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Iridoviridae

Edited by Dawn B. Willis

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Preface

In 1976 the International Committee on Taxonomy of Viruses (ICTV) created the family Iridoviridae to encompass several different vertebrate and invertebrate viruses that did not fit into any of the other established groups. The unifying features of this new family were (1) polyhedral symmetry; (2) large (approximately 170 kilobase pairs), linear, double-stranded DNA genomes; and (37) a cytoplasmic site of replication.

The name "iridovirus" was derived from the observation that larvae infected with many of the insect viruses, as well as purified pellets of these viruses, glowed with a blue or green iridescence – presumably due to the Bragg effect of the viral crystals. However, none of the vertebrate "iridoviruses" displayed this particular characteristic. An attempt was made to substitute the more descriptive name of "icosahedral cytoplasmic deoxyribovirus", but not only was this term too unwieldy, it also did not conform to the latinized nomenclature the ICTV wished to adopt. So, for both historical and esthetic reasons, "Iridoviridae" was adopted as a family name, with *Iridovirus* as the genus represented by the type 1 iridescent insect virus, *Tipula* iridescent virus. At the 1982 ICTV Meeting, enough biochemical data had accumulated to permit the establishment of the following five genera in the family Iridoviridae:

English vernacular name	International name	Type species
1. Small iridescent insect virus	*Iridovirus*	*Tipula* iridescent virus (Type 1)
2. Large iridescent insect virus	*Chloriridovirus*	Mosquito iridescent virus (Type 2)
3. Amphibian icosahedral deoxyribovirus	*Ranavirus*	Frog virus 3 (FV3)
4. Fish lymphocystis disease virus	*Lymphocystivirus*	Flounder lymphocystis disease virus (FLCDV)
5. African swine fever virus	–	African swine fever virus, Kenya strain (ASFV)

Investigators both in and out of the field of Iridoviridae research have voiced objections to this schema, saying that the two insect genera ought to be only one, and that the vertebrate viruses are so distantly related to the insect viruses and to each other as to be placed in separate families, but no one has proposed an acceptable alternative. Moreover, certain recent discoveries, e.g., the methylation of both frog virus (FV3) and fish lymphocystis disease virus (LCDV) DNAs, and the circular permutation of FV3, LCDV, and *Chilo* iridescent virus DNA, suggest that these genera are more closely related than was originally thought. The sole exception is African swine fever virus (ASFV), the only mammalian virus of the family. Except for its morphology, ASFV has structural and biochemical features identical to those of poxviruses – in particular, cross-linked DNA and an active DNA-dependent RNA polymerase.

Although the number of laboratories engaged in Iridoviridae research remains small, the enthusiasm generated by each new revelation is high. Does circular permutation of DNA mean that there is an evolutionary link with bacteriophage? How is highly methylated DNA transcribed by the host polymerase? The wide host range, selective toxicity on host cell macromolecular synthesis, and unique mode of replication of FV3 make it an exciting model in which to study virus infection, gene expresseion, and the infinite variety of nature. ASFV and LCDV cause commercially important diseases, and the insect viruses have been touted as a means of pest control. A number of excellent reviews on Iridoviridae have appeared in recent years, but this is the first single topic volume to be devoted to the subject. I thank each author for his contribution and I hope that this volume will serve to encourage further investigation of these unusual and interesting viruses.

Memphis, Tennessee, Spring 1985 DAWN B. WILLIS

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Comparative Ultrastructure of Iridoviridae

G. Devauchelle[1], D.B. Stoltz[2], and F. Darcy-Tripier[3]

1 Introduction

Electron microscopic observations of large icosahedral viral particles whose morphogenesis takes place in the cytoplasm of infected cells allow their classification into the Iridoviridae family. Members of this family are widely distributed in nature in a variety of vertebrate and invertebrate hosts. Today this family is divided into five genera (Fenner and Gibbs 1983).

A number of these viruses have been the main subject of specific reviews (Bellet 1968; Granoff 1969; Hess 1971; Wardley et al. 1983) or more general ones (Kelly and Robertson 1973; Mc Auslan and Armentrout 1974; Tinsley and Harraps 1978; Goorha and Granoff 1979).

In this chapter we shall review the information available on the structure and morphogenesis of Iridoviridae, concentrating mainly on the most studied members:

Iridovirus: *Tipula* iridescent virus (TIV), type 1
Sericesthis iridescent virus (SIV), type 2
Chilo iridescent virus (CIV), type 6

Chloriridovirus: Mosquito iridescent virus (MIV), type 3

Ranavirus: Frog virus 3 (FV 3)

African swine fever virus genus: ASFV

Lymphocystis disease virus group: FLDV

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2 Structure

Iridoviridae are by far the largest of the known isometric viruses, with reported diameters ranging from 120 to 300 nm. Icosahedral form was first demonstrated by double-shadowing of TIV particles in 1958 (WILLIAMS and SMITH); however, Iridoviridae are sufficiently large to allow unambiguous determination of three-dimensional form by observing sectional profiles (Fig. 1) of particles lying in different orientations (STOLTZ 1971). Measurements of icosahedral diameter vary depending on particle orientation, so that many of the reported values for Iridoviridae particle diameters (Table 1) are probably not comparable (Fig. 2). Nevertheless, it is clear that some Iridoviridae are considerably larger than others (STOLTZ 1971, 1973; TINSLEY and HARRAP 1978). In addition, some Iridoviridae which have been released by budding at the cell membrane (see below) can possess an outer envelope (HUKUARA and HASHIMOTO 1967; STOLTZ 1969; YULE and LEE 1973; WEBB et al. 1976; BRAUNWALD et al. 1979; CARRASCOSA et al. 1984).

Beside their shape, virtually nothing was known about the structure of Iridoviridae particles until 1969, when WRIGLEY demonstrated the existence of an icosahedral lattice of hexagonally packed subunits on the surface of SIV. Since then, surface subunits have been described for many Iridoviridae. They display a cylindrical shape with a central hole. Their diameter is remarkably constant (about 7 nm), whereas their height ranges from 7 to 13 nm (WRIGLEY 1969, 1970; TRIPIER-DARCY et al. 1982; CARRASCOSA et al. 1984).

After disruption of several *Iridovirus* and *Chloriridovirus* isolates (STOLTZ 1969, 1971, 1973; WRIGLEY 1970; BAILEY et al. 1976; MANYAKOV 1977), the lattice was shown to be built up of triangular (Fig. 3b) and in some cases pentagonal subunit aggregates named, respectively, tri- and pentasymmetrons. Knowing the number of subunits per trisymmetron (55), and assuming that each trisymmetron constitutes the major part of a face of the icosahedral particle, WRIGLEY (1969, 1970) suggested several possible triangulation numbers (T) for the *Iridovirus* particles; the value T = 147 (1472 subunits), assuming 31 subunits per pentasymmetron, now seems most probable for TIV and SIV (WRIGLEY 1970; MANYAKOV 1977). As one might expect, *Chloriridovirus* particles appear to have larger trisymmetrons (STOLTZ 1971, 1973) with, presumably, higher triangulation numbers. It is of considerable interest that Iridoviridae differing in size by at least 30 nm are nevertheless constructed from (or disrupted into) similar subunit aggregates. Disintegration into trisymmetrons and pentasymmetrons has never been observed for some other Iridoviridae such as FV3 and ASFV. Another type of approach using freeze-etching studies has led to possible triangulation numbers for ASFV ranging from 189 to 217 (1892–2172 subunits; CARRASCOSA et al. 1984).

The presence of a "fringe" (Fig. 3a) at the surface of some insect Iridoviridae has been well documented by STOLTZ (1971, 1973); the available evidence suggests that this fringe is probably microfibrillar, and that each subunit bears a single fibril (Fig. 3c). Fibrillar fringes are also obvious on certain other Iridoviridae, e.g., FLDV (YAMAMOTO et al. 1976) (Fig. 5) and CIV, but seem to be absent from others, such as ASFV and FV3 (Fig. 4a, b). The lattice and

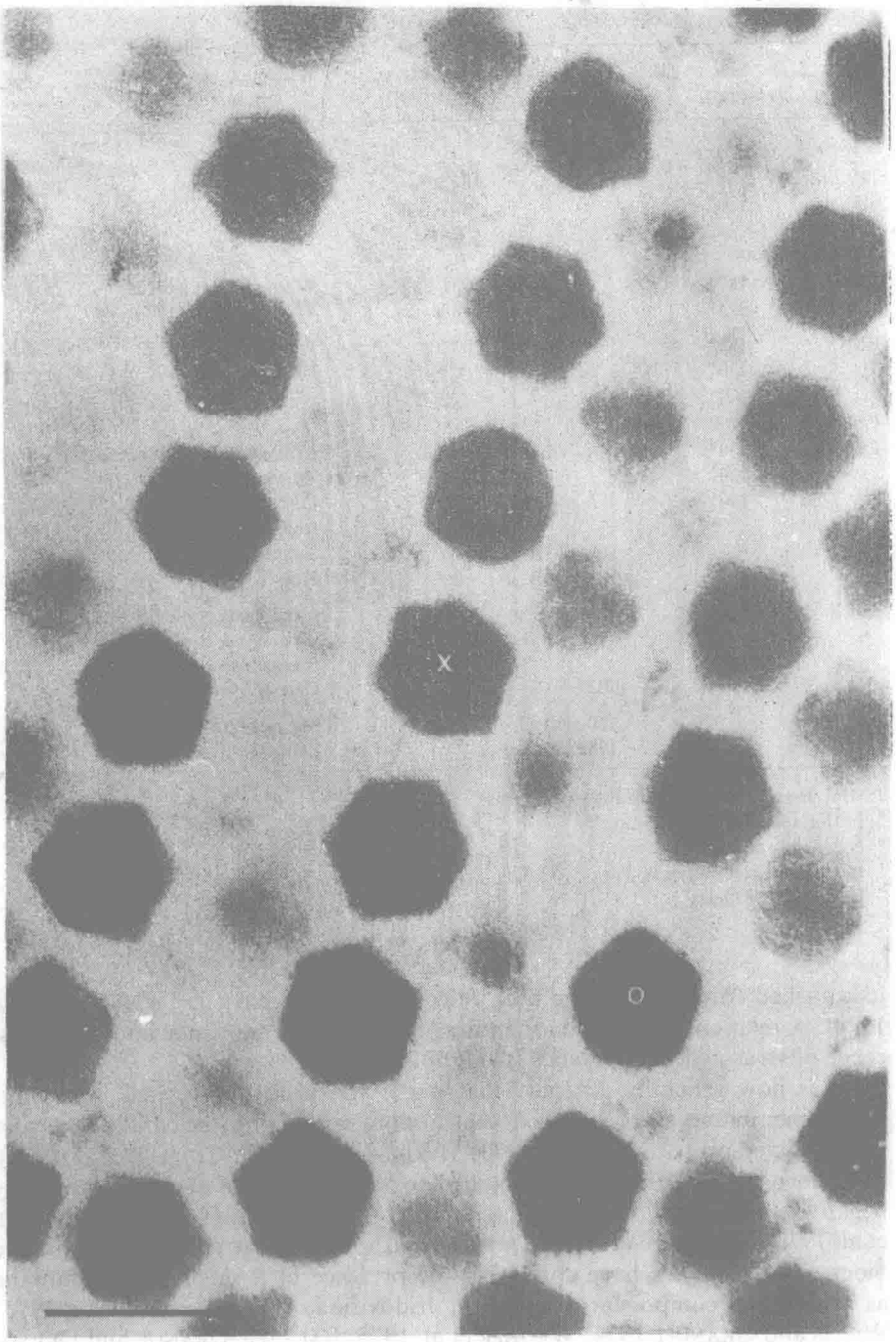

Fig. 1. A paracrystalline array of iridovirus type 27 in the cytoplasm of an infected cell of *Nereis diversicolor*. The virus particles are regularly ordered in rows. Cross sections in different planes (essentially hexagonal (*x*) or pentagonal (*o*) outlines) provide further evidence for the icosahedral symmetry of the capsid. *Bar*, 0.2 μm

Table 1. Dimensions of Iridoviridae

Virus	Side to side (nm)	Diameter (nm)	Vertex to vertex (nm)	References
Iridovirus				
TIV and SIV	130[a]		145[a]	THOMAS (1961)
SIV	130[b]		145[b]	GLITZ et al. (1968)
Chloriridovirus				
TMIV	187[a]		203[a]	WAGNER et al. (1973)
	203[b]		218[b]	WAGNER et al. (1973)
RMIV	195[a]		209[a]	WAGNER et al. (1973)
	230[b]		250[b]	WAGNER et al. (1973)
Ranavirus				
FV3	130[a]		145[a]	LUNGER and CAME (1966)
	130[b]		145[b]	TRIPIER and KIRN (1973)
	136[c]		162[c]	DARCY-TRIPIER and NERMUT (to be published)
		(165–200)[d]		TRIPIER and KIRN (1973)
ASFV	191[a]		210[a]	CARASCOSA et al. (1984)
	172[b]		191[b]	CARASCOSA et al. (1984)
	191[c]		228[c]	CARASCOSA et al. (1984)
		203[d]		CARASCOSA et al. (1984)
FLDV		130–300		MC ALLISTER (1979)
		198–227		DARAI et al. (1983)

RMIV, R strain of mosquito iridescent virus
[a] In thin sections
[b] Negatively stained
[c] In freeze-etching replicas
[d] Enveloped particles

its attached fringe layer have been referred to as the particle "shell" (STOLTZ 1973). A relationship between the presence of an external fringe and the iridescence of virus pellets or crystals is still not ascertained.

It is now generally accepted that under the Iridoviridae shell lies a unit lipidic membrane surrounding a central core containing the viral DNA and its associated proteins (STOLTZ 1973; WAGNER et al. 1975; CUILLEL et al. 1979). The inner membrane is closely applied to the icosahedral shell, as shown by negative staining and thin sectioning (STOLTZ 1973), neutron scattering (CUILLEL et al. 1979), and freeze-etching (TRIPIER-DARCY and NERMUT 1983). Quantitative biochemical analyses have confirmed the presence of a single unit membrane as an integral component of different Iridoviridae (KELLY and VANCE 1973; WILLIS and GRANOFF 1974; WAGNER et al. 1975; BALANGE-ORANGE and DEVAUCHELLE 1982; BLACK et al. 1981).

The observation of replicas of FV3 and CIV freeze-fractures through this membrane (Fig. 4c) showed the presence of intramembrane particles associated

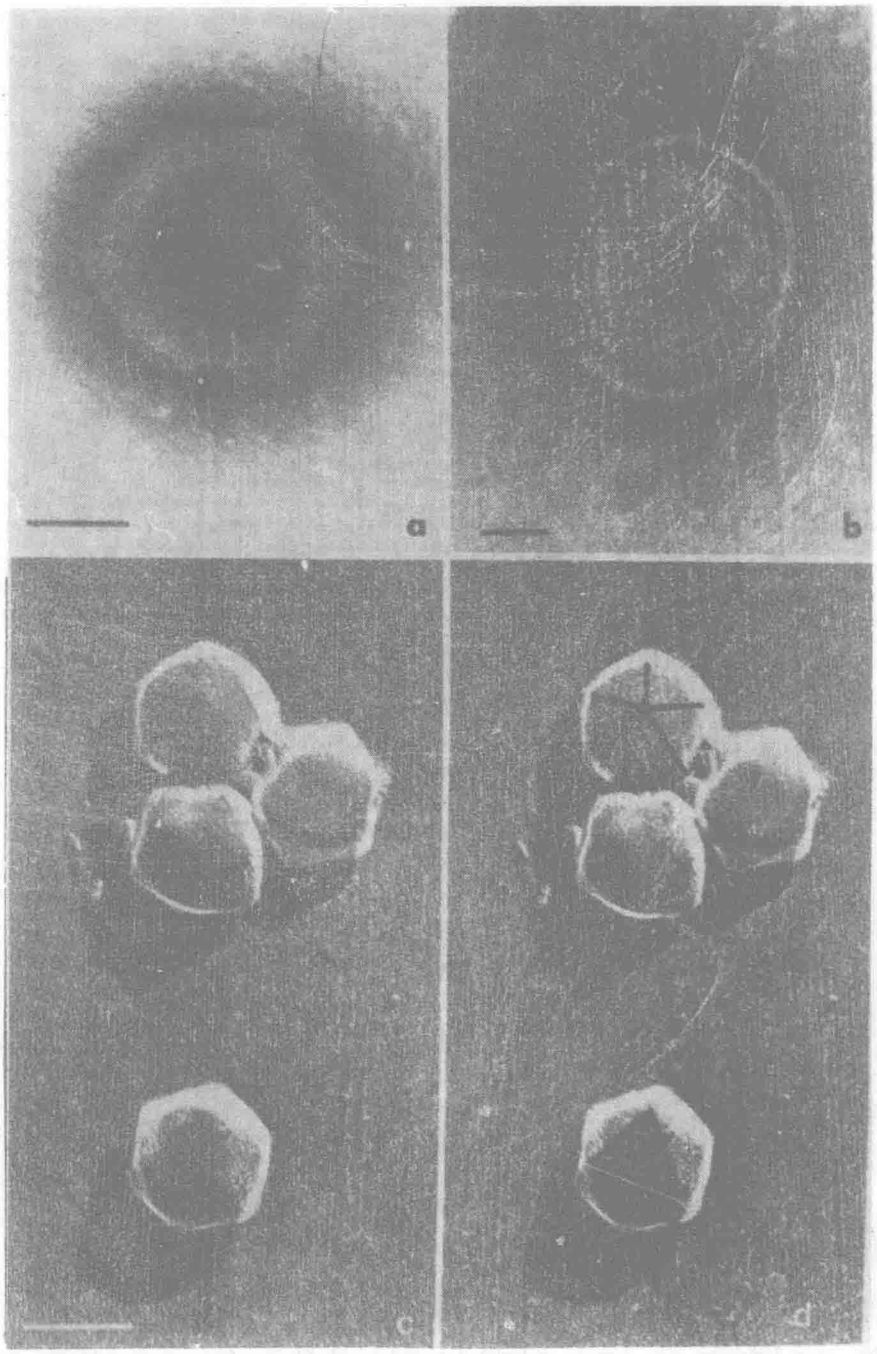

Fig. 2. a Negative staining (uranyl acetate 1%, 15 s) of purified CIV. *Bar*, 0.05 μm. **b** Enveloped FV3 particle, fixed for 2 h with 5% formol and observed after negative staining (2% sodium phosphotungstate, 45 s). The viral nucleocapsid (*arrow*) can be seen under the envelope (*arrowhead*). *Bar*, 0.05 μm. **c, d** FV3 prepared by freeze-etching after glutaraldehyde and osmium tetroxide fixation (1 min). The icosahedral shape is well preserved; some edges are clearly visible (**d**). *Bar*, 0.1 μm

Fig. 3a–c. Iridescent virus type 35. **a** Isolated particles with fibrillar fringe in hemolymph of the midge, *Chironomus plumosus*. *Bar*, 0.2 μm. **b** Trisymmetrons. *Bar*, 0.1 μm. **c** Individual subunits (*s*) with attached fibrils. *Bar*, 0.1 μm

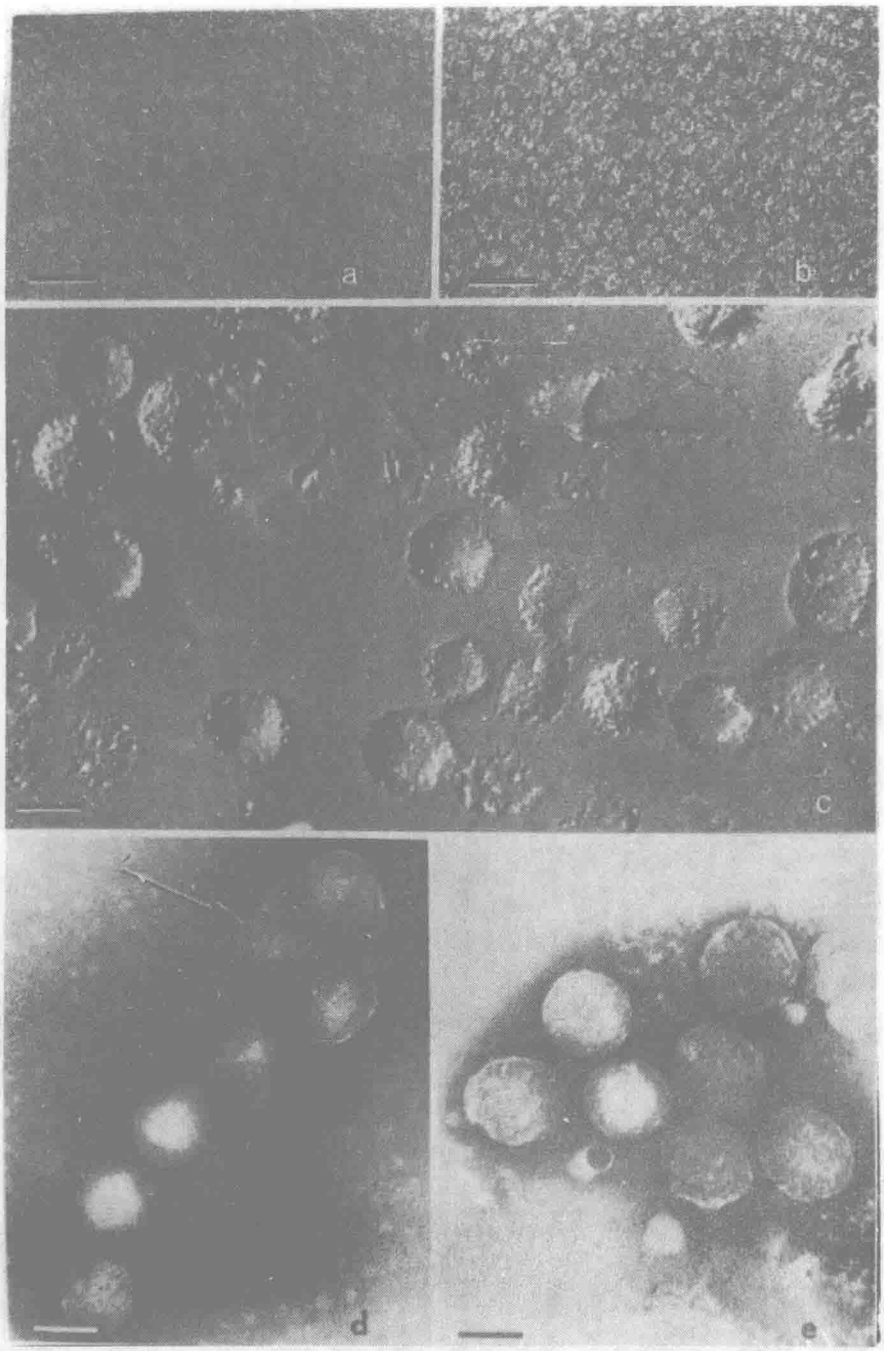

Fig. 4. **a** CIV surface subunits released by freeze-thawing and observed after negative staining (1% uranyl acetate). *Bar*, 0.03 μm. **b** FV3 surface subunits released by Nonidet P40 treatment (0.5%, 1 h, 37° C) and observed after negative staining (2% uranyl sulphate). *Bar*, 0.03 μm. **c** Freeze-etching of unfixed FV3. Many virus particles are membrane-fractured, revealing the presence of intramembrane particles. *Bar*, 0.1 μm. **d** CIV treated with pronase (25 μg/ml, 5 min, 37° C) and negatively stained (2% sodium phosphotungstate, 45 s). After the removal of the virus capsid the subparticles limited by the inner membrane appear spherical; sometimes fusion can occur (*arrow*). *Bar*, 0.1 μm. **e** FV3 treated with pronase (25 μg/ml, 5 min, 37° C) and negatively stained (2% sodium phosphotungstate, 45 s). FV3 is also degraded into spherical subparticles; unlike with CIV, fusion between adjacent subparticles never occurs. *Bar*, 0.1 μm

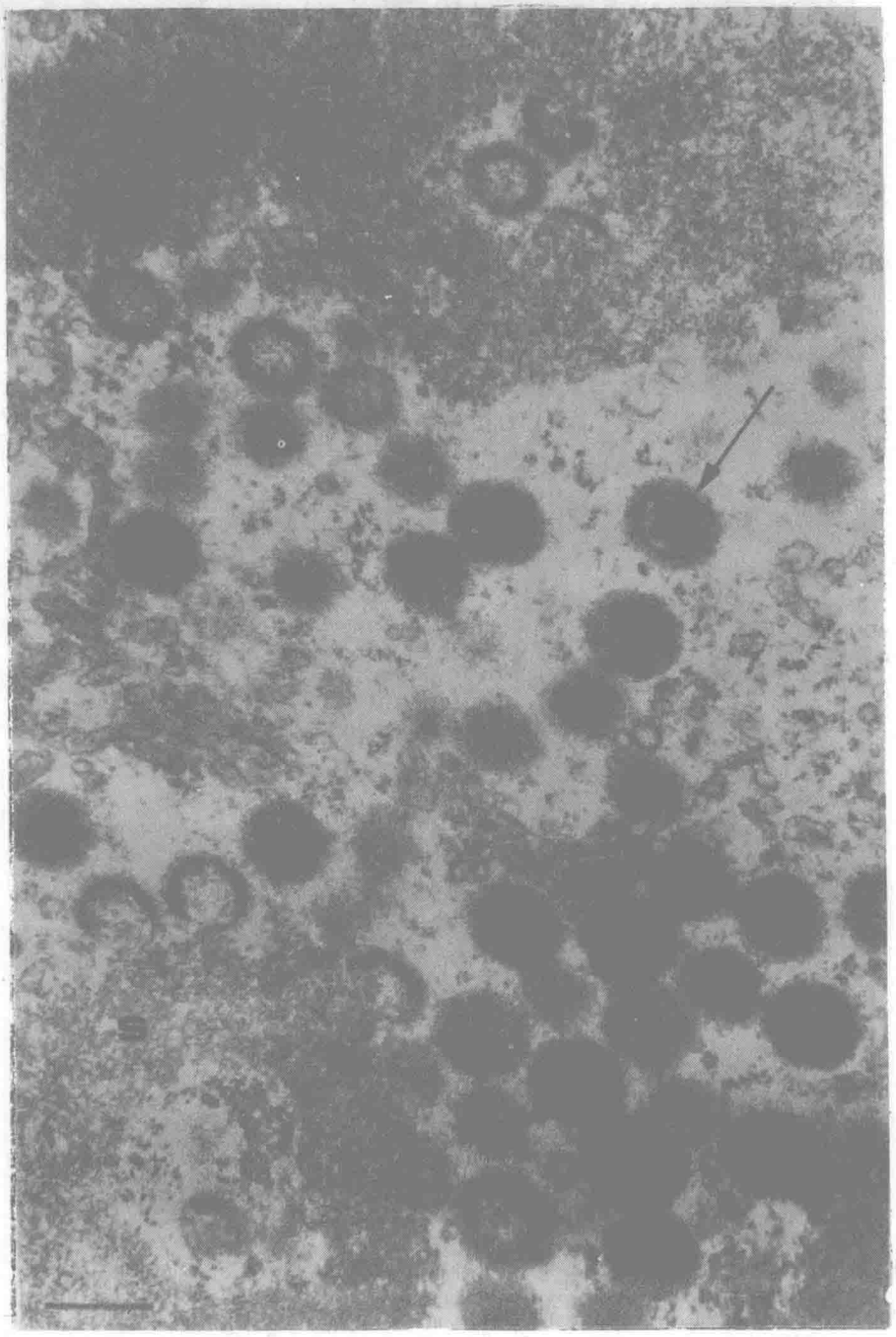

Fig. 5. Fish lymphocystis disease virus, portion of the cytoplasm showing several virogenic stroma (*s*) with virus particles in the process of formation. Fringes are visible at the periphery of the virion (*arrow*). (Courtesy of M. BERGOIN) *Bar*, 0.4 μm

with both the external and internal leaflets of the lipid bilayer (TRIPIER-DARCY and NERMUT 1983; STELZ, personal communication). Spherical subparticles bound by the lipidic membrane could be obtained from FV3 (Fig. 4e) and CIV after removal of the icosahedral shell by mild treatments with pronase (TRIPIER-DARCY et al. 1981). The observation of negatively stained preparations of CIV spherical subparticles showed that they were able to fuse (Fig. 4d); such a characteristic has not yet been reported for any other virus.

The term "core" represents the internal structural entity comprising the DNA and its associated protein(s) (Fig. 6a, b). Cores could be obtained by treatment of viral suspensions with Nonidet P40 (AUBERTIN et al. 1971; WILLIS and GRANOFF 1974; BLACK and BROWN 1976; WARDLEY et al. 1983) or chymotrypsin (WAGNER et al. 1975). In the case of FV3, after negative staining the cores appear roughly spherical and devoid of an outer membrane (Fig. 6c). Neutron scattering (CUILLEL et al. 1979) demonstrated that in FV3 cores, the distribution of both DNA and protein is uniform, without any discontinuity at the periphery indicative of a core shell, and that their degree of hydration is high. Ultrasonic absorption measurements (ROBACH et al. 1983) showed that the FV3 core exhibits a dynamic effect that does not exist in its dissociated components, thus pointing to the existence of some organized structure of the core. When the core is surrounded by the peripheral lipidic membrane, as in the case of spherical subparticles, the dynamic effect is markedly reduced; this may be due to the lipid bilayer hindering the motions of the core components or to the rigidification of the core periphery by the intramembrane particles that interact with it.

Freeze-etching of infected cells across paracrystalline arrays enables the observation of cross sections of viral particles (Fig. 6d). Stereo micrographs help to understand the organization of the core, where rods about 10 nm in width and randomly oriented can be seen. These rods might represent sections of the DNA-protein complex organized in the form of a long, convoluted filament (TRIPIER-DARCY and NERMUT 1983). In the case of the core of CIV, a thermodynamic approach suggested that the nucleoprotein complex could be organized into a nucleosomal structure (KLUMP et al. 1983).

When crude virus suspensions are purified by centrifugation in sucrose gradients, two components (bands) are often detected (GLITZ et al. 1968; KALMAKOFF and TREMAINE 1968; MATTA 1970; STOLZ 1973; KRELL and LEE 1974; WAGNER et al. 1977). The lower band represents intact virions, while the upper one consists of less dense particles, collectively referred to as "top component" (TC). Except for T strain of mosquito iridescent virus type 3 (TMIV), little detailed information concerning iridovirus TCs is presently available. Ultrastructurally, TC particles, at least in the case of TMIV, differ from virions in two respects: (1) they are permeable to negative stains, and tend to disrupt during negative staining; and (2) they contain less nucleoprotein. When fixed, however, they retain icosahedral form (STOLTZ 1973). Biochemical studies (WAGNER et al. 1977) have confirmed that TMIV TC particles contain less DNA than do virions, although the total amount of lipid is unchanged. Polyacrylamide gel profiles appear to be identical; only quantitative antigenic differences are observed (WAGNER et al. 1977). TC could represent defective particles formed