Isolation and Properties of the DNA of African Swine Fever (ASF) Virus

By L. ENJUANES, A. L. CARRASCOSA AND E. VIÑUELA

Centro de Biologia Molecular (C.S.I.C.-U.A.M.),
Velázquez 144, Madrid-6, Spain

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SUMMARY

African swine fever (ASF) virus was grown either in swine macrophages or in VERO cells and purified free of cell DNA. Virus DNA was isolated from virions as a molecule with a sedimentation coefficient of 60S and a contour of $58 \pm 3 \mu m$. These two values give a mol. wt. of $102 \pm 5 \times 10^6$ and $107 \pm 5 \times 10^6$, respectively, for the genome of ASF virus. Denatured DNA fragments from ASF virus re-associate with a $C_{ot}$ value of $0.60 \pm 0.05$ MS, which compared with the corresponding value for T4 DNA gives for the molecular mass of ASF virus DNA a value of $102 \pm 8 \times 10^6$ daltons. Only virus DNA is synthesized in ASF virus-infected swine macrophages.

INTRODUCTION

African swine fever (ASF) virus produces in domestic swine either an acute, contagious and fatal disease (Hess, 1971) or a chronic infection with some features common to other infections produced by slow viruses (Coggins, 1974).

Although ASF virus has been tentatively classified with the genus Iridovirus (Fenner et al. 1974), the molecular properties of the virion, of its double stranded DNA (Haag, Larenaudie & Gonzalvo, 1965; Addinger et al. 1966; Plowright, Brown & Parker, 1966; Moulton & Coggins, 1968) and the other structural components, as well as the biochemistry of the infection, are largely unknown.

We have determined the growth curve of ASF virus and the kinetics of DNA synthesis in infected swine macrophages, and the virus, grown either in swine macrophages or in VERO cells, has been purified free of cell DNA. This has enabled us to obtain homogeneous virus DNA and, in turn, to carry out determinations of the mol. wt. of the virus genome by zone centrifugation, electron microscopy and reassociation kinetics. These methods give similar values for the mol. wt. of ASF virus DNA, of about $100 \times 10^6$. We also show that the only DNA synthesized in ASF virus-infected macrophages is the virus DNA.

METHODS

Viruses, bacteria and cells. The source and the assay of ASF virus propagated about 80 times in swine macrophages and the virus adapted to VERO have been described by Enjuanes et al. (1976; see previous paper).

Bacteriophage φ29 and T4 were grown on Bacillus amyloliquefaciens and Escherichia coli as indicated by Méndez et al. (1971) and Cowie, Avery & Champe (1971), respectively.
Swine macrophages for virus production were obtained by adding about $1 \times 10^8$ purified leukocytes (Enjuanes et al. 1976, previous paper) per roller bottle of 750 cm$^3$ in 100 ml of Dulbecco’s minimal essential medium (DME) with 40% swine serum. After 3 days at 0.5 rev/min and 37 °C, the monocytes attached to the glass and differentiated to macrophages. After removal of the medium with unattached cells, the culture of macrophages was infected with ASF virus as indicated below.

VERO cells, obtained from the American Type Culture Collection, Rockville, Md., U.S.A., were grown at 37 °C in plastic plates (Falcon) or roller bottles with DME containing 10% calf serum. Primary and secondary cultures of pig kidney cells were obtained by standard techniques (Holper, 1969) and grown under the same conditions as VERO cells.

Infection and radioactive labelling. Macrophage cultures in 35 mm Petri dishes ($1 \times 10^6$ cells/plate) were prepared as described elsewhere (Enjuanes et al. 1976, previous paper). After removal of the medium, 0.3 ml of ASF virus in PBS ($1.5 \times 10^7$ H.A.D.U.) were added to each plate and the virus adsorption was allowed to proceed for 1 h at 37 °C. The inoculum was removed, the cells were washed three times with 2 ml of DME per washing, and 2 ml of DME with 40% swine serum dialysed against PBS and $0.02$ ml of $^3$H-thymidine ($0.2$ mm; 1 mCi/ml) were added to each plate. A series of control plates were mock infected in a similar way. At different times after infection, the cells were detached from the plates with a rubber policeman, resuspended in the culture medium, sedimented by low speed centrifugation and the pellet was resuspended in 2 ml of PBS. Acid-insoluble radioactivity was determined in 0.01 ml samples of the resuspended sediment and in the clarified culture medium. The samples were diluted with 1 ml of PBS, precipitated with 5% (w/w) cold trichloroacetic acid and filtered through glass-fibre filters. The filters were washed and dried and the radioactivity determined in a Packard TriCarb liquid scintillation spectrometer.

Cultures of swine macrophages in roller bottles, prepared as indicated before, were infected with 4 ml of ASF virus in phosphate-buffered saline (PBS) at a multiplicity of 0.2 to 0.4 haemadsorption units (H.A.D.U.) per attached cell, or mock infected. After an incubation of 2 h, 100 ml of DME containing 40% swine serum dialysed against PBS and 250 to 1000 µCi of $^3$H-thymidine (1 mCi/ml; 20 Ci/mmol) were added per bottle and the incubation continued for about 3 days, when extensive c.p.e. and cell detachment in the infected cultures were clear.

Pre-confluent cultures of VERO in roller bottles were infected with 4 ml of adapted virus in PBS at an m.o.i. of about 10 H.A.D.U./cell, or mock infected. After 2 h at 37 °C, 80 ml of DME containing 1% dialysed calf serum and 200 µCi of $^3$H-thymidine (1 mCi/ml; 20 Ci/mmol) were added per bottle and the incubation continued for 2 days, when most of the cells in the infected cultures were detached from the glass.

Virus purification

From swine macrophages. About 500 ml of infected culture medium with a virus titre of about $1 \times 10^8$ H.A.D.U./ml were clarified by low speed centrifugation. The pellet was resuspended in a small vol. of the supernatant and sonicated for 1 min at 4 °C at the maximal output of a MSE ultrasonic disintegrator. The suspension was centrifuged and the supernatant fluid added to the clarified culture medium. The virus was concentrated with 2% (w/v) polyethylene glycol 6000 in PBS, by gently stirring for 2 h at 4 °C and centrifuging at 4 °C in a Sorvall GS-3 rotor at 7500 rev/min for 20 min. The pellet was resuspended in 5 to 10 ml of PBS and stored at 4 °C until the next step of purification was carried out. The virus stored under these conditions did not lose infectivity for several months.

The virus suspension was treated at 37 °C with pancreatic RNase (2 µg/ml) for 15 min in
the presence of 5 mM-EDTA, DNase (2 µg/ml) for 15 min in the presence of 20 mM-MgCl₂ and finally, with trypsin (300 µg/ml) for 2-5 h. The suspension was clarified by low speed centrifugation and the supernatant fluid was centrifuged at 4 °C through three layers of 50, 35 and 25 % (w/v) sucrose in PBS (7 ml/layer) for 90 min at 20000 rev/min in a Beckman SW 25.1 rotor. The gradient was fractionated and the radioactivity, extinction and infectivity was determined in each fraction. The density of representative fractions of the gradient was determined picnometrically.

From VERO cells. About 1-5 l of infected culture medium were clarified by low speed centrifugation and the cells still attached to the glass were recovered by washing with 0.6 mM-EDTA in PBS and centrifugation. The two cell pellets were resuspended in 0.01 M-NaCl, 0.02 M-tris (pH 7.4) and 1.5 mM-MgCl₂ (RSB) and the cell concentration was adjusted to 3 x 10⁷ cells/ml. The cell suspension was cooled at 4 °C for 20 min and homogenized in a Dounce by 10 strokes. Immediately after homogenization sucrose was added to a final concentration of 0.25 M and the suspension was centrifuged at 4 °C and 2500 rev/min for 10 min in a Sorvall SS-34 rotor. The supernatant fluid was sonicated for 30 s at 4 °C at the maximal output of a MSE ultrasonic disintegrator, clarified by low speed centrifugation and treated with DNase (10 µg/ml) for 30 min at 37 °C in the presence of 5 mM-MgCl₂.

The virus suspension (16 ml) was layered on the top of 20 ml of a discontinuous gradient of sucrose formed by three layers with equal volumes of 50, 35 and 25 % (w/v) sucrose in 0.2 x PBS, and centrifuged for 90 min at 20000 rev/min and 4 °C in a Beckman SW 27.1 rotor. The gradient was fractionated and analysed as indicated before.

DNA extraction. DNA was released from purified ASF virus by lysis in a buffer containing 0.01 M-tris (pH 8.0), 0.01 M-EDTA, 0.01 M-NaCl and 0.5 % (w/v) SDS. The clear lysate was treated for 2 h at 37 °C with 50 µg/ml of proteinase K (Gross-Bellard, Dudet & Chambon, 1973) and deproteinized with phenol. The aqueous phase was dialysed against 1 x SSC (0.15 M-NaCl and 0.015 M-sodium citrate) for 4 h at room temperature and finally against 0.1 x SSC till the extinction at 260 nm in the dialysate was < 0.01. The recovery of DNA was 30 to 50 %.

Linear DNA from phages φ29 and T4 were prepared as described previously (Ortínez et al. 1971; Cowie et al. 1971). DNA from either ASF virus-infected swine macrophages or from uninfected primary pig kidney cultures, was extracted according to Gross-Bellard et al. (1973).

DNA concentrations were determined either spectrophotometrically by assuming that a solution of 1 mg/ml of DNA in 1 x sac has an E₂₆₀ = 20, or by the diphenylamine method (Burton, 1956). For renaturation studies of radioactive DNA, the concentrations were determined from the specific radioactivity.

Equilibrium centrifugation. ASF virus (0.1 ml), labelled with ³H-thymidine, was lysed in a buffer containing 0.05 M-tris (pH 7.8), 0.01 M-EDTA, 0.1 M-NaCl, 0.5 % (w/v) SDS and 2 % (v/v) sarkosyl. The lysate was digested for 12 h at 37 °C with 100 µg/ml of proteinase K, mixed with ¹⁴C-DNA of phage φ29 and diluted to 8 ml with a solution of CsCl in 0.01 M-tris (pH 7.8), 1 mM-EDTA, to a final density of 1.702 g/ml. The sample was centrifuged for 48 h at 20 °C and 39000 rev/min in a Beckman Ti-50 rotor. Fractions of 0.2 ml were collected and the radioactivity was determined as described before. The density of selected fractions of the gradient was determined picnometrically.

Sedimentation rate in sucrose gradients. ASF virus labelled with ³H-thymidine was treated as indicated before to release DNA and digest the virus protein with proteinase K. The sample was mixed with ¹⁴C-DNA of phage T4 and the mixture (0.1 to 0.2 ml) layered on to 4.8 ml of a linear 5 to 20 % (w/v) sucrose gradient in 0.05 M-phosphate (pH 7.6), 0.1 M-
NaCl (Burgi & Hershey, 1963). Sedimentation was carried out for 3.5 h at 25 °C at 25000 rev/min in a Beckman SW 50.1 rotor. Fractions were collected from the top with an ISCO model 83 fractionator operating at a constant flow, and the radioactive DNA in each fraction was analysed as indicated before.

Electron microscopy. A mixture of DNA molecules of ASF virus and phage φ29 prepared as indicated before were co-spread for electron microscopy according to Kleinschmidt (1968) and stained according to Gordon & Kleinschmidt (1968). The molecules were examined in a JEOL 100B electron microscope calibrated with a carbon grating replica (Fullam Inc.). In each composition obtained from the projection of one or more plates, one ASF virus DNA molecule and one φ29 DNA molecule were measured.

DNA reassociation kinetics. Reassociation of denatured radioactive DNA was followed by determining at different times the fraction resistant to S1 nuclease (Ando, 1966; Vogt, 1973; Kacian & Spiegelman, 1974).

Mixtures of radioactive and non radioactive DNAs were prepared and their specific radioactivity carefully determined. The DNA was fragmented according to Petterson et al. (1973) in 0.2 M-NaOH and 0.05% (w/v) SDS, followed by neutralization with 5 M-NaH2PO4 and extensive dialysis against 1 × SSC containing 0.05% (w/v) SDS. The DNA concentration in the dialysed samples was determined from the corresponding specific radioactivity.

The samples were heated for 10 min in a bath of boiling water and cooled in ice, covered by a layer of mineral oil and incubated at 60 °C. At different times two samples were taken and diluted tenfold in 0.03 M-sodium acetate (pH 4.6), 0.05 M-NaCl, 1 mM-ZnSO4 and 5% (v/v) glycerol, containing 20 µg/ml of heat-denatured calf thymus DNA (S1 buffer). One sample was treated 60 min at 37 °C with nuclease S1 (2.4 µg/ml) and the other samples in the same conditions omitting the nuclease. Under these conditions more than 98% of the denatured DNA was sensitive to the enzyme, whereas native DNA was fully resistant. The incubation was stopped by cooling in ice and addition of 25 µg of calf thymus DNA and 5% (w/v) cold trichloroacetic acid. The radioactivity in the precipitate was determined as indicated before. The percentage renaturation was determined by dividing the counts obtained after nuclease treatment by those in the sample not treated with the enzyme.

Reagents. 3H-thymidine (20 Ci/mmol) and 2-14C-thymidine (60 mCi/mmol) were obtained from the Radiochemical Centre, Amersham. Polyethyleneglycol 6000 (mol. wt. 6000 to 7500) was obtained from Pharmacia. Pancreatic ribonuclease (EC 2.7.7.16), deoxyribonuclease I (EC 3.1.4.5) and trypsin (EC 3.4.4.4) were purchased from Worthington. Aspergillus nuclease S1 was purified from amylase powder from Sigma, according to Vogt (1973).

RESULTS

Multiplication of ASF virus

The multiplication of ASF virus in swine macrophages was followed at different times after infection by studying the appearance of total and extracellular virus and the incorporation of 3H-thymidine into acid-insoluble material (Fig. 1). The appearance of progeny virus started at about 10 h p.i. and it reached its maximal value at about 24 h. Uninfected macrophages showed a low incorporation of radioactivity, as expected for non-dividing cells. 3H-thymidine incorporation in macrophages infected by ASF virus started at about 4 h p.i. before the appearance of intracellular virus and continued increasing till about 10 h, before most of the infective virus was formed. During the first hours of the infection most of the acid-insoluble radioactive material was intracellular; later, acid-insoluble radioactive material appeared in the extracellular medium.
DNA of ASF virus

Fig. 1. Time-course of \(^{3}H\)-thymidine incorporation and virus production in swine macrophages infected with ASF virus: \(\bullet-\bullet\): total and \(\bigcirc-\bigcirc\), acid-insoluble radioactivity in infected cultures; \(\blacksquare-\blacksquare\), total acid-insoluble radioactivity in uninfected cultures; \(\Delta-\Delta\), total and \(\bigtriangleup-\bigtriangleup\) extracellular virus.

Purification of ASF virus

From swine macrophages

To obtain enough purified virus DNA for physical studies, swine macrophages were cultured in roller bottles as indicated in Methods. The pool of extracellular and cell-associated virus was concentrated with polyethyleneglycol and treated with RNAse, DNase and trypsin. Fig. 2 shows the distribution of radioactivity and infectivity after sedimentation through a step gradient of sucrose. The heavier infectious material (band A) had a density of 1.20 g/ml and contained 20\% of the input infectious virus, whereas the lighter component (band B) had a density of 1.15 g/ml and contained 60\% of the virus in the sample. Both bands of infectivity coincided with peaks of radioactivity, although the specific infectivity relative to radioactivity was about twice as large for the lighter material than for the heavier one. The radioactive material under each peak was DNA, because after phenol treatment, it was sensitive to pancreatic DNase and, as will be shown later, the labelled DNA was only virus DNA. The DNA under either band had the same sedimentation rate in sucrose, buoyant density in CsCl and molecular length as determined by electron microscopy. Table I shows a summary of the purification of ASF virus grown in macrophages.

From VERO cells

ASF virus associated with infected cells was partially purified, after removal of the nuclei, by sedimentation through a sucrose step gradient (Fig. 3). Most of the infectivity banded at a density of 1.21 g/ml, coincident with a maximum of radioactive material (band A). A lighter component (band B) also contained some infectious material but much less radioactivity.

After phenol extraction, the radioactive material under peak A was fully sensitive to
DNase and had a sedimentation rate similar to that of the DNA isolated from virus grown in swine macrophages.

**Sedimentation rate of ASF virus DNA**

Fig. 4 shows the sedimentation rates of ³H-DNA from ASF virus grown either in swine macrophages (band A in Fig. 2) or in VERO cells (band A in Fig. 3), compared with T4 ¹⁴C-DNA. In both cases the ³H-DNA sedimented slightly slower than T4 DNA. Similar results were obtained with the DNA isolated from band B in Fig. 2.

By using the relationship in Burgi & Hershey (1963) and taking $s_{0,0}^0 = 62 \pm 0.7$ as the sedimentation coefficient of T4 DNA (Gray & Hearst, 1968), the corresponding value for the DNA of ASF virus was 60S. From this value and the empirical relationship between sedimentation coefficient and mol. wt. derived by Freifelder (1970), the mol. wt. of ASF virus DNA is $1.02 \pm 5 \times 10^8$.

**Density of ASF virus in neutral CsCl**

The densities in CsCl of ASF virus DNA from bands A or B in Fig. 2 were determined in the preparative ultracentrifuge by using φ29 DNA as an internal marker (Borst 1971). In both cases a density equal to $1.700 \pm 0.003$ g/ml was obtained. From this value, the fraction of (G+C) in ASF virus DNA is 41% (Schildkraut, Marmur & Doty, 1962) and the Tm, 86 °C (Marmur & Doty, 1962).

**Contour length of ASF virus DNA**

ASF virus grown in swine macrophages was purified, lysed and the DNA prepared as described in Methods. DNA from either band A or B (Fig. 2) was co-spread separately with phage φ29 DNA and examined under the electron microscope. In either case two clearly different groups of DNA molecules were obtained, one with the same length of φ29 DNA...
Table 1. Purification of ASF virus

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Infectivity (H.A.D.U. × 10⁻⁹)</th>
<th>Radioactivity (ct/min × 10⁻⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial*</td>
<td>2.5</td>
<td>21.2</td>
</tr>
<tr>
<td>Polyethylene glycol</td>
<td>1.8</td>
<td>12.2</td>
</tr>
<tr>
<td>Enzyme treatment</td>
<td>2.7</td>
<td>6.5</td>
</tr>
<tr>
<td>Sucrose gradient (peaks A+B)</td>
<td>1.7</td>
<td>1.7</td>
</tr>
</tbody>
</table>

* Starting with 250 ml of infected culture.

Fig. 3. Equilibrium centrifugation of ³H-thymidine-labelled ASF virus grown in VERO cells. Virus particles associated with ASF virus-infected VERO cells were centrifuged in a step gradient of sucrose as indicated in the legend to Fig. 2. •, virus titre; ○—○, acid insoluble radioactivity; □—□, density.

and the other with a length of 58 ± 3 μm. Fig. 5 shows the mol. wt. distribution of ASF virus DNA molecules isolated from band A or B in Fig. 2, and Fig. 6 shows a typical picture of an ASF virus DNA molecule. Assuming for φ29 DNA a contour length of 6.7 ± 0.2 μm (A. Talavera, personal communication), the mol. wt. of ASF virus DNA is 10⁷ ± 5 × 10⁶.

Reassociation kinetics of ASF virus DNA

Fragmented and denatured DNA preparations were allowed to reanneal in 1 × SSC with 0.05% SDS at a temperature close to 25 °C below their Tm. The renaturation of ASF virus DNA fragments was compared with that of T4 DNA of the same size and in the same salt conditions.

Fig. 7(a) shows representative kinetics of reannealing plotted semilogarithmically as a function of Cot. The extent and the profile of the reassociation observed strongly suggests that we are dealing with DNA of a single component.

Fig. 7(b) is a reciprocal plot for the initial kinetics of the experiment shown in Fig. 7(a). From the linearity of the experimental points and the extrapolation to 1.0 there was no
Fig. 4. Sedimentation rate in a sucrose gradient of $^3$H-thymidine-labelled ASF virus DNA produced in (a) swine macrophages or (b) VERO cells. Purified ASF virus $^3$H-DNA (●-●) co-sedimented with T4 phage $^{14}$C-DNA (○-○). Direction of sedimentation is to the left.

Fig. 5. Frequency distribution of mol. wt. of DNA molecules from purified ASF virus. The distribution of mol. wt. of the DNA from (a) peak A and (b) peak B in Fig. 2 is shown.

evidence for rapidly renaturing components in ASF virus DNA, within the limits of detection of the assay with the nuclease S1.

ASF virus DNA renatures slightly faster than T4 DNA. The $C_{ot}$ values obtained for half renaturation of ASF virus and T4 DNA were $0.60 \pm 0.05$ and $0.65 \pm 0.05$ ms, respectively. From these values and the mol. wt. of T4 DNA (Schmid & Hearst, 1969; Freifelder, 1970), the mol. wt. of ASF DNA was calculated to be $102 \pm 8 \times 10^6$ (Britten & Kohne, 1968; Gillis, De Ley & De Cleene, 1970).
DNA of ASF virus

Fig. 6. Electron micrograph of a typical ASF virus DNA molecule. The molecule has a length of 58 μm.

Nature of the DNA synthesized in swine macrophages infected with ASF virus

To study whether the DNA synthesized in macrophages infected with ASF virus (Fig. 1) was exclusively virus DNA or whether there was also a significant synthesis of cellular DNA, the kinetics of reassociation of 3H-DNA isolated from either ASF virus-infected swine macrophages or from uninfected primary cultures of swine kidney cells were compared. Fig. 8 shows the second order rate plots of the initial renaturation of both fragmented DNAs. In the case of the DNA isolated from infected cells, 60% or more renatures as a species with...
a single component, whereas in the case of the uninfected cells the DNA shows at least two components, a minor one reannealing rapidly and a major one reannealing very slowly.

To ascertain the nature of the DNA isolated from infected macrophages, which reanneals in the beginning of the reaction, several annealing reactions containing the same amount of $^3$H-DNA from infected cells and different amounts of non-radioactive virus DNA isolated from purified virions, were carried out.

By addition of a concentration $C_i$ of homologous non-radioactive DNA to a reannealing
Fig. 9. (a) Reannealing of \(^3\)H-DNA isolated from ASF virus-infected swine macrophages in the presence of increasing amounts of non-radioactive virus DNA: \(\bullet-\bullet\), 0; \(\Delta-\Delta\), \(C_i\); \(\square-\square\), \(2C_i\) and \(\circ-\circ\), \(4C_i\). (b) The \(t_i\) values obtained in (a) were plotted against the relative amounts of non-radioactive virus DNA added to the annealing mixture.

mixture containing an unknown concentration, \(C_v\), of radioactive virus DNA, the equation of Britten & Kohne (1968) becomes

\[
\frac{C_t + C_v}{C} = 1 + K_v(C_t + C_v)t
\]

where \(C\) = concentration of unpaired virus DNA strands; \(C_t + C_v\) = total concentration of virus DNA; \(K_v\) = rate constant for virus DNA, and \(t\) = time.

If all the radioactive DNA in the DNA sample isolated from infected macrophages is virus DNA, when half radioactive DNA has renatured at \(t = (t_i)_i\), after addition of \(C_i\) non-radioactive virus DNA,

\[
C_i = \frac{1}{K_v} \cdot \frac{1}{(t_i)_i} - C_v.
\]

Therefore, a plot of \(C_i\) versus \((1/(t_i)_i\) should give a straight line.

Fig. 9 shows the result of such an experiment. In the absence of non-labelled virus DNA, the radioactive DNA isolated from ASF virus-infected macrophages renatures with a \(t_i = 55773\) s (Fig. 9a). The linearity of the experimental points in Fig. 9(b) allows the determination of the values of \(K_v\) and \(C_v\). By substituting these figures in equation (2) for \(C_i = 0\), \(t_i = 57471\), a value is obtained which is very close to that obtained in the absence of added virus DNA. This result indicates that the DNA synthesized in ASF virus-infected swine macrophages is only virus DNA, and not host DNA.

**DISCUSSION**

ASF virus grown either in swine macrophages (band A and B of Fig. 2) or in VERO cells (band A of Fig. 3) was purified free of host DNA. Two kinds of infectious components with different buoyant densities in sucrose were obtained in macrophages but the DNA in both components had the same sedimentation coefficient, buoyant density in CsCl and contour length. Most probably, the infectious material in either component represents particles with
different amounts of protein and/or lipid in the outer envelope, or particles associated to different membrane fractions.

The mol. wt. of ASF virus DNA was determined by two methods requiring intact molecules. One is based on the determination of the sedimentation rate of the DNA in a neutral sucrose gradient. Using T4 DNA as reference, a value of $102 \pm 5 \times 10^6$ was obtained. Another was based on length measurements made in the electron microscope. Several problems associated with the calculation of DNA mol. wt. by this method seem to be overcome by using an internal standard DNA of known mol. wt. (Inman, 1967; Lang et al. 1967; Lang, 1970; Davis, Simon & Davidson, 1971). For this purpose we have used the DNA of bacteriophage φ29. With this internal standard and the length distribution of ASF virus DNA molecules, the mol. wt. of ASF virus DNA is $107 \pm 5 \times 10^6$.

Provided that a DNA is of high degree of purity and does not contain rapidly reannealing sequences, the mol. wt. value of non intact DNA molecules can be obtained from a study of its reassociation kinetics, by comparison with that of a well known DNA used as an internal standard (Britten & Kohne, 1968; Gillis et al. 1970; Wagner et al. 1974). For this purpose we have compared the renaturation rates of ASF virus and T4 DNAs. In all the experiments ASF virus DNA renatured slightly faster than T4 DNA, corresponding to a genetic complexity for ASF virus DNA of $102 \pm 8 \times 10^6$, a value very close to those obtained from the sedimentation coefficient and the molecular length. Another independent estimation of the molecular mass of ASF virus DNA, grown in VERO cells, based on the relative mobilities of the 16 fragments produced by the restriction endonuclease EcoRI, gives a value of $107 \times 10^6$ daltons (unpublished data).

The closeness of the genetic complexity of ASF virus DNA and the mol. wt. of the virus DNA strongly suggests that the genetic information of ASF virus is present in a single DNA molecule of about $100 \times 10^6$ daltons.

ASF virus has been tentatively classified as an *Iridovirus* (Fenner et al. 1974). The genome size of the DNAs from iridescent virus types 2, 6, 9 and 18 has been determined by studies of the kinetics of DNA renaturation, taking for T4 DNA, used as a standard, a molecular weight of $126 \times 10^6$. In this way the mol. wt. obtained for iridovirus DNAs ranged between $114 \times 10^6$ and $152 \times 10^6$ (Kelly & Avery, 1974). If we take for T4 DNA a mol. wt. value of $126 \times 10^6$, instead of $110 \times 10^6$ (Freifelder, 1970), the genetic complexity of ASF virus DNA would be about $117 \times 10^6$, a value close to the one obtained by Kelly & Avery (1974) for the DNAs of iridovirus types 9 and 18. Although it would be interesting to study DNA homology of ASF virus and *Iridovirus* DNAs, a negative result could be meaningless, since type 6 iridovirus has no sequence homology with the other iridovirus types (Kelly & Avery, 1974).

The DNA from ASF virus grown in swine macrophages has a buoyant density in CsCl of 1.700 g/ml, corresponding to a Tm value of 86 °C (Marmur & Doty, 1962). This value is 3 °C higher than that obtained by Adldinger et al. (1966) using partially purified ASF virus grown in PK, an established cell line derived from pig kidney cells.

The macrophages are non-dividing cells, which do not synthesize DNA (Cohn, 1968). To ascertain the nature of the DNA synthesized in ASF virus-infected swine macrophages, radioactive DNA isolated from infected cells was annealed in the presence of increasing amounts of non-radioactive viral DNA. The annealing rate in the different cases was the one expected if the only labelled DNA in the infected cells were virus DNA. This result indicated that no host DNA synthesis occurs in ASF virus-infected macrophages, and that the only DNA synthesized is virus DNA.
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