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The Structure of Togaviruses

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I. Introduction

In WEBSTER's dictionary a *toga* is defined as 'the loose outer garment worn by citizens when appearing in public'. The roman cloak gave its name to RNA viruses with a lipoprotein envelope which have taxonomic characteristics like those of CASAL's serological groups A and B[1] of arboviruses [1].

1 Members of the arbovirus group A will subsequently be referred to as *alphaviruses*, those of group B as *flavoviruses* [2].

Since 'arbovirus' is to be used in a purely ecological sense to denote the vast variety of viruses having a biological cycle in both arthropods and vertebrates, 'togavirus' met the demand for a structural term. A fundamental characteristic of the new family togaviridae proposed by the Vertebrate Virus Subcommittee [2] is the nucleocapsid symmetry; togaviruses will probably close the last gap in the structural classification system [3], into which enveloped RNA viruses with icosahedral symmetry of the nucleocapsids must be fitted.

Accumulating evidence suggested that particles with this architecture should be found among the ether-sensitive arboviruses. However, transmission by vectors is definitely not correlated with virus architecture since representatives of the rhabdo-, picorna- and reovirus groups, for example, have been identified as arthropod-borne while most members of the same groups spread conventionally. Therefore, it could be foreseen that agents with the structure of togaviruses would be identified also among the hitherto unclassified animal viruses without known arthropod transmission; rubella virus was regarded as a candidate for this group by many authors. Recently, the viruses of equine arteritis (EA), rubella of man (RU), mucosal disease/viral diarrhea of cattle (VD), and European swine fever/hog cholera (HC) have been compared electron microscopically with special respect to their internal structures; based on the morphological findings, these viruses were grouped together as belonging to the togavirus family [4]. In addition, the lactic dehydrogenase (LDH) virus [5] has been classified as a member of the group [6], on the basis of published evidence [7, 8].

The virus nature of the causative agents of EA, RU, VD, and HC has been known for a long time. EA virus was isolated in 1956 by Doll *et al.* [9] from a fetus aborted during an outbreak of an infectious disease in a group of pregnant mares. Its name is based on the specific lesions which can be found in the small arteries of fatally infected horses. The virus is not related to epizootic hemorrhagic disease of deer [10] and does not multiply in *Aedes* and *Anopheles* mosquitoes [11]. A review of the virological aspects of EA has been presented by Bürki [12].

RU virus, although producing only a mild disease of children and adults, has received much attention by clinicians and virologists because of the observation that an infection in early pregnancy could subsequently result in congenital malformations [13]. No antigenic cross-reaction between RU virus and a great number of arboviruses has been found [14]. The literature on RU virus has been reviewed by Norrby [15] and Banatvala [16].

In 1946, Olafson *et al.* [17] described a transmissible disease of dairy

cattle which had a striking resemblance to rinderpest, except that it was much milder; it was named virus diarrhea. It was subsequently proven that this virus was identical with the agent causing mucosal disease of cattle – an infection characterized by erosive and ulcerative lesions of the alimentary canal with comparatively high mortality [18, 19]. VD virus has been associated with teratological and lethal effects on the bovine fetus, including cerebellar hypoplasia, ocular defects, mummification and abortion. It has been shown to cross the placenta, causing concurrent maternal and fetal infection [20]. This virus diarrhea/mucosal disease topic has been discussed in a literature review by PRITCHARD [21].

In 1960, a serological relationship between the viruses of VD and HC was discovered by DARBYSHIRE [22]. Hog cholera in its classic form is an acute, highly contagious disease of swine with multiple hemorrhages as well as necrosis and infarctions in the internal organs. The viral etiology of the disease was proven by DE SCHWEINITZ and DORSET in 1903 [23]. Although its economic importance is gradually diminishing, HC still receives much attention because of its resemblance to African swine fever, which is menacing the world's swine industry. The embryopathic activity of HC virus has been known for a long time [24]. Papers reviewing the recent literature have been published by VAN AERT *et al.* [25] and SASAHARA [26].

While studying the relationship between tumor growth and the plasma LDH levels in mice, it was found that inoculation of plasma or cell-free tissue preparations from tumor-bearing mice into normal animals caused a significant increase in enzyme activity within two days [5]. Impaired enzyme clearance was found to be due to the multiplication of an infectious agent termed LDH virus, which has been encountered in many passaged mouse tumors; the virus does not produce lesions in the animals except for splenomegaly and slight lymph node enlargement [27]. Contact (cage to cage) infection does not seem to play a role in epidemiology [28], but a parenteral mode of infection by biting has been indicated by the studies of NOTKINS *et al.* [29]; arthropod transmission has not been published to our knowledge. The earlier literature on the properties of LDH virus has been reviewed by NOTKINS [30].

From this short review on the significance of non-arthropod-borne togaviruses as pathogens (with the exception of LDH virus, where no pertinent information is available), it is apparent that they all may affect the developing embryo, causing death or malformation. Although this property is not confined to togaviruses, the published observations suggest a common denominator which will probably be found in the structure, replication and

pathogenetic pattern of viruses belonging to this family. Some of the aspects concerning the role of viruses as causes of congenital defects have been reviewed by CATALANO and SEVER [31].

The present paper tries to compile, in a comparative way, information regarding the structural details of togaviruses not transmitted by arthropods. Since reviews of published work on arbovirus structure [32] and multiplication [33] exist, only the recent data on alpha- and flavoviruses are discussed insofar as they appear relevant for the comparison. Of the genus alphavirus Chikungunya (CHIK), Eastern equine encephalomyelitis (EEE), Semliki forest (SF), Sindbis (SIN), Venezuelan (VEE) and Western equine encephalomyelitis (WEE) viruses are considered; the dengue viruses (DEN), Japanese B encephalitis (JBE), tick-borne encephalitis (TBE), St. Louis encephalitis (SLE), and West Nile (WN) viruses represent the flavovirus group. For the sake of conciseness, 'non-arbo togaviruses' is introduced as a collective term for RU, EA, VD, HC, and LDH viruses.

II. Sensitivity to Chemical Treatment

First attempts in classifying new viruses usually involve treatment with organic solvents or detergents, since the presence of an envelope containing structural lipids can be inferred from sensitivity to these substances. Ether, chloroform and sodium deoxycholate (DOC) are most commonly used.

EA virus was shown by BÜRKI [34] to be readily inactivated by solvent treatment. DOC, saponin and Nonidet NP 40 – an octyl phenol condensate (Shell Chemical Co.) – had a similar effect on virus infectivity [HORZINEK, unpublished observations]. The strains Bucyrus and Bibuna proved resistant to treatment with trypsin [35]. An inactivating effect of ether, chloroform and DOC on RU virus infectivity was already mentioned in the first reports on isolation *in vitro* [36, 37]. Preparations showed a reduction of 2.5–4.5 log in infectivity after solvent treatment; a fluorocarbon (Genetron 113) had a less pronounced but still significant effect [38]. DOC efficiently destroyed virus infectivity [39]. The presence of structural lipids in the VD virus particle was indicated by studies using ether, chloroform and DOC [40–46]; the virus was readily inactivated by trypsin [41, 45–47]. Similar results have been obtained with HC virus (ether, chloroform, DOC [41, 48–50]; trypsin [41]). Also LDH virus has been found to lose its viability after treatment with ether, chloroform or butanol [51].

With respect to inactivation of infectivity by organic solvents and

detergents, the non-arbo togaviruses behave like all alpha- and flavoviruses tested [32]. The substances disintegrate the togavirion envelope thereby mediating release of the nucleic acid, which can be obtained in a biologically active form under certain circumstances.

III. Physical Properties

A. Effect of Temperature on Virus Infectivity

At temperatures below –20 °C, EA [52] and RU viruses [38, 53, 54] can be stored for years. The infectivity titer of RU virus remained unchanged at this temperature when sucrose, gelatin and calf serum had been added as stabilizers [55]. Loss of infectivity observed in togaviruses after freezing and thawing (e.g. TBE [56]) can be reduced by the addition of dimethyl sulfoxide to the material (SIN [57]; HC [AYNAUD, cited from 25]). At refrigerator temperatures EA [52], RU [38, 58], and the arthropod-borne togaviruses remain viable for months, in liquid medium or after lyophilization (LDH [51]), the inactivation depending upon the composition of the medium in which the virus is suspended.

Stability of alpha- and flavoviruses in protein-free salt solutions is poor [59] and can be enhanced by the addition of bovine serum albumin, amino acids and glutamine [60], for example. LDH virus could be stabilized in Eagle's medium supplemented with veal infusion broth [51].

Thermal inactivation at 37 °C of RU virus in the presence of 2% chicken serum proceeds at a rate of 0.3–0.4 log ID_{50}/h [38]; a half-life of 135 min has been calculated by others [61]. At this temperature, EA virus lost about 6.0 log ID_{50} between 2 and 3 days [52]. Incubation of LDH virus at 37 °C resulted in a decrease of more than 2.0 log ID_{50} in 24 h [51]. Similar inactivation rates have been found for alpha- and flavoviruses [62].

Rapid inactivation at 56–60 °C has been reported for RU [36], EA [52], HC [50], and LDH viruses [51]. A relationship between resistance to heating at 56 °C and pathogenicity of HC virus for the pig has been reported by AYNAUD *et al.* [63]; decreases of 1.6, 2.7, and 4.0 log in infectivity titer have been found within 30 min for highly virulent, attenuated and avirulent HC virus strains, respectively. In thermal inactivation experiments molar $MgCl_2$ did not exert a protective effect on EA [35], RU [64], VD, HC [41], and LDH viruses [65] nor on alpha- and flavoviruses [66]; in contrast to SIN virus, however, the infectivity of RU virus was stabilized in the presence of $MgSO_4$ [64].

The mechanism of thermal inactivation was studied with SF virus. It appears to be determined by two processes, one of which predominates at temperatures below 41 °C and the other at higher temperatures; it was suggested that at higher temperatures inactivation is the consequence of a structural breakdown of a surface unit in the virion whereas at the lower temperatures a more subtle change in the substructure occurs [67].

B. Effect of pH on Virus Infectivity

No loss of infectivity of RU virus was found over the pH range between 6.8 and 8.1, whereas rapid decrease in titer occurs at pH 5.9 and less [38, 68, 69]. At pH 3.0 EA [35], VD [46], and HC viruses [48, 50] are inactivated. Optimal stability of three VD virus strains has been found at pH 7.4; no significant inactivation was noted between pH 5.7 and 9.3 [47]. Comparison of infectivity titers of LDH virus suspended in different buffers showed significant losses at pH 4.0, whereas at pH 10.3 only moderate inactivation occurred [51]. The detrimental effect of low pH [60, 66] and the advantage of alkaline buffers are well known to arbovirologists who routinely employ a bovine albumin borate saline solution, pH 9.0, for virus dilution in infectivity and hemagglutination assays [70].

C. Buoyant Density

Isopycnic banding of viruses in density gradients is frequently used in physical studies because it represents a method for purification and for determination of a virion parameter at the same time. The densities determined in centrifugation experiments with different togaviruses are listed in table I.

Many studies have been performed with RU virus, for which initially an uncommonly low density was reported [61, 105–107]. The variation in the data published has been ascribed by McCombs and Rawls [71] to a difference in hydration states of the RU virion. In Tris-buffered sucrose gradients containing EDTA it appears to lose a certain amount of water thus assuming a density of 1.18 g/ml, whereas, after removal of this buffer and exchange against distilled water, the virus rehydrates and bands at 1.16 g/ml. A similar phenomenon, termed water extrusion, has been observed in various subcellular structures [108]. The reversibility of this reaction excludes the

possibility of a loss of some lipid-containing component, as described for alpha- and flavoviruses in salt gradients. Few experiments have been made with RU virus in CsCl gradients – apparently because of the inactivating effect of the salt [38] – but then consistently higher values than those obtained in sucrose and potassium salts were reported.

The buoyant densities of non-arbo togaviruses are found in the range between 1.15 and 1.20 g/ml; also LDH virus, which is not included in table I, has been shown to band at 1.168 g/ml in sucrose gradients [109]. Most alpha- and flavoviruses show peak titers of infectivity at 1.24 g/ml in CsCl gradients. However, it is questionable whether these data reflect an actual virus parameter or rather that of a salt-induced artifact. Lower values have been obtained after short centrifugation periods in preformed potassium salt and sucrose gradients (table I). It was demonstrated by AASLESTAD *et al.* [95] that a denser EEE virus particle (1.23 g/ml) resulted from CsCl-induced alteration of the virion which bands at 1.20 g/ml when being intact. Similar observations have been made for EA [81] and VD viruses [DINTER and HYLLSETH, cited from 81] in potassium tartrate gradients. Bimodal distributions of infectivity have been observed in density gradient analyses of alpha- and flavoviruses, which were frequently accompanied by additional peaks of virus-specific hemagglutinating and complement fixing activity at densities around 1.18 g/ml; they were shown to contain envelope material (EEE [95], SIN [100], VEE [96], DEN [104], JBE [110], Wesselsbron [111]).

Variations in the published density data and the uncommon wide density ranges observed by individual authors are certainly not due to technical reasons but reflect an intrinsic property of the togavirus envelope, its fragility, its state of hydration and its tendency to adsorb non-specific inhibitors of low density.

D. Sedimentation Coefficient

The sedimentation coefficients calculated for some togaviruses are listed in table II. A considerable variation is apparent which, however, may not reflect the true situation since the analytical ultracentrifuge was employed in only a few determinations. A reasonable correlation with results from rate zonal centrifugation in sucrose gradients exists for SIN and SF virus, where values of about 270 and 300 S, respectively, have been found. Unusually low coefficients from less than 100–150 S have been reported for VD, HC, and LDH viruses.

Table I. Buoyant densities determined for togaviruses

Non-arbo togaviruses							
Rubella		Equine arteritis		Virus diarrhea		Hog cholera	
density g/ml	references	density g/ml	references	density g/ml	references	density g/ml	references
				P 1.115	MAESS and RECZKO [84]		
				S 1.09–1.15	MOENNIG [85]		
				C 1.14	HAFEZ [47]	C 1.14–1.15	CUNLIFFE and REBERS [87, 88]
				P 1.145	DINTER and HYLLSETH (cited from HYLLSETH [81])	C 1.15–1.17	MAYR *et al.* [89]
				C, P, S 1.15	FERNELIUS [86]		
						S 1.15–1.17	RITCHIE and FERNELIUS [90]
S 1.16	MCCOMBS and RAWLS [71]					C 1.16	USHIMI *et al.* [91]
						C 1.15–1.20	HORZINEK [92]
F 1.16	SCHMIDT *et al.* [72]						
S 1.175	VAHERI *et al.* [73]	P, S 1.17	HYLLSETH [81]				
S 1.18	MCCOMBS and RAWLS [71]						
	SCHELL and WONG [69]						
	FURUKAWA *et al.* [74]						

S 1.19	Liebhaber *et al.* [75]	S 1.18–1.20	Maess *et al.* [82, 83]		
S 1.19–1.23	Schmidt *et al.* [72, 76]	C 1.180–1.215	Hyllseth [81]		
P 1.198	Walder and Munz [77]				
C,S 1.20	Thomssen *et al.* [78]				
P 1.20	Magnusson and Skaaret [79]				
C 1.22	Smith and Hobbins [80]				
C 1.23	Schmidt *et al.* [72]	P 1.24	Hyllseth [81]	P 1.235	Dinter and Hyllseth (cited from Hyllseth [81])

C = cesium chloride; F = ficol; P = potassium citrate or tartrate; S = sucrose.

Table I (continued)

Arthropod-borne togaviruses					
Alphaviruses			*Flavoviruses*		
virus	density, g/ml	references	virus	density, g/ml	references
EEE	S 1.18	Fuscaldo *et al.* [93]	WN		
SIN	P 1.19	Compans [94]	Ilheus	S 1.19	Stinski and Gruber [102]
EEE	C 1.20	Aaslestad *et al.* [95]	Rio Bravo		
VEE	P 1.20	Horzinek and Munz [96]			
EEE	C 1.23	Aaslestad *et al.* [95]	DEN	C 1.22	Smith *et al.* [103]
CHIK	C 1.24	Igarashi [97]	DEN	C 1.24	Smith *et al.* [103]
SF		Faulkner and McGee-Russell [98] Osterrieth [99]			Stevens and Schlesinger [104]
SIN		Mussgay and Rott [100]			
VEE	C 1.25	Uryvayev *et al.* [101]			

C = cesium chloride; F = ficol; P = potassium citrate or tartrate; S = sucrose.

Table II. Sedimentation coefficients determined for togaviruses

Non-arbo togaviruses			Arthropod-borne togaviruses		
virus	S_{20}	references	virus	S_{20}	references
VD	>90	HERMODSSON and DINTER [40]			
HC	108	HORZINEK [112]			
LDH	150	RILEY (cited from NOTKINS [30])	*Flavo-viruses*		
			DEN	175	BRANDT *et al.* [113]
EA	227[1]	HORZINEK *et al.* [4]	TBE	218	STROHMAIER *et al.* [114]
			Alphaviruses		
RU	240	THOMSSEN *et al.* [78]	EEE	240	FUSCALDO *et al.* [93]
				260	AASLESTAD *et al.* [95]
			SIN	273[1]	HORZINEK and MUSSGAY [115]
				280	ARIF and FAULKNER [116]
			SF	285	FAULKNER and McGEE-RUSSELL [98]
				303[1]	OSTERRIETH [99]
				320[1]	CHENG [117]
RU	342	RUSSELL *et al.* [107]	VEE	380	URYVAYEV *et al.* [101]

1 Determined with the analytical ultracentrifuge.

E. Size

Since the non-arbo togaviruses multiply to only moderate titers in tissue culture, indirect attempts for determining their particle size have employed filtration and sedimentation techniques. EA [34, 35] and RU viruses [118] were shown to pass filters with a pore diameter of 100 nm; from sedimentation and buoyant density data, diameters of 85–90 nm [107] and 48 ± 4 nm [78] were calculated for the infectious RU virion. Discrepant results with values greater than 100 nm have been obtained by others [36–38, 54, 119]. Filtration experiments with VD virus suggested a size between 40 and 100 nm [40, 46, 120, 121] and also HC virus was shown to pass 50 nm pores [41, 122–124]. From the sedimentation coefficient a diameter of 28–39

nm was calculated for the HC virion [112] and a value of 40 ± 5 nm was estimated from gel electrophoresis experiments [125]. Filtration of the LDH agent through gradocol and millipore membranes indicated a diameter between 30 and 55 nm [51, 126, 127]. The sedimentation data led to the estimation of an average diameter of approximately 40 nm for the virion [Riley, cited from 30].

Direct measurements of particles in negatively stained purified virus preparations or thin sections of infected cells are listed in table III. EA, RU, and LDH viruses, together with most alphaviruses, show the highest diameter values published; VD and HC viruses and the members of the flavovirus group are slightly smaller; the size difference between alpha- and flavoviruses has already been discussed by Mussgay [33] and has led to the assumption that antigenic interrelatedness also implies morphological similarity. Essentially higher values than those included in table III have been reported for RU (120–280 nm [105, 184], 50–250 nm [185]) and VD viruses (80–>100 nm [146]). The wide variations in virion diameter observed in the individual viruses again reflect a property of the envelope. It was shown to be non-rigid [117] and delicate, as evidenced by the frequency of deviations from the spherical form, finger-like protrusions or 'bleb' artifacts, described for all of these viruses by numerous authors [73, 91, 105, 134, 143, 144, 164, 165]. Indications for a true variation in size between genetically different clones of VEE virus have been found, the particle diameter being inversely correlated with plaque size of the respective virus population [186].

IV. Virion Morphology and Substructure

In most purified preparations and thin sections of infected cells toga-viruses appear as spherical particles. In some instances, however, when the thin section technique had been applied to fixed virus pellets or infected cells, elliptical or oblong particles have also been observed (RU [138], LDH [7], CHIK [157], SF [117, 163, 187]). In general the togavirion consists of an isometric internal structure (termed nucleocapsid, core, nucleoid or – when encountered in thin sections before being enveloped – precursor particle) and an envelope with the electron microscopic characteristics of a membrane. The envelope is essential for attachment of the virion to the cell surface early in infection; the nucleocapsid alone, although containing in-fectious RNA, is not infectious under the conditions used to assay either virions [164, 188–190] or infectious RNA [191]. The envelope may display

a smooth surface or may carry more or less prominent projections. Only togaviruses endowed with hemagglutinating activity have been unequivocally shown to possess surface projections (RU, alpha-, and flavoviruses). Treatment of alphaviruses with proteases has resulted in a decrease in hemagglutinating activity of the preparation which was accompanied by the appearance of smooth-surfaced virus particles in the electron microscope [161]. From these and other observations [100] it was concluded that the projections are the morphological substrate for the specific hemagglutinating activity of togaviruses.

A. Envelope

In preparations of EA virus from sucrose and CsCl gradients negatively stained with sodium tungstosilicate, projections 3–5 nm in length have been reported [143] which were not detected in gradient material contrasted with uranyl acetate [82, 83]; a comparison of EA and SIN virion surfaces in freeze-etched preparations disclosed projections only in the latter (fig. 1). Projections 5–6 nm in length have been described for the RU virion [80, 134] and can be identified also in electron micrographs of purified hemagglutinin [192]. It has been claimed that HC virus, too, carries surface projections [90, 91], whereas smooth envelopes have been observed by most authors.

Particles of alpha- and flavoviruses present themselves as 'fuzzy' spheres, the appearance being caused by a fine, net-like [161] coating. However, the projections may appear different with the various techniques of preparation, ranging from a diffuse halo of filamentous material to rod-shaped structures nearly as defined as myxovirus 'spikes'. Pretreatment such as heating may result in a change to the latter morphology [193]. On closer examination of negatively contrasted togavirions, an uneven stain distribution within the peripheral fringe can be observed: a stain density maximum is frequently encountered near the membrane (at the bases of the projections) and a minimum appears at the particle periphery [96, 99, 100, 165, 169, 192, 194–196]. Particle images may then resemble concentric circles (fig. 2). Based on these observations, a 'loop' hypothesis has been developed which is illustrated by a two-dimensional model (fig. 3). When two arbitrary but identical loop patterns are superimposed upon each other at different phase shifts the areas occupied by the background along the loop axis can be plotted against the corresponding levels of the axis. The background distribution profiles

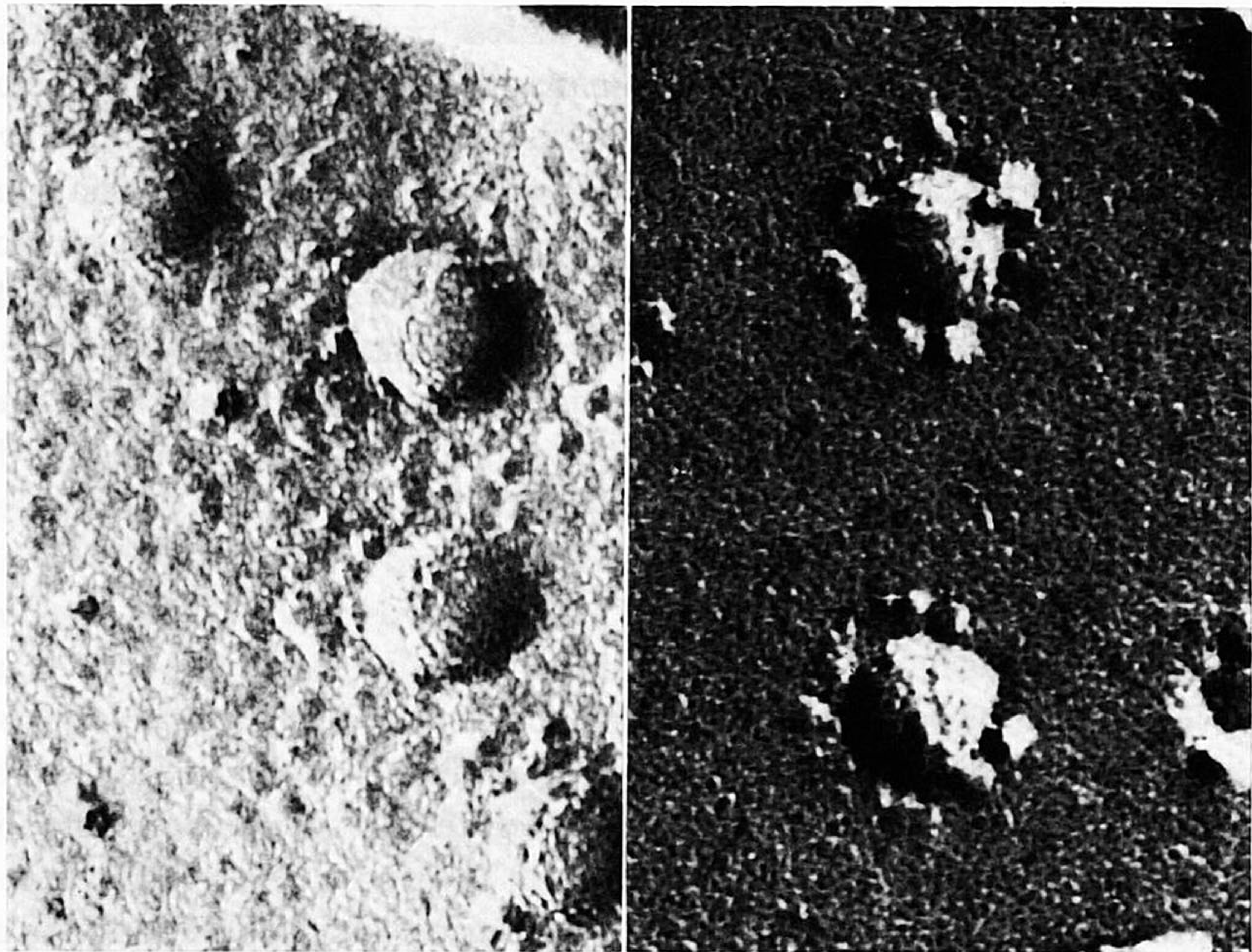

Fig. 1. Equine arteritis (left) and Sindbis virus (right) in freeze-etched preparations; note smooth periphery of the EA virion and ragged appearance of the SIN virion surface indicative for membrane projections (EA: × 230,000; SIN: × 320,000).

obtained in the model reflect the distribution of negative stain between loop-shaped viral surface projections. Densitometer tracings across micrographs of SIN and influenza A_2 equi virions, stained in an identical manner, were in agreement with the model [197]. In the DEN virion, ring-like 7-nm surface subunits were observed dissociating from spontaneously disintegrating particles. After sucrose gradient centrifugation, a peak of 'soluble' complement-fixing activity was observed, which upon electron microscopic examination revealed 7-nm structures indistinguishable from those on the virion surface. This material was devoid of virus-specific hemagglutinating activity, which was found accumulating in a peak of slower sedimentation than the virion; electron microscopy of the fractions disclosed ring-like or 'doughnut' structures with a diameter of about 14 nm [103]. Similar particles have been repeatedly found in preparations of different arboviruses irrespective of the antigenic group [117, 194, 195]. For non-arbo togaviruses, 12- to 15-nm particles have been described in HC virus preparations, where they aggregated in the presence of homologous antibody; here, too, their 'soluble'

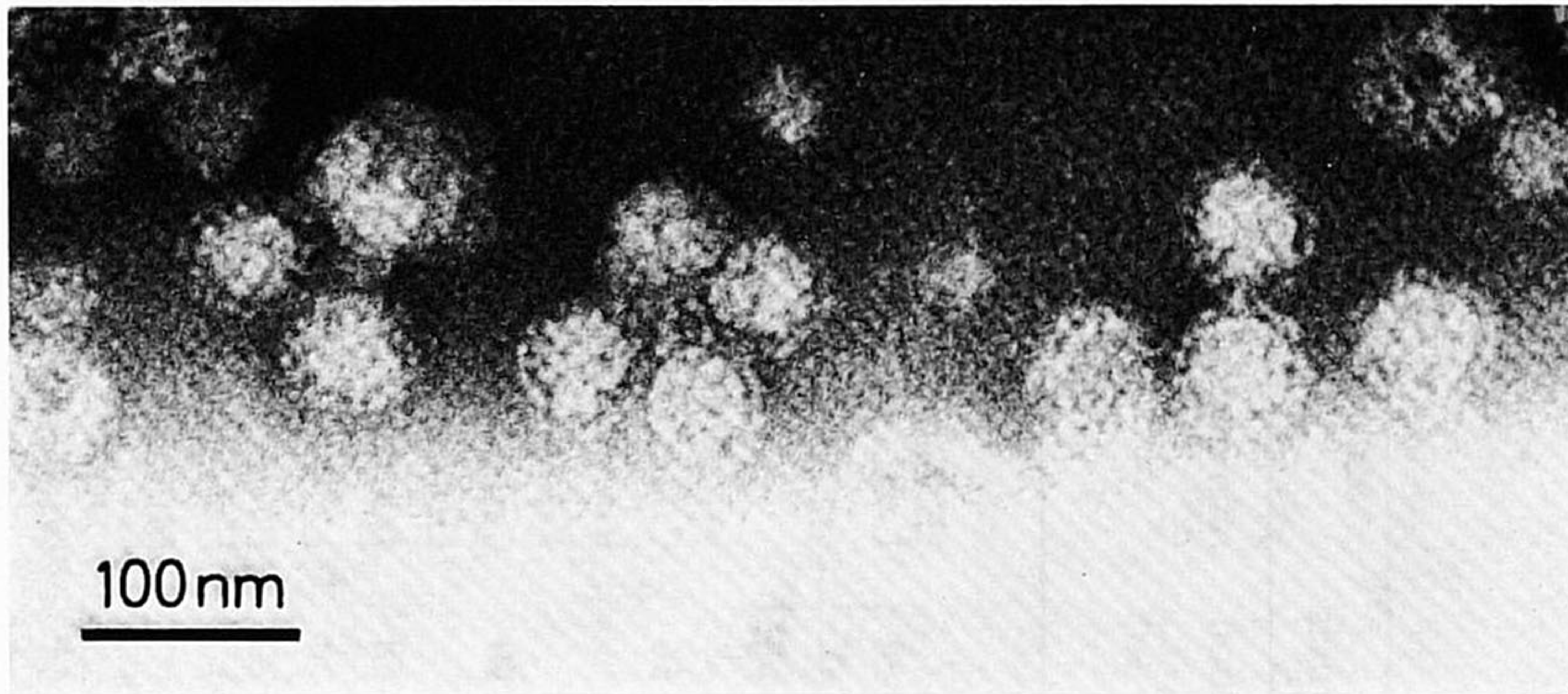

Fig. 2. Sindbis virus particles negatively stained with PTA (spray droplet preparation). An electron-dense fringe is observed between particle periphery and viral membrane.

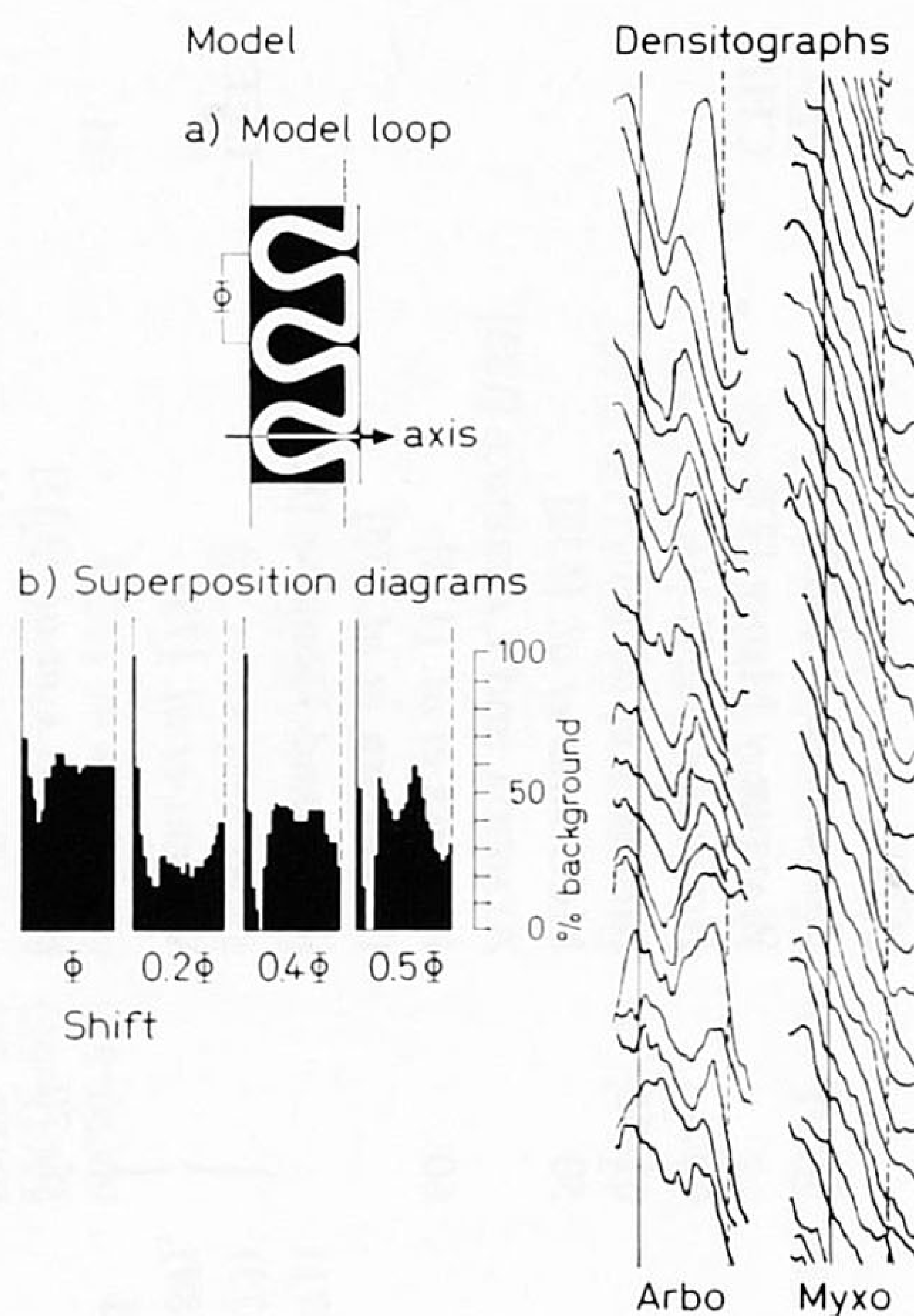

Fig. 3. Comparison between a loop superposition model (left) and densitometer tracings along negatively stained (PTA) viral projections (right). Two identical sets of arbitrary loops (a) were superimposed upon each other at different phase shifts (Φ) and the respective background areas were determined at distinct levels of the loop axis (b). The patterns which show a characteristic background minimum in the region of superimposed loop arcs resemble densitographs across the periphery of Sindbis virions (Arbo) but not of influenza A_2 equi virions (Myxo).

Table III. Diameter values of togaviruses as measured in electron micrographs

Non-arbo togaviruses				Arthropod-borne togaviruses			
virus	neg. staining nm	section nm	references	virus	neg. staining nm	section nm	references
Rubella				*Alphaviruses*			
	50–75		BEST *et al.* [128]	AURA		50–54	LASCANO *et al.* [152]
	60–80	60	HOLMES and WARBURTON [129]				
		38–70	KISTLER *et al.* [130]				
	52± 5		WALDER y MUNZ [77]	CHIK	54–58	50–56	CHAIN *et al.* [153]
		60	HOLMES *et al.* [131]		45–50		IGARASHI *et al.* [154]
		61(56–73)	MURPHY *et al.* [8]		50–60		YOSHINAKA and HOTTA [155]
		50	MCCOMBS *et al.* [132]			38–60	CHATTERJEE and SARKAR [156]
	50–60		ALMEIDA and LAURENCE [133]			42	HIGASHI *et al.* [157]
	60–80	60	HOLMES *et al.* [134]			50–58	JANZEN *et al.* [158]
	60		LIEBHABER *et al.* [75]				
	55(42–71)		SMITH and HOBBINS [80]				
	61(50–73)			EEE	54± 5		FUSCALDO *et al.* [93]
	74(55–89),	}	VAHERI *et al.* [73]			55	MURPHY and WHITFIELD [159]
	unfixed						
		38–70	BANATVALA *et al.* [135]			52–55	WHITFIELD *et al.* [160]
		59(50–74)	VON BONSDORFF and VAHERI [136]				

		56(45–65)	Edwards *et al.* [137]				
		60(50–85)	Oshiro *et al.* [138]	SF	55–59		Osterrieth and Calberg-Bacq [161]
	58± 7		Horzinek *et al.* [4]		70–80		Acheson and Tamm [162]
	50–60		Laufs *et al.* [139]			42–50	Erlandson *et al.* [163]
					50–53		Friedman and Berezesky [164]
					75		Faulkner and McGee-Russell [98]
Equine arteritis		43± 2	Breese and McCollum [140]		68		Osterrieth [99]
		45–55	Crawford and Davis [141]				
		58	Estes and Cheville [142]				
	55(45–65)	50(43–60)	Magnusson *et al.* [143]	SIN	60–70		Mussgay and Rott [100]
	60±13		Maess *et al.* [82]		62–69	67–69	Simpson and Hauser [165,166]
	65± 7		Maess *et al.* [83]		62–80, unfixed		
	60±13		Horzinek *et al.* [4]		61		Horzinek and Mussgay [115]
Lactic dehydrogenase	69–76		Bladen and Notkins [144]	VEE	60–75		Klimenko *et al.* [167]
	60–85, unfixed	40(36–75)	de Thé and Notkins [7]	*Flavoviruses*			
Virus diarrhea		40	Hermodsson and Dinter [40]	DEN	48–50		Smith *et al.* [103]
	50±20 46±16		Hafez [47]		50	45	Matsumura *et al.* [169]
	60±26		Hafez *et al.* [145]		50–55		Yoshinaka and Hotta [155]
				JBE	35–40		Nozima *et al.* [170]
	15–20 30–50		Ritchie and Fernelius [146]			37	Yasuzumi *et al.* [171]

Table III (continued)

Non-arbo togaviruses				Arthropod-borne togaviruses			
virus	neg. staining nm	section nm	references	virus	neg. staining nm	section nm	references
	57± 7		Maess and Reczko [84]			40	Ota [172]
	57± 7		Horzinek *et al.* [4]			40	Filshie and Rehacek [173]
					45–50		Yoshinaka and Hotta [155]
				Powassan	40–45	36–38	Abdelwahab *et al.* [174]
Hog cholera				SLE		38	Murphy *et al.* [175]
	40± 3		Horzinek *et al.* [147]	TBE	30–40		Weckström and Nyholm [176]
	39–40		Mayr *et al.* [89,148]			35–42	Tikhomirova *et al.* [177]
	40–50		Ritchie and Fernelius [90, 149]			50±10	Lotte *et al.* [178]
	40–50		Cunliffe and Rebers [87, 88]		60–80		Slavik *et al.* [179]
	46(41–54)		Ushimi *et al.* [91]				
		44	Scherrer *et al.* [150]	Wesselsbron		34	Parker and Stannard [180]
		40	Schulze [151]			45	Lecatsas and Weiss [181]
	53±14		Horzinek *et al.* [4]	WN		30	Southam *et al.* [182]
				Yellow fever		42	McGavran and White [183]
					40		David-West *et al.* [168]

For alpha- and flavoviruses only size estimations published later than 1963 are tabulated, since the earlier literature was reviewed by Mussgay [33].

antigen nature has been discussed [90, 149]. These authors also encountered 15- to 20-nm particles in VD virus material [146]. In purified VD and HC virions lacking some surface material, a 'rosary' envelope substructure has been described, which consisted of 6-nm spherules [4].

The envelopes of RU [136, 137], EA [142], HC [150], and LDH viruses [7], of alpha- [162] and flavoviruses [169] were described to have a membrane structure in thin sections, sometimes showing 'unit membrane' characteristics. Treatment of togaviruses with urea resulted in swelling and an increase in thickness of this membrane thus allowing demonstration of the nucleocapsid within the envelope in negatively-stained preparations; DOC appears to detach an outer and an inner layer of the envelope, before the core is liberated [4, 198].

B. Nucleocapsid

The membrane usually envelops one nucleocapsid per virion. Occasionally, however, aberrant forms 120–300 nm in diameter have been observed in suspensions of genetically heterogeneous VEE virus material; these contained two or more capsids enclosed in a common envelope [167, 199, 200]. Similar particles were detected in cells infected with CHIK virus [157] and with members of the flavovirus group [169, 177].

The nucleocapsid of togaviruses has been studied *in situ* in infected cells and in purified preparations derived from virus suspensions. The size measurements listed in table IV again reflect the difference between RU, EA, LDH viruses and the members of the alphavirus group on one hand and VD, HC viruses and the flavoviruses on the other, the former having diameters of about 30–40 nm, the latter between 20 and 30 nm. It must be emphasized that the capsid diameter may be influenced by the conditions of preparation of the specimen for electron microscopy. At low pH an irreversible contraction of SF virus nucleocapsids has been described [206], which has to be taken into consideration when uranyl acetate is used for negative staining.

In general, the togavirion nucleocapsid shows a circular outline in electron micrographs. During recent years, evidence for cubic symmetry of this structure has accumulated. Polygonal virion and/or core profiles have been indicated for alpha- and flavoviruses (SF [98]; SIN, Middelburg [165]; VEE [199]; JBE [172]; TBE [176, 177]). SIMPSON and HAUSER [165] published pictures of SIN virus particles from suspensions pretreated with

Table IV. Diameter values of togavirus nucleocapsid 'cores' as measured in electron micrographs

Non-arbo togaviruses				Arthropod-borne togaviruses			
virus	neg. staining nm	section nm	references	virus	neg. staining nm	section nm	references
Rubella				*Alphaviruses*			
	40		BEST *et al.* [128]	AURA		28–31	LASCANO *et al.* [152]
		30	HOLMES and WARBURTON [129]				
		20–40	KISTLER *et al.* [130]	CHIK		26–28	CHAIN *et al.* [153]
		30	HOLMES *et al.* [131]		28–32		IGARASHI *et al.* [154]
		28	MURPHY *et al.* [8]			25–30	HIGASHI *et al.* [157]
		35	MCCOMBS *et al.* [132]			25–31	JANZEN *et al.* [158]
		30	HOLMES *et al.* [134]				
	30		LIEBHABER *et al.* [75]	EEE		28	MURPHY and WHITFIELD [159]
		30–35	VON BONSDORFF and VAHERI [136]			28–30	WHITFIELD *et al.* [160]
		41	EDWARDS *et al.* [137]				
	33± 1		HORZINEK *et al.* [4]	SF	38–39		OSTERRIETH and CALBERG-BACQ [161]
						28	ACHESON and TAMM [162]
						25	ERLANDSON *et al.* [163]
Equine arteritis							
		35± 2	BREESE and MCCOLLUM [140]			25–30	GRIMLEY *et al.* [202]

		25–35	Crawford and Davis [141]		38		Osterrieth [99]
		25	Estes and Cheville [142]		35–42		Kääriäinen *et al.* [203]
		25–30	Magnusson *et al.* [143]		40		Acheson and Tamm [204]
	35± 9		Maess *et al.* [82, 83]				
	36± 3		Horzinek *et al.* [4]	SIN	32–40	32–40	Simpson and Hauser [165, 166]
					35		Horzinek and Mussgay [115]
Lactic dehydrogenase							
		26–29	de Thé and Notkins [7]	VEE	30–40		Klimenko *et al.* [167]
Virus diarrhea				*Flavoviruses*			
	25± 7		Hafez *et al.* [145]	DEN	26	20–30	Matsumura *et al.* [169]
	24± 4		Maess and Reczko [84]				
	28± 3		Horzinek *et al.* [4]	JBE		25	Ota [172]
						23	Yasuzumi and Tsubo [205]
						25	Filshie and Rehacek [173]
Hog cholera[1]							
	29± 3		Horzinek *et al.* [147]	SLE		27	Murphy *et al.* [175]
	28–29		Mayr *et al.* [89, 148]				
	33		Ushimi *et al.* [91]	Wessels-bron		25	Lecatsas and Weiss [181]
		36	Scherrer *et al.* [150]				
	27± 3		Horzinek *et al.* [4]	Yellow fever		35	David-West *et al.* [168]

1 By means of γ-ray inactivation a diameter of 31 nm has been calculated for the core of HC virus [201].

formaldehyde exhibiting a sixfold radial symmetry due to a regular arrangement of spherical units about 12.5 nm in diameter. Osterrieth [99], too, has demonstrated core images of SF virus with polygons 12 nm across on their surface; in this instance the virion had been decoated by the action of proteolytic enzymes. A mottled surface appearance of the SF virus nucleocapsid suggestive of a subunit structure has been observed by Acheson and Tamm [204]. Horzinek and Mussgay [115] have shown that the nucleocapsid of the SIN virion is built up of 32 morphological subunits 12–14 nm across, which are arranged within a symmetrical surface lattice of the T = 3 class. For a flavovirus (Powassan) indications for a similar nucleocapsid substructure have been published: in particles in which the envelope had become discontinuous, geometrically arranged morphological units compatible with a cubic symmetry were observed [174].

Of the non-arbo togaviruses, RU virus gave the first hints for an icosahedral nucleocapsid symmetry. After brief exposure of virus material to DOC, particles have been observed which had lost most of their envelopes, revealing cores with an angular outline [134]. The construction of the VD virus core out of subunits and its probable cubic symmetry have been discussed [84] and in HC virus-infected tissue cells, hexagonal contours of the viral core have often been observed in thin sections [150]. Also, negatively-stained LDH virus in some of the electron micrographs published by Bladen and Notkins [144] displays a regular hexagonal silhouette. In a comparative analysis of EA, RU, VD, and HC viruses, Horzinek *et al.* [4] have demonstrated isometric cores 27–36 nm in diameter which after staining with uranyl acetate showed ring-like subunits on their surface. Treatment with urea or detergents such as saponin, DOC and NP 40 has been found useful for enhancing the penetration of negative stain into the particle or removal of the envelope. DOC and NP 40 have been frequently used and permitted electron microscopic analysis of isolated and purified viral nucleocapsids (EA [4]; and alphaviruses: SF [203, 204, 207]; SIN [115]; CHIK [154]). Saponin treatment results in dissociation of the envelope which is accompanied by the appearance of disturbing cholesterol-saponin micelles; after fixation of the preparations with OsO_4 vapor, however, these structures disappear and clean nucleocapsids become visible [198].

Within the nucleocapsids of SIN virus a central spherical structure 12–16 nm in diameter ('central core component') has been detected [115]. In ultrathin sections through Middelburg virus, another alphavirus, a central body 8.5–12.5 nm across has been described within the core [166]; indications for an internal substructure of the nucleocapsid have been found for SF [162],

EEE [159, 160], and VEE viruses [96, 199], and for flavoviruses [177]. In the non-arbo togaviruses studied, the central core component was also disclosed [4]. In sections, RU [136, 137], EA [141], and LDH viruses [7] often show an electron-lucid center, thereby causing a ring-shaped appearance of the densely staining nucleoid.

Some physical properties of isolated togavirus nucleocapsids have been determined. The core of RU virus has been found to sediment with about 150 S [208, 209], which is in good agreement with the data reported for various alphaviruses (SIN [210, 215], SF [203, 204, 207], VEE [101], WEE [188]). In experiments employing CsCl equilibrium centrifugation, the ribonucleoprotein banded at densities of 1.30–1.34 (SIN [211, 212]), 1.32 (CHIK [97]), 1.42 (VEE [101]), and 1.47 g/ml (SF [204]). SF virus nucleocapsids which contain RNA indistinguishable from that of the intact virion, are frequently penetrated by negative stain and apparently also by pancreatic RNase, since treatment of isolated viral cores with this enzyme resulted in a reduction of their sedimentation coefficient and RNA content and digestion of the RNA into acid-soluble fragments [207, 213]. Capsids of SIN virus without RNA have been reported to sediment at 135 S [116]. Untreated nucleocapsids have a 260:280 UV absorbance ratio of 1.75–1.80 and an RNA content higher than 30% of their total mass [204–207]. When purified SF virus nucleocapsids were dialyzed against slightly acid buffers, an irreversible decrease in their size accompanied by an increase in sedimentation coefficient was noted, which had no consequence on the susceptibility of the enclosed RNA to pancreatic RNase [206].

V. Structural Constituents of the Togavirion

A. Protein and Glycoprotein

Inconsistent results have been published with respect to the number and significance of structural proteins of alphaviruses. In CHIK [196], Mayaro [214], SIN [210, 215], and WEE virus [188] a single protein has been reported for the envelope and the capsid, respectively; other investigators found an additional polypeptide associated with the membrane in SIN [216] and VEE viruses [217] or the core in SIN [115, 218] and SF viruses [219, 220]. Also flavoviruses (DEN [221], JBE [222], SLE [223]), and non-arbo togaviruses such as RU [209] and VD viruses [224] were reported to contain three structural proteins. A report by Liebhaber and Gross [225] on the structural

proteins of RU virus, too, describes accumulation of virus-specific label in three major electrophoretic zones; after dissociation and reduction under more vigorous conditions, however, eight species of partially resolved polypeptides were identified. The molecular weights of the respective proteins of togaviruses published so far are listed in table V. From the non-arbo togaviruses only RU virus has been analyzed; as discussed by VAHERI and HOVI [209] its envelope and nucleocapsid polypeptides have molecular weights close to those of the corresponding alphavirus structural proteins.

The highest molecular weight protein identified in the membrane of SIN virus is a glycoprotein containing about 14% carbohydrate by weight, whereas the core protein is devoid of carbohydrate [227]. The location of the glycoprotein within the viral membrane has been determined after ^{3}H-glucosamine labelling and incubation with bromelain. This treatment resulted in a selective degradation of the membrane projections accompanied by a loss of radioactivity from the remaining structure. From the finding that the glycoprotein can be completely removed by proteolytic enzyme treatment without destroying the integrity of the viral membrane it was inferred that the glycoprotein is situated outside of, rather than within, the lipid layer [94]. Concanavalin A, a hemagglutinating protein from the jack bean *(Canavalia ensiformis)*, also agglutinated SF virus. After the membrane glycoprotein had been removed from the virus, leaving the nucleocapsid surrounded by a lipid layer, the virion was no longer agglutinable; viral envelopes alone – but not the cores – were equally agglutinated by the substance [228]. The sugars in the envelope of SIN virus (which include glucosamine, mannose, galactose, fucose and sialic acid) are covalently linked with the membrane protein and are distributed over three principal glycopeptides. The smallest one (1,800 daltons) was estimated to contain nine sugar residues (only mannose and glucosamine) while the larger glycopeptides (2,800 and 3,200 daltons) are composed of mannose, glucosamine, galactose, fucose and, presumably, sialic acid. Glycopeptides from virus grown in BHK21 cells have a somewhat different size distribution but seem grossly similar to those of chick-cell-grown SIN virus [229]. From a comparison of glycopeptides isolated from SIN and vesicular stomatitis virus membranes it appeared that the host cell is principally responsible for the carbohydrate moiety covalently bound to the different virus-specified membrane proteins [230]. When the sialyl and fucosyl transferases from uninfected and SIN virus-infected cells were compared, no difference in either specific activity or acceptor specificity of the enzymes was found [231].

B. Lipid

Chemical analysis of purified SIN virus showed a high content of sphingomyelin and a high cholesterol:phospholipid ratio [232, 233]. The phospholipid and fatty acid compositions of the viral envelope were distinct from the compositions encountered in whole extracts of cells in which the virus had been grown [234, 235]. However, the lipids of SF virus were similar to those of the plasma membrane isolated from infected and uninfected cells, but less fatty acids and a little more cholesterol were present in the viral envelope [235]. Virus grown in chick embryo and BHK21 cells had a very similar lipid composition which has led to the suggestion that the lipid affinities of the envelope peptide(s) may determine their composition [234].

In contrast to these results, quantitative differences in the phospholipid moiety of VEE virus propagated in L-cells (mouse origin) and in chick fibroblasts have been determined. The biological significance is illustrated by the observation that L-cell virus, containing more sphingomyelin and less phosphatidylcholine than chick fibroblast virus, possesses a greater thermal stability [236]. From experiments employing treatment of SF virus with phospholipase C it was concluded that phospholipids, while not necessary for viral infectivity, do stabilize the structure of the virion [237]. The structural lipids of SIN virus are organized in a bilayer with a radius of about 23.2 nm according to electron-density profiles obtained by X-ray diffraction. The polar groups of the bilayer oriented towards the center of the virion interact directly with the nucleocapsid protein and the outer polar groups with the peripheral envelope glycoprotein. It seems unlikely that protein bridges are present across the lipid bilayer [238]. A total number of about 10,000 cholesterol-phospholipid pairs has been estimated per virion [235].

C. Ribonucleic Acid

First evidence for the RNA type of nucleic acid present in non-arbo togaviruses has been derived from studies of the kinetics of virus multiplication in the presence of halogenated deoxyuridines. No inhibition of the multiplication of EA [34], RU [38, 239–245], VD [40, 41, 43, 45, 46], and HC viruses [41, 48, 50] has been observed. Furthermore, no inhibition of togavirus multiplication has been noted in the presence of actinomycin D in the case of RU [244–249] and HC viruses [250]. Labelled uridine was incorporated into the nucleic acids of RU [248, 249, 251] and HC viruses [250].

Electron microscopy of thin sections through EA virus showed it to contain an RNA core after differential staining [252]. Acridine orange staining of VD virus preparations resulted in a red fluorescence which was sensitive to RNase [47].

Direct demonstration of the RNA nature of the genome of RU virus has been obtained by the extraction of infectious nucleic acid [249, 253]. Also EA [254], VD [85, 254, 255], and LDH [256] viruses yielded infectious RNA; apart from the usual extraction procedure employing phenol, different detergents such as SDS, DOC and NP 40 have been successfully used for this purpose. When precautions had been taken to remove RNase from pools of LDH virus, extraction with ether, butanol and chloroform, too, led to recovery of an infectious nucleic acid [30, 257].

Arthropod-borne togaviruses have repeatedly been shown to yield infectious RNA upon phenol treatment (alphaviruses: CHIK [258], EEE [259], SF [260], SIN [261]; flavoviruses: DEN [262], JBE [263], MVE [264], TBE [265], yellow fever [266]). Successful extraction with detergents alone has also been achieved. RICHTER and WECKER [267] showed that from EEE virus treated with DOC an infectious principle with the characteristics of free viral RNA was liberated; for other alphaviruses (SF [268]) and flavoviruses (MVE [269]) similar results have been published.

The subject of extraction, assay and properties of infectious nucleic acids has been reviewed by PAGANO [270]. As pointed out by BALTIMORE [271], the infectivity of a viral RNA suggests that it must be of the same strand as the mRNA; a polymerase then cannot exist within the virion or at least it does not serve an obligate role for the initiation of infection. According to a proposal made by FENNER, definition of the togavirus family should therefore include the infectious nature of the genome as an essential characteristic [PORTERFIELD, personal communication].

Some physicochemical properties of togavirus RNAs have been determined resulting in good agreement of the data. On the basis of their sensitivity to the degrading action of RNase, their sedimentation behavior and their buoyant density, togavirus RNAs are essentially single-stranded. A certain degree of secondary hydrogen-bonded structure of SIN and WEE virus RNAs, which is necessary for their infectivity, has been indicated since they undergo hyperchromic shift upon heating and appear to be almost completely reannealed upon slow cooling [261, 272]. However, secondary structure was considered minimal on the basis of experiments involving chromatography on benzoylated-diethylaminoethyl cellulose [273].

For RU virus RNA, sedimentation coefficients of 38–40 S in sucrose

gradients [248, 249, 253] and a buoyant density of 1.634 g/ml in Cs_2SO_4 [253] have been found. Similar values were obtained with EA and VD viruses. In the presence of 1 mM saline the sedimentation coefficients were reduced to about 30 S, and in 1 mM $MgCl_2$ they increased to about 55 S, indicating conformation changes dependent upon ion environment [85, 253, 254].

Comparable sedimentation data have been published for alphavirus [258, 274, 275] and flavovirus RNAs [276–278].

In addition to the 40 S molecule, a second single-stranded RNA species with a sedimentation coefficient of 26 S has been detected in extracts of cells infected with RU virus [247, 248] and several alphaviruses. RNA from the virion could be converted to this species upon heating followed by rapid cooling, centrifugation in sucrose gradients prepared in DMSO or after treatment with urea at high molarities [272, 279, 280]. These induced changes in a biophysical property have led to the speculation that viral RNA may exist in two conformations which sediment differently on sucrose gradients. An alternative hypothesis considered the viral genome as an aggregate of two or more RNA fragments having equal size and approximately the same sedimentation properties. However, if sufficient precautions had been taken to obtain RNA free of hidden breaks (after treatment with acid or formaldehyde), no conversion to the 26 S RNA species was observed after heating, DMSO or urea treatment. The genome consequently consists of a continuous length of single-stranded polynucleotide [281]. An adenylate-rich segment, about 50–100 nucleotides in length, has been demonstrated in the genome of SIN virus; such oligonucleotides were also present in Columbia SK virus, another positive-strand RNA virus, but not in vesicular stomatitis virus [282], which is negative-stranded.

In conclusion, all togaviruses studied contain single-stranded RNA with a molecular weight of 3 to 4×10^6 daltons, which is infectious when assayed under suitable conditions.

VI. Discussion

During recent years investigations of the architecture of viruses now included in the togavirus family were stimulated by the expectation that a unique group of spherical, lipid-containing RNA viruses with structural characters different from myxo-, corona-, arena- and leukoviruses would emerge. Since convincing electron microscopic analysis largely depends on the available virus concentration, most studies employed alphaviruses, which

grow to high titers in tissue culture. Consequently, most of our present knowledge on togavirus structure and multiplication has been accumulated by workers in this field and is indexed under the key word 'arbovirus'.

The general interest in RU virus, which emerged since its embryopathic activity had become established, directed research activity also towards studies of its structure; then the taxonomic dilemma arose which culminated in the rhetorical question, 'Is rubella an arbovirus?' [129, 134] – independently asked for EA [12, 143], VD, and HC viruses [41]. The need for a term devoid of ecological implications and endowed with only structural significance led to FENNER's proposal of 'encephalovirus' [283] as a designation of the 'classic' arboviruses; BÜRKI [34] suggested that EA, VD, and HC viruses should be included in a 'hemovirus' group. To end the confusion, the Subcommittee on Viruses of Vertebrates has coined the term 'togavirus', which was accepted on the basis of a concept developed by the International Committee on Nomenclature of Viruses [284].

Essential for the identification of a togavirus is the demonstration of a spherical nucleocapsid forming the viral core. Although core subunits have repeatedly been observed, their steric arrangement has been recognized only in SIN virus [115]. The difficulty in studying the symmetry of nucleocapsid surface patterns is due to the scarcity of one-sided images in negatively contrasted preparations of purified core particles and to their poor staining; since the viral lipid interacts directly with the capsid protein [238], remaining hydrophobic groups probably interfere with exhaustive embedding. The morphological subunits of togavirus nucleocapsids have a ring- or cup-like appearance and resemble those of the genus Calicivirus of the Picornaviridae (vesicular exanthema of swine [285], feline picornavirus [286]). The available evidence is in agreement with the concept of icosahedral symmetry of the nucleocapsid. An alternative symmetry type has been claimed for VEE virus by KLIMENKO *et al.* [167] who found helical structures in the native supernatant of infected chick embryo cell cultures; BYKOVSKI *et al.* [287] have assumed that a long ribonucleoprotein strand 1.5–1.7 nm thick is packed within the virion in such a way that it simulates cubic symmetry. For HC virus, also, a helical nucleocapsid has been postulated [148]. However, the incidental demonstration of such structures in crude material and the questionable topographic correlation of electron microscopic details in pictures of intact virions [159] seriously limit the value of these observations. It must be emphasized that the anatomy of enveloped viruses can only be studied after careful dissection, applied to concentrated and purified material. Detergent treatment has been found especially useful for the *in vitro* decoating of the

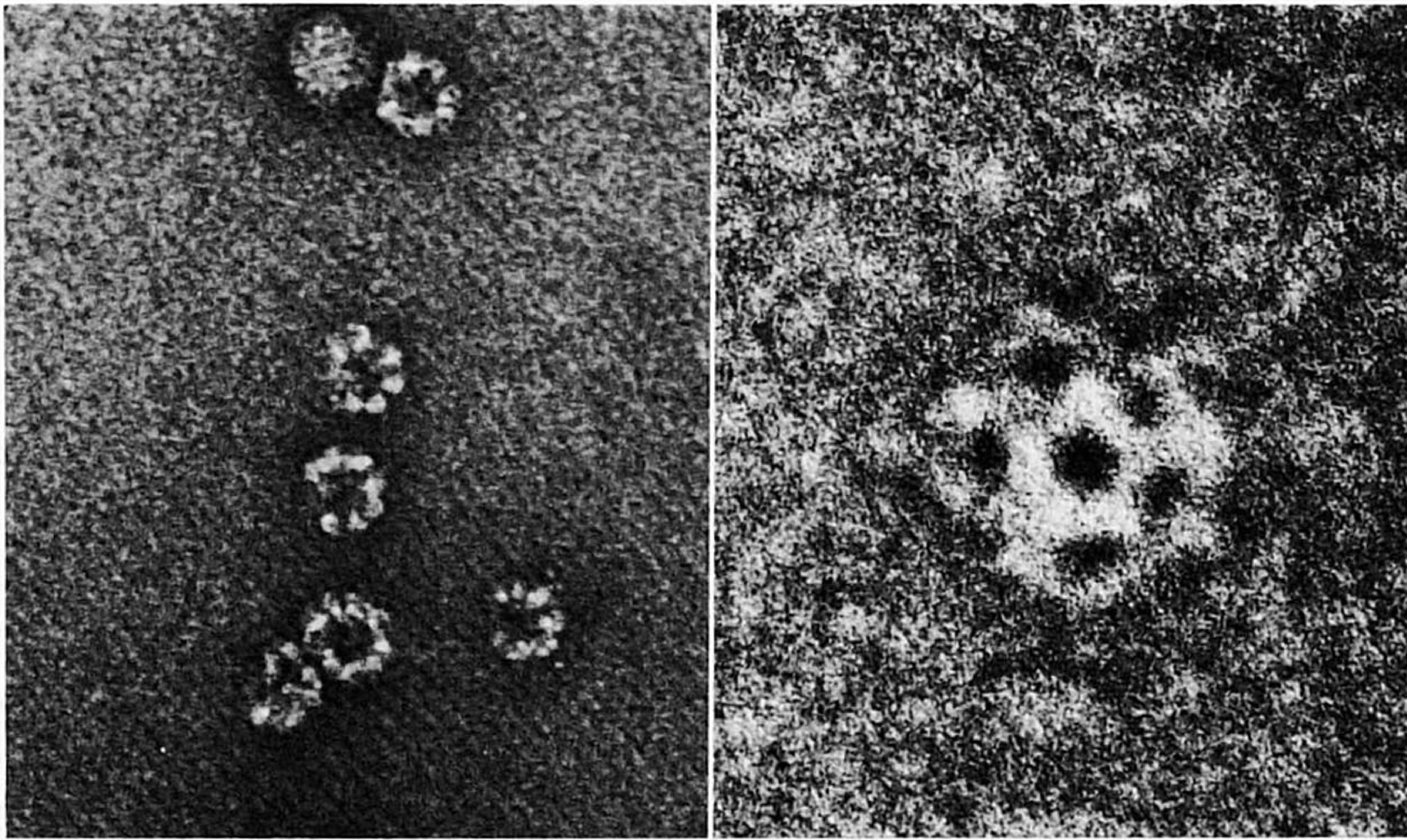

Fig. 4. Capsid-like artifacts obtained after saponin-ether treatment of purified Sindbis virus (left); the particles have a size similar to the viral nucleocapsid (uppermost spherule, × 180,000). At higher magnification (right, × 600,000) their regular architecture is evident.

spherical cores in togaviruses. On the other hand, in Uukuniemi virus and arboviruses of the Bunyamwera supergroup, the same treatment led to release and uncoiling of helical nucleocapsid strands [288].

Caution must be observed in the interpretation of electron micrographs of pretreated virus material since artifacts may occur. The coils and loops appearing after incubation of togaviruses with saponin [4, 198] are aggregates of cholesterol-saponin micelles [289]; our attempts to dissolve the micellar complex by treatment with ether resulted in the appearance of very regular spherical structures about 35 nm in diameter with the symmetry of a pentakis-dodecahedron (fig. 4). Their artifact nature became evident when saponin-ether treated influenza A_2 equi virus and egg yolk were examined in the electron microscope and identical particles emerged [HORZINEK, unpublished results].

Occasionally, elongated particles containing core structures of about the same width and staining density as the nucleocapsid were identified in infected cells (RU [138], CHIK [157, 163], yellow fever [168]); these particles may have resulted from a polyhead-like assembly of capsid subunits comparable to the aberrant rod-shaped forms detected in other viruses with cubic symmetry. On the other hand, TAN [187] has described tubular SF virions which contained more than one spherical nucleocapsid.

The replication of togaviruses, which is beyond the scope of this article, also shows some common features. In infected cells a heterogeneously sedimenting RNA species with a main peak at about 20 S appears which is partially resistant to RNase digestion and is considered as the replicative intermediate form of the RNA (RU [247–249], alphaviruses [274, 290], flavoviruses [291]). It has been found infectious in CHIK virus [292]. Two frequently noted morphogenetic characteristics are the intense vacuolization of infected cells and the maturation of viral particles in association with membrane structures either intracellular (e.g. of the Golgi apparatus – RU [134, 136], EA [143], HC [150]) or at the marginal cell membrane (RU [8, 131]). Alpha- and flavoviruses appear to differ with respect to their morphogenesis, the former budding preferentially from the marginal membrane, the latter from internal membranes [169, 173, 175]. In alphavirus-infected cells paracrystalline arrays of precursor particles have been observed by many workers. This arrangement does not necessarily indicate icosahedral symmetry of the crystal-forming entity but it actually reflects uniformity in particle size which is illustrated by the observation that enveloped mature DEN [169] and SLE (fig. 5) virions tend to form similar crystalloids. No cytoplasmic precursor particles have been reported so far for non-arbo togaviruses, but probably occur in flavovirus-infected cells [168, 169]. However, the formation of nucleoids may depend upon the host cell species and its efficiency of envelopment; for example, accumulations of EEE virus nucleocapsids in mosquito salivary gland cells are minimal, whereas they are common in mammalian cells [160]. A different type of crystalline inclusion consisting of elements smaller than nucleoids has been detected in cells infected with RU [134, 293], HC [151], and flavoviruses [180, 182]. The significance of these structures and their possible relationship to the annulate lamellae frequently present in togavirus-infected cells (RU [293], EA [140]) remains to be established [175].

In summarizing the structural properties of the togaviruses discussed, the following general characteristics can be ascribed to them. They are solvent- and detergent-sensitive RNA viruses 40–70 nm in diameter with buoyant densities varying between 1.15 and 1.25 g/ml and with sedimentation coefficients between 100 and 300 S. They are constructed of a spherical nucleocapsid 20–40 nm across, wrapped into an envelope of unit membrane structure which may or may not carry surface projections. Single-stranded infectious RNA sedimenting at about 40 S under standard conditions (0.1 M NaCl) can be isolated from the virion, detergent treatment being sufficient for extraction.

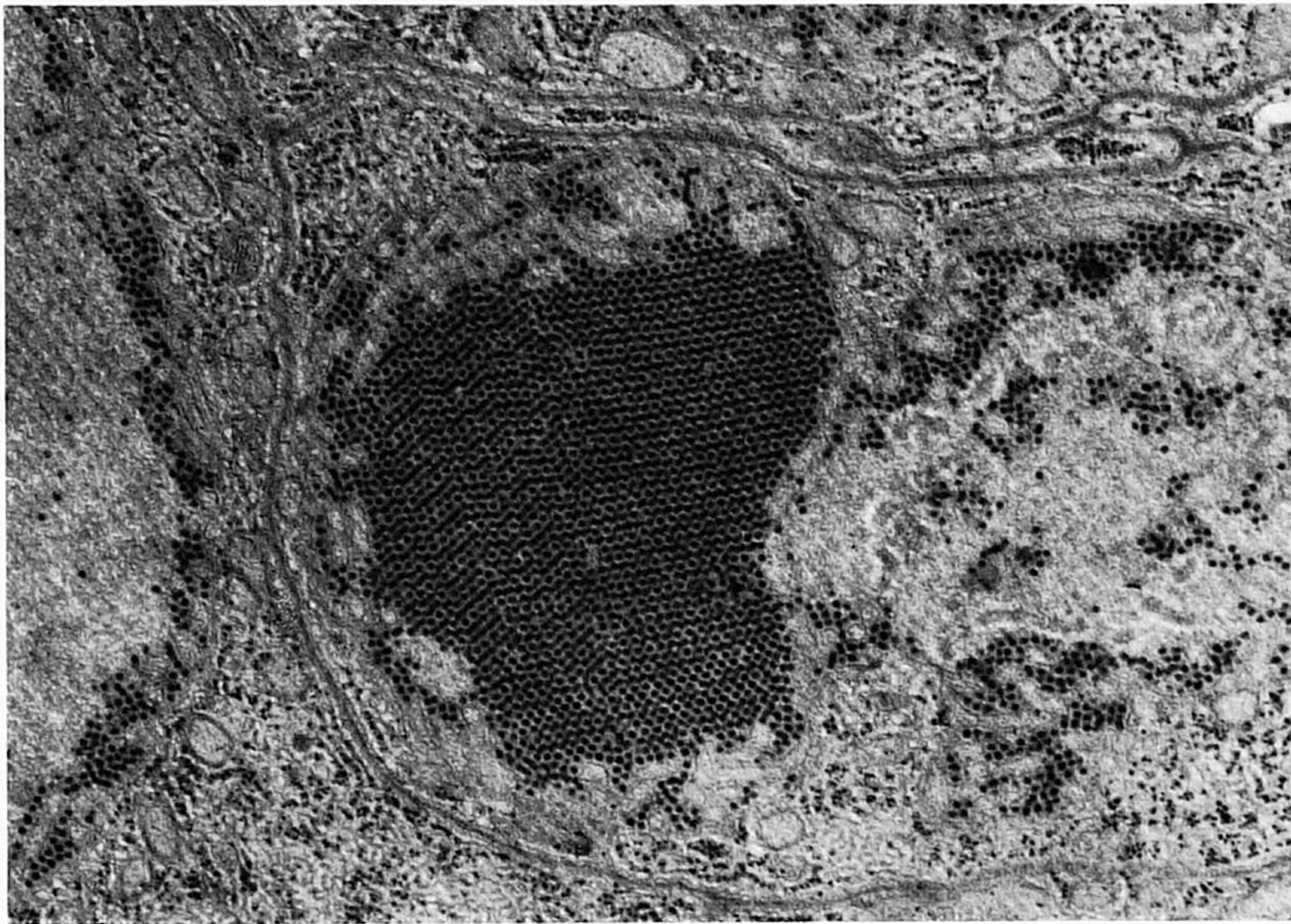

Fig. 5. St. Louis encephalitis virus particles accumulating within the lumen of the salivary gland of a *Culex pipiens* mosquito 25 days after infection via feeding upon a viremic chick. Virus particles are concentrated at the luminal margin and where their concentration is especially high they are amassed into paracrystalline array. × 20,500. Courtesy FREDERICK A. MURPHY.

The togavirus family at present includes two genera. The antigenic interrelatedness of the members of the alpha- and flavovirus groups is reflected by morphological similarities (tables III, IV) and by the molecular weights of their respective non-glycosylated capsid polypeptides (table V). Flavoviruses are inactivated by the action of trypsin while alphaviruses are not [294]. VD and HC viruses structurally appear 'flavovirus-like' [41] but should be considered as a third genus of the togavirus family on the basis of the proposed scheme [2] since they also possess a common antigen; the term 'pestiviruses' is proposed for these agents. Although subdivision of the togavirus family will be facilitated by antigenic relationships it must be essentially oriented according to structural criteria. This might imply that flavo- and pestiviruses, for example, are reduced to the rank of antigenic types within a common genus, when sufficient experimental data have become available.

It can be anticipated that other unclassified or newly isolated animal viruses will be identified as togaviruses in the near future. Since many of them

Table V. Structural proteins of togaviruses analyzed by polyacrylamide gel electrophoresis

Virus	Molecular weights, daltons								References
Alphaviruses									
CHIK			53,000 E	30,000 C					Igarashi [196]
SF			51,000 E	32,000 C					Acheson and Tamm [220]
SIN			53,000 E	30,000 C					Strauss *et al.* [210, 215]
		63,000 E		35,000 C	28,000 C				Nicoli *et al.* [218]
VEE		61,000–59,000 C		38,000–34,000 E		18,000–15,000 C			Uryvaev *et al.* [217]
Flavoviruses									
DEN			59,000 E				13,500 C	7,700	Stollar [221]
JBE			53,000 E				13,500 C	8,700	Shapiro *et al.* [222]
Kunjin	120,000 E	65,000 E				18,000 C	13,000 C		Westaway and Reedman [226]
SLE		63,000 E				18,000 C		8,500	Trent and Qureshi [223]
Non-arbo togaviruses									
RU		62,500 E	50,000–45,000	35,000 C					Vaheri and Hovi [209]
		63,000	56,000; 51,800	32,000	29,800				Liebhaber and Gross [225]
		60,000	54,000; 47,100						

C = capsid protein; E = envelope protein.

have been shown to be cytopathogenic either under defined culture conditions or not at all (RU, VD, HC, LDH, and some flavoviruses), togaviruses are likely to escape detection in the virological routine. Only recently, a plant virus (carrot mottle virus) has been identified as a member of the togavirus family by MURANT [295]. The ability to multiply in plant, invertebrate and vertebrate cells sheds a light on the versatility of these viruses and their distribution in nature. More information about their properties, especially of non-arbo togaviruses, is required so that their structural relationships can be better defined, thereby forming a rational basis for classification.

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