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*NUCLEIC ACID OF THE RAUSCHER MOUSE LEUKEMIA VIRUS**

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We wish to report the first successful extraction of an intact RNA from the Rauscher murine leukemia virus, in amounts sufficient for characterization by physicochemical means. As far as we know, this virus contains the largest RNA ever observed. It exhibits a sedimentation coefficient of 73*S* in 0.2 *M* salt, commensurate with a molecular weight of 13×10^6. The virus was obtained from the highest infectious titer source known, the plasma of viremic mice. A preliminary account of these results was given at the Conference on Murine Leukemia, held in October 1965.[1]

Mixed mouse spleen and thymus cell cultures support the growth of virus particles after infection with the Rauscher virus from mouse plasma.[2] A radioactive, RNase-sensitive, 70*S* component was recently detected by rate zonal centrifugation of the extract of fluids from P^{32}-labeled Rauscher virus-infected tissue cultures.[3] Caution must be exercised, however, in equating this RNA, or the RNA obtained from purified Rauscher virus produced in tissue culture, with the RNA of the virus in the plasma of viremic mice. The infectivity of the tissue culture fluids, containing large numbers of virus particles, is 3–5 orders of magnitude below that of the "plasma" virus.[2, 4] The relationship between the particles obtained from an infected tissue culture, and those obtained from the plasma of viremic mice, is not clear. Thus, although we detected RNA more than a year ago as the main nucleic acid component of the purified particles from infected tissue cultures,[5] we decided to concentrate instead upon obtaining an undegraded RNA in good yield from the highly infectious plasma virus.

A suitable method for the extraction of undegraded RNA from animal viruses has long eluded interested investigators. Recently, however, there have been reports

of successful extractions from Rous sarcoma[6] and from Newcastle disease viruses,[7] from which apparently intact RNA was obtained. These viruses and the murine leukemia viruses have several structural features in common, apparently because their surface is coated with the membrane components of the cells of their origin. As such cellular structures may very well contain RNase, and we had observed the extreme sensitivity of the Rauscher virus RNA to degradation,[8] we became aware of the necessity of employing conditions which would minimize the chance of degradation of the viral RNA.

We have devised a rapid purification and extraction procedure for the extraction of the RNA in the presence of an RNase inhibitor, and have now succeeded in obtaining appreciable quantities of undegraded RNA in our extract. We have been able to characterize both the RNA extract and the purified, intact virus from which the extract was made, by following the sedimentation of their UV absorbancies in the analytical ultracentrifuge. We have further determined the effect of heat upon the UV-absorption spectrum of the RNA, and obtained a "melting curve" similar to that to be expected from a single-stranded RNA molecule.

Materials and Methods.—Source of virus: Concentrated stocks of the Rauscher murine leukemia virus were prepared by the method referred to previously,[8] from the plasma of viremic mice, at Charles Pfizer and Company, Inc., Maywood, N. J., under the supervision of Dr. F. J. Rauscher.[9] The virus from 10 ml of plasma was resuspended in 1 ml of 0.05 *M* sodium citrate-citric acid buffer, pH 6.8, and was delivered to us frozen in sealed glass vials. Special precautions were taken during shipment so that the vials would remain approximately at solid CO_2 temperature. Once these solutions were thawed, the purification, extraction, and analytical ultracentrifugation were carried out without pause.

Buffer solution: The buffer solution used throughout this work contained 0.18 *M* NaCl, 0.02 *M* sodium phosphate buffer, pH 7, and 10^{-3} *M* magnesium chloride.

Electron microscopy: Dr. Guy de-Thé, of our laboratory, performed the electron microscopy of the Rauscher virus for us.[10] An aliquot of the virus zone, obtained from the discontinuous sucrose density gradient (see below), was diluted with buffer to reduce the solution density, and the virus was sedimented from the solution. The pellet so obtained was fixed for 1 hr with 2.5% glutaraldehyde buffered with sodium cacodylate, pH 7.4,[11] washed in cacodylate buffer, pH 7.2, for a few hours, postfixed for 40 min with 1% OsO_4 buffered following Palade,[12] dehydrated in ethanol, and embedded in epon-araldite mixture.[13] Thin sections were stained with uranyl acetate[14] and lead citrate,[15] and finally examined in the Siemens IA electron microscope.

Analytical ultracentrifugation: All analytical ultracentrifugations were performed at room temperature in the Spinco model E analytical ultracentrifuge, with UV optics. The solvent for the RNA was the 0.2 *M* buffer solution, containing 50 μg/ml polyglucose sulfate. The latter substance has no detectable absorbancy at the concentration used. An aliquot of the virus zone obtained from the sucrose gradient was dialyzed against the buffer solution before ultracentrifugation. Both the "intact virus" solution thus obtained, and the RNA extract, were adjusted to absorbancies of 0.75–0.90, at 260 mμ, for velocity sedimentation.

Temperature dependence of spectra: Measurement of absorbancy changes with temperature was performed in the Zeiss PMQII spectrophotometer equipped with a thermostatic cell holder, with a calibrated thermister immersed in a cuvette. Temperature control and measurement were performed using an apparatus built in our laboratory[16] according to a design previously described.[17] Absorbancy readings at every temperature, after a steady value was obtained, were corrected for volume expansion of water, and for light scattering. The scattering correction was made[18] from absorbancy readings in the nonabsorbing region, 310–350 mμ.

Procedure: Figure 1 outlines the procedure adopted to purify rapidly and concentrate the virus, utilizing its known physical parameter, a buoyant density of 1.16, reported[19, 20] as the density of the isodense band of viral infectivity in a sucrose gradient. Utilizing the discontinuous sucrose density gradient shown, virus was concentrated from relatively large volumes of fluid, at the 1.16–1.18 density interface. The virus zone isolated after centrifugation had a density of 1.154

gm/ml. An aliquot of this zone was set aside for characterization of the purified virus by electron microscopy and by analytical ultracentrifugation. The main portion of the virus zone was diluted with buffer to reduce the solution density, brought to a concentration of 5 μg/ml sodium polyglucose sulfate,[21] and the virus sedimented from the solution. The resultant pellet was taken up in a small volume of buffer, which contained 50 μg/ml polyglucose sulfate, and the RNA, extracted by treatment with sodium lauryl sulfate, phenol, ether, and nitrogen, as previously described.[1, 8] The molecular integrity of the RNA in the extract thus obtained was investigated immediately by analytical ultracentrifugation.

From an initial 30 ml of virus concentrate, 1.3 ml of aqueous extract was obtained, in addition to the samples set aside for virus characterization. This extract exhibited a characteristic nucleic acid UV-absorption spectrum, shown undiluted in Figure 2.

Results.—Virus characterization: The electron micrographs (Figs. 3 and 4) of virus recovered from the virus zone (Fig. 1) reveal a homogeneous population of virtually pure "*c*-type" virus particles,[23] characteristic of murine leukemia virus.[10, 24] The diameter of these particles is about 100 mμ. Many of these particles have "tail-like" elongated appendices.

The sedimentation pattern of the intact virus in the analytical ultracentrifuge, after dialysis against the 0.2 *M* buffer solution, is shown in Figure 5. The sharp boundary of the slowest-moving component sediments at a rate of 640*S*. Heterogeneous fast-moving material amounts to about 35 per cent of the material.

Characterization of the RNA extract: Immediately after completion of the extraction procedure, the analytical ultracentrifugation of the extract revealed (Fig. 6) a sharp 73*S* sedimenting boundary, representing about 57 per cent of the total material. A small amount of 4–8*S* material is also present, the remainder being non-sedimentable.

Upon completion of the sedimentation experiment, the ultracentrifuge cell was refrigerated and gently shaken to resuspend the sedimented material. The same cell was rerun in the ultracentrifuge the following morning in order to investigate the extent of breakdown of the RNA. A very diffuse boundary was observed, which represented only 30 per cent of the total UV absorbancy, and sedimented at

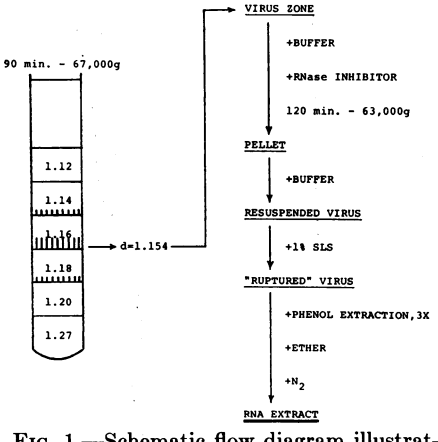

FIG. 1.—Schematic flow diagram illustrating the procedure used to concentrate and purify the Rauscher murine leukemia virus and extract its RNA (*ca.* 5°C).

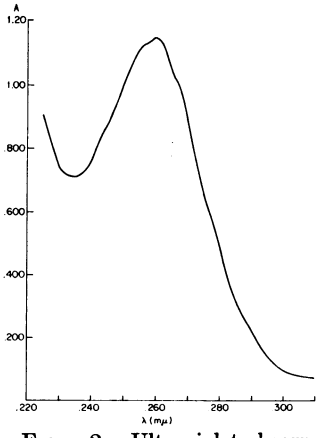

FIG. 2.—Ultraviolet-absorption spectrum of the nucleic acid extract of the Rauscher virus. Virus purification and extraction conditions as shown in Fig. 1.

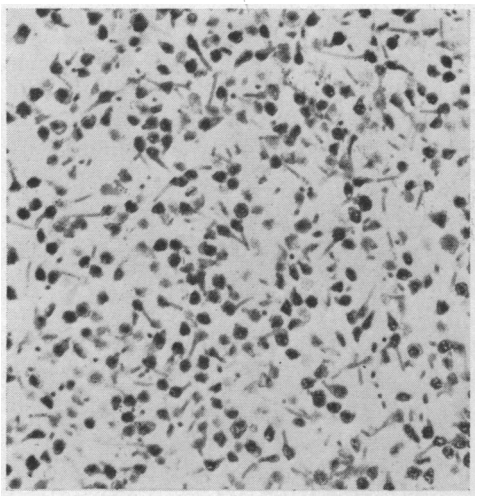

FIG. 3.—Electron micrograph of a thin section of a Rauscher plasma virus pellet obtained from the main virus band after sucrose density gradient centrifugation (described in text). Note the homogeneity of the preparation. Magnification 10,500×.

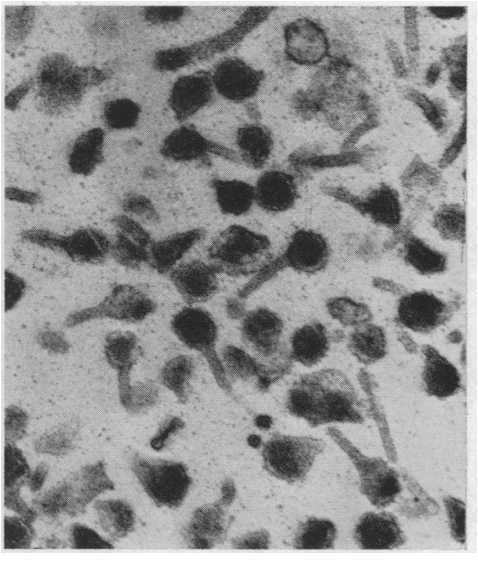

FIG. 4.—Same as Fig. 3. Tail-like appendices are well visualized, but no cell debris is visible. Magnification 60,000×.

67*S*. Apparently, there had been extensive degradation of the material in the ultracentrifuge cell.

A second aliquot, which had been refrigerated for approximately 18 hr, was subjected to ultracentrifugation and found to be still essentially identical to the original extract. The 73*S* material constituted 50 per cent of the total. Simultaneously, an experiment was initiated to examine the secondary structure of the RNA. We

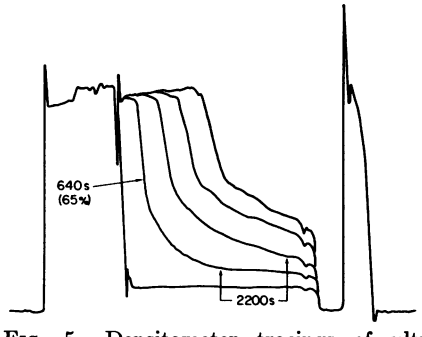

FIG. 5.—Densitometer tracings of ultraviolet absorption photographs taken during analytical ultracentrifugation of the Rauscher virus after density gradient purification (described in text). Tracings show the boundary during acceleration and at 2-min intervals thereafter (*left to right*) at 15,220 rpm. The sharp sedimentation boundary of the slowest-moving component (640*S*) represents about 65% of the total ultraviolet-absorbing material. An upper limit (2200*S*) to the sedimentation (of presumed aggregates) was estimated between the two arrows as the slowest position still apparently in the plateau region.

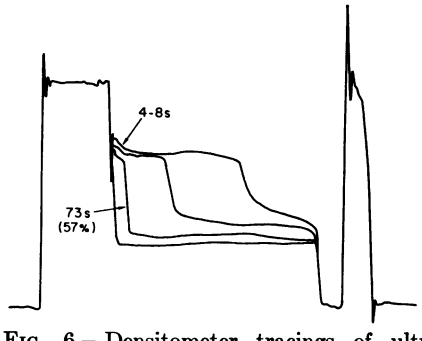

FIG. 6.—Densitometer tracings of ultraviolet absorption photographs taken during analytical ultracentrifugation of the Rauscher virus nucleic acid extract immediately after extraction (described in text). Tracings show the boundary at 1, 3, 7, and 11 min of centrifugation (*left to right*) at 44,770 rpm. The sharp 73*S* sedimenting boundary represents about 57% of the total ultraviolet-absorbing material. A small amount of 4–8*S* material is also present, the remainder being nonsedimentable.

determined the effect of heat upon the spectrum of the remaining aliquot of the extract.

Thermal denaturation of the RNA extract: The absorbancy changes at 260 mμ due to heating and to cooling show (Fig. 7), after correction for light scattering, a gradual hyperchromic transition which exhibits a relatively low median temperature (T_m = 59°) in the 0.2 *M* solvent used. The absorbancy begins to rise at relatively low temperatures. Upon cooling, the hyperchromicity is lost and the original absorbancy recovered. After the heating and cooling cycles (*ca.* 19 hr), ultracentrifugation of this sample revealed only 50 per cent of 4.4*S*, and 50 per cent nonsedimentable, material.

Temperature dependence of RNA spectrum: The extent to which the original lower absorbancy is regained can be seen from Figure 8. This figure shows spectra of the RNA extract, after correction for light scattering, at room temperature, at the maximum temperature of heating, and after cooling again to room temperature. The original absorbancy is essentially regained across the entire spectrum. These data are being analyzed by methods developed by Felsenfeld[17, 25] in an attempt to determine the composition of the material involved in the "melting" transition.

Additional information concerning composition can be derived from an analysis of the spectral changes after hydrolysis. A large additional hyperchromism was obtained after hydrolysis with 0.3 *N* KOH for 18 hr at 37°C. This is, of course, consistent with the fact that the Rauscher murine leukemia virus is an RNA virus.[8] This analysis is under way, using computer techniques developed by Pratt.[26]

Discussion.—Physical characterization of the virus: The major component present in the purified virus zone which sediments as a relatively homogeneous species at 640*S* (Fig. 5) is thought to be the virus particle. The other, more rapidly sedimenting fraction of the preparation contains a heterogeneous population of par-

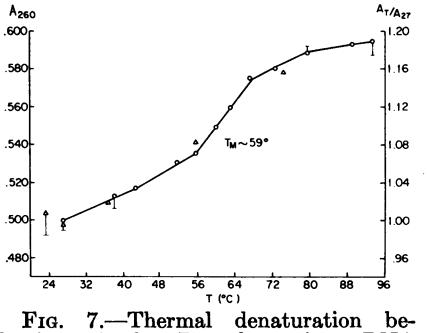

FIG. 7.—Thermal denaturation behavior of the Rauscher virus RNA extract. Absorbancy at 260 mμ as a function of temperature is shown for the heating cycle (*solid line, open circles*) and for the cooling cycle (*triangles*). The bars indicate the estimated limits of uncertainty introduced by the long extrapolation required to determine the scattering correction. T_m is the median transition temperature of the hyperchromicity. Details of experiment given in text. Solvent: 0.02 *M* sodium phosphate buffer, pH 7, 0.18 *M* sodium chloride, 0.001 *M* magnesium chloride.

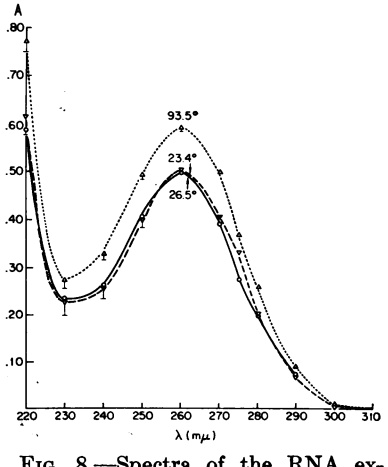

FIG. 8.—Spectra of the RNA extract at room temperature (○), at the highest temperature of the heating cycle (△), and after cooling again to room temperature (▽). Details of experiment given in legend to Fig. 7, and in text.

ticles, presumably including aggregates. The heterogeneity in structure of two other large, lipid-containing viruses, Newcastle disease virus[7] and Rous sarcoma virus,[6, 27] has been described. This heterogeneity has been attributed to imprecision in the acquisition of cell membrane material during the formation of the outer viral envelope at the cell surface. The sedimentation heterogeneity of the population may also be affected, however, by the past history of the virus preparation, which, in addition to affecting the extent of aggregation of the virus particles, can, of course, affect the extent of deformation of the virus envelope. Considering its origin, from the cell membrane, this envelope is presumably a loose, nonrigid structure, easily deformed by osmotic, shear, or other forces.

The electron micrographs obtained from the pellet of purified virus show many particles with "tails." The diameter (100 mμ) estimated for these particles (Fig. 4) agrees with that of spherical particles seen in micrographs obtained[28] using virus directly from the Pfizer preparations. Perhaps the nonrigidity of the virus envelope, suggested above, is indeed the correct basis for explaining the differences in shape noted for this virus.

The Rauscher virus particle weight can be estimated to be 2.2×10^8 from the sedimentation coefficient of the purified virus (640*S*), the diameter (100 mμ), and the value of the buoyant density (1.16 gm/ml)—assuming the particles are spherical. Since any anisotropy would reduce the *S* value, this viral particle weight may only be a minimum value.

Characterization of the RNA extract: The nucleic acid extract of the virus gave an ultraviolet spectrum (Fig. 2) characteristic of nucleic acid, with a maximum 260 mμ. The absorbancy ratios 280/260 and 230/260, were 0.43 and 0.64, respectively, indicating satisfactory purity from protein.

The optical density-temperature profile (Fig. 7) of the assuredly high molecular weight extract, in 0.2 *M* solution, is very similar to that exhibited by the single-stranded, assuredly large RNA molecule, TMV RNA.[29] Especially relevant is the increase in absorbancy at the lower temperature points, obtained at early times in our experiment, before much degradation could have occurred. Double-stranded RNA, such as wound tumor[30] or reovirus RNA,[31] exhibits essentially unchanged absorbancies until over 85°C; then the entire increase is observed within a 5°C range. We may surmise, therefore, that the Rauscher virus RNA occurs substantially in a single-stranded form.

One cannot rule out, however, an effect on the hyperchromicity of the nonsedimentable portion of the UV-absorbing material present in the extract (Fig. 6). Some of this material may be composed of nonviral RNA, oligonucleotides, and/or other ultraviolet-absorbing small impurities. Oligonucleotides have in fact been shown to possess hyperchromicity.[32] It is hard to imagine that a sharp, high-temperature transition like that exhibited by the double-stranded RNA preparations mentioned above could be so completely obscured by the hyperchromic contribution of such oligonucleotide material.

The fast-sedimenting 73*S* component in the extract exhibits a sharp sedimentation profile (Fig. 6) indicative of molecules of homogeneous size distribution. Thus, it is unlikely to represent a random degradation product of the viral RNA. The molecular weight of the RNA can be estimated from the sedimentation coefficient of 73*S* to be 13×10^6, using an empirical equation[33] for single-stranded TMV RNA

in 0.1 *M* salt, 0.01 *M* EDTA—assuming that this data applies to other single-stranded RNA molecules. This molecular weight estimate is probably an upper limit, since our experiments were done at a concentration of 0.2 *M* salt which contained 10^{-3} *M* $MgCl_2$. Under our conditions, *S* values should be higher for a single-stranded RNA molecule[29] than they would be under the conditions used[33] to relate *S* to molecular weight empirically. The above reservations concerning the estimated molecular weights of the virus particle and of the RNA suggest that the RNA constitutes less than 6 per cent of the weight of the virus. Since similar RNA membrane viruses contain[34] about 2 per cent RNA, the fast-sedimenting 73*S* component present in the Rauscher RNA extract is probably the intact viral nucleic acid, and one such molecule per virus particle would account for all the RNA in this virus.

We have obtained a high molecular weight RNA extract, using an RNase inhibitor, in the attempt to minimize the chance of degradation of the viral RNA. The successful viral RNA extractions which have recently been described[3, 6, 7] can be viewed in this light. A 64*S* RNA was detected[6] in the extract of P^{32}-labeled Rous sarcoma Rous-associated virus mixture by following sedimentation of radioactivity. Excess carrier RNA was added during extraction, which presumably helped protect the virus RNA by competition for nuclease. A 57*S* RNA from Newcastle disease virus was observed[7] in the analytical ultracentrifuge, after extraction performed in the presence of mercaptoethanol. In conjunction with the sodium dodecylsulfate used in the extraction, mercaptoethanol should[35] inhibit RNase action. A 70*S* P^{32}-labeled RNA was detected[3] after extraction of the supernatant fluids of P^{32}-labeled tissue culture cells infected with the Rauscher virus. Uninfected cells were added, to provide carrier, prior to extraction. In some experiments, a polyanionic RNase inhibitor,[22, 36] polyvinyl sulfate, was also added. The 70*S* value agrees well with our value of 73*S* obtained from purified plasma virus RNA.[37]

Additional physical, chemical, and biological analyses on the Rauscher murine leukemia virus and its RNA extract are planned.

Summary.—The RNA occurs in the infectious Rauscher murine leukemia virion in the single-stranded form, as a single molecule of about 13×10^6 mol wt.

* A preliminary account of this work, presented at the Conference on Murine Leukemia held October 13–15, 1965, at Philadelphia, will appear as part of the proceedings of the Conference.[1]

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[10] We thank Dr. Guy de-Thé for carrying out the electron microscopy, and for the interpretation of the micrographs.

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[37] Duesberg and Robinson kindly sent us a copy of a manuscript [Duesberg, P. H., and W. S. Robinson, these PROCEEDINGS, **55**, 219 (1966)]. They have recently obtained results essentially confirming those we previously reported[1] for the RNA of the Rauscher virus.

DEPENDENCE OF THE CHEMICAL NATURE OF ANTIBODIES ON THE NET ELECTRICAL CHARGE OF ANTIGENS

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Communicated by C. B. Anfinsen, December 23, 1965

It was reported previously that antibodies from different rabbit antisera distributed unequally among two immunoglobulin G[1] fractions obtained upon chromatography on DEAE-Sephadex.[2] Thus, antibodies to an acidic synthetic polypeptide antigen were mostly found in the first fraction, and anti-lysozyme was present exclusively in the second fraction. The present investigation was initiated in order to elucidate whether this difference is due to chemical differences between antigens, variations in the course of immunization, the span of time elapsed between the immunization and the collection of the antisera, or allotypic differences between animals.