Properties of Mouse Leukemia Viruses
XV. Electron Microscopic Studies on the Organization of Friend Leukemia Virus and Other Mammalian C-Type Viruses

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Further investigations on the structure of Friend murine leukemia virus (FLV) revealed that the transition from the "immature" (now termed "native") to the structurally less organized "mature" (now termed "collapsed") form occurs mainly as a result of the preparation for the electron microscope. A short pretreatment of virus with the detergent NP40 prior to negative staining with uranyl acetate is able to preserve the "native" structure of a high percentage of virus particles from standard preparations. Treatment with conventional fixatives was found to be ineffective.

Using this preparation technique, a more detailed electron microscopic analysis of the viral internal organization became possible. A thin layer designated "inner coat" was newly detected in close apposition to the viral membrane between the viral envelope and core. Removal of the unit membrane by more intensive detergent treatment suggests the existence of material extending between the viral surface knobs and the viral interior. The icosahedral core shell has an opening at one side and this opening matches with the hole in the apparently beehive-like arranged ribonucleoprotein (RNP) strand which represents the innermost structure.

A comparative study of a series of representative mammalian C-type viruses with the same technique indicated a close similarity to Friend virus in fine structure, although differences in stability were observed.

Based on these and earlier findings a model of the structure of mammalian C-type viruses is presented.

Introduction

The concepts on the structural organization of mammalian C-type viruses are still controversial. Some of the discrepancies stem from the occurrence of different internal structures seen in free virus particles. During the budding process particles with concentrically arranged layers and an electron-lucent center are formed. Free viruses of this type have been called "immature" C-type particles. Free viruses with an electron-dense core of irregular, often polygonal shape, have been defined as "mature" C-type particles; this type of particle constitutes the overwhelming majority [1, 2, for review see 3]. It was explicitly stated by the former authors that "these terms are purely morphological and no biological meaning should be implied". Indeed, no correlation between immature to mature ratio and infectivity has been found [4]. Moreover, it was reported "that the structure of the mature virion results from the collapse of the two shells inside the envelope" [2]. It therefore appears questionable to designate a transition to a state of apparently lower order as a maturation process.

By varying the conditions for the preparation of virus samples for electron microscopy we found that gentle treatment of standard FLV suspensions with NP40 with subsequent negative staining, leads to the preservation of the "immature" structure in a high percentage of the virus particles. Using this and other preparation methods the internal organization of FLV was reinvestigated. In addition, other mammalian C-type viruses were studied. Finally, based on the present and earlier findings, a model of mammalian C-type viruses will be proposed.

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Material and Methods

1. Viruses

Friend leukemia virus (FLV) was produced in the Eveline murine suspension cell line [5]. *Pig C-type virus (PLCP)* was grown in the V38A-1 porcine cell line [6]. *Feline leukemia virus* (Rickard)

(FeLV) was propagated in the cat F 422 cell line [7]. *RD114-virus* was produced in the RD114 rhabdomyosarcoma cell line [8]. *SSV-1/SSAV-1 woolly monkey virus* was obtained from the HF-SSV/Jü marmoset cell line [9, 10]. *Bovine leukemia virus (BLV)* was grown in primary bovine leucotic cell cultures (kindly provided by Dr. Überschär) [11]. All viruses were concentrated and purified immediately after harvesting by sedimenting them twice at pi 10 [12] and were freshly used for electron microscopy. The *rat* (RaLV) and *hamster* (HaLV) C-type viruses were kindly provided by Dr. Gilden, Bethesda. They were purified by density gradient centrifugation and shipped deep frozen. Isolation of cores after treatment with ether in the cold was performed as described by Lange *et al.* [13].

2. Preparation for electron microscopy

Negative staining was done with 1.0% uranyl acetate (UA) pH 4.3 or 1.0% sodium phosphotungstate (PTA) pH 4.3 – 7.2. Carbon coated Pioloform films [14] were used throughout. They were glow discharged immediately before use. A drop of virus suspension to be tested was placed on the grid for adsorption. After one minute the nonadsorbed material was removed by filter paper, the grid washed twice with distilled water (if UA was used as a stain) and then stained by adding a drop of the stain. After an adequate staining time which was varied between 5 seconds and 5 minutes the excess of stain was removed by filter paper also.

Freeze-drying and shadowing was done as described by Nermut *et al.* [15].

Fixation. For fixation either 2% glutaraldehyde (GA), 2% formaldehyde or 1.0% osmiumtetroxide in phosphate buffered saline (PBS) pH 7.2 were used. After adsorption of the material on the grid the latter was placed for 2 – 5 minutes on a drop of the fixative, washed three times with distilled water and stained thereafter.

Pretreatment with detergent. The non ionic detergent Nonidet P40 (NP40) (Shell Chem. Corp.) was preferentially used. A grid with virus material adsorbed as described for negative staining was placed for a given time onto a drop of an aqueous solution of the detergent. Thereafter the sample was washed three times with distilled water and used for freeze-drying or for negative staining.

For the preparation of thin sections NP40 was added at a concentration of 0.1% 5 minutes before fixation and this concentration of the detergent was present also during the following preparation steps (fixation, washing and dehydration).

For *embedding in Epon 812* the samples were prefixed with 2.5% glutaraldehyde in PBS pH 7.2 at 4 °C for 1 h and postfixed with 1% osmiumtetroxide in PBS pH 7.2 at room temperature. Staining was performed with UA and lead citrate.

Immuno electron microscopy. The method and the antiserum to the envelope glycoprotein (gp71) of FLV described by Schwarz *et al.* [16] were used.

3. Electron microscopy

Specimens were observed at 80 kV with Siemens Elmiskop 1a and 102 electron microscopes at nominal magnifications of 20,000 and 40,000×. The real magnification was evaluated as described by Frank and Day [17].

Results

A. Friend virus

The viral core

Native and collapsed cores. In *thin sections* of budding viruses cores always have a round shape, forming horseshoe or ring-like structures (Fig. 1a). In contrast, only 3 – 5% particles with a round core can be found in thin sections of virus pellets (see arrows in Fig. 1b) while the majority of the particles contain an electrondense core of irregular shape. For reasons given in the introduction and as will become evident in the course of this work, we would like to introduce here the terms "*native*" core particles and "*collapsed*" core particles, respectively. A particle with a native core structure, as seen by thin sectioning, is exemplified in Fig. 1c. Typically, the core has an electron light center and two inner layers which are concentrically arranged in close apposition to the outer layer. A particle with a shrunken core is shown in Fig. 1d. The electrondense core seems to be collapsed and the two inner layers are no longer discernible. The cores were also often located excentrically in the particle. The ball-like structure frequently seen within the core might represent part of the condensed RNP complex.

As found by thin sectioning, two types of particles were also found after *negative staining* with UA. Two to three concentrically arranged, circular layers are seen to form the native core (Fig. 2a). In contrast, collapsed cores show no structural details and

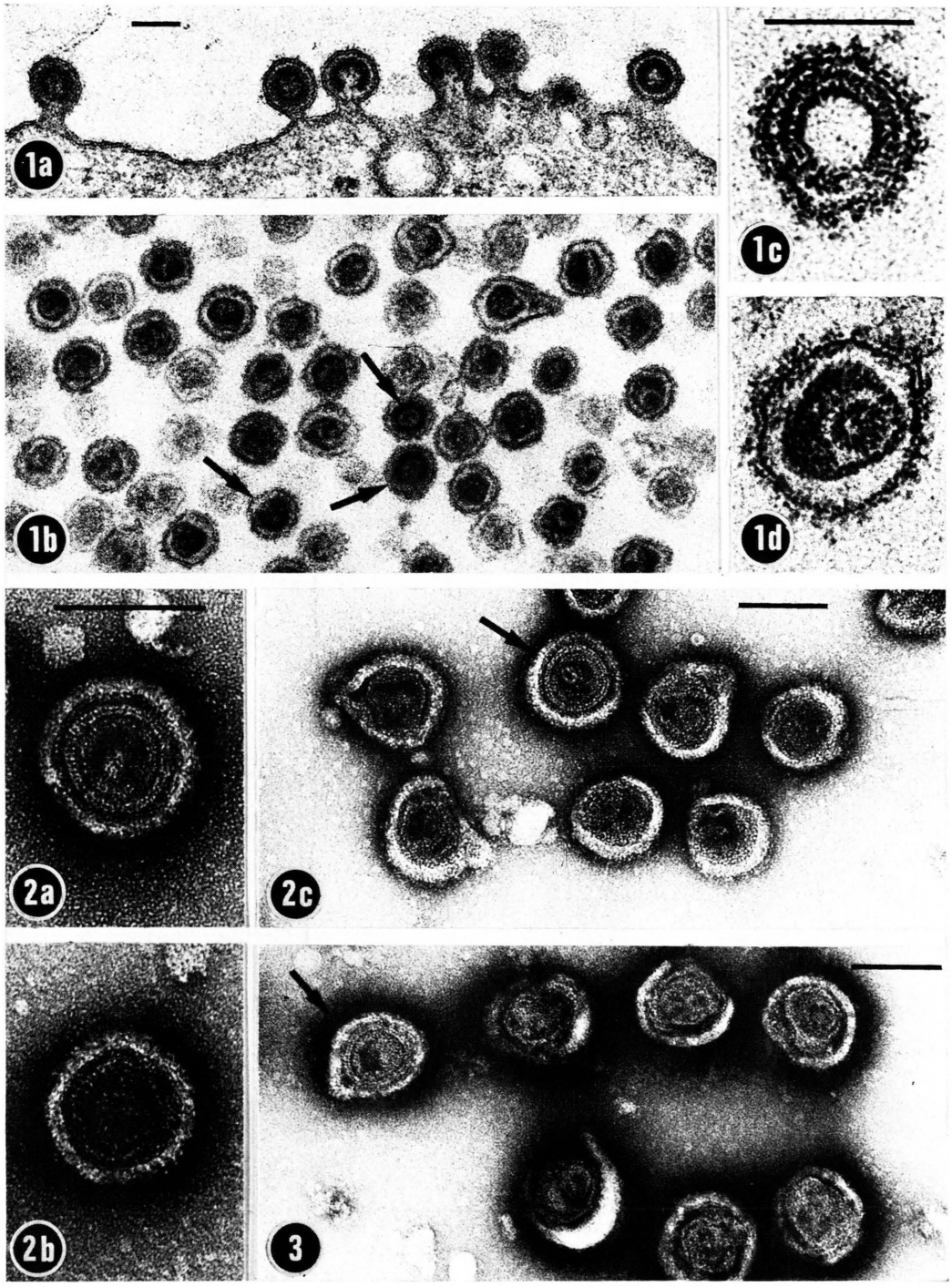


Fig. 1. Thin sections of FLV-producing Eveline cells and pellets of FLV. a) budding viruses, b) free viruses; particles with native cores are indicated by arrows, c) particle with native core and d) particle with collapsed core at higher magnification. Magnification of a) and b): × 65 000, of c) and d): × 220 000. Bars represent 100 nm.

Fig. 2. FLV particles negatively stained with uranyl acetate (UA) (5 minutes staining time), a) virus particle with native core, b) virus particle with collapsed core, c) survey micrograph; a particle with native core is indicated by arrow. Magnification of a) and b): × 220 000, of c): × 130 000. Bars represent 100 nm.

Fig. 3. FLV particles negatively stained with UA after 2 minutes fixation with 2% glutaraldehyde (staining time about 30 seconds). The arrow indicates a particle with native core. Magnification: × 130 000. Bar represents 100 nm.

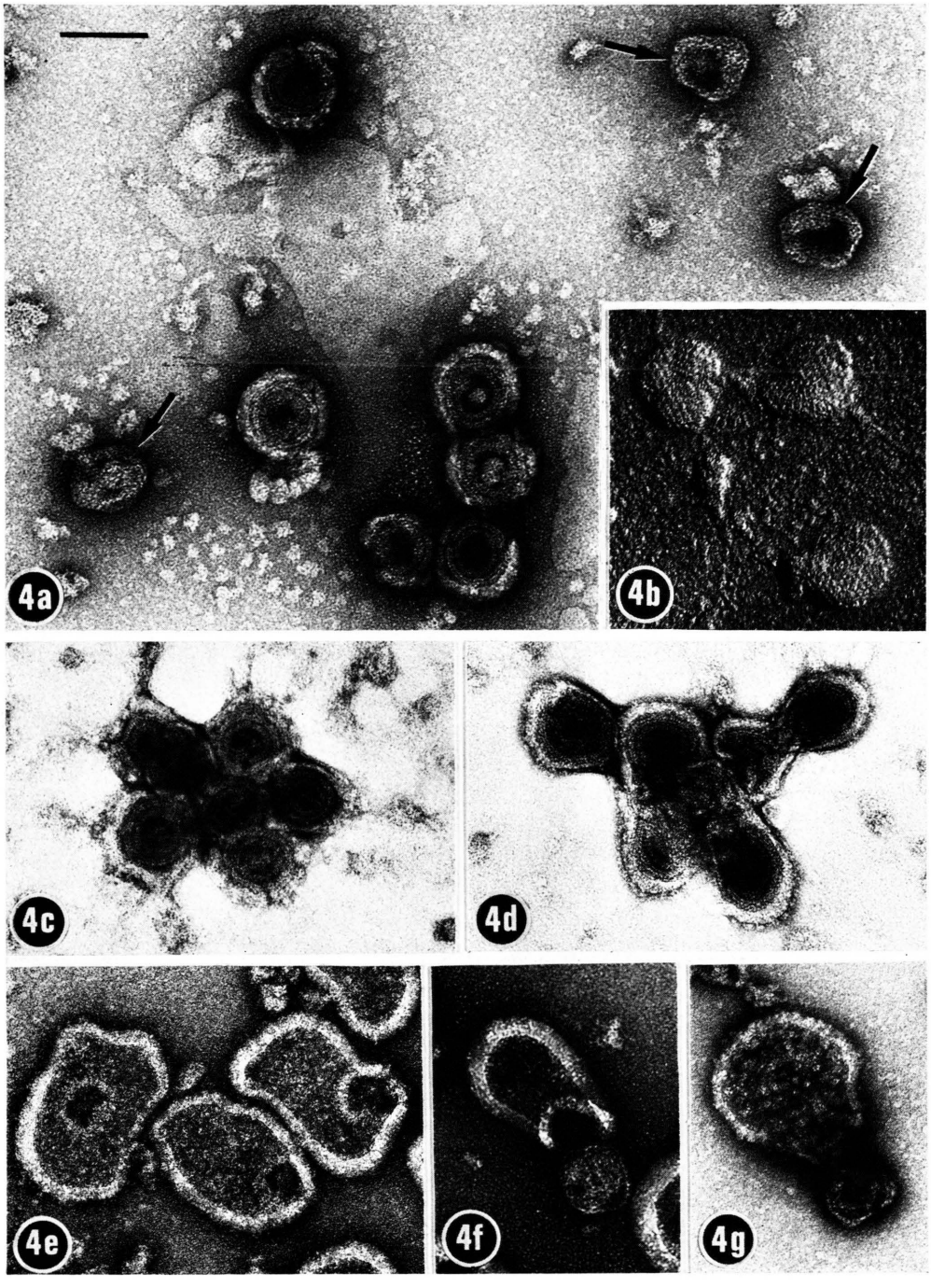


Fig. 4. FLV particles negatively stained with UA (a and c–g) (staining time about 5 seconds) or freeze-dried and shadowed (b). Particles pretreated for 5 seconds with NP40; a–d) with 0.1% NP40; e–g) with 0.02% NP40. In the survey micrograph (a) free collapsed cores are indicated by arrows; b) envelope-free, collapsed cores, c) and d) cluster of well preserved particles, e–g) empty virus envelopes with a hole. Such structures were sometimes found after pretreatment with low concentrations of NP40. Magnification: × 130 000. Bar represents 100 nm.

often have an angular outline (Fig. 2b). In this type of preparation 4 – 6% of the particles were of the native core type (Fig. 2c). This percentage did not differ significantly within preparations with widely different proportions of unpenetrated, and therefore unclassificable, particles (the range of unpenetrated particles observed was between 2.6 and 30%).

In order to determine whether or not preparation artifacts were responsible for the appearance of collapsed cores after virus release, the following strategy was applied: Freshly harvested virus was gently concentrated, adsorbed to the grid and stained after treatment with various detergents and fixatives to see if a preparation method could be found by which the ratio of native to collapsed cores could be increased. Virus was preferentially stained with UA since, according to earlier studies [15] this results in the best resolution of internal virus structures.

Pretreatment of the virus samples prior to staining with various fixatives such as glutaraldehyde, formaldehyde or osmiumtetroxide did not lead to an increased proportion of native particles (Fig. 3), but reduced the time required to achieve a good penetration of UA from 3 minutes to about 30 seconds.

A similar effect was obtained when the virus preparations were pretreated for 5 seconds with 0.1% of the detergent NP40, reducing the penetration time to only about 5 seconds. Even more striking, however, was the effect of NP40-treatment on the morphology of the virus particles (Fig. 4, Table I). First, a dramatic increase of the proportion of particles with native cores was observed (Fig. 4a), preferentially in virus clusters which nearly always were found to be composed of native core particles only (Figs 4c and d). Second, all collapsed cores had lost the envelope. These free collapsed cores revealed, after freeze-drying and shadowing, a surface pattern of hexagonally arranged subunits (Fig. 4b) as described earlier for cores isolated by ether treatment of FLV and other murine C-type viruses [13, 15]. The increase in the proportion of apparently native core particles suggests that NP40 helps to preserve the structure of the native particles. As can be deduced from the data shown in Table I, it is unlikely that this increase was due to the selective loss of particles with collapsed cores from the grid due to the NP40 treatment. Even if only particles with collapsed cores were lost, there remains a three to fourfold increase of native core particles.

Using NP40 at lower concentrations (0.01 – 0.02%) its preserving action decreased and, besides native core particles and free collapsed cores, also whole particles with collapsed cores were found (Tables I and II). In addition, in some preparations, empty envelopes were also observable, revealing a hole of about 50 – 70 nm in diameter (Fig. 4e). In some of the particles the collapsed

Table I. Effect of NP40 on FLV harvested after 72 h of culturing.

NP40 (%)	Proportion of particles (%) a)	b)	c)	d)	Total number of particl. counted	Loss due to treatment (%)
untreated	4.1	90.3	0	5.6	340	0
0.02	13.5	0.7	0.3	85.5	599	40
0.1	31.0	0	0.7	68.3	435	40
1.0	47.5 (*)	0	0.7	51.8	286	75

The numbers shown stem from a single virus preparation screened in the same experiment. a) Native core particles, b) collapsed core particles, c) free native cores, d) free collapsed cores, (*) these particles showed the presence of a fuzzy material surrounding the core (see text), also demonstrated in Fig. 5 a. Loss of particles due to the detergent was calculated by comparing the number of particles per square area with the number of untreated particles in a similar area on a different grid.

Table II. Effect of NP40 on FLV harvested after 4 h of culturing.

NP40 (%)	Proportion of particles (%) a)	b)	c)	d)	Total number of particl. counted	Loss due to treatment (%)
untreated	5.9	88.2	0	5.9	101	0
0.02	27.5	2.7	0.5	69.3	186	30
0.1	35.0	0.1	0	64.9	139	61

For explanation see legend of Table I.

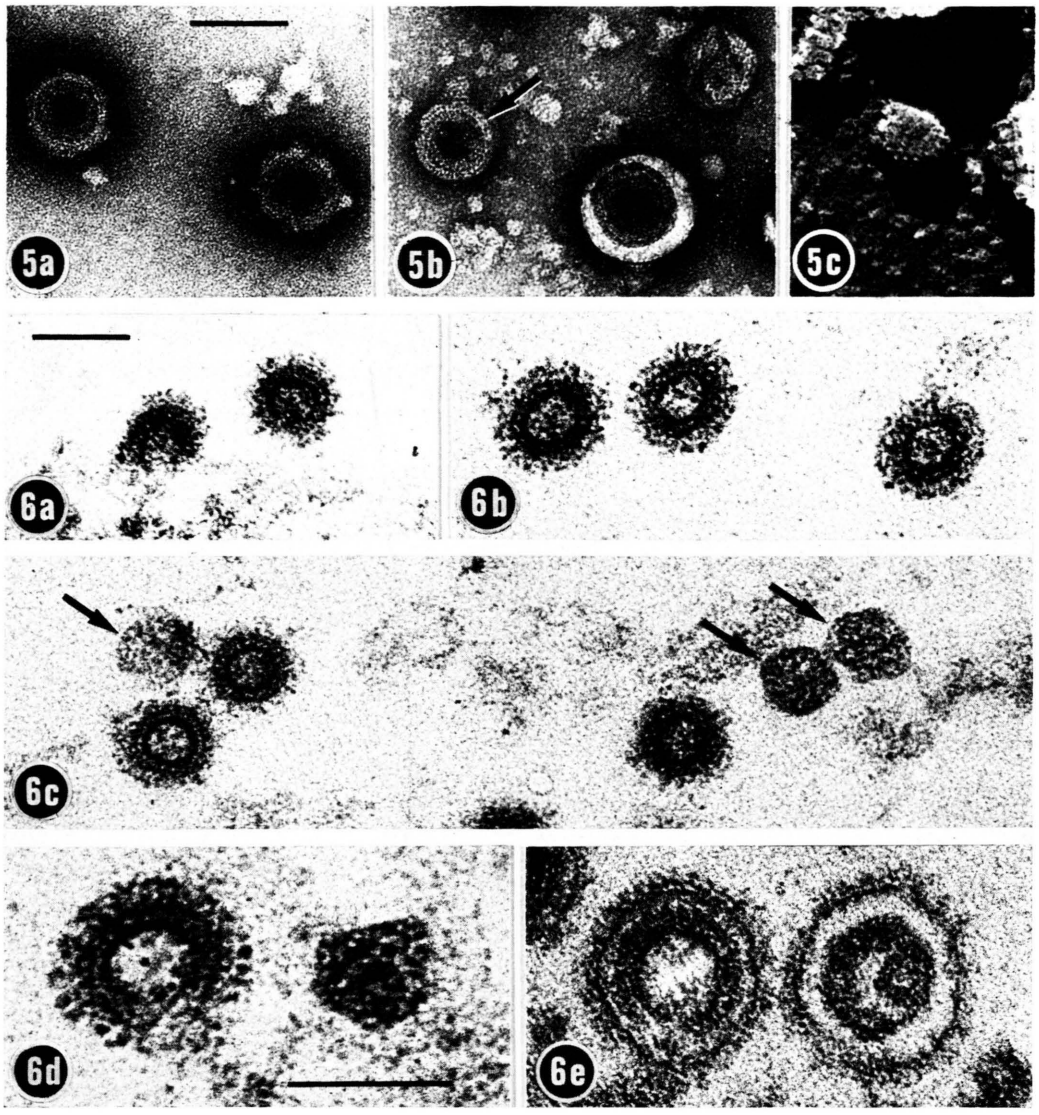


Fig. 5. FLV particles after 5 seconds pretreatment with 1.0% NP40 (a) and 0.1% NP40 (b and c). a) and b) negatively stained with UA, c) freeze dried and shadowed. The particle in c) and the particle indicated by the arrow in b) are rarely found envelope-free, native cores. Magnification: × 130 000. Bar represents 100 nm.
Fig. 6. Thin sections of budding (a) and free FLV particles (b–e). Virus-producing cells and free viruses in a–d) were fixed and dehydrated in the presence of 0.1% NP40. Free collapsed cores in c) are indicated by arrows. Magnification of a–c): × 130 000, of d) and e): × 220 000. Bars represent 100 nm.

core seemed to be "slipping" out of the envelope (Figs 4f and g).

At higher NP40 concentrations (1.0%) a new type of structure was obtained: particles which appear to contain fuzzy material replacing the envelope membrane (Fig. 5a and Table I). These particles always contained native cores. Similar particles were also obtained when treatment with 0.1% NP40 was extended to 5 or more minutes. Details of the fuzzy material remaining on native core particles after the more intensive detergent treatment are best seen in thin sectioned preparations. As shown in Fig. 6, this treatment removed the unit membrane of the cell and the virus but the cores of budding particles (Fig. 6a) and of free, native particles (Figs 6b – d) were still surrounded by fuzzy material which was

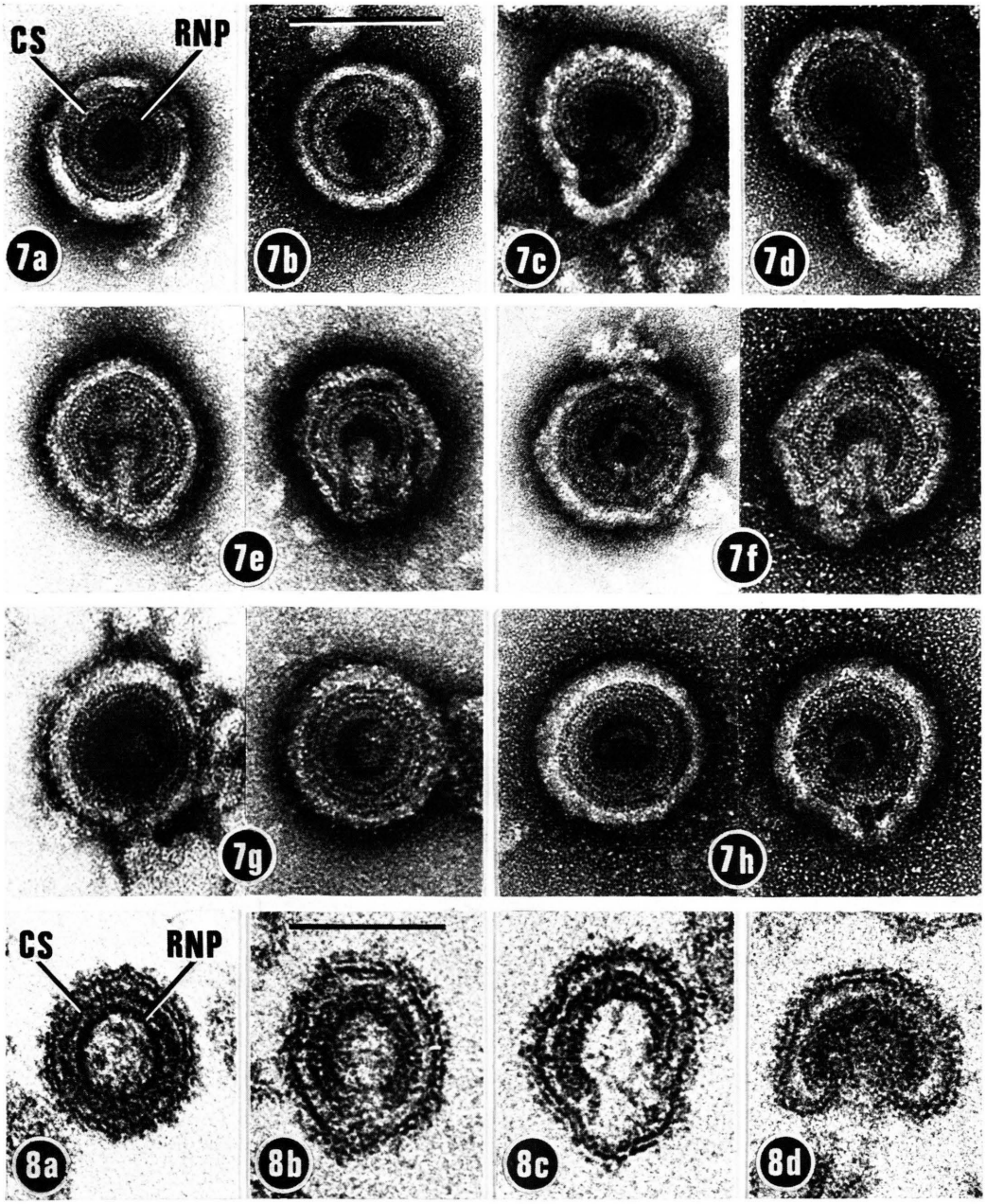


Fig. 7. FLV particles negatively stained with UA after 5 seconds pretreatment with 0.1% NP40. The particles show evaginations (c, d), invaginations (e, f) and inner vesicles (g, h). CS, core shell, RNP, ribonucleoprotein. Magnification: × 220 000. Bar represents 100 nm.

Fig. 8. Thin sections of FLV particles showing evaginations (b, c) and invagination (d). CS, core shell, RNP, ribonucleoprotein complex. Magnification: × 220 000. Bar represents 100 nm.

absent from collapsed cores (see arrows in Fig. 6c). As can best be observed in Figs 6b and d, this material seems to extend from the core to the area of the knobs of the virus envelope. These structures might also be responsible for the diffuse staining observed in the interspace between native core and envelope in particles not treated with NP40. Remarkably, they were never seen between collapsed

cores and envelope (Fig. 6e). Similar results as described in Table I were obtained with several different virus preparations.

Free native cores were only occasionally seen in the NP40 treated samples (Table I, arrow in Fig. 5b). After freeze drying and shadowing they revealed a hexagonal outline and a hexagonal arrangement of subunits. In addition, their shadow suggests the structure of an icosahedron (Fig. 5c).

An increase of native particles was also observed when preparations pretreated with NP40 were stained with PTA at pH 3.0 – 6.0. Further experiments showed that NP40 could be replaced by Sterox SE, Sterox SL and Brij 58 but not Brij 35. If the successive NP40-UA treatment was combined with fixation, the effect depended on whether the fixation was performed before or after incubation with NP40. Postfixation did not reduce the high percentage of particles with native cores whereas prefixation did.

For the experiments so far reported, virus preparations harvested after 72 h of culturing were used. To check whether virus harvested after a shorter period of cultivation behaves similarly, particles which were isolated 4 h after medium change of the cultures were investigated. As shown in Table II, again a striking increase of particles with native cores was obtained after NP40 treatment. This proportion was, however, not higher than in the normal harvest preparations (see also Table I).

Structure of the native core. In well preserved and stained cores obtained after short NP40 treatment, the following structural features were recognizable:

1) Two concentrically arranged interconnected lines representing the core shell (CS) [15], also described as outer shell [2] and intermediate layer [18].

2) An inner layer probably representing a wound up RNP strand which is assumed to be helicaly arranged [15]. This structure has also been designated as inner shell [2] or inner layer of the nucleoid [18].

Both structural elements are discernible in negative staining (Fig. 7a) and in thin sections (Fig. 8a). Examining a large number (over 300) of native cores in virus pretreated with NP40 and negatively stained it was found that many of them (25 – 30%) were not ring- but horseshoe-shaped (Fig. 7), suggesting that the core contains an opening. Similarly, of the low proportion of particles with native cores (71 out of 2010) in virus preparations not pretreated with detergent about 50% showed cores with horseshoe-like morphology, as determined by thin sectioning (Figs 1b and 8). In all cases the core shell as well as the RNP showed openings which matched each other. In the region of these openings the viral envelope was sometimes found to be evaginated (Figs 7c and d) or in-

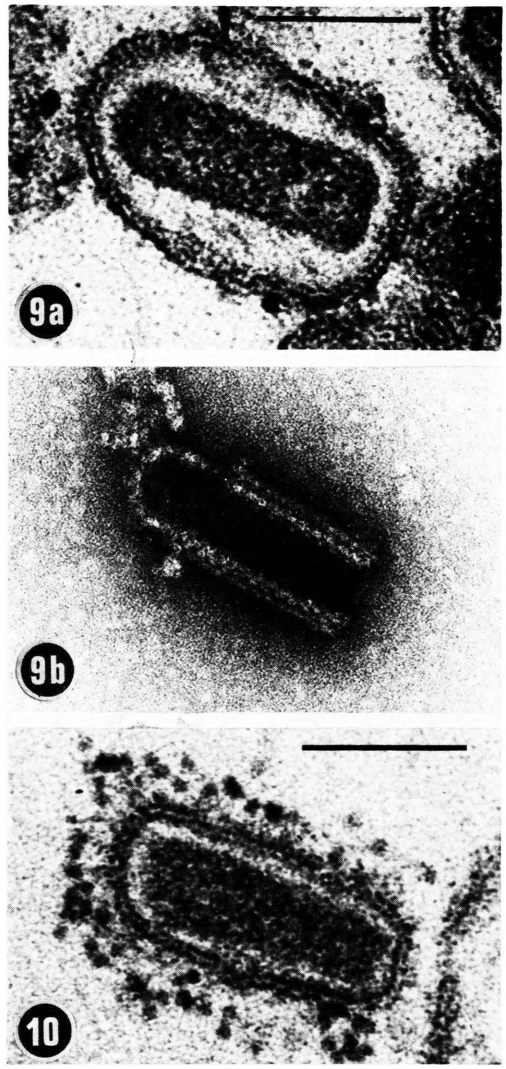


Fig. 9. Aberrant particles of FLV with tubular core a) thin sectioned, b) free tubular core after negative staining with UA. Magnification: × 220 000. Bar represents 100 nm.
Fig. 10. Aberrant FLV particle labelled with ferritin coupled anti gp71 antibody. Magnification: × 220 000. Bar represents 100 nm.

vaginated (Figs 7e and f). Depending on how far the stain penetrated and depending from which side the virus was viewed, the invaginated material appeared as a solid stalked knob (Fig. 7e), as a stalked vesicle (Fig. 7f), as a solid knob (Fig. 7g, see also Fig. 4a), or as a vesicle (Fig. 7h). Similar evaginations and invaginations were also found in thin sectioned material but much more rarely (Figs 8c and d). The fact that evaginations and invaginations were only rarely found in particles with native cores in thin sectioned materials suggests that they represent artifacts due to negative staining. These structures, however, are useful indicators of the opening in the core.

A few particles with tubular cores were also found. The cores (Figs 9a and b) were similar in structure and size (160/60 nm) to those of rhabdoviruses. However, in contrast to rhabdoviruses their envelope was not in close contact with the core. That they were indeed related to murine leukemia viruses is indicated by the observation that they could be tagged with ferritin-coupled antibodies to the gp 71 envelope antigen of FLV (Fig. 10). Occasionally, smaller particles of tubular type were also seen.

So far, very little is known about the structure of the innermost component, the RNP. In preparations of FLV we have occasionally found thread-like structures which seem to have helical symmetry and which we suspect represent released RNP (Fig. 11). Their incidence could be somewhat increased by treating core preparations [13] with acetic acid at pH 3.5. Attempts to obtain larger yields of pure RNP-like preparations were unsuccesful so far. At the moment, it cannot be excluded that the RNP-like structures represent products of self assembly of virus components formed after breakdown of the virus.

The inner coat of the envelope

In well preserved and well stained FLV particles a fine layer between the core shell and the viral membrane is recognizable as a further structural element (Fig. 12a, see also Fig. 2a). That this was not an artifact due to electron interference fringes [19], was indicated by the observation that the layer did not disappear in a focal series of pictures. In thin sections it is not visible as a distinct structure, but the inner line of the unit membrane appears to be thicker than the outer one (Fig. 12b, see also Figs 1c and d). In particles with collapsed cores this layer remains with the viral membrane (Fig. 12c, see also Fig. 2b) and in particles with several cores, the layer, whenever discernible, follows the

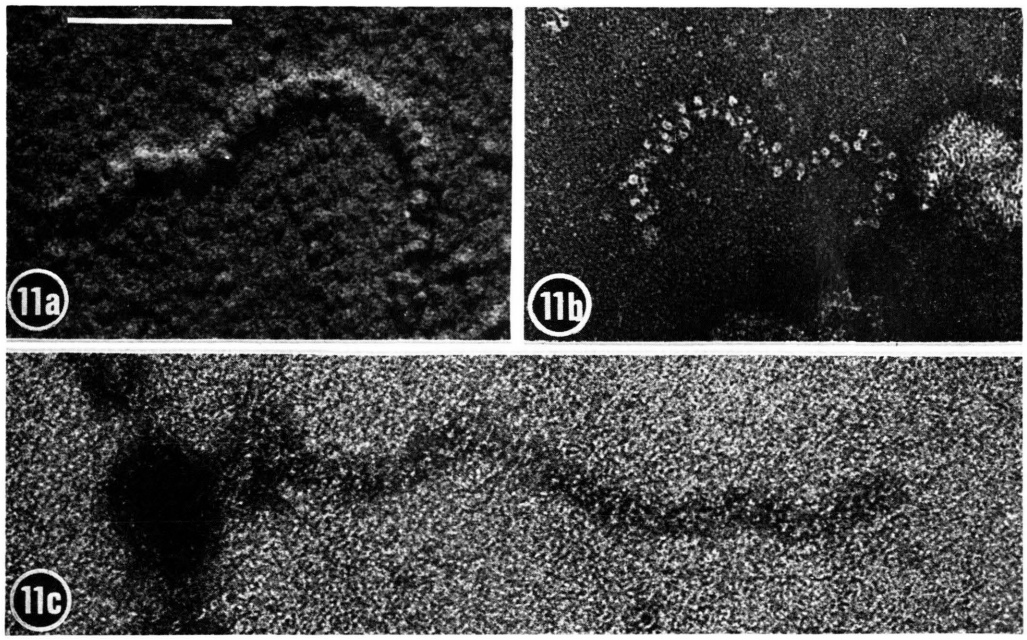


Fig. 11. Filamentous structures of helical symmetry probably representing RNP in untreated FLV preparations (a, b) and after slow degradation at pH 3.5 of cores prepared by the method of Lange *et al.* (1973) (c). a) Freeze dried and shadowed, b) and c) negatively stained with UA. Magnification: × 220 000. Bar represents 100 nm.

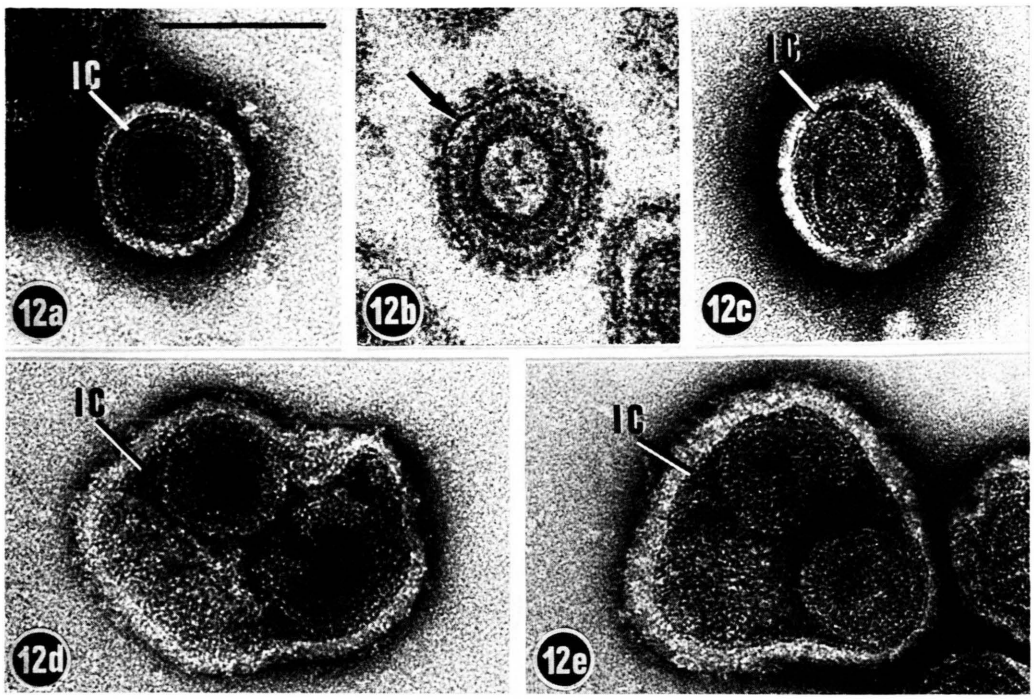


Fig. 12. Demonstration of the inner coat of the envelope (IC) of FLV. Particles pretreated with 0.1% NP40 and negatively stained with UA (a) and thin sectioned (b). Untreated particles stained with UA (c, d, e). Magnification: × 220 000. Bar represents 100 nm.

path of the viral envelope (Figs 12d and e). We propose for this new structure the term "inner coat of the envelope" or shortly "inner coat". Recently, we have discerned a similar structure in influenza viruses [20].

B. Other mammalian C-type viruses

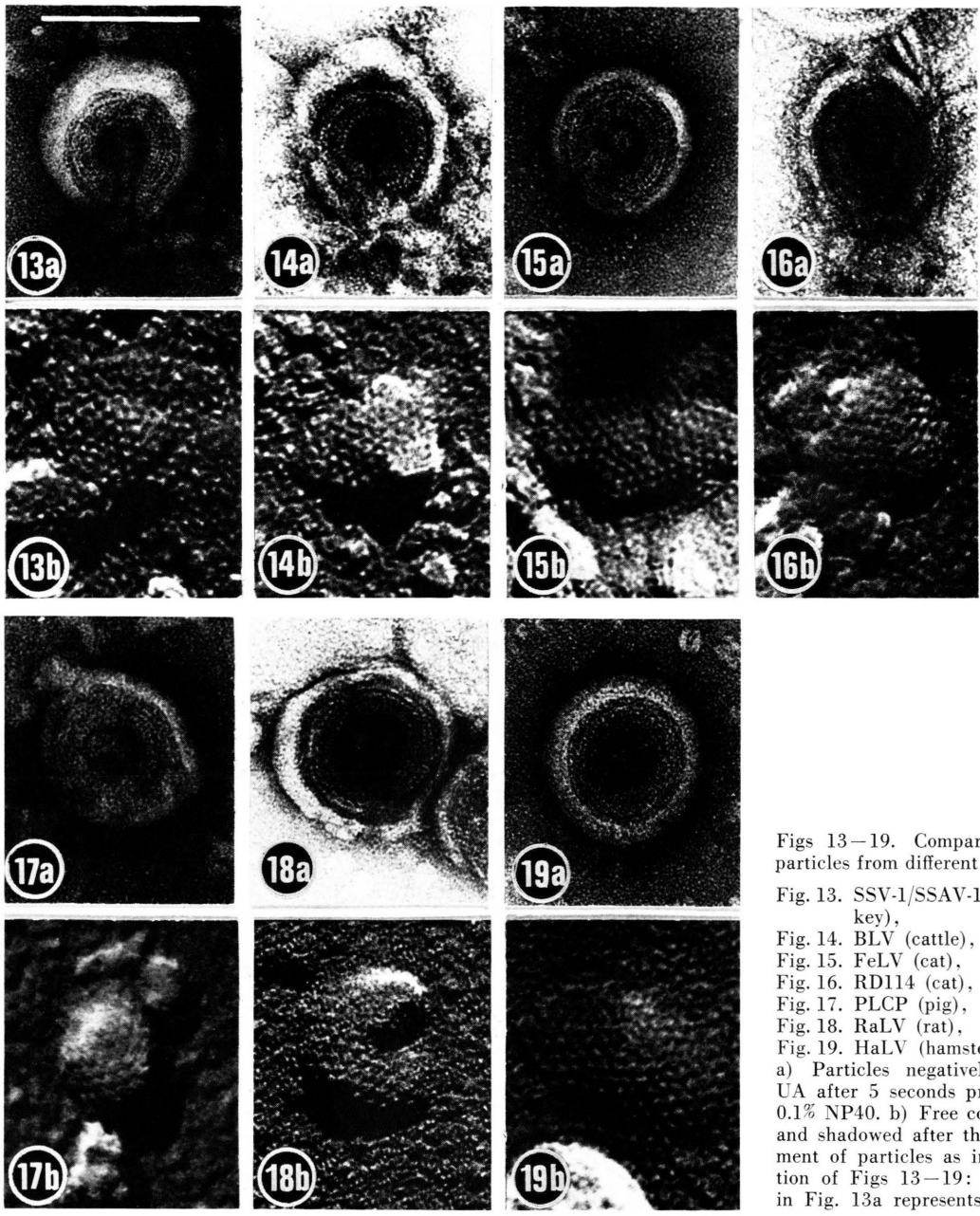
The fine structure of a variety of other mammalian C-type viruses was investigated in comparison to FLV. One series of preparations was pretreated with NP40 and negatively stained with UA in order to visualize the internal organization of the particles with native cores. In another series, detergent-treated preparations were freeze-dried and shadowed in order to visualize the surface of the liberated cores. No significant internal structural differences could be detected between FLV and C-type particles obtained from a woolly monkey (Fig. 13), cattle (Fig. 14), cat (Figs 15 and 16), pig (Fig. 17), rat (Fig. 18) and hamster (Fig. 19). The proportion of particles with an opening in the core or with an invaginated envelope was similar to the proportion of such particles in FLV preparations. Most of these virus strains have also been studied with regard to their envelope structure and were found to contain knob-like protrusions on their surface as found with murine viruses [15, 21]. The knobs, however, seem to be attached with variable strength depending on the virus strain and preparation. Thus, in the SSV-1/SSAV-1 woolly monkey virus only few particles were found to be covered with knobs even if the same isolation procedure was used as for FLV [5, 21]. Contrary to this, in the porcine virus, the knobs were found to be more firmly attached than in FLV [6].

Discussion

Preservation of the native core structure

Our findings with FLV showed that the proportion of particles with native (formerly called immature) cores can be considerably increased if the virus particles were gently pretreated with NP40 or other detergents before negative staining. This unexpected action of the detergent could be explained by the following possibilities:

(i) selective removal of particles with collapsed cores,
(ii) reconstitution of the native core structure or
(iii) preservation of the native structure.



Figs 13—19. Comparison of C-type particles from different mammals.

Fig. 13. SSV-1/SSAV-1 (woolly monkey),
Fig. 14. BLV (cattle),
Fig. 15. FeLV (cat),
Fig. 16. RD114 (cat),
Fig. 17. PLCP (pig),
Fig. 18. RaLV (rat),
Fig. 19. HaLV (hamster).
a) Particles negatively stained with UA after 5 seconds pretreatment with 0.1% NP40. b) Free cores, freeze-dried and shadowed after the same pretreatment of particles as in a). Magnification of Figs 13—19: × 220 000. Bar in Fig. 13a represents 100 nm.

As demonstrated in Tables I and II, a selective loss of a distinct form of particles due to the detergent treatment does not seem to occur. Reconstitution as an acting principle appears to be rather improbable considering the nonphysiological conditions employed as well as the complex structure of these viruses. That the native structure was preserved by the detergent treatment is supported by different observations. a) Small virus clusters, where a selective removal of one core type is unlikely to occur, revealed almost exclusively native core particles. b) The penetration time of UA into FLV particles was different for untreated (3 – 5 minutes), prefixed (20 – 30 seconds) and detergent-pretreated virus (3 – 5 seconds). This suggests that by quickly making the virus envelope permeable to the

stain aids in the preservation of the native core structure. The detergent in this sense acts as a sort of fixative. An intercalation of detergent molecules in the lipid bilayer without destruction of the membrane organization has been described earlier [22]. This interpretation agrees well with the finding that the stabilizing effect of the detergent treatment was not abolished by postfixation, whereas in prefixed virus preparations a stabilizing action of the detergent was no longer detectable.

No significant differences in the ratio of native to collapsed core particles were recognizable in virus preparations harvested in 72 h and 4 h intervals (Tables I and II). Hence, a transition of the majority of virus particles from a stable core to an unstable but still native core structure probably occurs shortly after termination of the budding process during which they all appear to be stable. The results obtained with the various preparation conditions indicate that concentrated FLV samples are composed of virus particles with different stabilities. 1) About 4 – 6% of the particles retain the stability of budding viruses, revealing the native structure independently of the preparation technique used. 2) At least 30 – 50% of the particles still possess the native structure but are labile and tend to collapse when prepared for electron microscopy. Most of these particles are preservable by the detergent treatment. 3) Finally, in a further fraction the cores may have collapsed before preparation for the electron microscope.

Structure of the native core

The shell of the native core and the RNP generally appear either ring-or horseshoe-shaped depending on the angle of observation. We suggest that the presumably filamentous RNP is spiraled into a beehive-like structure. The covering core shell which was already reported earlier to form an icosahedron [15] seems to lack one portion at the side where the opening of the RNP "beehive" is. This is strongly suggested by the high percentage of horseshoe-like cores as well as by the frequently found invaginations of the viral membrane. The question arose why the native core shell usually does not show a polygonal but a round shape. One possibility is that it is "blown up" by the closely apposed RNP like a leather soccer ball by its inner skin. Its polygonal appearance might also be smoothened by a superposition effect, as observed with other viral icosahedra after penetration of negative stain, *e. g.* with adenoviruses (Frank, unpublished results). The concept of the core shell as a polygonal structure is supported by the thin sectioned particle in Fig. 20. In this particular case the section was in the right plane and thin enough to avoid superposition effects. The picture shows additionally that

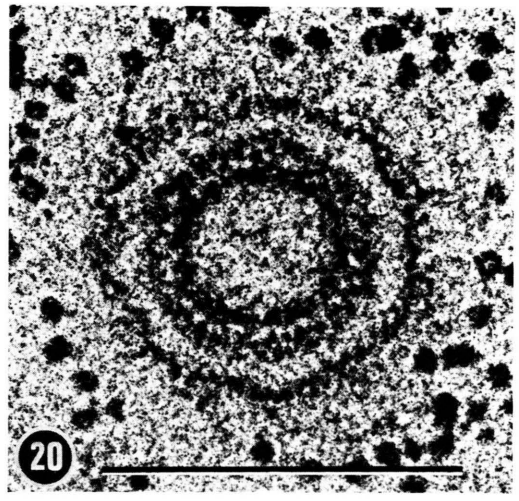


Fig. 20. FLV particle thin sectioned in a plane enabling the recognition of the hexagonal outline of the core shell. The particle shown stems from a preparation which was incubated with ferritin-labelled anti gp71 antibody and which was only slightly fixed [16]. The latter fact is probably also the reason why the envelope is only poorly discerned. Magnification: × 440 000. Bar represents 100 nm.

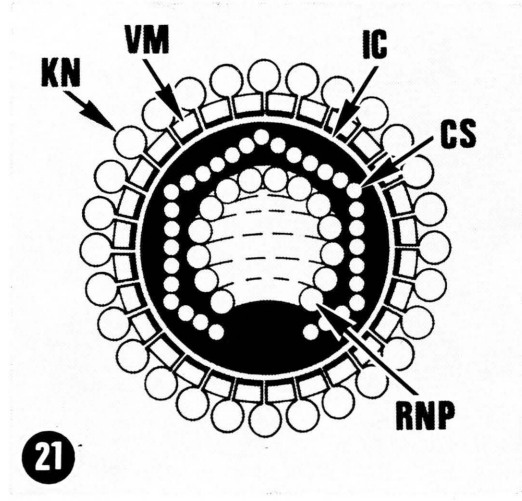


Fig. 21. Diagram of a model of mammalian C-type viruses. Abbreviations: KN, knobs; VM, viral membrane; IC, inner coat; CS, core shell; RNP, ribonucleoprotein.

the RNP strand is in close proximity to the core shell and may thus cause a smoothening of the polygonal outline of the latter.

The origin of the particles containing rhabdovirus-like cores is not yet clear. The most likely explanation is that they represent rare cases of an abnormal assembly like in polyheads of phages forming particles similar to rhabdoviruses. The finding that they contain the gp71 antigen of FLV on their surface supports this suggestion. However, this would also be the case if phenotypic mixing with a rhabdovirus-like agent would occur. So far, no indication for the presence of such a contaminating agent in the FLV-producing Eveline cell cultures has been obtained. Recently, we found very similar particles with aberrant, tubular cores also in thin sections of SSV-1/SSAV-1 particles produced by the marmoset cell line HF-SSV/Jü [10].

Transition from the native to the collapsed core form

An icosahedron which has an opening at one side is likely to be less stable than a closed icosahedron. This could account in part for the observed instability of a large proportion of FLV cores. Here, a stabilization of the icosahedral shell is possibly achieved by their internal RNP-"beehive". The changes observed after collapsing of the core seem to indicate that this results mainly from alterations of the latter structure: it becomes irregularly distributed and condensed in a smaller volume whereas the core shell still shows a regular arrangement of its subunits and its polygonal outline becomes generally more clearly recognizable. The latter could be due to the loss of the internal "pressure" by the intact RNP. The processes which lead to the observed disorganisation of the RNP are not yet understood. One might speculate that an enzyme like RNAse can penetrate through the opening of the core and become destructive for the RNP.

Another open question is, what the reason for the occurrence of particles with varying degrees of stability is. In this regard it is interesting that in particles where the viral envelopes were removed by a stronger NP40 treatment, native cores were obtained which were always surrounded by material which seemed to extend from the viral surface knobs to the viral interior and which might possess a stabilizing function of the viral structure. This is supported by the observation that it has never been found associated with collapsed cores and that the latter are much more easily liberated from whole particles by detergent treatment than native cores (see Table I and II). The possibility exists that this material correspond to the viral "gag" and "env" precursor molecules which have been suggested to be radially aligned during viral assembly at the budding sites and to be cleaved into the viral structural proteins [23]. In favor of this interpretation is the recent observation that an inverse relationship exists between the amount of particles with collapsed cores and the presence of the "gag"' precursor molecule p70 [24]. The difference in the stability of the various types of particles could thus be related to the degree of processing of precursor molecules.

Comparison with other mammalian C-type viruses

Analogous morphological structures as described for FLV were also recognizable in other mammalian C-type viruses, especially after gentle NP40 treatment as described above. The demonstration of these structures is thus of high diagnostic value for the detection of these viruses. The technique also offers the advantage that it is rapid and that only small amounts of virus material are necessary.

Mammalian C-type viruses of different species are very similar in structure, but are clearly distinguishable from avian oncornaviruses. For reasons which are not clear, among the latter, free particles with native cores are only very rarely found [25] and cannot be revealed by NP40 treatment either (Frank, unpublished results). In addition, the hexagonal structure of avian virus cores has not yet been definitely demonstrated by freeze drying and shadowing (Frank, unpublished results).

Despite their similarities, mammalian viruses vary considerably with regard to the stability of their core. This probably depends on the virus and host cell system used. Of exceptional stability are the cores of the ESP-1 virus [3], FLV produced by a particular cell line [4, 26] and the cores of endogenous pig virus [6]. In addition, differences were observed in the stability of attachment of the knobs (containing the envelope antigen gp71) to the viral surface. In some cases such as with the SSV-1/SSAV-1 virus most of the envelope antigen was found in "soluble" form in the tissue culture supernatant [10]. Since the envelope antigen serves as target in viral neutralisation and complement de-

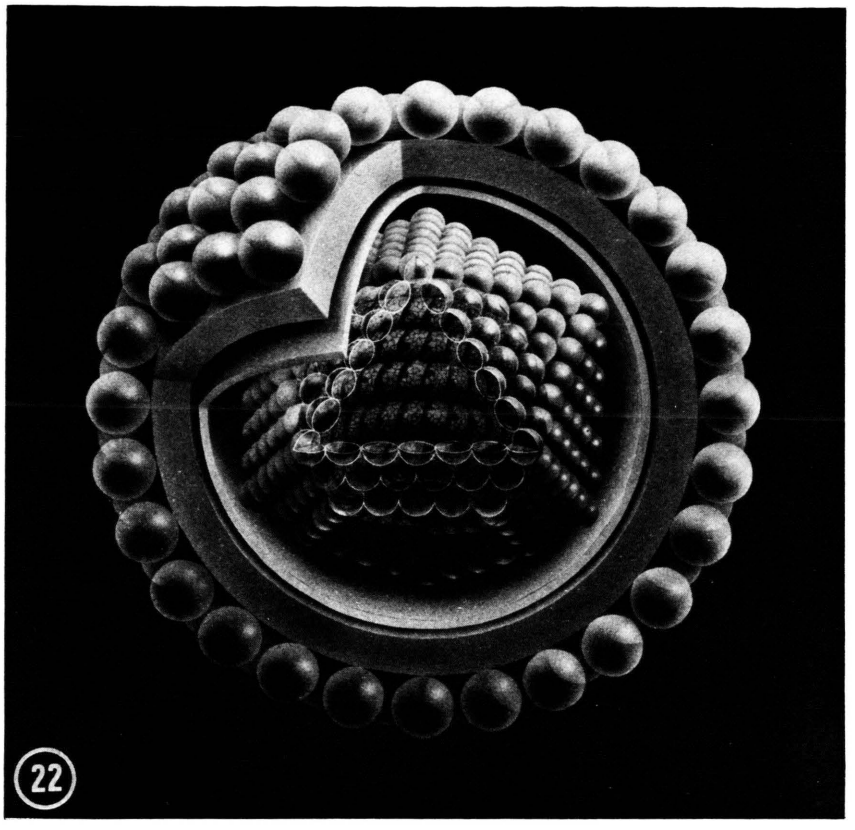


Fig. 22.
Three dimensional model of mammalian C-type viruses. The connections between knobs and inner coat are not drawn here. The removal of the front portion of the icosahedron permits visualization of the ribonucleoprotein.

pendent cytotoxic reactions it is likely that its easy release has immunological consequences in the host animal.

Model for the structure of mammalian C-type viruses

Several models for the structure of C-type viruses have been proposed previously [2, 15, 25, 27 – 29]. These models vary more in the interpretation of the structures observed than in the description of the different structures. All of these models have the drawback that they were based on the evaluation of a very low percentage of well preserved particles in the virus preparations used.

Because of the isolation of a mouse cell line producing large amounts of Friend leukemia virus, improvement of virus purification procedures, and the detergent method described here we were able to obtain homogenous virus preparations containing large amount of well preserved particles. This enabled us to do the studies described and to construct a more detailed and somewhat modified version of the model of C-type virus structure proposed earlier [15]. A schematic drawing of the model is shown in Fig. 21 and a three dimensional artist's view in Fig. 22.

The virus envelope contains the knobs known to represent the virus glycoprotein [21, 30]. These knobs are anchored in the viral membrane. The presence of knobs in murine leukemia viruses has already been proposed earlier [15]. The envelope also contains another structure which we have described here for the first time, designated as the inner coat. It is not clear whether this structure is a layer separate from the viral membrane as suggested in negatively stained preparations or whether it represents a part of the viral membrane as it appears in thin sections. Between the envelope and the core lies the material which is seen after extensive detergent treatment of the virus and which sems to connect the knobs with the inner coat or the core.

The finding of an icosahedral structure of the core shell confirms our previous studies [13, 15]. In addition, we have now found that the core shell contains an opening. As suggested by the structure of the core in budding viruses, this opening repre-

sents the site of the shell which was synthesized last during the process of budding. We would like to suggest that the innermost component, the ribonucleoprotein represents a wound up thread forming a beehive-like structure. Its opening is closely apposed to the opening in the core shell.

The location of the virus structural proteins within the virus particle has been discussed in separate communications [23, 31, 32].

Our studies with detergent treated C-type virus particles of mammalian species other than the mouse suggest that the model proposed is generally applicable to all mammalian C-type viruses.

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