Correlation Between Buoyant Density and Ribonucleic Acid Content in Viruses

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ABSTRACT

The relationship between buoyant density (ρ) in CsCl and ribonucleic acid (RNA) content of one bacterial and eight plant viruses was examined. The viruses selected for this study varied in their RNA content from 4 to 42%. A highly significant correlation ($R^2 = 0.99$) was found between ρ and the absolute amount of RNA in these viruses. The functional relationship indicated that the ρ of viruses increased at a greater than linear rate as the RNA content increased, but about 89% of this variation was directly attributable to the amount of RNA in the virions. Based on these observations, a mathematical formula was derived to estimate the RNA content of a virus from its ρ value. Data on the correlation between ρ values and nucleic acid contents of several other RNA viruses was reviewed and its significance discussed. Phytopathology 60:1778-1784.

Estimations of protein and nucleic acid content of viruses can be made by chemical and colorimetric analyses (33) and ultraviolet light absorption spectrophotometry (14, 28). Such methods are only reliable, however, when highly purified viral preparations are used. A procedure for estimating the nucleic acid content of isometric viruses based on the sedimentation coefficients of nucleic acid-free and complete virions in partially purified preparations was described by Reichmann (30). Estimations of the nucleic acid and protein contents of cellular organelles and macromolecules can also be made by the use of isotopic precursors and subsequent chemical fractionation (38). Alternatively, the protein to nucleic acid ratio of hydrated nucleoproteins can be deduced from their buoyant density (ρ) by equilibrium centrifugation in CsCl (47). A distinct advantage of the equilibrium centrifugation procedure is that significant information on the physicochemical properties of macromolecules can be obtained while conserving the material for other investigations. The use of equilibrium centrifugation methods for characterization of viruses, proteins, and nucleic acids by analytical and preparative ultracentrifugation has recently been reviewed (44). We report results of our investigations, under quasi-equilibrium conditions, to assess the extent and validity of the relationship between ρ values and RNA content of one bacterial and eight plant viruses.

MATERIALS AND METHODS.—Information on the sources of virus cultures, propagative and assay hosts, and viral purification procedures is given in Table 1. CsCl (99.9%) was purchased from Pierce Chemical Co., Rockford, Ill.

Leaf tissue, 20-24 days after inoculation, was used for purification of plant viruses. Extracts from comparable healthy plants were similarly processed, but in none of these preparations were any particulate components similar to virus particles observed upon equilibrium centrifugation. Viral concn were estimated spectrophotometrically based on their known extinction coefficients (5, 19, 43).

Equilibrium centrifugations were performed by a slight modification of the "step" isopycnic CsCl method (7) in the swinging bucket rotor, SB-206, in an I.E.C. Ultracentrifuge Model B-35. Five ml of CsCl solution ($\rho = 1.500$) was introduced into a 95 × 14.5 mm tube and an equal volume of the CsCl solution ($\rho = 1.252$) was carefully layered on the denser CsCl solution. Aliquots of the purified viruses (0.3 ml containing 400-700 μg) were then floated on the "step" gradient, and the tubes were immediately centrifuged. In a slight variation of this method, the viral preparations were thoroughly mixed with the lighter CsCl solution, and the resultant mixture was then layered on the denser CsCl solution. The density gradient tubes were generally centrifuged at 160,000 *g* for 18-20 hr at 15 C. During this period, an essentially linear and stable density gradient was engendered, and prolonging the centrifugation period to 30-35 hr did not affect the CsCl density-gradient profile in the centrifuge tubes. At the end of the centrifugation period, the rotor was allowed to stop by coasting. The visible zones were removed, either by a hypodermic syringe from the side of the centrifuge tube, or the density gradient column was fractionated by an ISCO fractionator (Model D) at a flow rate of 0.2 ml/min, and the column was monitored at 254 nm by a UA-2 analyzer. In some cases, 0.05-ml fractions were collected manually to resolve those viral components that banded closely in the density-gradient column. Alternatively, in multicomponent viruses, e.g., turnip yellow mosaic virus (TYMV) and tobacco ringspot virus (TRSV), the visible bands after isopycnic centrifugation were individually removed from several tubes, pooled, dialyzed, concd (by ultracentrifugation) and resubmitted separately to the isopycnic centrifugation procedure, and the density-gradient columns were fractionated. The refractive indices of the various fractions were determined by a Bausch and Lomb Abbe 3L refractometer at 25 C and converted into density values. Aliquots of the fractions were diluted with distilled water, and their ultraviolet light absorption spectra characterized. For infectivity tests, diluted frac-

TABLE 1. Sources of virus cultures, propagative and assay hosts, and references to the purification procedures[a]

Virus	Original source	Propagative host	Assay host	Purification procedure
Barley stripe mosaic (BSMV)	H. H. McKinney	Barley, Atlas	Barley, Atlas	(15)
Bromegrass mosaic (BMV)	W. H. Sill, Jr.	Barley, Atlas	Barley, Atlas	(8)
Cucumber mosaic (CMV)	R. W. Fulton	Tobacco, Xanthi-nc	Tobacco, Xanthi-nc Cowpea	(34)
Southern bean mosaic (SBMV)	M. K. Corbett	Bean, Bountiful	Bean, Pinto	(48)
Tobacco mosaic strain U_1 ($TMV\text{-}U_1$)	A. Siegel	Tobacco, Samsun	Tobacco, Xanthi-nc	(36)
Tobacco ring spot (TRSV)	R. W. Fulton	Tobacco, Xanthi-nc	Tobacco, Xanthi-nc Cowpea	(42)
Turnip yellow mosaic (TYMV)	J. M. Kaper	Chinese cabbage, Chi-hi-li	Chinese cabbage Chi-hi-li	(10)

[a] Purified pea enation mosaic virus (PEMV) was supplied by R. W. Bozarth and was assayed on pea, Perfection Wales. Purified MS2 virus was purchased from Miles Laboratory, Elkhart, Indiana.

tions were assayed on appropriate test plants according to the previously described procedure (35).

In some experiments, the purified viruses were fixed with formaldehyde (16) prior to the equilibrium centrifugation to prevent the dissociative effect of CsCl on the viral nucleoproteins. Additional experiments with bromegrass mosaic virus (BMV) and southern bean mosaic virus (SBMV) for isopycnic centrifugation were conducted in presence of Mg^{++}, since these ions are known to stabilize BMV and SBMV in vitro (4, 48).

The ρ of viruses were determined in several experiments employing two or more preparations, except in the case of pea enation mosaic virus (PEMV) and barley stripe mosaic virus (BSMV) in which three determinations on single preparations were made. Under the experimental conditions described, very reproducible results were obtained in the estimation of the ρ of viruses; in all instances, the differences were within $\pm$ 0.001 g/ml of the individual ρ values of viruses or viral components.

The relationship between ρ values and RNA content was examined using a second degree polynomial regression analysis, $Y = a + bx + cx^2$, where Y = estimated ρ, x = RNA content (%), a = intercept value, and b and c are regression coefficients (41).

RESULTS.—The OD profiles of the viruses upon isopycnic CsCl centrifugation and fractionation are presented in Fig. 1. The relationship between ρ and nucleic acid contents of several RNA viruses is represented in Fig. 2. Biological and physico-chemical properties of the various viruses and viral components are summarized in Table 2.

BSMV, tobacco mosaic virus (TMV), cucumber mosaic virus (CMV), and BMV formed single sharp peaks in the CsCl density-gradient columns and banded at the ρ of 1.309, 1.324, 1.336, and 1.352, respectively. Only fractions under these peaks were infectious. SBMV formed a slightly broad peak, $\rho = 1.359$, and all the viral infectivity was associated with this peak.

The presence of two components, a and b, was indicated in MS2. Because of its low ρ value (1.288) but high 280:260 ratio, component a appears to be the dissociated coat protein subunits. The component b formed a somewhat skewed peak at a ρ of 1.408 and, based on its 280:260 ratio of 0.57, appears to contain 32% RNA (28), the published value of the RNA content of mature MS2 virions (43). PEMV also formed two components in the CsCl density-gradient column. Component a, $\rho = 1.277$, appears to be the viral protein devoid of nucleic acid. Based on 280:260 ratio (0.58), $\rho = 1.418$, and infectivity test, component b appears to contain the full complement of the PEMV genome.

At least four components were detected in the OD profile of TYMV in the CsCl gradient columns. Component a appears to be the viral capsid shell devoid of nucleic acid on the basis of its low ρ (1.288) and relatively high 280:260 ratio (0.90). Components b, c, and d banded at the ρ of 1.403, 1.410, and 1.440, respectively. Infectivity tests of the separately centrifuged components indicated that only component d was infectious. Based on the ρ values, the RNA contents of components b and c were calculated to be 30 and 32%, respectively. Component d contained the full complement (35% RNA) of the viral genome.

TRSV also produced four components, a, b, c, and d, and their respective ρ values were 1.290, 1.410, 1.495, and 1.506. Component a appears to be the RNA-deficient capsid shell, while the RNA contents of components b and c were calculated to be 32 and 41%, respectively. Only component d proved infectious, and apparently contained the full viral genome (42% RNA) in the intact state.

Treatment of viral preparations with formaldehyde prior to isopycnic centrifugation did not affect their ρ values in CsCl. Similarly, no significant differences were observed in the ρ values of BMV and SBMV in presence or absence of Mg^{++}.

Our data (Fig. 2) on the dependence of ρ upon RNA content of viruses indicated a highly significant relationship. Goodness of fit was extremely high, as indicated by the sample coefficient of determination, 0.99. The functional relationship indicated that the ρ

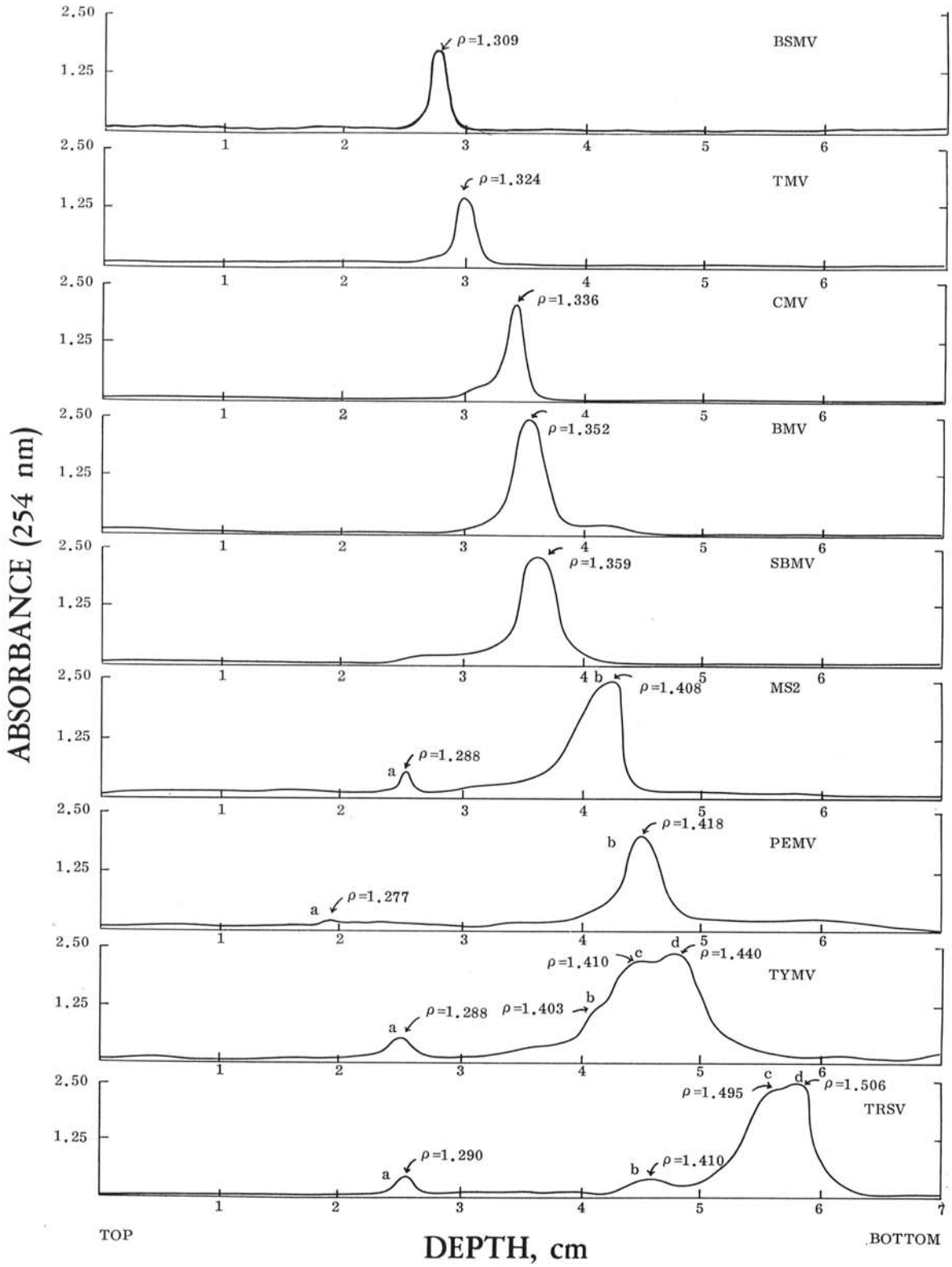

Fig. 1. Optical density profiles of viruses upon isopycnic CsCl centrifugation.

TABLE 2. Biological and physico-chemical properties of viruses and viral components after isopycnic CsCl centrifugation

Virus and viral components	Intensity of bands in the density-gradient column	Corrected 280:260 ratio[a]	Infectivity[b]	RNA content[c]	Buoyant density (g/ml)				
					Our data	Other reports[d]	Calculated from additive ρ of RNA and protein[e]	Calculated from experimental $\bar{v}$ of viruses[f]	Calculated from $\bar{v}$ of protein and RNA[g]
BSMV	Distinct	0.94	+	4.0	1.309		1.304	1.365	
TMV-U_1	Distinct	0.86	+	5.1	1.324	1.325	1.307	1.300, 1.365	1.373
CMV	Distinct	0.60	+	18.5	1.336		1.355		1.426
BMV	Distinct	0.60	+	21.4	1.352	1.361	1.365		1.428
SBMV	Distinct	0.65	+	21.0	1.359	1.354-1.366	1.363	1.426, 1.436	1.440
MS2									
Component a	Faint	1.10	nc	0	1.288				1.371
Component b	Distinct	0.57	nc	32.0	1.408	1.38, 1.42, 1.46	1.401		1.490
PEMV									
Component a	Faint		—	0	1.277				
Component b	Distinct	0.58	+	28-30	1.418	1.42	1.384		
TYMV									
Component a	Faint	0.90	—	0	1.288			1.351	1.323
Component b	Faint	0.58	—	30	1.403				
Component c	Distinct	0.58	—	32	1.410	1.422-1.499			
Component d	Distinct	0.56	+	35	1.440		1.412	1.501	1.491
TRSV									
Component a	Faint	0.84	—	0	1.290				1.358
Component b	Faint	0.65	—	32	1.410				
Component c	Distinct	0.56	—	41	1.495				
Component d	Distinct	0.54	+	42	1.506		1.437		1.521

[a] Corrected 280:260 ratio of the dialyzed preparations.
[b] (+) = Infectious; (—) = noninfectious; nc = not checked.
[c] Data compiled from reports by Atabekov & Novikov (1), Kaper (18), Shepherd et al. (39), and Strauss & Sinsheimer (43). The RNA content of components b and c of TYMV and TRSV was estimated from Fig. 2.
[d] Based on reports by Siegel & Hudson (40), Bancroft et al. (2), Strauss & Sinsheimer (43), Nathans (26), Magdoff-Fairchild (22), Bozarth & Chow (3), and Derosier & Haselkorn (13).
[e] Calculated from the protein and RNA content of viruses assuming ρ of RNA and protein as 1.64 and 1.29, respectively.
[f] Calculated from the relationship, $\bar{v} = 1/\rho$ (6). Data on the $\bar{v}$ of viruses was compiled from reports by Markham (23) and Lauffer & Benedet (21).
[g] Calculated from $\bar{v}$ of viral coat proteins based on amino acid composition (9, 46), and assuming $\bar{v}$ of RNA as 0.55 (18).

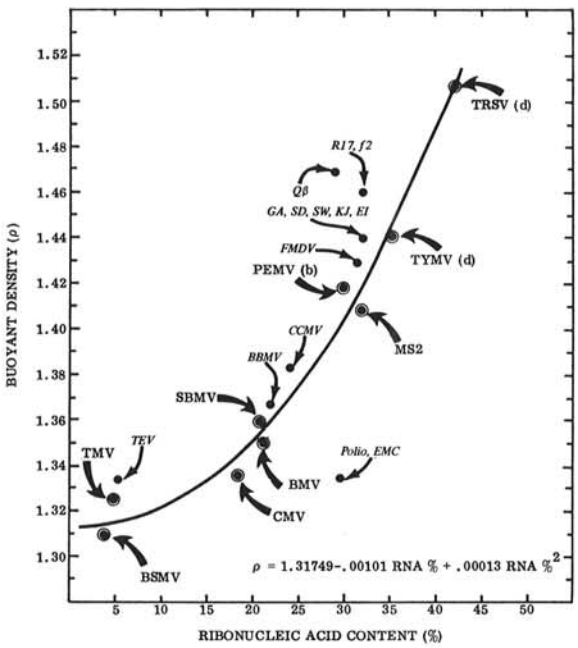

Fig. 2. The correlation between buoyant density and ribonucleic acid contents of representative viruses. The curve was drawn from the indicated relationship from data employing experimental (⊙) viruses only. See text to ascertain the abbreviated names of the viruses.

determinations by this procedure increased at a greater than linear rate as the viral RNA content increased; however, ca. 89% of this variation was associated with a direct linear response to the increase in the viral RNA contents. From the known ρ value of a virus, its RNA content can be estimated by the formula, RNA content =

$$3.88462 + \frac{\sqrt{(0.00101)^2 - (0.00052)\,(1.31749 - \rho)}}{0.00026}.$$

The experimental ρ values of the viruses examined were closer to those calculated from the additive densities of protein and RNA (Table 2). These values were, however, considerably lower than those calculated from the partial specific volume (v/v) of viruses, an observation consistent with the other reports (2).

Discussion.—Our estimates of the ρ values of TMV, BMV, and PEMV are essentially similar to those reported by other investigators (2, 3, 40). Five electrophoretic variants of SBMV that differed slightly in their ρ in CsCl (range 1.354-1.366) were isolated by Magdoff-Fairchild (22). Each of these variants was homogenous electrophoretically and in ρ values, and possessed identical specific infectivities. In our studies, SBMV formed a slightly broader peak in the CsCl density-gradient column in comparison to other single component viruses, but no indication of its density heterogeneity was obtained. SBMV has a marked dependence on metal ions for its "structural cohesion" (48). It seems likely that the electrophoretic or density SBMV variants arise by preferential binding of Cs+ or Cl− by the capsid protein rather than by actual differences in the composition or absolute content of the SBMV-RNA.

Density heterogeneity and the affinity of TYMV to bind large amounts or mono- and divalent ions is also evident from other studies (13, 17, 24). Although the exact mechanism by which density TYMV components arise is not known, Kaper (18) has suggested that preferential binding of Cs+ by virus particles or loss of some coat protein subunits during the equilibrium centrifugation may be the factors responsible. It seems probable also that a portion of the viral genome was lost from some virus particles during centrifugation in CsCl, resulting in the infectivity loss. Whether or not similar explanations would be applicable for the observed density heterogeneity of TRSV is not known.

Discrepancy exists in the reported values of MS2 virions. Strauss & Sinsheimer (43) observed that in concd viral preparations, MS2 banded at a ρ of 1.38, whereas, in diluted suspensions it banded at a ρ of 1.46. Nishihara & Watanabe (27) reported its ρ as 1.46. In our studies, no concn-dependent differences in the ρ of MS2 were observed employing either 2.5 mg or 90 µg of this virus. Our estimate of the ρ of MS2 (1.408) is closer to the value (1.42) reported by Nathans (26).

The relationship between the experimental ρ values of tobacco etch, (TEV) (11, 12), broad bean mottle (BBMV), and cowpea chlorotic mottle (CCMV) (2), and foot-and-mouth disease (FMDV) (45) viruses is generally in accord with the data in Fig. 2. The ρ values of several RNA phages; e.g., Qβ, R17, f2, GA, SD, SW, KJ, EI (27), and large, double-stranded RNA viruses, e.g., reo and wound tumor (37), are, however, greater than expected from their chemical composition. The reason for this discrepancy is not clear, but it may be due to the attachment of Cs+ with the viral capsid or permeation of Cs+ into the hollow portions of these virions. Striking anomaly is also evident between the RNA content and ρ values of two animal viruses, e.g., polio and encephalomyocarditis (EMC) (18, 32); the experimental ρ values of these viruses are significantly lower than other viruses containing 30% nucleic acid. Presence of lipids and carbohydrates as structural viral components can markedly reduce the ρ of viruses in CsCl (25, 29, 31), but no evidence of the presence of such components in polio and EMC viruses exists (18). Probably the degree of solvation of these virions or as yet some undiscovered biophysical properties of these viruses may be the contributing factors.

Physical alterations in the macromolecular configuration of virions (without affecting the protein to nucleic acid ratio) can also affect that ρ of viruses in CsCl. For instance, the ρ of a temp-resistant strain of a DNA phage, T_5st, is somewhat lower (1.534) than the wild-type strain, T_5 (1.546). This change has been interpreted to be due to a mutational event resulting in a more compact (or less hydrated) form of the T_5st virions (20).

Ideally, for the relationship between ρ and RNA content to be fully valid, there should be no interaction between Cs+ and the virus particles. As discussed

earlier, several intrinsic viral features can markedly affect the ρ of viruses in CsCl. Our results, nevertheless, suggest that the ρ of viruses in CsCl is primarily dependent upon their protein and nucleic acid content. Gross deviations from the expected relationship between ρ and RNA content could be indicative of configurational or compositional peculiarities of viruses or interaction of Cs+ with the virions.

A particularly useful feature of the isopycnic CsCl centrifugation procedure is that reliable estimates can be made of the RNA content of viruses which cannot otherwise withstand rigorous purification procedures, provided these experiments are supplemented by appropriate infectivity assays (35).

LITERATURE CITED

1. ATABEKOV, I. G., & V. K. NOVIKOV. 1966. The properties of barley stripe mosaic virus nucleoprotein and its structural components. Biokhimiya (USSR) 31: 157-166.
2. BANCROFT, J. B., E. HIEBERT, M. W. REES, & R. MARKHAM. 1968. Properties of cowpea chlorotic mottle virus, its protein and nucleic acid. Virology 34:224-239.
3. BOZARTH, R. F., & C. C. CHOW. 1966. Pea enation mosaic virus; purification and properties. Contrib. Boyce Thompson Inst., 23:301-309.
4. BRAKKE, M. K. 1963. Stabilization of brome mosaic virus by magnesium and calcium. Virology 19:367-374.
5. BRAKKE, M. K. 1967. Ultraviolet extinction coefficients of some representative viruses. *In* Iscotables; a handbook of data for biological and physical scientists. Instrumentation Specialities Co., Lincoln, Nebraska. 32 p.
6. BREEDIS, C., L. BERWICK, & T. F. ANDERSON. 1962. Fractionation of shope papilloma virus in cesium chloride density gradients. Virology 17:84-94.
7. BRUNCK, C. F., & V. LEICK. 1969. Rapid equilibrium isopycnic CsCl gradients. Biochim. Biophys. Acta 179:136-144.
8. CHIU, R. J., & W. H. SILL, JR. 1963. Purification and properties of brome grass mosaic virus. Phytopathology 53:1285-1291.
9. COHN, E. J., & J. T. EDSALL. 1943. Proteins, amino acids, and peptides. Reinhold, N.Y. 686 p.
10. CONSENTINO, V., K. PAIGEN, & R. L. STEERE. 1956. Electron microscopy of turnip yellow mosaic virus and its associated abnormal protein. Virology 2:139-149.
11. DAMIRDAGH, I. S., & R. J. SHEPHERD. 1970. Purification of the tobacco etch virus and other viruses of the potato virus Y group. Phytopathology 60: 132-142.
12. DAMIRDAGH, I. S., & R. J. SHEPHERD. 1970. Some of the chemical properties of the tobacco etch virus and its protein and nucleic acid components. Virology 40:84-89.
13. DEROSIER, D., & R. HASELKORN. 1966. Minor components associated with turnip yellow mosaic virus. Virology 30:705-715.
14. ENGLANDER, S. W., & H. T. EPSTEIN. 1957. Optical methods for measuring nucleoprotein and nucleic acid concentrations. Arch. Biochem. Biophys. 68: 144-149.
15. HARRISON, B. D., H. L. NIXON, & R. W. WOODS. 1965. Length and structure of particles of barley stripe mosaic virus. Virology 26:284-289.
16. HUXLEY, H. E., & G. ZUBAY. 1960. Electron microscope observations on the structure of microsomal particles from Escherichia coli. J. Mol. Biol. 2:10-18.
17. JOHNSON, M. W. 1964. The binding of metals by turnip yellow mosaic virus. Virology 24:26-35.
18. KAPER, J. M. 1968. The small RNA viruses of plants, animals and bacteria. A. Physical properties, p. 1-133. *In* H. Fraenkel-Conrat [ed.] Molecular basis of virology. Reinhold, N.Y.
19. KAPER, J. M., T. O. DIENER, & H. A. SCOTT. 1965. Some physical and chemical properties of cucumber mosaic virus (strain Y) and its isolated ribonucleic acid. Virology 27:54-72.
20. LARK, K. G. 1962. Physical alterations in the structure of phage T_5 associated with mutation to heat resistance. Biochim. Biophys. Acta 61:832-834.
21. LAUFFER, M. A., & I. J. BENEDET. 1954. The hydration of viruses. Advances Virus Res. 2:241-287.
22. MAGDOFF-FAIRCHILD, B. S. 1967. Electrophoretic and buoyant density variants of southern bean mosaic virus. Virology 31:142-153.
23. MARKHAM, R. 1951. Physicochemical studies of the turnip yellow mosaic virus. Discussions Faraday Soc., 11:221-227.
24. MATHEWS, R. E. F. 1960. Properties of nucleoprotein fractions isolated from turnip yellow mosaic virus. Virology 12:521-539.
25. MURANT, A. F., R. A. GOOLD, I. M. ROBERTS, & J. CATHRO. 1969. Carrot mottle—a persistent aphid-borne virus with unusual properties. J. Gen. Virol. 4:329-341.
26. NATHANS, D. 1968. Natural RNA coding of bacterial protein synthesis, p. 787-791. *In* L. Grossman & K. Moldave [ed.] Methods in enzymology, Vol. XIIB. Academic Press, Inc., N.Y.
27. NISHIHARA, T., & I. WATANABE. 1969. Discrete buoyant density distribution among RNA phages. Virology 39:360-362.
28. PAUL, H. L. 1959. Die Bestimmung des Nucleinsauregehaltes pflanzlicher Viren mit Hilfe einer spektrophotometrischen Methode. Z. Naturforschg. 14b: 427-432.
29. PFEFFERKORN, E. R. 1968. The large RNA viruses C. Arboviruses and vesicular stomatitis virus. p. 332-350. *In* H. Fraenkel-Conrat [ed.] Molecular basis of virology. Reinhold, N.Y.
30. REICHMANN, M. E. 1965. Determination of ribonucleic acid content of spherical viruses from sedimentation coefficients of full and empty particles. Virology 20: 166-169.
31. ROBINSON, W. S., & P. H. DUESBERG. 1968. The large RNA viruses. B. The chemistry of RNA tumor viruses, p. 306-331. *In* H. Fraenkel-Conrat [ed.] Molecular basis of virology. Reinhold, N.Y.
32. SCHAFFER, F. L., & L. H. FROMMHAGEN. 1965. Similarities of biophysical properties of several human enteroviruses as shown by density gradient ultracentrifugation of mixture of viruses. Virology 25: 662-664.
33. SCHUSTER, H. 1964. The infective nucleic acids of plant viruses, p. 49-78. *In* R. J. C. Harris [ed.] Techniques in experimental virology. Academic Press, Inc. N.Y.
34. SCOTT, H. A. 1963. Purification of cucumber mosaic virus. Virology 20:103-106.
35. SEHGAL, O. P., & JONG-HO JEAN. 1970. Purification of maize dwarf mosaic virus by equilibrium centrifugation in cesium chloride. Phytopathology 60:189-190.
36. SEHGAL, O. P., & G. F. KRAUSE. 1968. Efficiency of nitrous acid as an inactivating and mutagenic agent for intact tobacco mosaic virus and its isolated nucleic acid. J. Virol. 2:966-971.
37. SHATKIN, A. J. 1968. The large RNA viruses. D. Viruses containing double stranded RNA, p. 351-392. *In* H. Fraenkel-Conrat [ed.] Molecular basis of virology. Reinhold, N.Y.
38. SHATKIN, A. J. 1969. Estimation of RNA, DNA, and protein by use of isotopic precursors followed by

chemical fractionation. V. Quantitative procedures for assay of RNA, DNA and protein. *In* K. Habel & N. P. Salzman [ed.] Fundamental techniques in virology. Academic Press, Inc., N.Y.

39. Shepherd, R. J., R. J. Wakeman, & S. A. Ghabrial. 1968. Preparation and properties of the protein and nucleic acid components of pea enation mosaic. Virology 35:255-267.
40. Siegel, A., & W. Hudson. 1959. Equilibrium centrifugation of two strains of tobacco mosaic virus in density gradients. Biochim. Biophys. Acta 34:254-255.
41. Snedecor, G. W., & W. G. Cochran. 1967. Statistical methods. [6th ed.] The Iowa State Univ. Press, Ames. 593 p.
42. Stace-Smith, R., M. E. Reichmann, & N. S. Wright. 1965. Purification and properties of tobacco ringspot virus and its two RNA-deficient components. Virology 25:487-494.
43. Strauss, J. H., Jr., & R. L. Sinsheimer. 1963. Purification and properties of bacteriophage MS2 and its ribonucleic acid. J. Mol. Biol. 7:43-54.
44. Szybalski, W. 1968. Equilibrium sedimentation of viruses, nucleic acids and other macromolecules in density gradients. Fractions, p. 1-15. Spinco Division, Beckman Instruments Inc. Palo Alto, Calif.
45. Trautman, R., & S. S. Breese. 1962. Isodensity ultracentrifugation of foot-and-mouth disease virus in caesium chloride. J. Gen. Microbiol. 27:231-239.
46. Tremaine, J. H., & D. E. Goldsack. 1968. The structure of regular viruses in relation to their subunit amino acid composition. Virology 35:227-237.
47. Weigle, J., M. Meselson, & K. Paigen. 1959. Density, alterations associated with transducing ability in the bacteriophage lambda. J. Mol. Biol. 1:379-386.
48. Wells, J. M., & H. D. Sisler. 1969. The effects of EDTA and Mg^{2+} on the infectivity and structure of southern bean mosaic virus. Virology 37:227-236.