Physicochemical Properties of *Tipula* Iridescent Virus¹

J. KALMAKOFF AND J. H. TREMAINE

Department of Microbiology, The University of British Columbia, and Canada Department of Agriculture Research Station, Vancouver, British Columbia, Canada

Received for publication 26 April 1968

The molecular weight of *Tipula* iridescent virus, based on sedimentation and diffusion coefficients, was 5.51×10^6 , with hydration of 0.57 g of water per g of virus. Deoxyribonucleic acid content, based on total inorganic phosphorus liberated, was $19 \pm 0.2\%$. At 260 $m\mu$, the virus gave an uncorrected absorbance of 18.2 cm^2/mg of virus and a light-scattering corrected absorbance of 9.8 cm^2/mg of virus. Amino acid analyses of the virus protein revealed a remarkable similarity to *Sericesthis* iridescent virus. The possibility is discussed that the four iridescent insect viruses reported to date bear a strain relationship.

Since the discovery of *Tipula* iridescent virus (TIV) by Xeros (28) at the Virus Research Unit at Cambridge, England, reports of other iridescent viruses have appeared in the literature. *Sericesthis* iridescent virus (SIV) was found (25) in a Scarab beetle larva in Australia, mosquito iridescent virus (MIV) was isolated in Florida (5), and *Chilo* iridescent virus (CIV) was found in the rice stem borer moth in Japan (8). These viruses are closely similar in most physical and biological properties, but they are not identical. It has been reported (7) that TIV and SIV are serologically related, and a study (3) of the deoxyribonucleic acid (DNA) of CIV, SIV, and TIV showed that the nucleic acids of these viruses were very similar, but not identical. These viruses have icosahedral symmetry, with reported diameters ranging from 1,800 Å for MIV to 1,300 Å for TIV. Extensive chemical analysis on TIV was carried out (26), and an amino acid analysis was done on SIV (20). Recently, Smith (20), in a monograph on insect viruses, summarized the properties of TIV and SIV.

In this investigation, we report further studies on the chemical and physical properties of TIV and the relationship of these results to those published for TIV and other iridescent viruses. Considering the wide experimental and natural host and geographical range of TIV (21) and

the similarity of the properties of all of the iridescent viruses, these viruses probably represent strains of a single iridescent virus.

MATERIALS AND METHODS

Virus purification. TIV was obtained from K. M. Smith of the University of Texas, Austin, and from C. F. Rivers of the Agricultural Research Council Virus Research Unit, Cambridge, England. Both virus preparations proved infectious when inoculated into larvae of *T. paludosa*, giving the characteristic symptomatology (20). The culture obtained from K. M. Smith was passaged in the laboratory by inoculation of 10 μ liters of a virus suspension containing about 1.8×10^9 virus particles with 250 units of penicillin and 0.25 mg of streptomycin per ml. In the mass production of TIV, the larvae were cut into fine pieces with scissors 6 to 10 days after inoculation. The pieces of larvae in distilled water were stirred magnetically at 4 C overnight. The resulting suspension was clarified by centrifugation at 1,000 rev/min for 10 min in a Sorvall SS-1 rotor, followed by centrifugation in a no. 30 rotor in the Spinco model L centrifuge at 20,000 rev/min for 30 min. The pellets were dissolved in 0.01 M phosphate buffer (pH 7.4) or in distilled water. The resuspended virus was further purified by centrifugation in a Spinco SW25 rotor at 15,000 rev/min for 20 min through a linear sucrose density gradient of 5 to 40% (24). The gradient was fractionated on an ISCO model D density gradient fractionator. The optical density of the fractions was measured at 260 $m\mu$ with a Beckman DU spectrophotometer. The virus fractions were dialyzed against buffer and concentrated by high-speed centrifugation if necessary. The virus was repurified by sucrose density gradient prior to use for physical studies, as it was found that virus suspensions tended to aggregate upon standing.

Amino acid analysis. Analyses were performed on

¹ Joint publication, Department of Microbiology, University of British Columbia, and Contribution no. 144, Canada Department of Agriculture Research Station, Vancouver, B.C., Canada. Taken in part from a thesis submitted to the Department of Microbiology, University of British Columbia, in partial fulfillment of the requirements for the Ph.D. degree.

purified virus from *Tipula* larvae inoculated with a pool of material received from C. F. Rivers and K. M. Smith. The protein was separated from the nucleic acid by mixing 5 ml of a 2 mg/ml virus preparation with 1 ml of 6 N HCl. The denatured protein was separated by centrifugation, and the protein pellet was hydrolyzed (12) with the use of 24- and 72-hr hydrolysis times.

Tryptophan content was determined by reaction of the whole virus with *p*-aminobenzaldehyde (23). Cysteine content was determined in a separate sample of virus protein oxidized with performic acid. The cysteic acid content was estimated by reference to aspartic acid and alanine, which were determined in the same analysis. The results were corrected for 10% loss of cysteic acid.

A correlation of protein content and absorbance at 260 m μ was made by placing a sample of whole virus in a hydrolysis tube with 6 N HCl, hydrolyzing for 24 hr, and analyzing the sample in the same manner as the virus protein. Adjustments for glycine, tryptophan, cysteine, threonine, and serine were made by comparison with values obtained for the virus protein.

Phosphorus. Inorganic phosphorus was determined (4) from samples of whole virus heated at 190 to 200 C for 15 min with 70% perchloric acid. The virus had previously been dialyzed at 4 C against 0.02 M tris(hydroxymethyl)aminomethane (Tris) - chloride buffer (pH 8.1) for 3 weeks.

Antiserum. A rabbit was injected intramuscularly with 2 mg of purified TIV emulsified with incomplete Freund's adjuvant. After 24 days, the animal was given a similar inoculation and was then bled 21 days after the second injection. The serum was mixed with an equal volume of glycerol and stored at 0 C until used.

Sedimentation and diffusion. Diffusion experiments were performed in a Spinco model H electrophoresis-diffusion apparatus using the 11-ml cell at 3.7 C with virus in 0.02 ionic strength buffer. The diffusion coefficients were calculated from photographs of the Rayleigh interference fringes taken over a period of 10 to 18 days (17). Measurements of fringe positions were made with a Gaertner two-dimensional micro-comparator.

Sedimentation velocity was determined at 0.02 ionic strength buffer in a Spinco model E analytical ultracentrifuge at 6,995 rev/min, the temperature of the rotor being regulated at 20 C.

Partial specific volume. The partial specific volume of TIV was calculated from its composition by use of the additive procedure (6). Therefore, $\bar{V} = \sum \bar{V}_i W_i + \bar{V}_{\text{DNA}} W_{\text{DNA}}$, where $\bar{V}_i$ is the partial specific volume of amino acid residue *i*, $\bar{V}_{\text{DNA}}$ is the partial specific volume of DNA (0.59 ml/g), and W_i and W_{DNA} are the percentage composition of each of the amino acid residues and the DNA, respectively.

RESULTS

Virus purification. A virus preparation purified by one cycle of ultracentrifugation was separated by sucrose density gradient into at

least three components (Fig. 1A). This pattern was not significantly altered by use of 0.01 M phosphate at pH 6.7, borate at pH 7.5 (7), 0.01 M Tris-chloride at pH 8.0, or distilled water, or by extraction of the virus with 5% chloroform at 4 C. When the main virus component was concentrated by centrifugation and subjected to another density gradient, the main and bottom components were present; the top component was absent. The nature of these components was studied by dialyzing appropriate fractions against distilled water and examining them in a Philips 200 electron microscope after negative staining with 2% uranyl acetate (Fig. 1B and 1C).

The main component consisted of virus particles with diameters of 1,300 A, showing an icosahedral shape (Fig. 1B). The appearance of chloroform-treated virus was not significantly different from that of untreated virus. Many aggregated virus particles were found in the bottom component (Fig. 1C). The view that these are aggregates is supported by the formation of this component upon pelleting the main virus component and repurification. The particles in the top component did not reveal any unique sizes or other detectable structural changes, but they may have a different DNA or protein composition from that of the virus. No further attempt was made to characterize the top component. Figure 1D shows the sedimentation pattern of the purified main component in the analytical ultracentrifuge. The schlieren optical patterns were visible only at concentrations below 0.5 mg of virus per ml because of the large light-scattering property of this virus. Inoculation into a rabbit of virus purified in this manner did not induce antibodies to healthy larval extracts.

Amino acid composition. The results of the amino acid analyses of virus protein are presented in Table 1. The values obtained in the 24-hr hydrolysis were used in calculation of the average value, unless otherwise indicated. Threonine and serine were calculated by extrapolating the values from the 24- and 72-hr hydrolysates to zero hydrolysis time. These values compared favorably with the 10.6% loss for serine and 0.8% loss of threonine at 24 hr observed for carnation ringspot virus protein (12). The 72-hr hydrolysis values were used in calculating the average for isoleucine, leucine, and valine because of the greater stability of their peptide bonds. The 24-hr hydrolysis value for methionine was corrected for the 5% loss reported (16). A decrease of methionine in the 72-hr hydrolyzate was correlated with an increase in ninhydrin-reactive components, presumably methionine sulfoxides, eluted in a position preceding aspartic acid.

FIG. 1. *A*: Photograph of a 5 to 40% sucrose density gradient showing separation of TIV into (T) top, (M) main, and (B) bottom components. *B*: Electron micrographs of TIV from main component negatively stained with 2% uranyl acetate; $\times 66,400$. *C*: TIV from bottom component stained with uranyl acetate; $\times 66,300$. *D*: Ultracentrifuge pattern of TIV (0.5 mg/ml) in 0.02 ionic strength Tris-chloride, pH 8.1. Photographs taken 10 and 14 min after reaching 6,995 rev/min; note the light-scattering effect of the sedimenting virus.

Table 2 gives the uncorrected average value from a 24-hr hydrolysis of whole virus and the average value obtained (13) from an 18-hr hydrolysis of whole virus. The whole virus and virus protein in our analyses were similar except for glycine, which was higher for the whole virus; there were also the expected variations in those amino acids which depend on hydrolysis time. The results of the other workers are discussed later.

The minimal molecular weights of the subunit protein calculated from the percentage composition are presented in Table 3. The lowest molecular weight giving a good fit for the protein was calculated to be 28,054. This protein molecular weight would be in error if two or more protein species were present. Calculation of amino acid residues based on a minimal molecular weight of 30,460 and the amino acid analysis for SIV (20) are included in Table 3 for comparison.

Phosphorus. Inorganic phosphorus liberated by 70% perchloric acid was analyzed from eight samples of whole virus. The quantity of virus

protein was determined by measuring the optical density at 260 m μ of the whole virus and the yield of amino acids after hydrolysis of the whole virus. By use of the optical density of 22.5 cm²/mg of virus protein, an average value of 0.02395 ± 0.0002 g of phosphorus per g of virus protein was obtained. If TIV is composed only of protein and DNA and the average nucleotide residue weight is 309 g/mole, the virus contains $19 \pm 0.2\%$ DNA.

Physical properties. A sedimentation coefficient of $S_{20}^w 2,147 \times 10^{-13}$ sec was obtained in Tris-chloride (pH 8.1) at 0.02 ionic strength buffer. This value does not differ significantly from the reported value of 2,200S (3, 27).

Several diffusion experiments were done in different buffers since TIV aggregated spontaneously in distilled water (14, 22) and SIV precipitated in phosphate buffer (7). A diffusion experiment was carried out at pH 8.1 in Tris-chloride buffer of 0.02 ionic strength over a period of 10 days. Because particles with large molecular weights have a considerable time cor-

rection, the relationship given by Gosting (9) was used:

$$D_A = D \left(1 + \frac{\Delta t}{t'} \right)$$

where Δt = time correction, t' = observed time, D_A = apparent diffusion coefficient, and D = diffusion coefficient. A plot of $D_{A'}'$ versus t' (Fig. 2) gave a linear relationship, and D was calculated from the slope which after correction to standard state was $D_{20}^s = 3.27 \times 10^{-8}$ cm²/sec. A diffusion experiment was carried out at pH 7.4 in phosphate buffer of 0.02 ionic strength, since a molecular weight for TIV had been determined (27) in phosphate buffer. A plot of D_A versus $1/t'$ (Fig. 3) shows that the virus aggregated rapidly after 5 days in phosphate buffer, and extremely low diffusion coefficients were obtained. Extrapolating the D_A values obtained in the first 5 days (Fig. 3) gave a value of 1.03×10^{-8} cm²/sec, which is similar to the diffusion coefficient of 1.07×10^{-8} cm²/sec calculated from the molecular weight and sedimentation coefficient determined (27) in an 18-day experi-

TABLE 2. *Amino acid composition from hydrolysis of whole Tipula iridescent virus*

Amino acid	Amino acid residues (g/100 g recovered)	
	Kawase and Hukuhara ^a	Kalmakoff and Tremaine ^b
Lysine.....	6.6	5.13
Histidine.....	2.85	1.96
Arginine.....	9.65	5.95
Aspartic acid.....	10.35	12.80
Threonine.....	6.1	6.85
Serine.....	8.35	9.17
Glutamic acid.....	7.1	8.11
Proline.....	7.45	8.19
Glycine.....	4.9	5.30
Alanine.....	5.6	5.83
Cysteine.....	trace	trace
Valine.....	4.85	4.69
Methionine.....	1.45	1.67
Isoleucine.....	4.7	4.56
Leucine.....	6.65	7.66
Tyrosine.....	5.7	6.23
Phenylalanine.....	5.15	5.46
Ammonia.....	2.7	—
Totals.....	100.15	99.56

^a Average from 18-hr hydrolysis (13).

^b Uncorrected average from 24-hr hydrolysis of virus in this investigation.

TABLE 1. *Amino acid residue recoveries from Tipula iridescent virus protein*

Amino acid	Amino acid residues (g/100 g recovered)								
	24 hr			72 hr			Avg value ^d		
Lysine.....	4.77	4.63	4.67	4.71	4.71	4.77	4.69		
Histi-									
dine.....	1.78	1.74	1.78	1.64	1.64	1.64	1.76		
Arginine.....	5.40	5.40	5.52	5.34	5.40	5.40	5.44		
Aspartic									
acid.....	11.43	11.77	11.61	11.71	11.51	11.61	11.61		
Threo-									
nine ^a	6.90	6.88	6.92	6.84	6.82	6.84	6.94		
Serine ^a	7.02	7.10	7.04	6.63	6.49	6.55	7.40		
Glutamic									
acid.....	7.42	7.50	7.46	7.54	7.69	7.44	7.46		
Proline.....	6.57	6.63	6.53	6.90	6.94	6.70	6.57		
Glycine.....	4.37	4.35	4.47	4.71	4.69	4.59	4.39		
Alanine.....	5.30	5.32	5.40	5.40	5.40	5.20	5.34		
Valine ^b	5.26	5.38	5.34	6.35	6.27	6.15	6.25		
Methio-									
nine ^c	1.88	1.88	1.88	trace			1.98		
Isoleucine ^b	5.50	5.54	5.60	6.61	6.39	6.41	6.45		
Leucine ^b	7.95	7.91	8.09	8.33	8.29	8.29	8.30		
Tyrosine.....	5.70	5.85	5.81	4.98	5.06	4.92	5.78		
Phenylala-									
nine.....	5.89	6.01	5.95	6.05	6.11	5.89	5.95		

^a Average was obtained by extrapolation to zero hydrolysis time and comparison to carnation ringspot virus losses.

^b The 72-hr values were used in average.

^c The 24-hr values were corrected for 5% loss.

^d The 24-hr values were used in calculation of average unless otherwise indicated.

ment with phosphate buffer. By use of the diffusion coefficient obtained in Tris-chloride buffer, the molecular weight, M was calculated from the equation:

$$M = \frac{RTS_{20}^w}{(1 - \bar{V}\rho)D_{20}^w}$$

where R = gas constant, T = absolute temperature, $\bar{V}$ = partial specific volume, ρ = density of water at 20 C, and S_{20}^w and D_{20}^w = sedimentation and diffusion coefficients corrected to standard state. The partial specific volume of TIV calculated from its composition was $\bar{V} = 0.703$. Although molecular weights require evaluation of the sedimentation and diffusion coefficients at infinite dilution, the assumption was made that these coefficients have a similar concentration dependence. A molecular weight of 5.51×10^8 was calculated from the data at 0.5 mg of virus per ml. A frictional ratio of 1.22 was calculated from the hypothetical diffusion coefficient of the anhydrous particle and the observed diffusion coefficient. The value of 0.57 g of water per g of virus was obtained, assuming the deviation of the frictional ratio from unity was due to

TABLE 3. *Composition and molecular weight of Tipula iridescent virus (TIV) protein*

Amino acid	Amino acid residue (g/100 recovered) ^a	Minimal mol wt	Assumed residues	Calculated mol wt	TIV residues ^b	SIV residues ^c	TIV residues ^d
Lysine.....	4.69	2,731	10	27,310	10.3 ± 0.6	11.7	11.2
Histidine.....	1.76	7,790	4	31,160	3.6 ± 0.04	4.0	3.9
Arginine.....	5.44	2,871	10	28,710	9.8 ± 0.2	18.1	10.6
Aspartic acid.....	11.61	991	28	27,748	28.3 ± 0.7	29.2	30.7
Threonine.....	6.94	1,457	19	27,683	19.3	18.5	21.0
Serine.....	7.40	1,177	24	28,248	23.8	26.4	25.8
Glutamic acid.....	7.46	1,731	16	27,696	16.2 ± 0.2	16.2	17.6
Proline.....	6.57	1,478	19	28,082	19.0 ± 0.3	23.9	20.6
Glycine.....	4.39	1,346	21	28,266	20.8 ± 0.5	23.7	22.6
Alanine.....	5.34	1,331	21	27,951	21.1 ± 0.4	21.9	22.9
Cysteine ^e	1.11	9,297	3	27,891	3.0	1.9+	3.3
Valine.....	6.25	1,586	18	28,548	17.7 ± 0.5	19.3	19.2
Methionine.....	1.98	6,629	4	26,516	4.2	5.0	4.6
Isoleucine.....	6.45	1,775	16	28,080	16.0 ± 0.5	15.5	17.4
Leucine.....	8.30	1,366	20	27,320	20.5 ± 0.1	19.7	22.3
Tyrosine.....	5.78	2,826	10	28,260	9.9 ± 0.2	10.7	10.8
Phenylalanine.....	5.95	2,474	11	27,214	11.3 ± 0.2	11.0	12.3
Tryptophan ^f	2.63	7,072	4	28,288	4.0	—	—
Totals.....	100.05		258	28,054 (avg)	258.8	276.7	276.8

^a From Table 1.^b Residues calculated from minimal molecular weight of 28,054. Includes t_{s_x} at $P = 0.05$, ($n - 1$) degrees of freedom.^c Amino acid analysis of *Sericesthis* iridescent virus (20).^d Residues calculated from minimum molecular weight of 30,460.^e By performic acid analysis plus 10% loss.^f As described in text.

hydration. The hydration of T2 bacteriophage, which is similar in size to TIV, is 0.60 g of water per g of virus (15).

DISCUSSION

The properties of TIV are in many respects similar to those of other iridescent insect viruses, indicating that all the iridescent viruses may be strains of a single virus species. Because of the wide geographic distribution of the discoveries of the isolates and the wide experimental host range, it would be surprising if many more isolates of iridescent viruses are not reported. An objection to the single virus theory is the wide variation in reported sizes, from 1,300 to 1,800 Å. This does not present so formidable an obstacle as it would seem, since it has been pointed out (11) that the diameter of the outline of an icosahedron can range from 0.851 to 1, depending on the orientation of the particle, and on whether the measurements are maximal or minimal diameters. Moreover, it has been shown (21) that inoculation of TIV into different hosts might result in slight variation in particle size with host species. These factors and different techniques in electron microscopy could account

for the apparent variability in the size of the iridescent viruses.

The similarity of amino acid composition for TIV and SIV (Table 3) strongly suggests a strain relationship. If the residues are calculated on the basis of a minimal molecular weight of 30,460, the values for all amino acids, with the exception of arginine, are within experimental error. The higher arginine in SIV may reflect a host modification of the virus, since the moth larva *Galleria mellonella* (L.) was used for virus passage (20). There is some precedent for host modification of viruses from work on bacteriophages, as reviewed by Arber (2). The data of Kawase and Hukuhara (13) show a similarity of composition between their TIV and the virus used in this investigation (Table 2). Their virus had a content of arginine similar to that in SIV and higher than that of our virus. It is interesting that the host modification concept is supported by the results (13), since they also used *G. mellonella* (L.) as host for TIV. It is likely that there was a host selection of strains already present in the TIV inoculum, rather than host modification.

Various estimates of DNA content for iri-

descent viruses have been reported: for TIV, 15% (19), 12.4% (26), and 16% (1); for SIV, 17.6% (7). Any estimation of DNA content depends directly on the accuracy of virus or protein weight determination. Since extraction with chloroform for TIV or with chloroform and ether for SIV (7) did not alter these viruses, the 5.2% lipid reported in TIV (26) was probably a host contaminant and would have affected the reported DNA percentage. Our estimate of $19 \pm 0.2\%$ DNA based on amino acid content should be valid, because extensive tests (26) revealed no major components other than DNA and protein.

Recently, Bellett and Inman (3) studied DNA isolated from CIV, SIV, and TIV, and found that DNA preparations from these viruses had sedimentation coefficients of about 62S and molecular weights of 1.30×10^8 . When molecular weights of 5.51×10^8 for TIV and 1.30×10^8 for DNA are used, a value of 23% DNA is obtained. Considering that the molecular weights of DNA were calculated with an empirical formula, 23% DNA is in good agreement with the chemical estimation of 19% DNA.

The molecular weight reported here of 5.51×10^8 is lower than the values of 10.5×10^8 (27) and 12.2×10^8 (26). If we assume the particles are spherical, the hydrodynamic diameter can be calculated from the molecular weight and

FIG. 2. Plot of $D_A \cdot t'$ versus t' values from diffusion experiment carried out at 3.7 C in 0.02 ionic strength Tris-chloride (pH 8.1), where D_A is the apparent diffusion coefficient and t' is the observed time. Diffusion coefficient was calculated from the slope.

FIG. 3. Plot of D_A versus $1/t'$ of diffusion experiment carried out at 3.7 C in 0.02 ionic strength phosphate buffer (pH 7.4). Point P is the diffusion coefficient at 18 days when D_A for the first 5 days is extrapolated. The downward curvature after 5 days indicates diffusion coefficients for aggregates of TIV.

frictional coefficient (18):

$$M = NfS/(1 - \bar{V}\rho)$$

$$f = 6\pi\eta r$$

where N = Avogadro's number, f = frictional coefficient, η = viscosity of water at 20 C, and r = hydrated radius of the particle. The other symbols were previously described. Hydrated diameters of 1,310, 2,500, and 2,910 Å were calculated from molecular weights of 5.5×10^8 , 10.5×10^8 , and 12.2×10^8 , respectively. The value of 1,310 Å is slightly greater than 1,300 Å, the diameter established for the frozen-dried particle by electron microscopy (14). The value of 1,310 Å is reasonable, since it has been pointed out (10) that much of the water of hydration probably occupies the depressions on the surface of virus particles. If the frozen-dried diameter of 1,300 Å is taken as the anhydrous diameter, the diameters of 2,500 and 2,910 Å, representing molecular weights of 10.5×10^8 and 12.2×10^8 , would give hydration values of 4.3 and 7.2 g of water per g of virus, respectively. Since most proteins have a total hydration not greater than about 1 g of water per g of protein, the large hydration values obtained are improbable, and hence the molecular weights used in their calculation are also improbable.

Considering that many of the properties of

CIV, MIV, SIV, and TIV are similar, if not identical, the molecular weight of 5.51×10^6 for TIV is probably a good estimate of the molecular weight of these other iridescent viruses.

ACKNOWLEDGMENTS

The senior author expresses thanks to R. E. Fitzpatrick, Research Station, Canada Department of Agriculture, for making the facilities of the Research Station available, and to J. J. R. Campbell, Department of Microbiology, University of British Columbia, for his kind cooperation and encouragement. This work was supported by a National Research Council Studentship.

LITERATURE CITED

- Allison, A. G., and D. G. Burke. 1962. The nucleic acid content of viruses. *J. Gen. Microbiol.* **27**:181-194.
- Arber, W. 1965. Host-controlled modification of bacteriophage. *Ann. Rev. Microbiol.* **19**:365-378.
- Bellett, A. J. D., and R. B. Inman. 1967. Some properties of deoxyribonucleic acid preparations from *Chilo*, *Sericesthis* and *Tipula* iridescent viruses. *J. Mol. Biol.* **25**:425-432.
- Chen, P. S., Jr., T. Y. Toribara, and H. Warner. 1956. Microdetermination of phosphorus. *Anal. Chem.* **28**:1756-1758.
- Clark, T. B., W. R. Kellen, and P. T. M. Lum. 1965. A mosquito iridescent virus (MIV) from *Aedes taeniorhynchus* (Wiedemann). *J. Invertebrate Pathol.* **7**:519-520.
- Cohn, E. J., and J. T. Edsall. 1943. Proteins, amino acids and peptides, p. 375. Reinhold Publishing Corp., New York.
- Day, M. F., and E. H. Mercer. 1964. Properties of an iridescent virus from the beetle, *Sericesthis pruinosa*. *Australian J. Biol. Sci.* **17**:892-902.
- Fukaya, M., and S. Nasu. 1966. A *Chilo* iridescent virus (CIV) from the rice stem borer, *Chilo suppressalis* Walker (Lepidoptera:Pyralidae). *Appl. Entomol. Zool.* **1**:69.
- Gosting, L. J. 1956. Measurement and interpretation of diffusion coefficients of proteins. *Advan. Protein Chem.* **11**:429-554.
- Harrison, B. D., and A. Klug. 1966. Relation between length and sedimentation coefficient for particles of tobacco rattle virus. *Virology* **30**:738-740.
- Hosaka, V. 1965. A criterion for evaluating the number of capsomeres of icosahedral capsids. *Biochim. Biophys. Acta* **104**:261-273.
- Kalmakoff, J., and J. H. Tremaine. 1967. Some physical and chemical properties of Carnation ringspot virus. *Virology* **33**:10-16.
- Kawase, S., and T. Hukuhara. 1967. Amino acid composition of the *Tipula* iridescent virus. *J. Invertebrate Pathol.* **9**:273-274.
- Klug, A., R. Franklin, and S. P. F. Humphreys-Owen. 1959. The crystal structure of *Tipula* iridescent virus as determined by Bragg reflection of visible light. *Biochim. Biophys. Acta* **32**:203-219.
- Lauffer, M. A., and I. J. Bendet. 1954. The hydration of viruses. *Advan. Virus Res.* **2**:241-287.
- Moore, S., D. H. Spackmann, and W. H. Stein. 1958. Chromatography of amino acids on sulfonated polystyrene resins. *Anal. Chem.* **30**:1185-1190.
- Schachman, H. K. 1957. Ultracentrifugation diffusion and viscometry, p. 78-95. In (S. P. Colowick and N. O. Kaplan (eds.), *Methods in enzymology*, vol. 4. Academic Press, Inc., New York.
- Schachman, H. K. 1959. Ultracentrifugation in biochemistry, p. 216. Academic Press, Inc., New York.
- Smith, K. M. 1958. A study of the early stages of infection with *Tipula* iridescent virus. *Parasitology* **48**:459-462.
- Smith, K. M. 1967. Insect virology, p. 74-89. Academic Press, Inc., New York.
- Smith, K. M., G. J. Mills, and C. F. Rivers. 1961. Studies on the cross-inoculation of the *Tipula* iridescent virus. *Virology* **13**:233-241.
- Smith, K. M., and R. C. Williams. 1958. Insect viruses and their structure. *Endeavour* **17**:12-21.
- Spies, J. R., and P. O. Chambers. 1949. Chemical determination of tryptophan in proteins. *Anal. Chem.* **21**:1249-1266.
- Stace-Smith, R. 1965. A simple apparatus for preparing sucrose density-gradient tubes. *Phytopathology* **55**:1031.
- Steinhaus, E. A., and R. Leutenegger. 1963. Icosahedral virus from a scarab (*Sericesthis*). *J. Insect Pathol.* **5**:266-270.
- Thomas, R. S. 1961. The chemical composition and particle weight of *Tipula* iridescent virus. *Virology* **14**:240-252.
- Weber, F. N., D. W. Kupke, and J. W. Beams. 1963. Molecular weights: measurements with gravity cells. *Science* **139**:837-838.
- Xeros, N. 1954. A second virus disease of the leatherjacket, *Tipula paludosa*. *Nature* **174**:562.