Markovitz, A. (1972), *Biochim. Biophys. Acta 281*, 522-534.
Nomota, M., Narahashi, Y., and Murakami, M. (1960), *J. Biochem. (Tokyo), 48*, 906-918.
Rossmann, M. G., and Liljas, A. (1974), *J. Mol. Biol. 85*, 177-181.
Rossmann, M. G., Moras, D., and Olson, K. W. (1974), *Nature (London) 250*, 194-199.
Schimmel, P. R. (1973), *Acc. Chem. Res. 6*, 299-305.
Schimmel, P. R., Uhlenbeck, O. C., Lewis, J. B., Dickson, L. A., Eldred, E. W., and Schreier, A. A. (1972), *Biochemistry 11*, 642-646.
Schoemaker, H. J. P., Budzik, G. P., Giegé, R. C., and Schimmel, P. R. (1975), *J. Biol. Chem. 250*, 4440-4444.
Schoemaker, H. J. P., and Schimmel, P. R. (1974), *J. Mol. Biol. 84*, 503-513.
Sperling, J. (1976), *Photochem. Photobiol. 23*, 323-326.
Sperling, J., and Havron, A. (1976), *Biochemistry 15*, 1489-1495.
Steinmaus, H., Rosenthal, I., and Elad, D. (1969), *J. Am. Chem. Soc. 91*, 4921-4923.
Strniste, G. F., and Smith, D. A. (1974), *Biochemistry 13*, 485-493.
Stroud, R. M. (July, 1974), *Sci. Am.*, 74-78.
Toth, B., and Dose, K. (1976), *Radiat. Environ. Biophys. 13*, 105-113.
Walsh, K. A., and Neurath, H. (1964), *Proc. Natl. Acad. Sci. U.S.A. 52*, 884-889.
Weintraub, H. (1973), *Cold Spring Harbor Symp. Quant. Biol. 38*, 247-256.

Molecular Weights of Particles and RNAs of Alfalfa Mosaic Virus. Number of Subunits in Protein Capsids†

R. A. Heijtink,‡ Corrie J. Houwing, and E. M. J. Jaspars*

ABSTRACT: The molecular weights of the four main RNAs of alfalfa mosaic virus, viz., the three genomic RNAs 1, 2, and 3 and the subgenomic RNA 4, which carries the coat protein cistron, were determined by two different methods. First, the weights of purified particles in which these RNAs occur were obtained by sedimentation equilibrium measurements. Since the particles are composed of RNA and a single species of protein subunits for which nucleotide and amino acid compositions are known, the weights of the RNA contents could be calculated from the particle weights and the phosphorus and nitrogen contents of the particles. They turned out to be 1.13, 0.80, 0.66, and 0.56×10^6, respectively. Second, sedimentation equilibrium distributions of the isolated and purified RNA species yielded molecular weights of the polyanions of 0.95, 0.66, 0.58, and 0.22×10^6, respectively. As a mean of these results, values of 1.04, 0.73, and 0.62×10^6 were accepted for RNAs 1, 2, and 3, respectively. It was concluded that two molecules of RNA 4 are encapsidated together. Thus a mean molecular weight of 0.25×10^6 was calculated for RNA 4. The molecular weights are lower than most values reported in the literature, which in many cases had been obtained from sedimentation coefficients and electrophoretic mobilities using empirical relationships. From the above values we calculated the alfalfa mosaic virus RNAs to consist of 3250, 2250, 1950, and 800 nucleotide residues, respectively. This leaves 240 and 140 extracistronic residues in the case of RNAs 3 and 4, respectively. With the aid of the particle weights, the RNA weight fractions, and the molecular weight of the coat protein (24 280), the numbers of protein subunits in the capsids were calculated. It is proposed that the RNAs are in capsids of 60 + ($n \times 18$) subunits, n being 10, 7, 5, and 4, respectively.

Alfalfa mosaic virus is an interesting virus from a biological and from a structural point of view. The genome of the virus consists of three RNA molecules called RNA 1, RNA 2, and RNA 3 in order of decreasing length. The genome parts are found in separate particles, thus giving rise to three different size classes, which in order of decreasing sedimentation velocity are referred to as components B, M, and Tb, respectively. Virus preparations contain also subgenomic RNAs. In general these occur in minor quantities, with the exception of a small RNA which encodes for the sole coat protein of the virus and is referred to as RNA 4. Most of this RNA is found in component Ta, which sediments slightly slower than component Tb (Van Vloten-Doting and Jaspars, 1977).

Host plants can be infected by a mixture of the three nucleoprotein components B, M, and Tb, but not by a mixture of their RNAs. The mixture of the three genomic RNAs becomes infectious after addition of a small amount of coat protein or RNA 4 (Bol et al., 1971).

In recent years the tripartite genome has been found to be a characteristic of several groups of plant viruses (for reviews, see: Jaspars, 1974; Van Vloten-Doting and Jaspars, 1977). All these viruses appear to have four major RNAs not differing very much in length from those of alfalfa mosaic virus. In some of these viruses the coat protein or its messenger is necessary for infectivity; with others it is not.

AMV[1] differs from other viruses with tripartite genomes by its particles being bacilliform instead of spherical. This is

†From the Department of Biochemistry, University of Leiden, Leiden, The Netherlands. *Received March 7, 1977; revised manuscript received June 29, 1977.* This work was sponsored in part by the Netherlands Foundation for Chemical Research (S.O.N.), with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

‡Present address: Department of Virology, Medical Faculty, Erasmus University, Rotterdam, The Netherlands.

[1]Abbreviations used: AMV, alfalfa mosaic virus; EDTA, ethylenediaminetetraacetate; Tris, tris(hydroxymethyl)aminomethane.

an unusual morphology among simple viruses. The components of AMV all have the same diameter but represent different length classes. Their structure has been the subject of several electron microscopic investigations (Hull et al., 1969b; Mellema and Van Den Berg, 1974; Mellema, 1975).

Since the knowledge of basic data like molecular weights of coat protein, nucleocapsids, and RNAs of a virus seems to be of primary importance for the study of its life cycle as well as for a balanced picture of the structure of the virus particles, we have undertaken physical and chemical measurements on AMV as representative of the rather large group of viruses with tripartite genomes. The molecular weight of the coat protein is known from earlier work from our laboratory (Kruseman et al., 1971). From the recently elucidated sequence of its 220 amino acid residues, a precise value of 24 280 can be deduced (Kraal et al., 1976; Van Beynum et al., 1977). Molecular weight data of RNAs in the literature are mostly derived from relative electrophoretic mobilities.

In the present study we report on the determination of the particle weights of the four nucleoprotein components of AMV and of their chemical compositions. From these data the molecular weights of the four major RNA species could be deduced. The molecular weights of the RNAs were also established directly by performing physical measurements on the isolated molecules. Acceptable agreement was obtained between the two sets of molecular weight values. The values were lower than those reported in the literature.

Materials and Methods

Virus Culture, Isolation, and Fractionation. AMV isolate 425 of Hagedorn and Hanson (1963) was cultured, isolated, and partially fractionated (using 0.03 M $MgSO_4$) according to the methods of Van Vloten-Doting and Jaspars (1972). From the Mg^{2+} precipitate (bottom fraction), components B, M, Tb, and the bacilliform Ta particles (Ta-b) were isolated. The supernatant of the Mg^{2+} precipitate (top "a" fraction) was used to isolate the spheroidal Ta particles (Ta-t) (Heijtink and Jaspars, 1976).

AMV isolate 15/64 (Hull, 1969) was cultured and isolated as described before (Heijtink and Jaspars, 1974).

Purification of Nucleoprotein Components. The nucleoprotein components were purified by means of one or two cycles of sucrose velocity gradient centrifugation in a MSE B XIV or a Beckman Ti-15 zonal rotor as described before (Heijtink and Jaspars, 1974). Fractions were selected for measurements or further purification on the basis of electrophoresis patterns. Selected fractions were concentrated by ultracentrifugation or dialyzed immediately against the appropriate buffer. Nucleoprotein preparations were stored and handled at 4 °C in 0.01 M sodium phosphate, 10^{-3} M EDTA, and 10^{-3} M NaN_3, pH 7.0, unless otherwise stated. Before use in chemical and/or physical experiments, the nucleoprotein and RNA composition of the purified preparations was analyzed electrophoretically on 3% polyacrylamide gels as described before (Heijtink and Jaspars, 1974).

Procedures of purification and fractionation for strains 15/64 and 425 were identical.

Isolation and Purification of RNA Species. RNA 1 was extracted from purified component B according to the method of Van Vloten-Doting and Jaspars (1972) in the presence of 2% pyrophosphate; crude RNA 4 was extracted from top "a" fraction by phenol/sodium dodecyl sulfate (Bol and Van Vloten-Doting, 1973). Thirty to fifty milligrams of these RNA preparations were heated for 5 min at 60 °C and subsequently subjected to sucrose velocity gradient centrifugation in an MSE B XIV zonal rotor. The buffer for gradient centrifugation was 0.01 M sodium phosphate, 10^{-3} M EDTA, and 0.05 M NaCl, pH 7.0. Centrifugation was for 12 and 22 h in 10–30% sucrose gradients at 45 000 rpm and 4 °C for RNAs 1 and 4, respectively. The RNA composition of the collected fractions was analyzed by polyacrylamide gel electrophoresis. Electrophoresis was carried out according to Loening (1967) in 3% polyacrylamide gels and buffer containing 0.1% sodium dodecyl sulfate. Selected fractions of the gradients were combined and samples of 1 mL were run through a Sephadex G-25 column to remove sucrose and to change buffer.

RNAs 2 and 3 were isolated from partially purified components M and Tb, respectively, since highly purified components M and Tb could not be obtained in sufficient amounts for RNA extraction and subsequent purification. The difference in sedimentation coefficient between RNAs 2 and 3 is too small to separate them in sucrose density gradients. Therefore we isolated these RNAs by preparative electrophoresis on polyacrylamide gels as reported previously (Heijtink, 1974). In short, a nucleoprotein preparation which contained at least 80% of the desired component (M or Tb) was applied to thirty 2.7% analytical gels after dissociation in 0.1% sodium dodecyl sulfate. Gel slices containing a single RNA species were cut from the gel. After a phenol extraction procedure, residual gel material was removed by centrifugation in a discontinuous sucrose density gradient. Finally the RNA solutions were extensively dialyzed against the appropriate buffer. From 12 mg of nucleoprotein material we obtained about 60 μg of highly purified RNA. RNA 4 was isolated in this way to have a check on the method.

All operations with RNA solutions were performed at 0 to 5 °C, except electrophoresis and most density measurements which were done at ambient temperature. RNA solutions were stored at −20 °C. To minimize aggregation, often seen in freshly thawed solutions of RNAs 3 and 4, preparations of these RNAs were heated for 5 min at 40 °C before use.

Determination of Dry Weight. Since prolonged dialysis against distilled water causes the nucleoproteins to precipitate irreversibly, samples for dry weight determination were dialyzed at a concentration of about 10 mg/mL against 10^{-4} M sodium phosphate, 10^{-4} M NaN_3, pH 7.0. After 1 day the buffer was renewed and the dialysis was continued for another 4 to 5 days. During dialysis the concentration decreased to 6 to 7 mg/mL. This treatment had no influence on the integrity of the nucleoproteins or their RNA contents as judged by electrophoresis in polyacrylamide gel and by assay of biological activity. Triplicate samples containing 0.5 to 3 mg of nucleoprotein each were dried in small Teflon cups in an oven at 60 °C and subsequently heated at 110 °C. In general constancy in weight was obtained in 24 h. Measurements were performed in vacuo with a Cahn RG electrobalance. The samples were not allowed to cool before they were put into the balance. The correction for the dry weight of the buffer was found to be below the accuracy limit of the method.

Determination of Nitrogen and Phosphorus. Samples of nucleoproteins were dialyzed as described for the dry weight samples since these conditions had proven to be excellent for keeping the nucleoproteins intact. However, this necessitated a buffer correction in the case of phosphorus determinations. At the nucleoprotein concentrations used (6 to 7 mg/mL), this correction was about 3%. Due to the Donnan equilibrium, the concentration inside the bag is less than the concentration outside (about 25%). The exact correction depends on the nucleoprotein concentration and was read from a calibration line made with the aid of [^{32}P]phosphate. Samples of RNA were always dialyzed for 5 to 6 days against distilled water, with a change after 1 day.

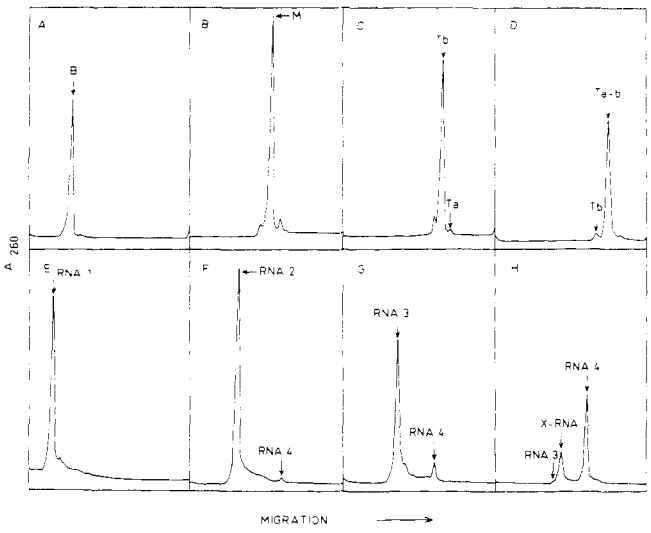

FIGURE 1: Densitograms of polyacrylamide gels showing the nucleoprotein (upper row) and the RNA composition (lower row) of highly purified nucleoprotein components B (A, E), M (B, F), Tb (C, G), and Ta-b (D, H). In the lower row panels, the nucleoprotein material was dissociated with 1% sodium dodecyl sulfate prior to electrophoresis.

The nitrogen content of the samples was determined by direct Nesslerization of Kjeldahl digests using the procedure of Minari and Zilversmit (1963). Phosphorus content was estimated by the method of Knight and Woody (1958). In all cases the determinations were performed in duplicate at three to four dilutions of the sample solution. Known concentrations of ammonium sulfate and KH_2PO_4 were run simultaneously as standards. In some experiments controls from a batch of component B for which nitrogen and phosphorus analyses were carried out by the Element Analytical Section of the Institute of Organic Chemistry TNO, Utrecht, The Netherlands, under supervision of Mr. W. J. Buis, were also run. Our determinations agreed within 2% with the element analyses.

Density Determinations. Densities of nucleoprotein and RNA solutions were determined with a pycnometer, a density cell of a Cahn RG electrobalance or a digital precision device for density measurements in liquids and gases DMA 02/C of Anton Paar (Austria). The latter apparatus measures the vibration frequency of a glass tube filled with the sample. It has to be calibrated with samples of known density. We used air and distilled water for this purpose.

In all cases the densities of the dialysis buffers were determined in parallel experiments.

Analytical Ultracentrifugation. Experiments were done in a Beckman Model E analytical ultracentrifuge equipped with ultraviolet optics, a photoelectric scanning system using monochromatic light of 265 nm wavelength, and a multiplexer attachment. Reference distances on scans and in counterbalances were determined according to Chervenka (1969).

Sedimentation velocity experiments were performed using 12-mm aluminum-filled Epon double-sector cells in an An-H rotor modified as recommended by Incardona et al. (1971). Sedimentation coefficients were corrected for the solvent, if necessary, with the use of measured values of density and viscosity of the buffer.

Diffusion coefficients were determined in low-speed centrifugation experiments using 12-mm double-sector synthetic boundary cells (capillary type) in the heavy An-J rotor.

Sedimentation equilibrium experiments were done according to the meniscus depletion method of Yphantis (1964). Both 12-mm aluminum-filled Epon double-sector cells and six-channel centerpieces were used with an An-J rotor. Column height was about 3 mm. Time required to reach equilibrium was determined by comparing the slope of ln c vs. r^2 at various times during the run.

Buoyant density determinations of nucleoprotein components in CsCl gradients were performed using 12-mm aluminum-filled Epon double-sector cells in an An-F (Ti) rotor according to procedures described before (Heijtink and Jaspars, 1974).

In all cases ultracentrifuge cells were equipped with sapphire windows. Centerpieces and windows intended for runs with nucleoprotein solutions were cleaned with synthetic soap, washed in distilled water, and dried; in the case of RNA solutions, this procedure was extended with successive incubation in a bentonite solution (2 mg/mL) and in a solution of 0.1% sodium dodecyl sulfate and 0.1% EDTA.

Viscosity measurements were performed in an Ubbelohde type viscometer with a flow time of about 300 s for water at 20 °C. Before each series of experiments the viscometer was cleaned with aqua regia, washed several times with distilled water, and dried with analytical grade acetone. The starting solution which contained 10–20 mg of nucleoprotein per mL and the diluent were filtered through a GF/C glass filter. After a series of ten experiments, the concentration of the nucleoprotein was determined spectrophotometrically. The remainder of the solution was centrifuged at 6000 rpm in a Sorvall SS-3 centrifuge to remove aggregates and was diluted for the next series of experiments.

Spectrophotometry. Light absorption was measured at 260 nm in cuvettes with a 1-cm light path in a Zeiss PMQ II spectrophotometer. Adjustments of the wave length scale were made by allowing the H_β line of the deuterium lamp to coincide with 486 nm on the scale. Measurements of both nucleoproteins and RNAs were made in 0.01 M sodium phosphate, pH 7.0, at room temperature which varied between 20 and 22 °C.

Electron Microscopy. Nucleoprotein preparations, concentration of about 0.1 mg/mL, were dialyzed overnight at 4 °C against a solution containing 10^{-4} M sodium phosphate, 1% formaldehyde, pH 7.0. They were then negatively stained with 2% phosphotungstic acid on carbon coated grids. Observations were made with a Philips EM 300 electron microscope. The magnification of the microscope was obtained with a carbon grating replica. Size distributions were measured on photographs (magnification 123 800×) with a Zeiss particle size analyzer (TGZ 3).

Radioactivity measurements were performed in a Nuclear Chicago Isocap/300 or in a Philips liquid scintillation spectrometer. [^{32}P]Orthophosphate (Philips Duphar, 5–20 mCi/mg) was determined in water or diluted buffer by Čerenkov counting. ^{22}NaCl (The Radiochemical Centre, Amersham, England, 13 mCi/mg) was counted in aliquots of 0.5 mL of buffer to which was added 10 mL of a scintillation liquid consisting of 600 mL of toluene, 300 mL of Triton-X-100, 100 mL of distilled water, 4.95 g of 2,5-diphenyloxazole, and 90 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene.

Results

Physical Studies and Particle Weights of Nucleoprotein Components

Characteristics of the Nucleoprotein Preparations. Figure 1A–D shows the nucleoprotein composition of the highly purified preparations of B, M, Tb, and Ta-b used in our experiments. They are contaminated with small amounts of minor nucleoprotein components which are always found in AMV preparations upon electrophoresis in polyacrylamide gel (Bol

TABLE I: Sedimentation Coefficients ($s^0_{20,w}$) of AMV Components.[a]

Component	$s^0_{20,w}$ (S)
B	94.3 ± 0.5 (7)
M	81.6 ± 0.7 (10)
Tb	73.2 ± 0.7 (7)
Ta-b	66.4 ± 0.4 (3)
Ta-t	66.0 ± 0.9 (9)

[a] Sedimentation coefficients with standard deviations and, between parentheses, the number of determinations. Centrifugation at 30 000 rpm and 20 °C in 0.01 M sodium phosphate, 10^{-3} M EDTA, 10^{-3} M NaN_3, 0.1 M NaCl, and 0.02% formaldehyde, pH 7.0.

et al., 1971; Bol and Lak-Kaashoek, 1974) and which are extremely difficult to remove entirely from the major components. A slight mutual Tb/Ta-b contamination is also observed. Figure 1E-H shows the RNA contents of the preparations. It is evident that the nucleoprotein components largely contain intact RNA molecules of the species expected. Contaminating RNA 4 may come partly from contaminating minor nucleoprotein components of different classes (Bol and Lak-Kaashoek, 1974). It is clear that component Ta-b contains a considerable amount of an RNA species with a mobility intermediate between RNA 3 and RNA 4. Minor RNA species migrating in this region are always found in AMV preparations and are called X-RNAs (Bol et al., 1971). Our preparations of component Ta-t had a purity comparable to those of component Ta-b, but they contained less X-RNA (result not shown, but compare Heijtink and Jaspars, 1976).

A mixture containing purified components B, M, and Tb was biologically active in infectivity tests on beans (Van Vloten-Doting and Jaspars, 1967), whereas single components were not.

Sedimentation Velocity Experiments. In Table I the sedimentation coefficients of B, M, Tb, Ta-b, and Ta-t are summarized. The boundaries in the sedimentation plots were very sharp. No trace of heterogeneity was detected. The presence of formaldehyde had no significant influence on the sedimentation coefficients (checked for components B and Tb). On the other hand, omission of NaCl and NaCl plus EDTA from the buffer resulted in statistically significant changes of the sedimentation coefficient of component B of about −1 S and +1 S, respectively (for detailed results, see Heijtink, 1974).

The sedimentation coefficient of component B of strain 15/64 (Hull, 1969), isolated and purified as described for strain 425, was found to be 93.6 ± 0.9 S (seven determinations).

Buoyant densities in CsCl of components B, M, Tb, and Ta-b fixed with formaldehyde were measured. First the density of component B was calculated from its position in the centrifuge cell to be 1.372 g/cm^3. The other components were mixed with component B and were found to band at slightly lower densities. Starting from the density of B we determined the densities of M, Tb, and Ta-b to be 1.368, 1.369, and 1.366 g/cm^3, respectively; Ta-b and Ta-t are known to have identical buoyant densities in CsCl (Heijtink and Jaspars, 1976).

Particle Lengths. The lengths of 271 to 284 particles of each of the four bacilliform components were measured. No selection was made, except that particles with abnormal morphology were rejected. The length distributions are close to Gaussian distributions, at least when 5% of the particles at either extreme are disregarded (Figure 2). The median values

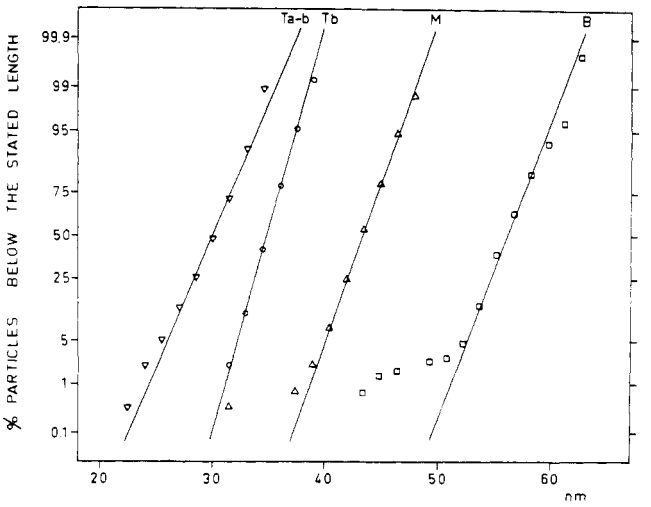

FIGURE 2: Integrated length distributions of 284 B, 271 M, 275 Tb and 276 Ta-b particles after fixation with formaldehyde and negative staining with phosphotungstic acid.

TABLE II: Partial Specific Volume of Nucleoprotein Components.[a]

Component	Prep no.	Concn[b] (mg/mL)	Method[c]	$\bar{\nu}$ (cm^3/g)
B	1	19.92	Pyc	0.707
B	1	10.25	Dens c	0.703
B[d]	1	10.39	Dens c	0.704
B	1	10.28	DMA	0.701
B[d]	1	10.44	DMA	0.703
B	2	10.33	DMA	0.703
B	2	2.05	DMA	0.700
"M"[e]	1	11.00	DMA	0.703
Ta-t	1	10.87	DMA	0.701
Ta-t	2	13.00	DMA	0.703
Ta-t	2	2.74	DMA	0.703

[a] Solutions were dialyzed for 2 days with a change after 1 day against 0.01 M sodium phosphate, 10^{-3} M EDTA, 10^{-3} M NaN_3, 0.1 M NaCl, pH 7.0. [b] Concentrations were determined with the extinction coefficients from Table IV. [c] Densities of solutions were determined with a 10-mL pycnometer (pyc) at 20.0 ± 0.2 °C; with a 2-mL density cell of an electrobalance (dens c) at 20.3 ± 0.1 °C; or with the density measuring apparatus of Anton Paar (DMA) at 20.01 ± 0.01 °C. [d] Formaldehyde (0.02%) was present in the dialysis buffer. [e] Contains at least 55% of component M.

and their standard deviations of the components B, M, Tb, and Ta-b are 56.1 ± 2.2, 43.3 ± 2.0, 34.8 ± 1.6, and 29.9 ± 2.4 nm, respectively. Measurements of the width of 50 component B particles yielded a mean of 15.5 ± 1.0 nm. Employing a similar method for fixation and staining as in the present study other authors found higher values in unfractionated preparations (see summary given by Hull, 1969). Our values are closest to the ones reported by Verhoyen (1967).

Partial Specific Volumes. The partial specific volume can be calculated from the density increment of a solution of known concentration with the aid of the equation: $\bar{\nu}$, $1/\rho_0 - 1/c[(\rho - \rho_0)/\rho_0]$. Concentrations were estimated from light absorbances of solutions at 260 nm using extinction coefficients which were based on nitrogen determinations (Table IV).

The partial specific volume of component B did not appear to vary markedly with the method of density determination, with the concentration or with the presence or absence of formaldehyde (Table II). For calculations of the particle weight of this component we used a value of $\bar{\nu}$ of 0.703 cm^3/g.

TABLE III: Particle Weights of Nucleoprotein Components by Sedimentation Equilibrium.[a]

Component	Time (h)	Speed (rpm)	Concn (mg/mL)	Particle wt $\times 10^{-6}$
B	85	2200	0.05	6.92 ± 0.15 (7)
M	100	2600	0.10	5.12 ± 0.06 (8)
Tb	75	2800	0.05	4.25 ± 0.07 (11)
Ta-b	72	3000	0.03	3.77 ± 0.12 (7)
Ta-t	72	3200	0.03	3.54 ± 0.08 (4)

[a] Experiments at 20 °C in 0.01 M sodium phosphate, 10^{-3} M EDTA, 10^{-3} M NaN_3, 0.1 M NaCl, and 0.02% formaldehyde (pH 7.0). Partial specific volume for all components is 0.703 cm^3/g.

The same value was adopted for the other components since their RNA/protein ratio is very similar to that of component B. Values determined with the apparatus of Anton Paar for two preparations of component Ta-t and for a preparation consisting of at least 55% of component M are in agreement with this.

Sedimentation Equilibrium and Particle Weights. A part of the preparations dialyzed for sedimentation velocity experiments (Table I) was used in the sedimentation equilibrium experiments. Formaldehyde fixation (0.02% formaldehyde) was performed as a preservative of the structure of the virus particles during the runs for days at room temperature (Bol and Veldstra, 1969). Experiments were done with B, M, Tb, Ta-b, and Ta-t. Table III summarizes the conditions and results. Plots of the logarithm of the concentration (obtained from recorder deflection) against the square of the distance from the axis of rotation were linear, which confirms the homogeneity of the preparations. Particle weights were calculated from the slope by a modified eq 11 of Svedberg and Pederson (1940):

$$M = \frac{2RT}{(1 - \bar{\nu}\rho)\omega^2} \frac{\mathrm{d} \ln c}{\mathrm{d}r^2}$$

Particle weights of component B were determined at different concentrations ranging from 0.03 to 0.12 mg/mL. As no significant differences were found we concluded that particle weights are concentration independent in this region.

Particle weight of component B of strain 15/64 was determined under comparable experimental conditions and found to be 6.85 ± 0.14 × 10^6 (three determinations).

Diffusion experiments with component B (about 0.15 mg/mL) were performed in an analytical ultracentrifuge rotor at about 3000 rpm and at 7 and 20 °C in the same buffer as used for sedimentation experiments with nucleoproteins. Plots of boundary spreading (corrected for sedimentation) against time were straight lines. Diffusion coefficients were calculated using a modified formula of Markham (1962): $D = \sigma^2(1 - s\omega^2 t)/2t$, where σ^2 is the variance of the distribution at the time t. The sedimentation coefficient required here was determined at low-speed centrifugation during the diffusion run. Values of $D^0_{7,b}$ and $D^0_{20,w}$ were 0.75 ± 0.01 (four determinations) and 1.13 ± 0.01 (five determinations), respectively.

Viscosity measurements on solutions of component B were done in 0.01 M sodium phosphate, 10^{-3} M EDTA, 10^{-3} M NaN_3, pH 7.0, at two series of solute concentrations at 20.00 ± 0.02 °C. Intrinsic viscosity was determined by extrapolating η_{sp}/c to infinite dilution. Values of 0.705 and 0.725 dL/g were found.

Molecular Weight from s and D. Using eq 3a of Svedberg and Pederson (1940), $M = RTs/(1 - \bar{\nu}\rho)D$, the diffusion coefficients mentioned above, the sedimentation coefficients obtained with the same preparation [$s^0_{7,b}$ = 65.9 ± 0.9 S (seven determinations); $s^0_{20,w}$ = 94.3 ± 0.5 S (seven determinations)], and the partial specific volume were combined to give the particle weight of component B. We calculated 6.86 ± 0.13 × 10^6 and 6.82 ± 0.07 × 10^6 from the values obtained at 7 and 20 °C, respectively.

Particle Weight from [η], s, and Particle Shape. Scheraga and Mandelkern (1953) have combined sedimentation coefficient, intrinsic viscosity, and molecular weight in the equation:

$$\frac{Ns[\eta]^{1/3}\eta_0}{100^{1/3}M^{2/3}(1 - \bar{\nu}\rho)} = \beta(a/b)$$

where a/b is a function of the shape of the particle. With the axial ratio (a/b = 3.62; a = major axis, b = minor axis) from electron micrographs and a table of β vs. a/b for a prolate ellipsoid of revolution given by the authors, we calculated particle weights from two experiments using the intrinsic viscosity mentioned above and the sedimentation coefficients of the same preparations measured in the same buffer [$s^0_{20,w}$ = 93.1 ± 1.2 S (six determinations) and 93.4 ± 0.5 S (eight determinations)]. Values of 6.88 and 6.98 × 10^6 were found.

Molecular Weights of RNAs from Particle Weights and Nitrogen and Phosphorus Determinations. Extinction Coefficients of Nucleoprotein Components

The mole fraction (X) of RNA in nucleoprotein can be calculated from the weight ratio of nitrogen and phosphorus (N/P) determined in a given sample using the equation:

$$\frac{N}{P} = \frac{(X\,\%\ \text{N in RNA}) + ((1 - X)\,\%\ \text{N in protein})}{X\,\%\ \text{P in RNA}}$$

A nitrogen content of 17.42% was derived for the protein from the primary structure (Van Beynum et al., 1977). The nitrogen and phosphorus contents of RNA 1 (anion) were calculated from its base composition (Van Vloten-Doting and Jaspars, 1967) to be 16.10% and 9.655%, respectively. The calculated values for RNA 4 (anion) were slightly different, viz., 16.08% and 9.650%, respectively. For RNA 2 and RNA 3 the values of RNA 1 were adopted.

Nitrogen and phosphorus analyses were performed in two or three different batches of each component. The RNA mole fractions calculated from them are listed in Table IV. They vary between 0.150 and 0.164. From the average mole fraction and the particle weight (Table III) the weight of the RNA contents of a given component can be calculated. This figure will be equal to the molecular weight of the RNA species (anion) of the component if the particle contains a single RNA molecule.

From the mole fractions of RNA and protein of the components the nitrogen contents of the components were deduced. This permitted the calculation of the concentration of nucleoprotein solutions which were used for dry weight and light absorbance determinations. Dry weights agreed favorably with concentrations on the basis of nitrogen. From concentrations and light absorbances of solutions at 260 nm, the extinction coefficients were deduced. These values are also listed in Table IV. As expected from the RNA mole fractions, the extinction coefficients were very similar to each other.

Physical Studies and Molecular Weights of RNA Species

Characteristics of RNA Preparations. Figures 3A and 3B show respectively the electrophoretic patterns of preparations of RNAs 1 and 4, isolated by zonal gradient centrifugation. Figures 3C, D, and E show respectively the composition of preparations of RNAs 2, 3, and 4, isolated by preparative gel

TABLE IV: RNA Mole Fractions and Extinction Coefficients of Nucleoprotein Components.[a]

Component	Prep no.	RNA mole fraction[b]		Particle wt × 10^{-6} [c]	Wt of RNA contents × 10^{-6} [d]	Nucleoprotein content[e] as % of dry wt	$A_{1cm}^{0.1\%}$ (260 nm)[f]	
B	3	0.1618	0.1629	6.92	1.13	100.5	4.98	5.05
B	4	0.1640				98.6	5.12	
M	2	0.1546	0.1554	5.12	0.80	99.7	4.81	4.84
M	3	0.1561					4.86	
Tb	1	0.1545	0.1550	4.25	0.66	99.6	4.76	4.79
Tb	2	0.1555					4.82	
Ta-b	1	0.1499	0.1521	3.77	0.57	97.5	4.67	4.69
Ta-b	2	0.1543					4.71	
Ta-t	1	0.1558	0.1563	3.54	0.55	99.3	4.73	4.69
Ta-t	3	0.1558				98.3	4.65	
Ta-t	4	0.1572				100.1	4.68	

[a] Solutions were dialyzed extensively against 10^{-4} M sodium phosphate, 10^{-4} M NaN_3, pH 7.0. [b] From determination of nitrogen/phosphorus ratios and known nitrogen contents of protein and RNA and known phosphorus content of RNA. [c] From Table III. [d] If a single molecule of RNA is present per particle, the figure is equal to the molecular weight of the RNA (anion). [e] From nitrogen content. [f] From nitrogen content and absorbance in 0.01 M sodium phosphate, pH 7.0, in the absorbance range of 0.4 to 0.8 and at 20 to 22 °C. The absorbance is not corrected for light scattering.

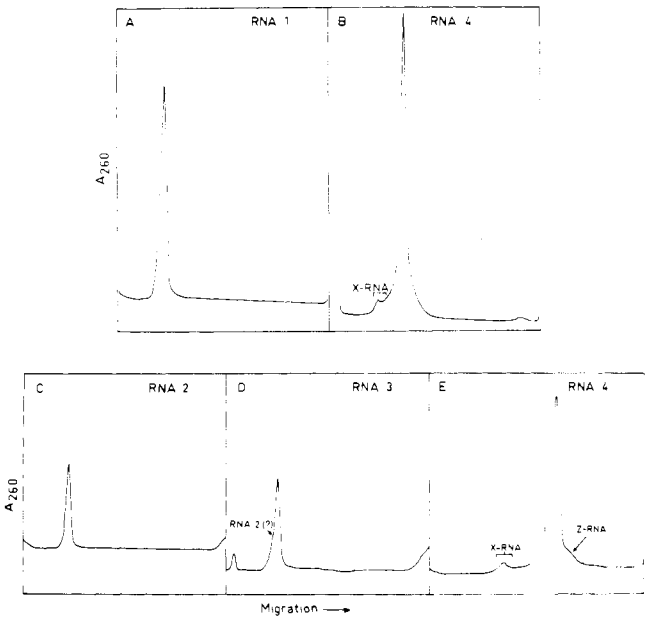

FIGURE 3: Densitograms of polyacrylamide gels showing the composition of preparations of purified RNAs 1 (A), 2 (C), 3 (D), and 4 (B, E). A and B were purified by zonal gradient centrifugation of phenol/sodium dodecyl sulfate extracts of purified components B and top a fraction, respectively; C, D, and E were purified by preparative gel electrophoresis of sodium dodecyl sulfate disrupted partially purified components M, Tb, and Ta, respectively.

electrophoresis. RNA 3 seems to be seriously contaminated with a slowly migrating RNA. However, this was absent when electrophoresis was carried out in the presence of 8 M urea. Thus this material may be an aggregate of RNA 3. A contamination of RNA 3 with RNA 2 seems likely, not only on basis of the gel patterns but also from infectivity experiments with different combinations of preparations. Combinations of all four RNAs were highly infectious. Preparations of RNA 4 are contaminated with X- and Z-RNAs. Since X- and Z-RNAs are present in different kinds of particles of the top "a" fraction (Bol and Lak-Kaashoek, 1974), small amounts may easily slip through the gradient purification procedure when RNA 4 is purified from extracts of top "a" fraction. Surprisingly they were also found in our gel-isolated preparations of RNA 4.

Extinction Coefficients and Partial Specific Volumes. Concentrations of RNA solutions were determined by measuring the light absorbance at 260 nm. Since an accurate knowledge of the concentration is required when partial specific volumes are calculated, extinction coefficients were determined for three preparations of RNA species 1 and 4 and for one preparation extracted from an unfractionated virus preparation. After extensive dialysis of the preparations against distilled water, samples were taken for phosphorus and/or nitrogen analysis and for light absorbance measurements. The latter samples were diluted with sodium phosphate buffer in such a way that the final buffer concentration and pH were 0.01 M and 7.0, respectively. Absorbance was measured in the range of 0.5 to 0.8. The extinction coefficient $A_{1cm}^{0.1\%}$ of RNA 1 (anion) was found to be 26.3 at 260 nm (average of four values ranging from 25.8 to 26.5). For RNA 4 (anion) $A_{1cm}^{0.1\%}$ (260 nm) was 25.8 (average of four values ranging from 25.5 to 26.0). Thus the sodium salts of RNAs 1 and 4 have extinction coefficients of 24.6 and 24.2, respectively. A single determination of the extinction coefficient of the unfractionated preparation (anions) yielded a value of 25.8, which is within the ranges of both RNA 1 and RNA 4.

When RNA preparations for density determinations are dialyzed against buffer, the question arises whether the highly negative charged polyanions will not, even in a buffer of moderate ionic strength, cause such an unequal distribution of the diffusible ions that the partial specific volume when calculated simply from the density difference of the RNA solution and the buffer outside the dialysis bag will be significantly in error. We have checked this possibility by performing control experiments in which preparations of RNA 1 underwent the usual dialysis procedure in the presence of $^{22}NaCl$ or [^{32}P]phosphate. It was found that at an RNA concentration of about 4.5 to 5.5 mg/mL the sodium ion concentration became about 8% higher inside the bag than in the dialysis buffer. The phosphate concentration differed in the opposite sense by

TABLE V: Partial Specific Volume of RNAs.[a]

RNA species	Prep no.	Concn[b] (mg/mL)	Method[c]	Temp (°C)	$\bar{\nu}$ (sodium salt) (cm^3/g)
1	1	4.68	Dens c	6	0.458
1	1	4.12	Pyc	6	0.46
1	1	3.41	Pyc	20	0.45
1	1	1.64	DMA	20	0.456
1	1	3.32	DMA	20	0.461
4	1	3.97	DMA	20	0.470
Mixture[d]	1	4.01	DMA	20	0.458
Mixture[d]	2	4.63	DMA	20	0.472

[a] Solutions were dialyzed for 2 days with a change after 1 day against 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.0. [b] Concentrations were determined with extinction coefficients of 24.6 for the sodium salts of RNA 1 and RNA mixtures and 24.2 for the sodium salt of RNA 4. [c] Densities of solutions were determined with a 5-mL pycnometer (pyc) at 20.0 or 6.0 ± 0.2 °C; with a 2-mL density cell of an electrobalance (dens c) at 5.9 ± 0.1 °C; or with the density measuring apparatus of Anton Paar (DMA) at 20.02 ± 0.01 °C. [d] Ratio of RNA species 1, 2, 3, and 4 was about 5:2:1:1.

TABLE VI: Sedimentation Velocity of RNA 1 and RNA 4 under Various Conditions.[a]

Buffer	RNA species	Temp (°C)	$s^0_{t,b}$ (S)	$s^0_{20,w}$ (S)
0.01 M Tris, 0.1 M KCl, pH 7.0	1	20		23.7 ± 0.5 (6)
0.01 M NaH_2PO_4, 10^{-3} M EDTA, 10^{-3} M NaN_3, pH 7.0	1	20		16.6 ± 0.2 (3)
0.01 M NaH_2PO_4, 0.15 M NaCl, pH 7.0	1	6	17.4 ± 0.4 (7)	25.8[b]
0.09 M Na_2HPO_4, 0.01 M NaH_2PO_4, 1.1 M formaldehyde	1	20		13.2 ± 0.4 (8)
0.01 M NaH_2PO_4, 0.15 M NaCl, pH 7.0	4[c]	7	8.77 ± 0.10 (8)	12.7[b]
0.09 M Na_2HPO_4, 0.01 M NaH_2PO_4, 1.1 M formaldehyde	4[c]	20		7.8 ± 0.1 (7)

[a] Sedimentation at 48 000 rpm for RNA 1 and 52 000 rpm for RNA 4. [b] Calculated. [c] Isolated by zonal gradient centrifugation.

about 2 to 3%. It was checked in experiments without RNA that the dialysis membrane alone had no influence on the distribution of the ions. Furthermore the exact ratios of sodium to phosphate ions found in each experiment agreed very well with ratios expected theoretically. Calculations of the Donnan equilibrium showed that the activity coefficient of the sodium counterions must be considerably less than that of the other sodium ions. Ratios of activity coefficients of 0.36 and 0.38 were found in two independent experiments. These data enabled us to calculate the ionic distributions and corresponding density differences in the experiments where partial specific volumes of RNA had to be calculated from density measurements. The values of $\bar{\nu}$ listed in Table V would have been 5 to 6% higher if no correction had been made for the density difference caused by the unequal distribution of diffusible ions. We considered the values obtained with the DMA apparatus of Anton Paar to be the most accurate. This led us to take for molecular weight calculations $\bar{\nu} = 0.459$ cm^3/g for the sodium salts of RNAs 1, 2, and 3 and $\bar{\nu} = 0.470$ cm^3/g for the sodium salt of RNA 4. The data in Table V further show that there is

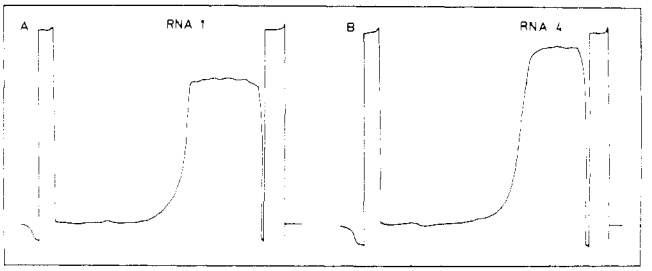

FIGURE 4: Sedimentation of RNA 1 (A) and RNA 4 (B) in 0.09 M Na_2HPO_4 + 0.01 M NaH_2PO_4, containing 1.1 M formaldehyde according to Boedtker (1968) at 20 °C. Sedimentation is from left to right. Scans were made 28 min (A) and 77 min (B) after the rotor reached a speed of 52 000 (A) and 56 000 rpm (B), respectively.

TABLE VII: Molecular Weights of RNA Species by Sedimentation Equilibrium.[a]

RNA species	Speed (rpm)	Mol wt × 10^{-6}
1[b]	4400	1.01 ± 0.01 (9)
2[c]	5200	0.70 ± 0.05 (13)
3[c]	5600	0.62 ± 0.02 (7)
4[b]	9000	0.24 ± 0.02 (13)
4[c]	9000	0.23 ± 0.01 (5)

[a] Experiments at 6 °C in 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.0, at concentrations varying between 7 and 25 μg/mL. Partial specific volumes used in the calculations were 0.459 (RNAs 1, 2, and 3) and 0.470 cm^3/g (RNA 4). [b] Purified by zonal gradient centrifugation. [c] Purified by preparative gel electrophoresis.

no marked dependence of partial specific volume on temperature.

Sedimentation velocity experiments were performed with RNAs 1 and 4 isolated by zonal centrifugation. Table VI gives the results under various conditions of temperature and buffer. Boundaries were sharp as could be expected from the gel electrophoretic patterns (Figures 3A and 3B). Figure 4 shows that under denaturing conditions of 1.1 M formaldehyde according to Boedtker (1968) homogeneity was maintained.

Diffusion measurements with RNAs 1 and 4 (both purified by zonal gradient centrifugation; concentration about 30 μg/mL) were performed in an analytical ultracentrifuge rotor at 4800 and 8000 rpm, and at 6 and 7 °C, respectively, in 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.0. Plots of boundary spreading (corrected for sedimentation) against time were straight lines. Diffusion coefficients were calculated as described for component B. Values of $D^0_{6,b}$ for RNA 1 and $D^0_{7,b}$ for RNA 4 were 0.78 ± 0.02 (four determinations) and 1.37 ± 0.03 (four determinations), respectively. Combination of these data in the Svedberg formula with the sedimentation coefficients in the same buffer and at the same temperature (Table VI) and the partial specific volumes of 0.459 for RNA 1 and 0.470 for RNA 4 yielded molecular weights of 0.97 ± 0.03 × 10^6 and 0.28 ± 0.01 × 10^6 for RNAs 1 and 4, respectively.

Sedimentation equilibrium experiments were done at low temperature with the five RNA preparations. Experimental procedure and subsequent calculations were essentially the same as those employed in the case of nucleoprotein components. Plots of the concentration distributions in the centrifuge cell were straight lines except that those of RNAs 3 and 4 exhibited a slight curvature at the highest r values, which points to some heterogeneity (Yphantis, 1964). The source of this heterogeneity has been discussed already. Only points in the

TABLE VIII: Molecular Weights and Number of Nucleotides of RNAs; Weights and Number of Subunits of Capsids.

RNA species	Mol wt × 10^{-6}			No. of nucleotides[c]	Nucleoprotein species	Wt × 10^{-6}		No. of subunits[f]
	Indirectly[a]	Directly[b]	Mean			Particle[d]	Capsid[e]	
1	1.13	0.95	1.04	3250	B	6.92	5.88	242
2	0.80	0.66	0.73	2250	M	5.12	4.39	181
3	0.66	0.58	0.62	1950	Tb	4.25	3.63	150
4	0.57[g]	0.22	0.25[i]	800	Ta-b	3.77	3.27[j]	135
	0.55[h]				Ta-t	3.54	3.04[j]	125

[a] From particle weight and RNA mole fraction (see Table IV). [b] From sedimentation equilibrium of free RNA species; values of Table VII corrected for 6.4% of weight contributed by sodium counterions. [c] From mean molecular weight of RNAs using a residue weight of 321, which was calculated from the base compositions of RNAs 1 and 4; values rounded off to fifties. [d] From sedimentation equilibrium (Table III). [e] From particle weights and mean molecular weights of RNAs. [f] From capsid weights using a protein subunit molecular weight of 24 280. [g] Derived from Ta-b. [h] Derived from Ta-t. [i] Assuming that the indirect value represents twice the molecular weight. [j] Subtracting twice the mean molecular weight of RNA 4 from the particle weight.

straight part of the line have been used to calculate the slope by least-squares treatment. Table VII summarizes the results of the sedimentation equilibrium experiments.

Discussion

In Table VIII the molecular weights of the four RNA species of AMV as obtained by two independent methods are summarized. In the first method the weights of the particle species which contain the RNAs were determined with the sedimentation equilibrium method and the weight fraction of RNA in the particles was determined by phosphorus and nitrogen analyses. We think that the results given by this method were reliable since other hydrodynamical methods gave virtually identical particle weights of component B and since phosphorus and nitrogen determinations were checked in a laboratory highly specialized in elementary analysis. Moreover we payed much attention to the correct determination of the partial specific volume of the particles (Table II). Nevertheless the molecular weights of the RNA species when determined directly with the sedimentation equilibrium method were significantly lower than those calculated from the particle weights and their RNA fractions, viz., 16%, 18%, and 12% in the case of RNAs 1, 2, and 3, respectively. Also with the RNA species the correspondence between the results obtained with different hydrodynamical methods was satisfactory. Here too the partial specific volumes were carefully determined. The values for the molecular weights of the RNAs derived from particle weights could be too high if the coat protein contained phosphorus or if the particles were contaminated with material of low nitrogen content or with inorganic phosphate. However, these possibilities can be ruled out. The coat protein does not contain phosphorylated amino acids (Van Beynum et al., 1977). The RNA molecular weights would have been virtually the same if they had been calculated from particle weights and RNA weight fractions derived from dry weights and phosphorus contents (Table IV). Finally, no accumulation of [^{32}P]phosphate took place when particles were dialyzed for a long time against 10^{-4} M phosphate buffer. As expected the presence of the negative charged particles led to a decrease of the phosphate concentration inside the dialysis bag.

Thus, the discrepancy between the indirectly and directly obtained values for the RNA molecular weights remains unexplained. A certain degree of heterogeneity might have influenced the equilibrium distribution in some cases. However, as there was no compulsory reason for a preference for either set of values, we took the mean values for the best possible. In the case of components Ta-t and Ta-b, it is clear that the particle contains more than a single molecule of RNA 4. Since the assumption of three RNA molecules per particle would mean that in this case the directly obtained value of the molecular weight would be the higher one, we found a particle contents of two RNA 4 molecules more plausible. This was already suggested by Hull et al. (1969b). It implies that the directly measured molecular weight is 21% lower than the indirectly obtained value. Such a difference is comparable with the differences found for the other RNA species.

Given the molecular weights of the RNAs and a mean nucleotide residue molecular weight of 321, which is calculated from the base compositions of RNAs 1 and 4 (Van Vloten-Doting and Jaspars, 1967), the numbers of nucleotide residues of RNAs 1 to 4 rounded off to fifties were found to be 3250, 2250, 1950, and 800, respectively. Assuming sequence homogeneity it is clear that RNA 4 cannot accommodate for a second cistron besides a coat protein cistron of 660 nucleotides. Genetic complementation studies have shown that RNA 3 contains the information for the coat protein (Dingjan-Versteegh et al., 1972), whereas in vitro translation shows that a protein with a molecular weight of 35 000 is also encoded for by RNA 3. This leaves about 240 nucleotides for extracistronic regions. The products translated from RNAs 1 and 2 are heterogeneous but the largest peptide chains have molecular weights of 110 000 to 90 000 and 90 000 to 70 000, respectively, which accounts for almost the entire length of the RNAs (Mohier et al., 1975, 1976; Thang et al., 1975, 1976; Van Vloten-Doting et al., 1975, 1977).

Molecular weights of AMV-RNAs in the literature have only in a few cases been derived from physical and chemical data of particles (Bancroft and Kaesberg, 1960; Kelley and Kaesberg, 1962; Hull et al., 1969b). The values reported for the particle weights of the components B and Ta-t did not deviate much from those found by us, but since the authors used different RNA fractions the molecular weights of the RNAs reported by them were very different.

Comparison of particle weights (Table III) and sedimentation coefficients (Table I) with those mentioned in the literature (Bancroft and Kaesberg, 1960; Kelley and Kaesberg, 1962; Gibbs et al., 1963; Verhoyen, 1967; Hull et al., 1969b) may lead to the suggestion that AMV strain 425 has somewhat lighter particles than other strains. However, measurements of the sedimentation coefficient and the particle weight of strain 15/64 of Hull gave in our hands values identical with those found for strain 425.

In all other cases the molecular weights of AMV-RNAs in the literature have been calculated from sedimentation coefficients or electrophoretic mobilities with the aid of empirical relationships (Hull et al., 1969a; Pinck, 1969; Bol, 1969; Hull,

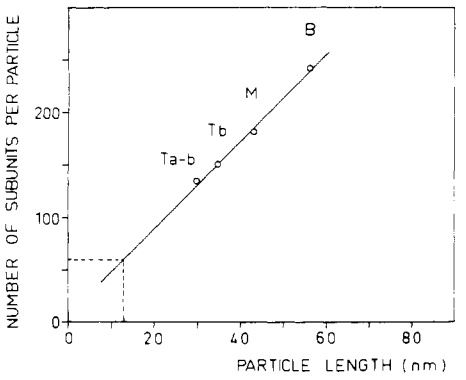

FIGURE 5: Number of protein subunits of the nucleoprotein components plotted against their lengths. A straight line fitted by least squares has a slope of 18 subunits/4.34 nm and reaches 60 subunits at a particle length of 12.8 nm.

1972; Pinck and Hirth, 1972). Mostly the values are higher than our mean values derived from physicochemical measurements. This is so, even when highly denaturing conditions are employed. In 8 M urea at 60 °C (Reijnders et al., 1973, 1974) values of 1.26, 0.88, 0.74, and 0.26 × 10^6 daltons for the molecular weights of the anions of RNAs 1 to 4 were found, respectively (Dr. L. Reijnders, personal communication). On the other hand, an excellent agreement exists between the mean values of RNAs 1 and 4 derived from physicochemical measurements and the values obtained when the sedimentation coefficients of these RNAs denatured in formaldehyde from Table VI are substituted in a relation which can be deduced from the work of Boedtker (1968); viz., $s_{20,w} = 0.0504M^{0.403}$. These values were 1.00 and 0.27 × 10^6 for RNAs 1 and 4, respectively.

A striking correspondence was found between the extinction coefficients of the nucleoprotein components determined experimentally and those calculated from the weight fractions and the extinction coefficients of the component parts. When corrected for light scattering according to Englander and Epstein (1957), the extinction coefficients [$A_{1cm}^{0.1\%}$ (260 nm)] of the components B, M, Tb, Ta-b, and Ta-t were 4.72, 4.52, 4.52, 4.46, and 4.47, respectively. Determinations of the extinction coefficient of the coat protein in 0.05 M sodium acetate, pH 5.5, at room temperature yielded values of $A_{1cm}^{0.1\%}$ of 0.70 at 280 nm and 0.49 at 260 nm after correction for light scattering. Using the weight fractions of Table IV and values of $A_{1cm}^{0.1\%}$ (260 nm) of 26.3 for RNAs 1, 2, and 3 and 25.8 for RNA 4, we calculated extinction coefficients of the components B, M, Tb, Ta-b, and Ta-t to be 4.70, 4.50, 4.49, 4.34, and 4.44, respectively. Except for component Ta-b these values agree within 1% with the values mentioned above. Thus, upon particle assembly either hypo- and hyperchromicity changes of the RNA molecules neutralize each other completely or the encapsidated RNAs have the same secondary structure as when free in solution.

The data collected for the determination of the molecular weights of the RNAs also provide information about the nucleoprotein composition of the virus particles. In the first place it is evident from the data in Table IV that the different components have very similar RNA/protein ratios. However, particles of component B appear to contain about 0.8% more RNA than the other components. This difference agrees with its banding at a slightly higher buoyant density in CsCl gradients. Small density differences between the unfixed AMV components, notably between components B and Ta in gradients of Cs_2SO_4 and Metrizamide were also recently reported by Hull (1976).

Knowledge of the weights of the particle species and their RNA contents enables us to calculate the number of subunits of the different capsids (Table VIII). The values listed here for the capsids of the components Ta-t and Ta-b are higher than those published elsewhere (Heijtink and Jaspars, 1976) since the molecular weight of the coat protein as derived from the primary structure (Van Beynum et al., 1977) was found to be somewhat less than that assumed earlier. This implied also a slight change in the nitrogen content of the coat protein.

The tubular part of AMV capsids has a hexagonal lattice structure with spacings of about 48 Å according to Hull et al. (1969b) or of about 40 Å according to Mellema and Van Den Berg (1974). If we plot the number of protein subunits found experimentally in the different capsids against their median lengths taken from Figure 2, we get a straight line with a slope of 18 subunits per 43.4 Å (Figure 5). Component Ta-t has not been used for constructing this line since it has an irregular structure (Heijtink and Jaspars, 1976). Extrapolation of the line yields a 60-subunit particle at 12.8 nm. Spherical particles formed in vitro from protein dimers under certain conditions have been found to be built from 60 subunits (Heijtink, 1974; Driedonks et al., 1976). These spheres have a diameter of 18 nm, which is close to the diameter found for AMV particles (Hull, 1969) (15.5 nm in this study), but when lying on a carbon layer over holes in the supporting film the diameter is found to be only about 12.6 nm (Driedonks et al., 1976). According to the authors this low value is due to the absence of flattening in the freely suspended particles. However, the agreement between this value of 12.6 nm in carbon layers and the extrapolated value of 12.8 nm of Figure 5 makes sense only if one assumes that the flattening has much more influence on the diameter than on the length of particles. Anyhow it is conceivable that the AMV capsids represent a series of structures based on a 60-subunit icosahedron cut across and having n rings of 18 subunits inserted between the halves. Such a series has already been proposed by Hull et al. (1969b). However, we must arrive at different n values since these authors used capsid weights not very different from ours but they divided them by a protein subunit molecular weight of 32 600. When n is taken to be 10, 7, 5, and 4 for the components B, M, Tb, and Ta-b, respectively, the calculated numbers of subunits come very close to the experimentally determined values. Probably other values of n are realized by the capsids of the minor components observed upon electrophoresis of unfractionated AMV preparations in polyacrylamide gels (Bol and Lak-Kaashoek, 1974).

Acknowledgments

Professor H. Veldstra created the climate in which this work was done. We are much indebted to him. We wish to thank Mr. C. E. G. C. Bakhuizen, Dr. J. A. M. Van Boxsel, Mr. C. D. De Jonge, and Mr. P. C. J. Krijgsman for their assistance in part of the experimental work. The help of Mrs. Marianne Lak-Kaashoek, Miss Lyda Neeleman, Mr. F. Th. Brederode, and Mr. A. G. Wesseling in obtaining virus material is gratefully acknowledged. Dr. J. A. M. Smit of the Department of Physical Chemistry permitted us to use the sensitive density measuring apparatus of Anton Paar in his laboratory which made the determination of the partial specific volumes much easier. Drs. Lous Van Vloten-Doting, J. F. Bol, and S. Srinivasan made useful comments on the manuscript.

References

Bancroft, J. B., and Kaesberg, P. (1960), *Biochim. Biophys. Acta 39*, 519.

Boedtker, H. (1968), *J. Mol. Biol. 35,* 61.
Bol, J. F. (1969), Ph.D. Thesis, University of Leiden.
Bol, J. F., and Lak-Kaashoek, M. (1974), *Virology 60,* 476.
Bol, J. F., and Van Vloten-Doting, L. (1973), *Virology 51,* 102.
Bol, J. F., Van Vloten-Doting, L., and Jaspars, E. M. J. (1971), *Virology 46,* 73.
Bol, J. F., and Veldstra, H. (1969), *Virology 37,* 74.
Chervenka, C. H. (1969), A Manual of Methods, Beckman Instruments, Inc., California.
Dingjan-Versteegh, A., Van Vloten-Doting, L., and Jaspars, E. M. J. (1972), *Virology 49,* 716.
Driedonks, R. A., Krijgsman, P. C. J., and Mellema, J. E. (1976), *Philos. Trans. R. Soc. London, Ser. B 276,* 131.
Englander, S. W., and Epstein, H. T. (1957), *Arch. Biochem. Biophys. 68,* 144.
Gibbs, A. J., Nixon, H. L., and Woods, R. D. (1963), *Virology 19,* 441.
Hagedorn, D. J., and Hanson, E. W. (1963), *Phytopathology 53,* 188.
Heijtink, R. A. (1974), Ph.D. Thesis, University of Leiden.
Heijtink, R. A., and Jaspars, E. M. J. (1974), *Virology 59,* 371.
Heijtink, R. A., and Jaspars, E. M. J. (1976), *Virology 69,* 75.
Hull, R. (1969), *Adv. Virus Res. 15,* 365.
Hull, R. (1972), *Proc. Int. Congr. Virol., 2nd, 1971,* 233.
Hull, R. (1976), *Virology 75,* 18.
Hull, R., Hills, G. J., and Markham, R. (1969b), *Virology 37,* 416.
Hull, R., Rees, M. W., and Short, M. N. (1969a), *Virology 37,* 404.
Incardona, N. L., Notarius, H., and Flanegan, J. B. (1971), *Anal. Biochem. 40,* 267.
Jaspars, E. M. J. (1974), *Adv. Virus Res. 19,* 37.
Kelley, J. J., and Kaesberg, P. (1962), *Biochim. Biophys. Acta 61,* 865.
Knight, C. A., and Woody, B. R. (1958), *Arch. Biochem. Biophys. 78,* 460.
Kraal, B., Van Beynum, G. M. A., De Graaf, J. M., Castel, A., and Bosch, L. (1976), *Virology 74,* 232.
Kruseman, J., Kraal, B., Jaspars, E. M. J., Bol, J. F., Brederode, F. Th., and Veldstra, H. (1971), *Biochemistry 10,* 447.
Loening, U. E. (1967), *Biochem. J. 102,* 251.
Markham, R. (1962), *Adv. Virus Res. 9,* 241.
Mellema, J. E. (1975), *J. Mol. Biol. 94,* 643.
Mellema, J. E., and Van Den Berg, H. J. N. (1974), *J. Supramol. Struct. 2,* 17.
Minari, O., and Zilversmit, D. B. (1963), *Anal. Biochem. 6,* 320.
Mohier, E., Hirth, L., Le Meur, M.-A., and Gerlinger, P. (1975), *Virology 68,* 349.
Mohier, E., Hirth, L., Le Meur, M.-A., and Gerlinger, P. (1976), *Virology 71,* 615.
Pinck, L. (1969), Ph.D. Thesis, University of Strasbourg.
Pinck, L., and Hirth, L. (1972), *Virology 49,* 413.
Reijnders, L., Aalbers, A. M. J., Van Kammen, A., and Thuring, R. W. J. (1974), *Virology 60,* 515.
Reijnders, L., Sloof, P., Sival, J., and Borst, P. (1973), *Biochim. Biophys. Acta 324,* 320.
Scheraga, H. A., and Mandelkern, L. (1953), *J. Am. Chem. Soc. 75,* 179.
Svedberg, T., and Pederson, K. O. (1940), Die Ultracentrifuge, Theodor Steinkopff, Dresden.
Thang, M. N., Dondon, L., and Mohier, E. (1976), *FEBS Lett. 61,* 85.
Thang, M. N., Dondon, L., Thang, D. C., Mohier, E., Hirth, L., Le Meur, M.-A., and Gerlinger, P. (1975), *INSERM Colloq. 47,* 225.
Van Beynum, G. M. A., De Graaf, J. M., Castel, A., Kraal, B., and Bosch, L. (1977), *Eur. J. Biochem. 72,* 63.
Van Vloten-Doting, L., Bol, J., Neeleman, L., Rutgers, T., Van Dalen, D., Castel, A., Bosch, L., Marbaix, G., Huez, G., Hubert, E., and Cleuter, Y. (1977), in Nucleic Acid and Protein Synthesis in Plants, Bogorad, L., and Weil, J. H., Ed., New York, N.Y., Plenum Press, p 387.
Van Vloten-Doting, L., and Jaspars, E. M. J. (1967), *Virology 33,* 684.
Van Vloten-Doting, L., and Jaspars, E. M. J. (1972), *Virology 48,* 699.
Van Vloten-Doting, L., and Jaspars, E. M. J. (1977), in Comprehensive Virology, Vol. 11, Fraenkel-Conrat, H., and Wagner, R. R., Ed., New York, N.Y., Plenum Press, p 1.
Van Vloten-Doting, L., Rutgers, T., Neeleman, L., and Bosch, L. (1975), *INSERM Colloq. 47,* 233.
Verhoyen, M. (1967), Ph.D. Thesis, University of Louvain.
Yphantis, D. A. (1964), *Biochemistry 3,* 297.