stable in contrast to the instability of B. This factor was obtained with high specific activity, and because of its link to GTP split, we use the term "G factor" for it. Mainly due to the purification of G, the background of unrelated GTP hydrolysis could be reduced to such an extent that an excess of phosphate release could be observed, apparently dependent on and equivalent to peptide synthesis. The blanks for phosphate liberation are of the same order as the increment seen with the completed system. Nevertheless, this seems to indicate that one mole of GTP is split for every peptide bond formed.

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[1] Allende, J. E., R. Monro, and F. Lipmann, these PROCEEDINGS, **51**, 1211 (1964).

[2] Arlinghaus, R., J. Shaeffer, and R. Schweet, these PROCEEDINGS, **51**, 1291 (1964).

[3] Fessenden, J. M., and K. Moldave, *J. Biol. Chem.*, **238**, 1479 (1963).

[4] Conway, T. W., and F. Lipmann, these PROCEEDINGS, **52**, 1462 (1964).

[5] Keller, E. B., and P. C. Zamecnik, *J. Biol. Chem.*, **221**, 45 (1956).

[6] Nirenberg, M. W., and J. H. Matthaei, these PROCEEDINGS, **47**, 1588 (1961).

[7] Nathans, D., and F. Lipmann, these PROCEEDINGS, **47**, 497 (1961).

[8] von Ehrenstein, G., and F. Lipmann, these PROCEEDINGS, **47**, 941 (1961).

[9] Salas, M., M. A. Smith, W. M. Stanley, Jr., A. J. Wahba, and S. Ochoa, *J. Biol. Chem.*, **240**, 3988 (1965).

[10] Boman, H. G., and S. Hjerten, *Arch. Biochem. Biophys.*, Suppl. I (1962), p. 276.

[11] Colowick, S. P., in *Methods in Enzymology*, ed. N. O. Kaplan and S. P. Colowick (New York: Academic Press, 1955), vol. 1, p. 90.

[12] Gardner, R. S., A. J. Wahba, C. Baslio, R. S. Miller, P. Lengyel, and J. F. Speyer, these PROCEEDINGS, **48**, 2087 (1962).

[13] Martin, J. B., and P. M. Doty, *Anal. Chem.*, **21**, 965 (1949).

[14] Nirenberg, M., and P. Leder, *Science*, **145**, 1399 (1964).

[15] Spyrides, G. J., and F. Lipmann, these PROCEEDINGS, **48**, 1977 (1962).

[16] Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

[17] Nakamoto, T., T. W. Conway, J. E. Allende, G. J. Spyrides, and F. Lipmann, in *Synthesis and Structure of Macromolecules*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 28 (1963), p. 227.

[18] Nathans, D., G. von Ehrenstein, R. Monro, and F. Lipmann, *Federation Proc.*, **21**, 127 (1962).

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## *NUCLEIC ACID AND PROTEINS ISOLATED FROM THE RAUSCHER MOUSE LEUKEMIA VIRUS (MLV)**

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The viral etiology of several mouse leukemias has been firmly demonstrated.[1] The biological effects of these viruses have been studied extensively, but little is known about their molecular structure. It is known, however, that the mouse leukemia viruses have several structural properties in common with the avian tumor

viruses and with the myxoviruses. Their viral nucleic acid is RNA.[2-4] They contain a significant amount of lipid making them disruptable by ether[2, 3, 5] and making their buoyant densities less than those of nonlipid-containing viruses.[3, 6-8] They have similar sizes and similar dense staining inner cores with less dense double outer "membrane" structures in electron micrographs.[3, 9, 10] However, nothing is known about the size and structure of the RNA in the mouse leukemia viruses. Obstacles to such studies have been the difficulty in obtaining large quantities of purified virus and the inability to extract intact RNA from the virus.[4]

Certain features of the mouse leukemia virus (MLV) described by Rauscher[11] appeared to us to make the study of its nucleic acid practical. Its biological effects are unusually rapid after inoculation into mice, so that only 1–3 weeks are required to assay viral infectivity.[12, 13] The virus can be obtained in relatively large amounts from the plasma of leukemic mice.[6] The virus can be grown in tissue culture[14] which facilitates use of radioactive precursors to label viral components. Finally, the structural features in common with the avian tumor viruses and the myxoviruses suggested that the mouse leukemia virus could be purified and its nucleic acid isolated in the manner recently described for the mixture Rous sarcoma virus and Rous associated virus (RSV + RAV),[15] Newcastle disease virus (NDV),[16] and avian myeloblastosis virus (AMV).[17]

The purification of the Rauscher MLV, the isolation and characterization of its nucleic acid, and some properties of the protein are described in this paper. The viral RNA was found to have a sedimentation constant and structure similar to those of the RNA's from RSV + RAV and AMV.[15, 17]

*Materials and Methods.—Virus:* The Rauscher virus was obtained from two sources. (*a*) The first source was medium from cultures of an established cell line JLS-V5 propagating the Rauscher virus, derived from cells of spleen and thymus of normal weanling BALB/c mice.[14] The cells were incubated at 36°C on Eagle's minimal essential medium in Hanks' balanced salt solution supplemented with 10% calf serum.[18] (*b*) The second source was 100 ml plasma from leukemic BALB/c mice, collected by pooling the mouse blood and adding $^1/_{10}$ vol of a 3% sodium citrate solution. This plasma was supplied by Dr. E. M. Jensen of the Pfizer Corp., Maywood, N.J.

*Virus assay: Assay of virus infectivity:* The virus was assayed by the "spleen-weight-assay"[12] using weanling (3–4 week-old) BALB/c mice. Ten mice for each virus sample to be assayed were inoculated intraperitoneally with 0.1 ml of the appropriate virus dilution. They were sacrificed 14 days later and the wet spleen weight was determined.

*Virus purification:* The virus was purified in two steps, both carried out in the cold (0–4°C). The same procedure was used for the purification of virus from plasma and tissue culture medium. Before purification the virus material was centrifuged for 10 min at 10,000 rpm in a Servall centrifuge to remove cellular debris.

(1) *Centrifugation to a density interface:* A sucrose solution with a density of approximately 1.32 gm per ml containing 65% (w/v) sucrose, 0.001 *M* Tris·HCl, 0.1 *M* NaCl, and 0.001 *M* EDTA was prepared in $D_2O$. Three ml of this solution were placed at the bottom of a 30-ml tube for the Spinco SW25 rotor and overlaid with 5 ml of a 15% (w/v) sucrose-$H_2O$ solution containing the same buffer. The remainder of the tube was then filled with the virus solution and it was centrifuged for 1 hr at 25,000 rpm. After centrifugation of plasma from leukemic mice a visible band was seen on top of the sucrose-$D_2O$ solution. This band was found to possess all of the MLV infectivity. Centrifugation of the tissue culture medium from the JLS-V5 cell line yielded a faint band, indicating that plasma contains a much higher concentration of virus than tissue culture medium. Infectivity studies also have shown that plasma contains much higher concentrations of virus than tissue culture medium.[18] After centrifugation, the medium over the virus band was removed by suction and replaced by more medium containing virus. After three

such centrifugations a heavier band of virus was present. The tube was punctured at the bottom and the virus band collected in approximately 2–3 ml. It was then diluted with 2 or 3 vol of buffer, containing 0.01 *M* Tris·HCl pH 7.4, 0.1 *M* NaCl, and 0.001 *M* EDTA to lower the solution density. With this virus solution a similar procedure was carried out in a 5.4-ml tube for the Spinco SW50 rotor, and centrifugations were at 50,000 rpm for 30 min. In this way the virus in 80 or 100 ml of plasma or tissue culture medium was concentrated into about 0.3 ml with considerable purification and without pelleting and resuspending the virus.

(2) *Sucrose density gradient:* This concentrated virus solution was then diluted with the previously described Tris-buffer pH 7.4 to a final volume around 1.0 ml to reduce the solution density. This was layered on top of a linear sucrose gradient (15–60% sucrose, 0.01 *M* Tris·HCl pH 7.4, 0.1 *M* NaCl, 0.001 *M* EDTA) and centrifuged in a Spinco SW50 rotor at 46,000 rpm for 3 hr. The virus band could be seen at about the middle of the gradient. When the virus was purified from tissue culture medium, a separate light scattering band of nonviral material usually appeared between the virus band and the top of the tube. The virus was collected by puncturing the tube from the bottom and collecting fractions. When $P^{32}$-labeled virus was purified from tissue culture medium, several $P^{32}$-labeled components in addition to virus appeared in the final sucrose gradient. None of these were found to contain RNA and they probably consist of cellular components containing phospholipid.

*Growth of $H^3$- and $P^{32}$-labeled virus:* Labeled virus was produced by placing 6 ml Eagle's medium containing 250 $\mu$c $P^{32}O_4$ or 100 $\mu$c uridine $H^3$ ($H^3$-U) on a confluent JLS-V5 cell layer in a 10-cm plastic tissue culture plate. The medium was collected from each of four such plates every 12 hr for 48–72 hr and replaced each time by fresh medium containing $P^{32}O_4$ or $H^3$-U. After centrifugation at 10,000 rpm for 10 min the medium was frozen at −80° C for later purification of the virus.

*Isolation of the RNA:* The appropriate fractions from the final sucrose density gradient step of the virus purification were pooled and the RNA was extracted by the phenol-SDS method as previously described.[16]

*Isolation of the protein for acrylamide gel electrophoresis:* After the first phenol extraction[16] of the virus solution, the protein was recovered from the phenol phase. The phenol was washed two times with the described Tris buffer pH 7.4. The protein was precipitated by the addition of 5 vol of 95% ethanol and several drops of a 2 *M* ammonium acetate solution. The precipitate was allowed to flocculate in the cold, collected by centrifugation, and washed with 50% ethanol to remove the remaining phenol. The protein was then dissolved in 8 *M* urea containing 0.1 *M* Tris·HCl pH 8.1 and 0.01 *M* EDTA and reduced at room temperature with Dithiothreitol (DTT) 1 mg per ml. The reduced protein was then reprecipitated with ethanol and redissolved in 8 *M* urea containing 0.01% DTT. This protein solution was examined by disk electrophoresis on polyacrylamide gel columns (6 × 0.3 cm) in three different pH systems containing 8 *M* urea. All gel-buffer stock solutions were 66% dilutions of those previously described[19] and only 0.25 mg $(NH_4)_2S_2O_8$ per ml final gel-solution was employed to polymerize the acrylamide.

*Results and Discussion.—Purification of virus and comparison of the plasma and tissue culture-grown virus:* Figure 1 shows the results of an experiment comparing the virus in the plasma of leukemic mice with the virus produced in tissue culture. Tissue culture medium (36 ml) containing U-$H^3$-labeled virus was added to plasma from leukemic mice (100 ml), and the virus was purified as described in *Methods*. An easily visible virus band appeared in the middle of the tube after the final sucrose gradient step of purification. When virus from 36 ml of tissue culture medium was purified alone, too little virus was present to produce a visible virus band in the final sucrose gradient. Consequently, most of the virus detected optically in this experiment represents virus from plasma and the tritium-labeled virus is from tissue culture. The viral infectivity of each fraction was determined at two concentrations of virus, and the results in Figure 1 indicate that, under the conditions used, spleen weight is a function of virus concentration. Viral infectivity, radioactivity, and absorbancy at 260 m$\mu$ are seen to coincide. This result indicates that all of the tritium-labeled material and all of the UV-absorbing

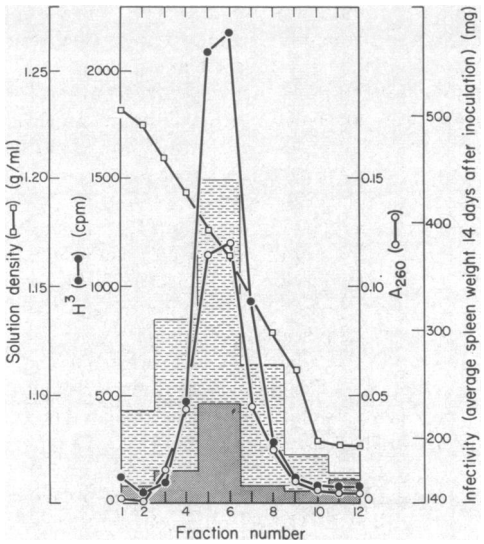


FIG. 1.—Distribution of $H^3$ (●—●—●), absorbancy (○—○—○), and infectivity (shaded areas) after equilibrium density gradient (□—□—□) centrifugation of purified U-$H^3$-labeled tissue culture and unlabeled plasma MLV. Virus solution (1.2 ml) was layered over a linear gradient of 4.6 ml sucrose 15–60% (w/v) containing 0.01 *M* Tris·HCl pH 7.4, 0.1 *M* NaCl, and 0.001 *M* EDTA and was centrifuged for 3 hr at 46,000 rpm in a Spinco SW50 rotor at 5°C. Fractions of 450 μl were collected. One aliquot (50 μl) of each fraction was diluted with an equal volume of water and counted in a Tricarb liquid scintillation counter using Bray's scintillation fluid.[24] Another aliquot (50 μl) was diluted with 1 ml of the described pH 7.4 Tris buffer to determine the optical density. Viral infectivity was then determined by pooling equal aliquots of consecutive fractions and diluting each 1:30 with tissue culture medium containing 1% calf serum (see *Methods*). Into each of 10 mice, 0.1 ml of each sample (light shaded area) and 0.1 ml of a 1:10 dilution of each (dark shaded area) was then inoculated. Spleen weights were determined as described in *Methods*. The average spleen weight of 10 uninfected mice (140 mg) was chosen as the base line of the infectivity scale.

material has the buoyant density of infectious virus. Further, the buoyant density of virus from tissue culture is identical with that of virus from plasma. The observed density of 1.17 gm per ml is in agreement with the previously reported buoyant density of MLV.[6, 20]

*Properties of the viral nucleic acid:* The sucrose gradient fractions containing tritium-labeled virus from tissue culture and unlabeled virus from plasma (fractions 4–7) obtained in the experiment shown in Figure 1 were pooled, and the nucleic acid was isolated as described in *Methods* using cellular sRNA as carrier. The nucleic acid was then fractionated by sucrose gradient centrifugation. The result shown in Figure 2 was obtained by layering 200 μl nucleic acid solution over a linear gradient of sucrose (5–20%) containing 0.1 *M* NaCl, 0.01 *M* Tris·HCl pH 7.4, and 0.001 *M* EDTA, and centrifuging for 2 hr and 10 min at 36,000 rpm in a Spinco SW39 rotor at 5°C.

It can be seen that there are two components of radioactively labeled material. One sediments rapidly and appears near the bottom of the gradient. The other sediments much more slowly and is near the top of the gradient. Both are rendered completely TCA-soluble by pancreatic ribonuclease (method described previously)[15] indicating that they consist of RNA. The fast-sedimenting component is con-

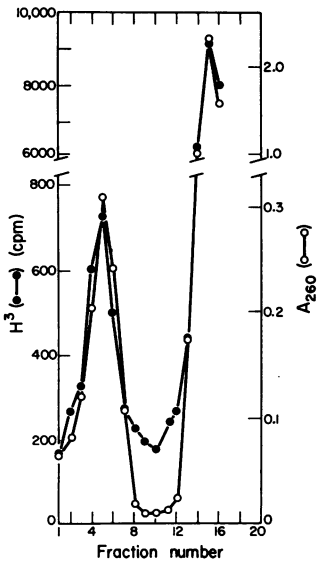


FIG. 2.—Distribution of $H^3$ (●—●—●) and absorbancy (○—○—○) at 260 mμ after velocity sedimentation of MLV-RNA obtained from U-$H^3$-labeled tissue culture and unlabeled plasma virus. As carrier 200 μg sRNA was added. A 0.2-ml RNA solution was layered on top of a 5-ml linear sucrose gradient (5–20% w/v) containing 0.01 *M* Tris·HCl pH 7.4, 0.1 *M* NaCl, and 0.001 *M* EDTA, and centrifuged for 2 hr 10 min at 36,000 rpm in a Spinco SW39 rotor at 5°C. An aliquot of each fraction was counted directly in Tricarb liquid scintillation counter using Bray's scintillation fluid. The absorbancy was measured in a Zeiss PQM II spectrophotometer.

sidered to be intact MLV-RNA. The slow-sedimenting component is probably degraded viral RNA, although the presence of RNA from other sources has not been excluded. The recovery of two distinct RNA components from purified virus was also found in the cases of RSV + RAV,[15] NDV,[16] and AMV,[17] and their significance is discussed in these papers. In a recent study of MLV[4] only heterogeneous RNA with an average sedimentation constant of 3.9*S* was recovered. This material may be the same as the slowly sedimenting RNA component in our RNA preparation which sediments with cellular 4*S* RNA. The UV-absorbing peak at the top of the gradient in Figure 2 represents sRNA used as carrier in the isolation of viral RNA. The UV-absorbing peak near the bottom of the gradient coincides exactly with the fast-sedimenting component of tritium-labeled viral RNA and represents the RNA from the virus in 100 ml of leukemic plasma (experiments have shown that too little RNA is obtained from the virus in 36 ml of tissue culture medium to be detected optically). Thus, not only are the viruses obtained from tissue culture and plasma identical in buoyant density but, as might be expected, their RNA's have identical sedimentation rates.

*RNA base composition:* MLV was labeled with $P^{32}O_4$ in tissue culture as described in *Methods*, the virus was partially purified, and the RNA was extracted and then fractionated by sucrose gradient centrifugation (as shown in Fig. 2). The fast-sedimenting component of $P^{32}$-labeled viral RNA was recovered and its base composition determined as previously described.[16] The values are shown in Table 1 and do not suggest complementary base pairing.

*Sedimentation velocity:* The sedimentation properties of MLV-RNA were determined by two different methods. First, it was compared with the RNA of RSV + RAV in the preparative ultracentrifuge. The RNA's of $H^3$-labeled MLV and $P^{32}$-labeled RSV + RAV were isolated together and fractionated simultaneously by sedimentation in a sucrose gradient using the conditions described in the legend for Figure 2. The results are shown in Figure 3. Tobacco mosaic virus (TMV)

RNA added as a carrier is represented by the $A_{260}$ tracing. The RNA of MLV (closed circles) has a slightly higher sedimentation rate than RNA of RSV + RAV (closed triangles) which has been shown to have a sedimentation constant of 71 $S_{20,w}$.[17] The small peak of tritium at fraction 18 represents association of tritium-labeled material with carrier TMV-RNA and does not represent a separate component of MLV-RNA. In the absence of TMV-RNA this peak is never present (Fig. 2).

Since dependence of the sedimentation constant on ionic strength has been shown to be characteristic for single-stranded RNA,[21, 22] a sedimentation experiment with $H^3$-U-labeled MLV and $P^{32}O_4$-labeled RSV + RAV-RNA in 0.001 $M$ salt was carried out. Both RNA's show (Fig. 4) a similar reduction in their sedimentation

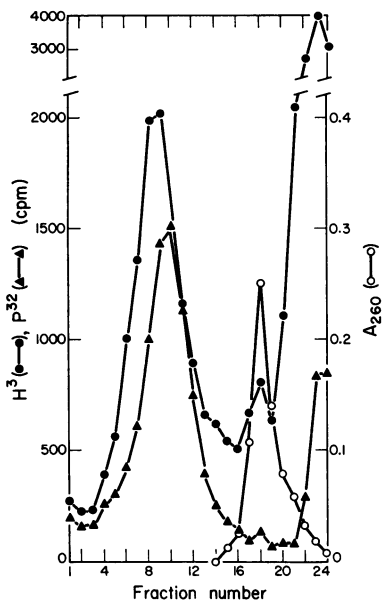


FIG. 3.—Distribution of $H^3$ (●—●—●), $P^{32}$ (▲—▲—▲), and absorbancy at 260 mμ (○—○—○) after velocity sedimentation in a sucrose gradient of $H^3$-labeled MLV-RNA, $P^{32}$-labeled RSV + RAV-RNA and TMV-RNA(15 μg). The RNA's were centrifuged for 2 hr 10 min as described for Fig. 2.

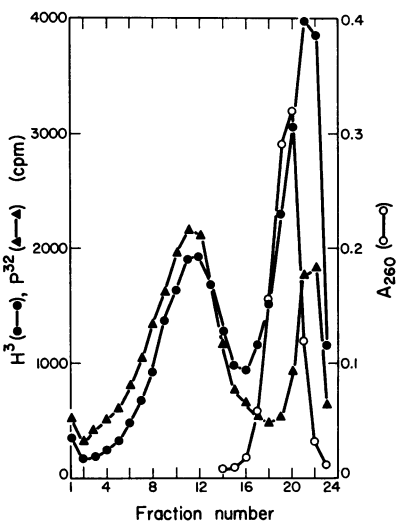


FIG. 4.—Distribution of $H^3$ (●—●—●), $P^{32}$ (▲—▲—▲), and absorbancy at 260 mμ (○—○—○) after velocity sedimentation of $H^3$-labeled MLV-RNA, $P^{32}$-labeled RSV + RAV-RNA and TMV-RNA(15 μg). The RNA's, dissolved in 0.150 ml water, were layered on top of a 5.4-ml linear sucrose gradient (5–20% w/v) containing 0.001 $M$ Tris·HCl pH 7.4 and 0.00025 $M$ EDTA, and centrifuged for 2 hr 10 min at 50,000 rpm in a Spinco SW50 rotor at 5°C.

constant in low salt. A value of 27 $S_{20,w}$ has been obtained for RSV-RNA under these conditions.[17] Thus, MLV-RNA is undoubtedly single-stranded and probably similar to RSV-RNA in size and secondary structure.

Second, the sedimentation properties of intact MLV-RNA were studied in the Spinco model E analytical ultracentrifuge using UV-optics. This experiment was done with a minimum of RNA because of the limited amount of material available. MLV-RNA was obtained by purification of virus from 90 ml plasma from leukemic BALB/c mice. The total fast-sedimenting RNA recovered after sucrose gradient fractionation (as shown in Fig. 2) was about 20–30 μg.

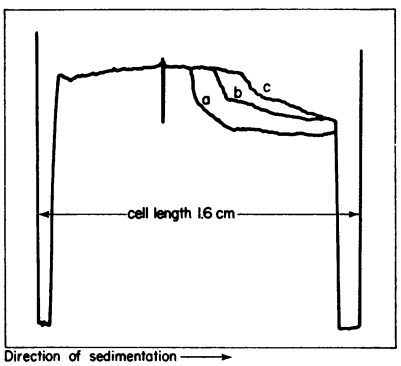


FIG. 5.—Ultraviolet patterns of MLV-RNA approximately 20 $\mu$g/ml in 0.01 $M$ Tris·HCl pH 7.4, 0.10 $M$ NaCl, and 0.001 $M$ EDTA sedimenting at 42,040 rpm in the Spinco model E ultracentrifuge at 3°C. Photographs were taken every 4 min for calculation of the sedimentation constant. (*a*) Tracing of the first picture taken at full speed; (*b*) and (*c*) the UV patterns 4 and 8 min later.

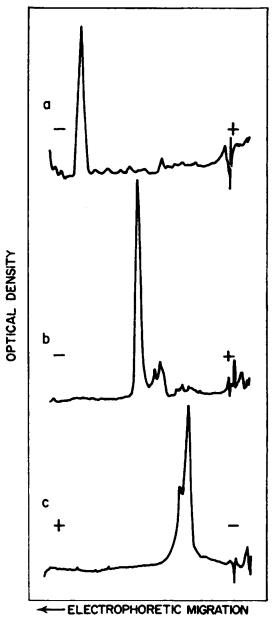


FIG. 6.—Densitometer tracings of MLV-protein after electrophoresis in acrylamide gel in 8 $M$ urea at three different pH's. After electrophoresis the proteins were stained with amido black and directly traced with a Joyce-Loebl densitometer. The right end of the abscissa represents the top of the gel columns. (*a*) The pattern obtained at pH 3.8; (*b*) the pattern at pH 4.5; (*c*) the distribution of the MLV-protein at pH 9.9.

The fast-sedimenting RNA component was precipitated with alcohol and redissolved in 0.5 ml buffer containing 0.01 $M$ Tris·HCl pH 7.4, 0.10 $M$ NaCl, and 0.001 $M$ EDTA for analysis in the ultracentrifuge. Densitometer tracings from the sedimentation of intact MLV-RNA are shown in Figure 5. About 70 per cent of the material sediments with a fairly sharp boundary. The remaining material appears to consist of an aggregate leading the sharp portion of the boundary. Very little material trails the sharp portion of the boundary. The sedimentation constant calculated using the sharp portion of the boundary was 74 $S_{20,w}$.

If it is assumed that the viral RNA is single-stranded, as suggested by the base composition (Table 1), susceptibility to ribonuclease, and the dependence of sedimentation constant on salt concentration, an estimate of its molecular weight can be made with the empirical relation: $M = 1550\ (S_{20,w})^{2.1}$ determined by Spirin[23] for TMV-RNA in 0.1 $M$ NaCl, 0.01 $M$ EDTA. A single-stranded RNA with $S_{20,w}$ of 74 in 0.1 $M$ NaCl would have a molecular weight of $13 \times 10^6$. It has been shown[21] that the relationship of sedimentation constant and molecular weight is not the same for all single-stranded RNA's so that the value calculated here must be considered an estimate.

No single-stranded RNA with a sedimentation constant as great as 74$S$ has been previously described. The avian tumor viruses, RSV + RAV and AMV, were recently shown to contain RNA's with a sedimentation constant of 71 $S_{20,w}$.[17] Thus, avian tumor viruses and the Rauscher virus contain single-stranded RNA's

TABLE 1

INTACT MLV-RNA*

| | |
|---|---|
| C | = 26.7 ± 0.4 |
| A | = 25.5 ± 0.4 |
| G | = 25.1 ± 0.4 |
| U | = 22.7 ± 0.3 |

The base composition was determined, using $P^{32}O_4$-labeled RNA from two different virus preparations. The viral RNA was fractionated by sucrose gradient centrifugation (Fig. 2). The appropriate fractions from the fast-sedimenting RNA peak were pooled, 100 μg carrier TMV-RNA was added, and the total RNA precipitated with ethanol. The RNA was then hydrolyzed, and the mononucleotides were separated by high-voltage paper electrophoresis as described previously.[16] All radioactivity on the paper was confined to the four nucleotide spots visible with the UV lamp.

* Av. 6 ± SE.

of similar size, and these RNA's are probably larger than the known single-stranded RNA's from other groups of viruses.

*Properties of the viral proteins:* The virus protein was recovered from purified virus (fractions 4–7 in Fig. 1) by phenol extraction (*Methods*). Figure 6*a* represents a densitometer tracing of the total virus protein resolved by electrophoresis at pH 3.8 in an acrylamide gel column and stained with amido black. Most of the protein appears in one single band near the bottom of the gel. A close examination of this band shows that the leading portion is much steeper than the trailing portion of the peak, indicating heterogeneity. This observation was confirmed by analyzing the protein at pH 4.5. Both acidic gels show several minor components which may represent minor viral components or contaminating proteins in the virus preparation.

The electrophoretic pattern of the viral protein at pH 9.9 shows a resolution of the main portion of the protein in two components. This result confirms the heterogeneity of the main band suggested by its asymmetric shape in the acidic system. Therefore, it is believed that the virus contains at least two major protein subunits.

*Summary.*—Rauscher mouse leukemia virus has been purified from plasma and tissue culture medium. The identity of the virus obtained from the two sources is demonstrated. Intact nucleic acid isolated using SDS and phenol extraction consists of single-stranded RNA with $S_{20,w}$ = 74 in 0.1 *M* salt. The purified virus was found to contain at least two major protein subunits.

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[1] Moloney, J. B., *Ann. Rev. Med.*, **15**, 383 (1964).

[2] Schäfer, W., in *The Viruses*, ed. F. M. Burnet and W. M. Stanley, vol. 1, p. 475 (1959).

[3] Vogt, P. K., *Advan. Virus Res.*, in press.

[4] Mora, P. T., and V. W. McFarland, these PROCEEDINGS, **54**, 756 (1965).

[5] O'Connor, T. E., F. J. Rauscher, G. deThe, P. Gerber, and M. A. Fink, to be published in *Natl. Cancer Inst. Monograph.*

[6] O'Connor, T. E., F. J. Rauscher, and R. F. Zeigel, *Science*, **144**, 1144 (1964).

[7] Barry, R. D., *Australian J. Exptl. Biol. Med. Sci.*, **38**, 499 (1960).

[8] Stenback, W. A., and D. P. Durand, *Virology*, **20**, 545 (1963).

[9] Dalton, A. J., and F. Haguenau, *Tumors Induced by Virus: Ultrastructural Studies* (New York: Academic Press, 1962).

[10] Waterson, A. P., in *Newcastle Disease Virus*, ed. R. P. Hanson (Univ. of Wisc. Press, 1964), p. 119.

[11] Rauscher, F. J., *J. Natl. Cancer Inst.*, **29**, 515 (1962).

[12] Rauscher, F. J., and B. V. Allen, *J. Natl. Cancer Inst.*, **32**, 269 (1964).

[13] Pluzink, D. H., and L. Sachs, *J. Natl. Cancer Inst.*, **33**, 535 (1964).
[14] Wright, B. S., and J. C. Lasfarques, *J. Natl. Cancer Inst.*, **35**, 319 (1965).
[15] Robinson, W. S., A. Pitkanen, and H. Rubin, these PROCEEDINGS, **54**, 137 (1965).
[16] Duesberg, P. H., and W. S. Robinson, these PROCEEDINGS, **54**, 794 (1965).
[17] Robinson, W. S., and M. Baluda, these PROCEEDINGS, **54**, 1686 (1965).
[18] Jensen, E. M., personal communication.
[19] Duesberg, P. H., and R. R. Rueckert, *Anal. Biochem.*, **11**, 342 (1965).
[20] Chenaille, P., F. P. Levy, M. Boiron, and J. Bernard, *Compt. Rend.*, **248**, 3129 (1964).
[21] Strauss, H. J., and R. Sinsheimer, *J. Mol. Biol.*, **7**, 43 (1963).
[22] Boedtker, H., *J. Mol. Biol.*, **2**, 171 (1960).
[23] 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.
[24] Bray, S. A., *Anal. Biochem.*, **1**, 279 (1960).

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## *ERRATUM*

In the article entitled "The Structural Gene for Deoxyribonucleic Acid Polymerase in Bacteriophages T4 and T5," by A. de Waard, A. V. Paul, and I. R. Lehman, which appeared in the October 1965 issue of these PROCEEDINGS (vol. 54, 1241–1248), in the legend to Table 1, the designation for mutant X5 should read "X5, N81 (gene 41), N122 (gene 42), B22 (gene 43), N82 (gene 44), and E10 (gene 45)."