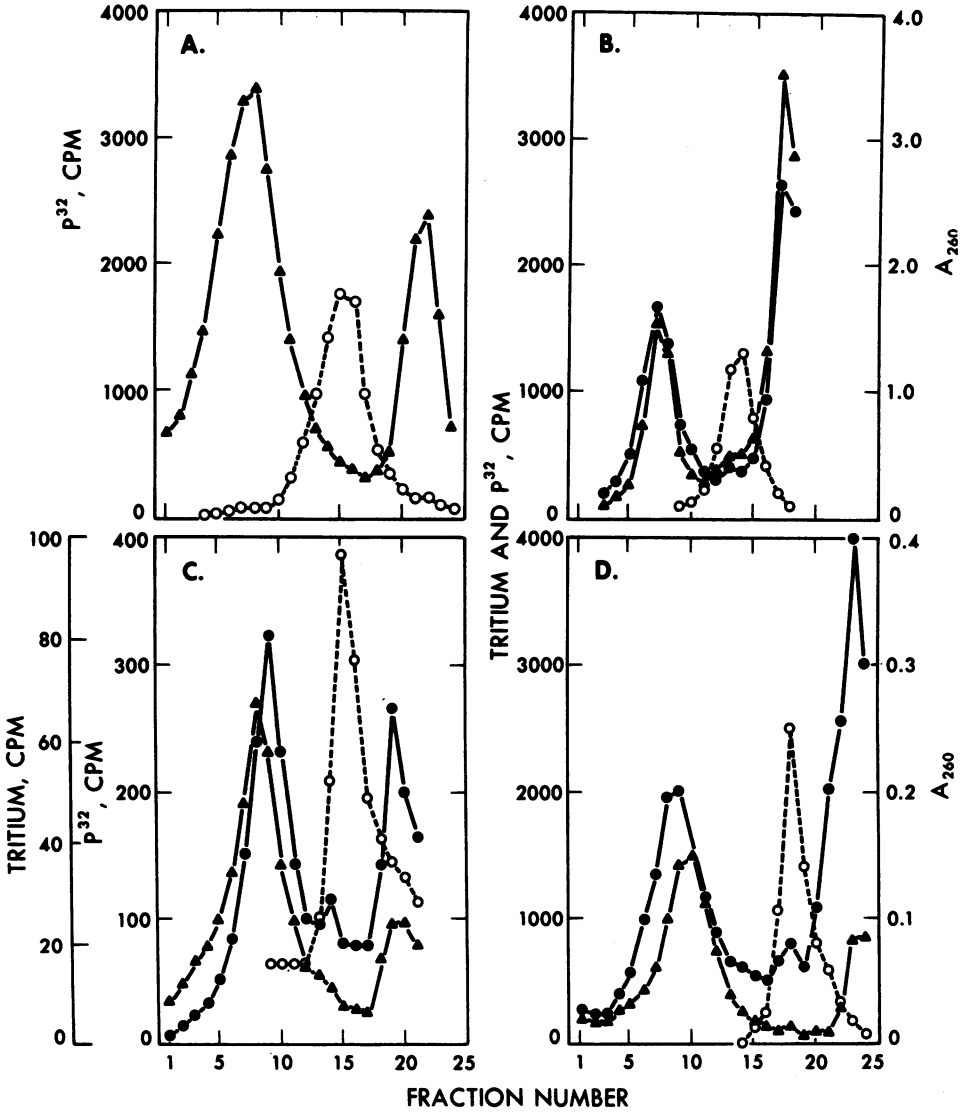

FIG. 1.—Sedimentation of tritium- or P^{32}-labeled viral RNA's with carrier TMV-RNA (A_{260}) in sucrose density gradients (5–20%) containing 0.1 *M* NaCl, 0.01 *M* Tris HCl, pH 7.4, and 0.001 *M* EDTA in a Spinco SW50 rotor at 4°C. (*A*) P^{32}-labeled RSV + RAV-RNA and TMV-RNA centrifuged at 36,000 rpm for 2.25 hr.[1] (*B*) H^3-labeled RSV + RAV-RNA, P^{32}-labeled AMV-RNA, and TMV-RNA centrifuged as described in (*A*).[2] (*C*) P^{32}-labeled RSV + RAV-RNA, H^3-labeled MTV-RNA, and TMV-RNA centrifuged at 50,000 rpm for 75 min.[4] (*D*) P^{32}-labeled RSV + RAV-RNA, H^3-labeled MLV-RNA, and TMV-RNA centrifuged for 2 hr and 10 min at 36,000 rpm.[3] P^{32} (▲——▲), tritium (●——●), and A_{260} (○----○) were determined on each fraction.

that the two viral RNA components are distinctly separated by velocity sedimentation would suggest that the breakdown of intact viral RNA into small fragments occurs in only a fraction of the particles in the virus preparation prior to isolation of the RNA. If the RNA breakdown occurred during isolation with SDS and phenol, the result would be partial or complete degradation of all RNA in the prepa-

ration and the appearance of a broad sedimentation band rather than two distinct components. The RNA tumor viruses appear to be quite fragile and a certain fraction of the virus particles may be disrupted in all virus preparations.

Figure 1*B* shows P^{32}-labeled AMV–RNA sedimenting with tritium-labeled RSV + RAV-RNA. The faster-sedimenting components of both appear to be identical in sedimentation rate under the conditions used.

Figure 1*C* shows tritium-labeled RNA from mouse mammary tumor virus sedimenting with P^{32}-labeled RNA from RSV + RAV. The faster-sedimenting component from MTV appears to sediment slightly less rapidly than RSV + RAV-RNA. Figure 1*D* shows that MLV-RNA labeled with tritium sediments slightly more rapidly than P^{32}-labeled RNA from RSV + RAV. In Figures 1*B*, *C*, and *D*, it can also be seen that the preparations of RNA from AMV, MTV, and MLV, like that from RSV + RAV, contain a slowly sedimenting component.

RNA's with similar behavior in sucrose gradient sedimentation have been recovered from AMV by J. Harel *et al.*,[7, 25, 26] from RSV by L. Harel *et al.*,[8] and from Rauscher MLV by Galibert *et al.*[9] and Mora *et al.*[10]

Sedimentation constants of 71*S* for both RSV + RAV-RNA and AMV-RNA and 74*S* for MLV-RNA in 0.11 *M* salt have been determined in the analytical ultracentrifuge (Table 1). These values are in agreement with the results in Figure 1 where RSV + RAV and AMV-RNA's sediment together in 0.11 *M* salt (Fig. 1*B*) and MLV-RNA sediments slightly more rapidly than RSV + RAV-RNA (Fig. 1*D*). MTV-RNA moves more slowly than RSV + RAV-RNA (Fig. 1*C*) and can be estimated to have a sedimentation constant of about 69*S* in 0.11 *M* salt.

The other data in Table 1 are the sedimentation coefficients of the RNA's at different ionic strengths. In the case of each RNA there is a marked dependence of sedimentation rate on ionic strength. Such behavior has been shown to be characteristic of single-stranded RNA. However, it is of interest that TMV-RNA has been shown to change around ninefold in sedimentation rate with a change in salt concentration from 150 to 0.2 mM.[11] The change in sedimentation rate with these RNA's is only about threefold with a similar change in ionic environment, suggesting that in comparison with TMV-RNA there may be some restriction to unfolding of the tumor virus RNA's in low salt.

Certain other characteristics besides dependence of sedimentation rate on ionic strength suggest that the tumor virus RNA's are single-stranded. Less than 2 per cent of any of the RNA's remains TCA-precipitable after digestion with pancreatic RNase (10 μg per ml for 1 hr at 37°) in 0.2 *M* salt under conditions where double-stranded RNA is not digested. Furthermore, the base compositions of the faster-

TABLE 1

SEDIMENTATION CONSTANTS ($s_{20,w}$) OF TUMOR VIRUS RNA'S

	Salt Concentration		
	0.21 *M*	0.11 *M*	0.001 *M*
RSV + RAV[1]	79	71	27
AMV[2]	79	71	27
MLV[3]	—	74	~26*
MTV[4]	—	~69*	~22*

Sedimentation constants of RNA's determined in the Spinco model E analytical ultracentrifuge.

* Estimated by comparison with RSV + RAV-RNA during zone sedimentation in sucrose gradients.

sedimenting components of RSV + RAV, AMV, and MLV-RNA do not suggest complementary base-pairing.

Using the relationship between sedimentation constant and molecular weight determined by Spirin for single-stranded TMV-RNA in 0.1 *M* salt,[12] it can be estimated that single-stranded RNA's with sedimentation constants around 70*S* in 0.1 *M* salt have molecular weights around 12 million. However, it should be emphasized that this value is only an estimate for the molecular weight of the tumor virus RNA's because the relationship between sedimentation constant and molecular weight may differ considerably for different single-stranded RNA's.[13]

The value of 10 million for the molecular weight of AMV-RNA has been recently estimated by electron microscopy of viral RNA.[14]

Thus, the RNA's recovered from the four tumor viruses studied here are single-stranded and consist of two major components. One component sediments at more than twice the rate of TMV-RNA in 0.11 *M* salt and is considered to be the intact high-molecular-weight viral RNA. The other component sediments around 4*S* and is thought to contain degraded viral RNA, probably broken down in the virus particles before RNA isolation. The RNA's of MLV and MTV are very similar to the two avian tumor virus RNA's in sedimentation behavior. This suggests that the RNA's of all RNA tumor viruses may be very similar in size and structure and probably quite different in size from the RNA's of other groups of viruses.

RSV-RNA.—Recently, one of us (H. R.) has isolated particles of the Bryan high-titer strain of RSV in the absence of its helper RAV and demonstrated that the RNA of RSV is indistinguishable from that of RAV in sedimentation constant and base composition. As mentioned above, stocks of the Bryan high-titer strain of RSV (BH-RSV) always contain a second virus[5] which may be one of several avian leukosis viruses. This apparently obligatory occurrence of a second virus in the Bryan stocks is due to the defectiveness of the BH-RSV described by Hanafusa *et al.*[6] When cells are infected with BH-RSV alone, the infected cells and their daughters assume the morphological and malignant characteristics of RSV-transformed cells but do not produce infectious virus. These cells have been called nonproducer (NP) cells.[6] When NP cells are superinfected with an avian leukosis virus such as RAV-1 or RAV-2, they not only produce RAV but also infectious RSV which has the same growth characteristics,[15] physical properties,[16] antigenic specificity,[17] and host range[18] as the superinfecting helper virus. Type-specific viral-coat antigen cannot be detected in NP cells,[6, 17] and it has been hypothesized that BH-RSV cannot direct the synthesis of coat antigen and that the role of the helper virus which initiates the production of RSV by NP cells is to provide coat antigen.

Recently, Dougherty and Di Stefano[19] described small numbers of viruslike particles in electron micrographs of NP cells. With this observation in mind, cultures of NP cells were incubated with radioactive precursors of RNA such as tritiated uridine. The culture medium was then recovered and carried through the procedures described above for virus purification. Figure 2 shows the distribution after sedimentation in a sucrose gradient of radioactively labeled particles from the medium of an NP culture (*a*), an NP culture which had been superinfected with RAV seven days before the experiment and which was producing infectious RSV and RAV (*b*), and an uninfected chick embryo cell culture (*c*).

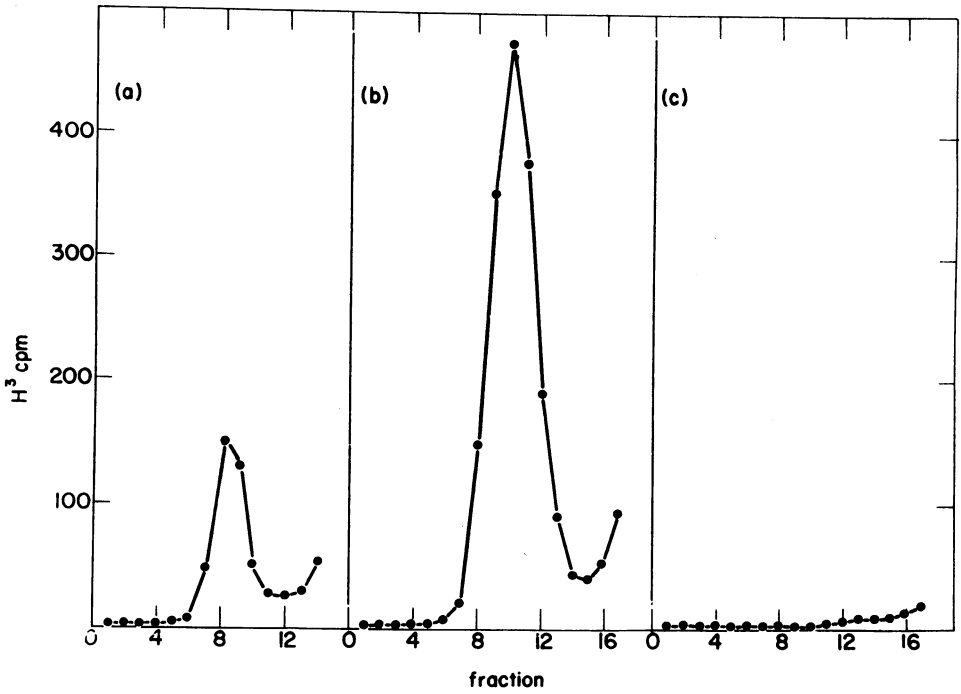

FIG. 2.—Incorporation of tritiated uridine into particles isolated from the medium of an NP culture, its RAV-challenged sister culture, and a culture of normal cells.[22] Approximately 10^6 NP cells isolated by the method of Trager and Rubin[20] (*a*), NP cells which had been challenged 1 week prior with 10^6 interfering units of RAV-1 (*b*), and normal chick embryo fibroblasts (*c*) were incubated in 5 ml medium containing 100 μc H^3-uridine. At 18 hr the medium was harvested and centrifuged at 15,000 rpm for 10 min in the Sorvall RC-2 to remove cell debris, following which 4 ml was layered over 7 ml of 10% sucrose which had in turn been layered over a 5-ml linear gradient from 15 to 60% sucrose. Centrifugation was for 4 hr at 25,000 rpm at 6°C in the SW25.3 rotor of the Spinco preparative ultracentrifuge. No focus-forming units were detected in 1 ml of medium from the nonproducer cells or the control cells. The medium from the RAV-challenged NP culture contained 2×10^6 focus-forming units/ml.

It can be seen that tritium-labeled material in the position expected for virus was recovered from the medium of the NP culture superinfected with RAV. In addition, particles in the same position in the gradient with about one fourth as much radioactivity were recovered from the medium of the NP culture. No such labeled particles were detected in the medium of the culture of uninfected cells. Similar experiments with 14 different clones of NP cells have yielded similar results. In each case no infectious RSV or RAV could be detected in the NP culture medium using the assay techniques of Rubin and co-workers.[21, 5]

Equilibrium and velocity sedimentation studies to characterize the particles produced by NP cells (NP particles) indicate that they are very similar, although not identical to infectious RSV + RAV in size and chemical composition.[22]

In order to examine the labeled RNA in the NP particle, a culture medium containing tritiated uridine-labeled NP particle was mixed with a medium containing P^{32}-labeled RSV + RAV, the labeled particles were purified together, and the total RNA was isolated from the particles using SDS and phenol in the presence of unlabeled cellular RNA. The RNA was then fractionated by zone sedimentation in a sucrose gradient. Figure 3 shows the results of such an experiment. Sedimen-

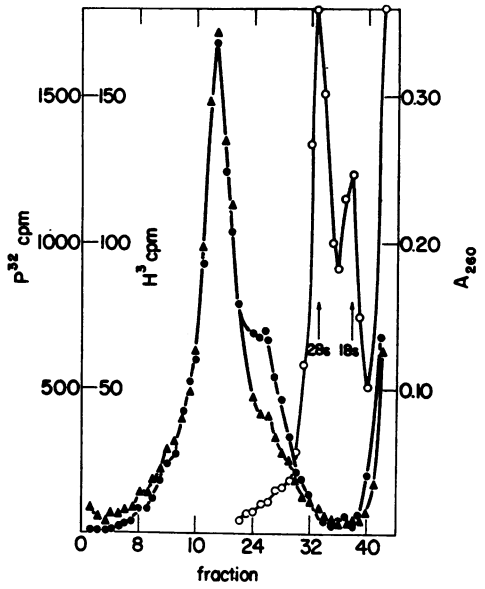

FIG. 3.—Velocity sedimentation of H^3-labeled RNA from the NP particle and P^{32}-labeled RNA from RSV + RAV.[22] A mixture of tritiated uridine-labeled NP particle RNA (●–●–●) and P^{32}-labeled RSV + RAV-RNA (▲–▲–▲) was fractionated by zone sedimentation in a 17-ml linear gradient from 10 to 30% sucrose prepared in buffer containing 0.1 *M* NaCl, 0.001 *M* EDTA, 0.01 *M* tris, 0.2% SDS, pH 7.4. Centrifugation was for 6 hr at 25,000 rpm at 25°C in the SW25.3 rotor of the Spinco ultracentrifuge. One ml of the original NP medium contained no detectable focus-forming units, while 6×10^6 focus-forming units/ml were present in the medium from the RAV-challenged NP culture. O–O–O, A_{260} of the 28 and 18*S* ribosomal RNA used as carrier.

tation is from right to left. The closed circles represent the tritium-labeled RNA from NP particles which can be seen to sediment exactly with the P^{32}-labeled 71*S* RNA from RSV + RAV (*closed triangles*). The open circles represent the unlabeled 28 and 18*S* cellular RNA components detected by absorbancy at 260 mμ. Both peaks of labeled RNA have small shoulders on their slower-sedimenting sides, which is thought to represent some breakdown of the high-molecular-weight RNA's.

Breakdown of the RNA in NP particles appears to occur more readily than RNA in RSV + RAV, suggesting that the NP particle is significantly less stable than infectious virus. For example, RSV + RAV can be precipitated with ammonium sulfate and recovered with little or no loss of intact RNA. On the other hand, after precipitation of the NP particle with ammonium sulfate, the RNA of the NP particle is mostly degraded indicating that the NP particles have been disrupted.

The base compositions of the 71*S* RNA from the NP particle is given in Table 2 along with the base compositions of the RNA from a 10:1 mixture of RAV + RSV and from the RNA of avian myeloblastosis virus (AMV), another avian leukosis virus which, like RAV, can function as a helper for RSV. The data in Table 2 indicate that these three viruses have RNA's with similar if not identical

TABLE 2

BASE COMPOSITIONS OF 71*S* RNA'S FROM AVIAN TUMOR VIRUSES

	AMV[2]	RAV(10 parts) + RSV (1 part)[1]	NP particle[22]
C	23.0 ± 0.2	24.2 ± 0.2	24.2 ± 0.3
A	25.3 ± 0.2	25.1 ± 0.3	24.8 ± 0.4
G	28.7 ± 0.2	28.3 ± 0.5	29.2 ± 0.5
U	23.0 ± 0.2	22.4 ± 0.4	21.7 ± 0.8

Base composition of P^{32}-labeled RNA's determined by alkaline hydrolysis and paper electrophoresis of intact RNA's isolated from virus and fractionated by sucrose gradient sedimentation.[1]

base compositions. Similarly, the RNA's are indistinguishable in susceptibility to digestion by pancreatic RNase. It is presumed that the RNA from the NP particle represents RSV-RNA in the absence of RAV-RNA.

Evidence that the NP particle does contain the RSV genome is provided by the next experiment. Following the demonstration[23] that poliovirus can be made to infect chick embryo cells under conditions where the cells are fusing (chick cells presumably do not have receptor sites for polio adsorption), similar experiments were undertaken with NP particles using UV-inactivated Newcastle disease virus (NDV) to fuse cells.[24] Table 3 shows the results of such an experiment using 12 chick embryo fibroblast cultures. Purified, concentrated NP particles were added to cultures 1, 2, 7, and 8, and RSV + RAV-1 to cultures 3, 4, 9, and 10. No virus was added to cultures 5, 6, 11, and 12. Highly concentrated UV-inactivated NDV was then added to cultures 1, 3, 5, 7, 9, and 11. After one hour the fluid was removed from each culture and the cells were incubated for 30 minutes with culture medium containing RAV-1-neutralizing antibody. The cultures were then washed repeatedly with medium and finally placed in medium containing NDV-neutralizing antibody. RAV-1 was added to the medium of cultures 1–6 (multiplicity about 1) and all 12 cultures were incubated for three days. The medium of each culture was assayed daily for infectious RSV.

It can be seen (Table 3) that focus-forming virus was produced by the cells incubated with NP particles during cell fusion when the cells were subsequently infected with RAV-1 (culture 1). No focus-forming virus was produced by cells incubated with NP particles without cell fusion (culture 2) or without superinfection of cells with RAV-1 (culture 7). As expected, cells infected with RSV + RAV (cultures 3, 4, 9, and 10) produced focus-forming virus with and without cell fusion or RAV-1 superinfection. Control cultures (5, 6, 11, and 12) exposed to neither NP particles nor RSV + RAV produced no focus-forming virus following cell fusion (cultures 5 and 11) or RAV-1 infection (cultures 5 and 6). Three sepa-

TABLE 3

INFECTION OF CHICK EMBRYO FIBROBLAST CULTURES WITH NP PARTICLE DURING CELL FUSION

Culture	Components of Reaction Mixture: Virus (0.3 ml)	UV-inactivated NDV (0.2 ml)	RSV in Culture Medium on Different Days after Fusion (Focus-Forming Units/ml): 0	1	2	3
1	NP particle	+	0	0	14	11
2	NP particle	0	0	0	0	0
3	RSV + RAV	+	52	3×10^4	4.3×10^5	6.1×10^5
4	RSV + RAV	0	116	5.4×10^4	—	—
5	0	+	0	0	0	0
6	0	0	0	0	0	0
7	NP particle	+	0	0	0	0
8	NP particle	0	0	0	0	—
9	RSV + RAV	+	240	7.0×10^3	2.1×10^5	8.1×10^5
10	RSV + RAV	0	70	3.6×10^4	2.8×10^5	—
11	0	+	0	0	0	0
12	0	0	0	0	0	—

Plates of 60 mm were seeded with 3×10^6 chick embryo fibroblasts 18 hr before the experiment. In order to fuse cells, approximately 5000 hemagglutinating units of the IM strain of NDV were added to cultures 1, 3, 5, 7, 9, and 11. At the end of the fusion reaction only about 1% of the cells contained more than one nucleus. Cultures 1–6 were superinfected with RAV-1 immediately following cell fusion, and cultures 7–12 were not superinfected with RAV-1. At the designated times medium was harvested from the cultures and centrifuged two times at 15,000 rpm for 5 min to remove whole cells and cell debris. NP particles and RSV + RAV were then concentrated and purified from the culture medium as previously described.[22] No focus-forming activity could be detected in 0.3 ml of the concentrated purified NP particles, whereas about 10^7 focus-forming units were present in 0.3 ml of the concentrated purified RSV + RAV. See text for other experimental details.

TABLE 4

PRODUCTION OF NP PARTICLE AND RSV + RAV BY TWO NP CLONES AND THEIR RAV-CHALLENGED SISTER CULTURES[22]

	Clone A*		Clone B*	
	NP cells	NP cells superinfected with RAV-1	NP cells	NP cells superinfected with RAV-1
Cell number	7×10^5	9×10^5	7×10^5	7×10^5
RSV, focus-forming units†	0	3.1×10^7	0	2.7×10^7
RAV-1, interfering units†	0	7.5×10^7	0	1.3×10^8
RAV-2, interfering units†	0	0	0	0
Cpm H^3 in NP particle or in RSV + RAV‡	4.0×10^3	7.1×10^3	3.1×10^3	4.6×10^3
Cpm H^3 in cell RNA‡	2.3×10^6	2.5×10^6	2.3×10^6	2.2×10^6

* Cultures were transferred and superinfected with RAV-1 4 days before the experiment.

† Focus-forming units and interfering units present in 5 ml medium after 18 hr of growth. The titers of RAV-1 and RAV-2 were determined in an end-point assay as the number of units which when grown with chick embryo fibroblasts for three passages interfered with superinfection of the fibroblasts by either RSV with RAV-1 coat antigen or RSV with RAV-2 coat antigen.

‡ Cultures were incubated with 200 μc H^3-uridine in 5 ml of growth medium for 18 hr. Under these conditions incorporation of H^3-uridine is linear for several hours, after which it falls off. NP particle and RSV + RAV were isolated as described in Fig. 2. Cell RNA was recovered in SDS solution added to the culture plate. Aliquots of the virus and cell material were analyzed for acid-precipitable, alkali-labile H^3.

rate experiments have given similar results. These experiments indicate that under conditions of cell fusion, NP particles can infect cells and initiate the production of infectious RSV when the cells are simultaneously infected with RAV. This is proof that the NP particle does contain the BH-RSV genome.

The quantitative aspects of NP particle production by two NP clones and their RAV-challenged sister cultures are shown in Table 4. All cultures were incubated with H^3-uridine for 18 hours and then the amount of radioactivity in particles and the titers of infectious RSV and RAV were determined for each culture. It can be seen that about one half as much radioactivity was incorporated into the particles produced by the NP culture as into particles produced by the RSV + RAV-infected culture. In addition, the ratio of infectious RAV (interfering units) to infectious RSV (focus-forming units) produced by the RSV + RAV-infected culture is about three to one. If the ratio of infectious RAV/RSV measured in the medium of the RAV-challenged cultures is roughly proportional to the ratio of radioactively labeled RAV/RSV produced by the same culture, then approximately the same amount of labeled RSV is being produced by the RSV + RAV-infected culture as labeled NP particle by the NP culture. Thus it would appear that the presence of helper virus does not effect the rate of production of particles with the RSV genome.

These experiments clarify the nature of the defectiveness of the Bryan strain of RSV. When defectiveness was first observed, it was proposed that NP cells failed to produce virus because the Bryan strain of RSV lacked some late viral function involved in the production of coat antigen.[6] From the experiments here, it seems clear that NP cells do produce virus particles which contain RSV-RNA and that the rate of particle production is probably the same as that of RSV production by RSV + RAV-infected cells. This suggests that many viral functions proceed normally in NP cells despite the absence of detectable coat antigen. However, the virus particles produced by NP cells are defective in the sense that they are not infectious. In addition, NP particles differ slightly from infectious RSV in buoyant density and sedimentation velocity and differ significantly in particle stability.

It has been suggested[6] that the very high efficiency of RSV in transforming infected cells might be related to its defectiveness. Tumor viruses such as avian leukosis viruses are not defective and apparently transform cells infrequently. However, it is not clear how the defectiveness of BH-RSV as defined in the experiments here would be related to cell transformation, especially since the cell-transforming efficiency of RSV is not changed by simultaneous infection with a helper virus which presumably supplies the missing function.

Finally, it is of interest to note the similarities between the RNA of RAV isolated from a 10:1 mixture of RAV + RSV, of AMV, and of BH-RSV isolated from the NP particle. The fact that these RNA's are indistinguishable in sedimentation constant, base composition, and ribonuclease sensitivity suggests that the three viruses are closely related and that RSV may have arisen by mutation(s) of an avian leukosis virus.

In summary, BH-RSV, RAV, AMV, MLV, and MTV contain single-stranded RNA which sediments between 69 and 74*S*. The RNA of the BH-RSV is indistinguishable from the RNA of RAV and AMV in sedimentation velocity and base composition, indicating that these viruses are closely related.

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[1] Robinson, W. S., A. Pitkanen, and H. Rubin, these PROCEEDINGS, **54**, 219 (1965).
[2] Robinson, W. S., and M. A. Baluda, these PROCEEDINGS, **54**, 1686 (1965).
[3] Duesberg, P. H., and W. S. Robinson, these PROCEEDINGS, **55**, 219 (1966).
[4] Duesberg, P. H., and P. Blair, these PROCEEDINGS, **55**, 1490 (1966).
[5] Rubin, H., and P. K. Vogt, *Virology*, **17**, 184 (1962).
[6] Hanafusa, H., T. Hanafusa, and H. Rubin, these PROCEEDINGS, **49**, 572 (1963).
[7] Harel, J., J. Huppert, F. Lacour, and L. Harel, *Compt. Rend.*, **261**, 2266 (1965).
[8] Harel, L., A. Golde, J. Harel, L. Montagnier, and P. Vigier, *Compt. Rend.*, **261**, 4559 (1965).
[9] Galibert, F., C. Bernard, P. Chenaille, and W. Boiron, *Compt. Rend.*, **261**, 1771 (1965).
[10] Mora, P. T., V. W. McFarland, and S. W. Luborsky, these PROCEEDINGS, **55**, 438 (1966).
[11] Boedtker, H., *J. Mol. Biol.*, **2**, 171 (1960).
[12] Spirin, A. S., in *Progress in Nucleic Acid Research*, ed. J. N. Davidson and W. E. Cohn (New York: Academic Press, 1963), vol. 1, p. 301.
[13] Strauss, J. H., and R. L. Sinsheimer, *J. Mol. Biol.*, **7**, 43 (1963).
[14] Granboulin, N., J. Huppert, and F. Lacour, *J. Mol. Biol.*, **16**, 571 (1966).
[15] Hanafusa, H., and T. Hanafusa, *Virology*, **28**, 369 (1966).
[16] Hanafusa, H., T. Hanafusa, and H. Rubin, *Virology*, **22**, 591 (1964).
[17] Hanafusa, H., T. Hanafusa, and H. Rubin, these PROCEEDINGS, **51**, 41 (1964).
[18] Hanafusa, H., *Virology*, **25**, 248 (1965).
[19] Dougherty, R. M., and H. S. Di Stefano, *Virology*, **27**, 351 (1965).
[20] Trager, G. W., and H. Rubin, *Virology*, **30**, 266 (1966).
[21] Rubin, H., these PROCEEDINGS, **46**, 1105 (1960).
[22] Robinson, H. L., these PROCEEDINGS, in press.
[23] Enders, J. F., A. Holloway, and E. A. Grogan, these PROCEEDINGS, **57**, 637 (1967).
[24] Kohn, A., *Virology*, **26**, 228 (1965).
[25] Bauer, H., and W. Schäfer, *Z. Naturforsch.*, **20b**, 815 (1965).
[26] Bauer, H., *Z. Naturforsch.*, **21b**, 453 (1966).