Electron Microscopy of the RNA of Foot-and-Mouth Disease Virus*

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Electron microscopic examination of molecules of single-stranded RNA requires their visualization as isolated and elongated strands. Inter- or intramolecular hydrogen bonding, which presumably is responsible for aggregation and tangling of single-stranded RNA, can be broken by the use of urea (Granboulan, Huppert & Lacour, 1966). Furthermore, incubation of purified reovirus with urea before spreading has been demonstrated as a gentle procedure for the release of double-stranded RNA (Granboulan & Niveleau, 1967; Vasquez & Kleinschmidt, 1968).

We describe here morphological studies on the RNA of foot-and-mouth disease virus. The method employed was the microdiffusion technique recently developed by Mayor & Jordan (1968) from the protein monolayer method of Kleinschmidt & Zahn (1959). A droplet of the virus suspension was mixed with a droplet of a 4 to 10 M-urea solution on an iced sheet of Teflon or dental wax. After 10 min. the surface of the droplet containing the mixture was touched with a needle previously wetted in a Protein solution consisting of equal volumes of water and crystals of cytochrome C (Schuchardt, Munich). During the following 30 min. at room temperature the RNA molecules diffused towards the protein monolayer which formed on the droplet surface. The monolayer was then transferred to formvar and carbon coated grids. After a short rinse with water the grids were dried by touching the surface with ethanol for 30 sec. The grids were then rotary-shadowed with a thin layer of a platinum-palladium-silver-gold alloy (Usine Genèveoise de Dégrossissage d’or, Genève, alloy No. 1003) at an angle of about 8°. Using purified and highly concentrated suspensions of virus particles (Fig. 1 a) this treatment gave micrographs in which densely packed, approximately parallel filaments were visible (Fig. 1 b). When the virus was diluted before treatment it was possible to visualize isolated filaments which were suitable for measurement (Fig. 2 a). Their length ranged from 0.1 to 5.0 μm. The majority of molecules had a length of less than 1 μm. but their relative concentration depended on the method of preparation which was thus probably responsible for this fragmentation.

Eighty filaments of 1 μm. and longer were measured; but it was not always possible to distinguish discontinuities within the strands as points of breakage or new filaments. The mean length of strands longer than 1 μm. was 2.22 μm. The distribution of lengths (Fig. 3) shows that threads exceeding 3.4 μm. were rare. In our opinion the occurrence of these strands may be explained by an end-to-end attachment of two filaments in which the junction is not detectable. The most frequent length was 1.6 to 2.0 μm., which is about half that of the extreme single length of 3.4 μm.; if greater lengths may be disregarded.

Two different interpretations of these results are feasible:

1) The native length is 3.4 μm. and the RNA filaments break preferentially near the middle to produce two fragments of about half the length.

2) The native length corresponds with the modal value of about 2 μm. and end-to-end combinations occur between fragments of different lengths.

If it is assumed, following the data of Granboulan & Franklin (1966), that the molecular
weight of single-stranded RNA is 100 units/Å, then a relative high molecular weight of $3.4 \times 10^6$ follows from the first interpretation. In the morphologically related rhinovirus McGregor & Mayor (1968) found an RNA molecular weight of $4 \times 10^6$, which corresponds

Fig. 1 (a) Purified preparation of foot-and-mouth disease virus negatively stained with 1 % uranyl acetate. (b) Densely packed filaments released from concentrated purified foot-and-mouth disease virus suspension by incubation with 8 M-urea.
to twice the value for poliovirus RNA. These authors explain their results in terms of the higher buoyant density of rhinovirus compared with that of poliovirus. Foot-and-mouth disease virus also has a high buoyant density of 1.43 g./cm.³ and this could account for the molecular weight of $3.4 \times 10^6$ for foot-and-mouth disease virus RNA. Electrophoretic data (Matheka, Trautman & Bachrach, 1967) further suggest that following the preparation of

![Fig. 2](image)

**Fig. 2** (a) Isolated filaments released from diluted purified virus suspension by incubation with 8 M-urea. (b), (c), (d) RNA molecules obtained from foot-and-mouth disease virus by phenol extraction.
RNA more than 90% of the RNA strands are disintegrated. Using the same calculation factor of 100 units/Å for the second interpretation a molecular weight of about $2 \times 10^6$ is obtained. This value approximates to the $2.2 \times 10^6$ calculated by Bachrach (1968) from the molecular weight of virus particles and their RNA content. In the absence of other data and in view of Bachrach's estimate of 32% RNA the second interpretation is favoured. It is therefore suggested that end-to-end attachments are formed relatively often during preparation.

Because of the preparation technique used the filaments observed may still have been associated with protein. If so, a further correction must be considered to obtain the molecular weight of RNA free of protein.

In contrast to the single-stranded RNA molecules of avian myeloblastosis virus (Granboulan, Huppert & Lacour, 1966) and of bacteriophage R17 (Granboulan & Franklin, 1966, 1968), the observed RNA filaments of foot-and-mouth disease virus were more rigid and frequently arched. RNA isolated from purified virus suspensions by phenol extraction was therefore examined for comparison. After ethanol precipitation, the phenol-extracted RNA was dissolved in 0.3 M-NaCl + 0.03 M-C$_6$H$_5$Na$_2$O$_5$ buffer, pH 7.6, incubated with urea and prepared for electron microscopy by the microdiffusion technique. The filaments in these preparations (Fig. 2b, c, d) were thinner, less rigid and more wavy than those released from the virus by incubation with urea (Fig. 1b, 2a). These differences may also be explained by the possibility that RNA prepared by the urea method is still associated with protein. Current experiments should resolve this problem.

**Fig. 3.** Histogram of lengths of filaments exceeding 1 μm. released from foot-and-mouth disease virus by incubation with 8 M-urea.

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