

RESEARCH NOTE

ELECTRON MICROSCOPY OF BLUETONGUE VIRUS RNA

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ABSTRACT

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RNA fragments were released from bluetongue virus particles using the Kleinschmidt spreading technique. Electron microscopy of viruses in protein monolayers spread on urea showed that each virion liberated an average of about 6 fragments although up to 10 fragments were occasionally observed. A large variation of filament lengths was found with an average of 3,5 μm total composite length and with a maximum of 6,5 μm leading to an estimated RNA molecular mass of $13,6 \times 10^6$. In addition, a background of loose dispersed fragments was invariably found.

Characterization of bluetongue virus (BTV) ribonucleic acid (RNA) has been carried out by Verwoerd, Louw & Oellermann (1970). A similarity has been found between the nucleic acid moieties of BTV and reovirus. Both genomes consist of 10 double-stranded RNA segments of a reproducible size distribution. The morphology of BTV has been studied by Els & Verwoerd (1969) and Verwoerd, Els, De Villiers & Huisman (1972).

In order to obtain more information on the nature of the BTV genome, the virus was studied by the spreading technique of Vasquez & Kleinschmidt (1968). This method permits the electron microscopic visualization of nucleic acid strands upon disintegration of the viral capsid and allows an estimation of the RNA molecular mass from the measured lengths of RNA filaments.

Purified BTV was obtained according to the improved purification procedure described by Verwoerd *et al.* (1972). Fractions of BTV used for electron microscopy were suspended in 0,002 M Tris buffer and diluted to give a concentration of $2 A_{280}$ units per ml. Samples for electron microscopy were prepared according to the one-step release method as described by Vasquez & Kleinschmidt (1968). In this procedure the intact virus (0,05 ml) was diluted in 0,05 ml of a solution of 0,02% cytochrome *c* in 1 M ammonium acetate. This mixture was spread onto a subphase of urea whose concentration varied in different experiments from 1 to 6 M.

After spreading, the protein monolayer was transferred to carbon-coated formvar grids at different intervals of time between 0 and 20 min. The specimens were rinsed on a drop of water for 15 seconds. The adherent water droplet was replaced by ethanol in the same way and the grid dried on filter paper. Rotary shadow casting of the grids was carried out at an angle of 8° with either Platinum or Palladium-Gold. Electron micrographs were taken using a Siemens Elmiskop 1A electron microscope at a calibrated magnification of 10 000 times. Negatives were projected onto paper, the filaments traced and measured with a map measuring device.

Virus particles spread on subphase concentrations of less than 1,5 M urea yielded no RNA fragments. The absence of loose fragments under such conditions indicated that no loose RNA contaminants were present in the purified virus preparations. The release of RNA occurred at higher urea concentrations. Concentrations of 3 M or higher are unsuitable since, under such conditions, a network is observed of dispersed filaments which break into shorter pieces with time.

The best spreading and release of RNA fragments were obtained with a urea concentration of between 1,75 and 2,5 M urea. At such concentrations, a gradual disintegration of virus particles trapped in the cytochrome *c* protein monolayer occurs. In general, the same pattern of events, as observed with reovirus by Vasquez & Kleinschmidt (1968), appears to take place in the release of BTV RNA. RNA fragments from BTV particles were released as a sequence of events over a period of time. Initially, the disruption of the capsid occurs between 0 and 2 min (Fig. 1a), followed by the formation of forms referred to as "spiders" and "splashes" by Vasquez & Kleinschmidt (1968). Between 2 and 5 min, loops and fragments appear around a virus particle (incomplete "spider", Fig. 1b), changing to a complete "spider" between 5 and 10 min (Fig. 1c). From 10 to 15 min the "spider" disintegrates to form a "splash" of separated filaments, (Fig. 1d). After 15 min, the RNA fragments become widely dispersed throughout the protein monolayer, and break into shorter segments.

The sequence of events during the period 2 to 15 min is, however, not always reproducible since all the described forms from the disrupted capsid to the "splash" occur, to some extent, at any particular instant. There is, in addition, an increasing background of scattered loose RNA filaments dispersed from the region of the "spiders" and "splashes".

The presence of these filaments remains an unsolved problem. Attempts to avoid their occurrence were unsuccessful. Experimental conditions were altered by changing the pH and concentration of the ammonium acetate, the temperature of the urea, the concentration of the cytochrome *c* and by using other spreading proteins. All these changes had, however, little or no effect on the occurrence of these loose filaments.

From the "spider" forms of the disintegrated viruses it is possible to estimate the number of RNA fragments released per virion. From the total length of the filaments one can also estimate its molecular mass. The calculation is based on the assumption that one μm of length corresponds to a molecular mass of $2,1 \times 10^6$ (Vasquez & Kleinschmidt, 1968).

The fragments were uniform in width but showed a great variation in length. The majority (about 90%) of the fragment-lengths were in the range between 2 and 4 μm . No clear trimodal length distribution could be demonstrated as has been shown for reovirus. Less than 3% of the "spiders" measured in a given experiment showed a total filament length of more than 5 μm up to

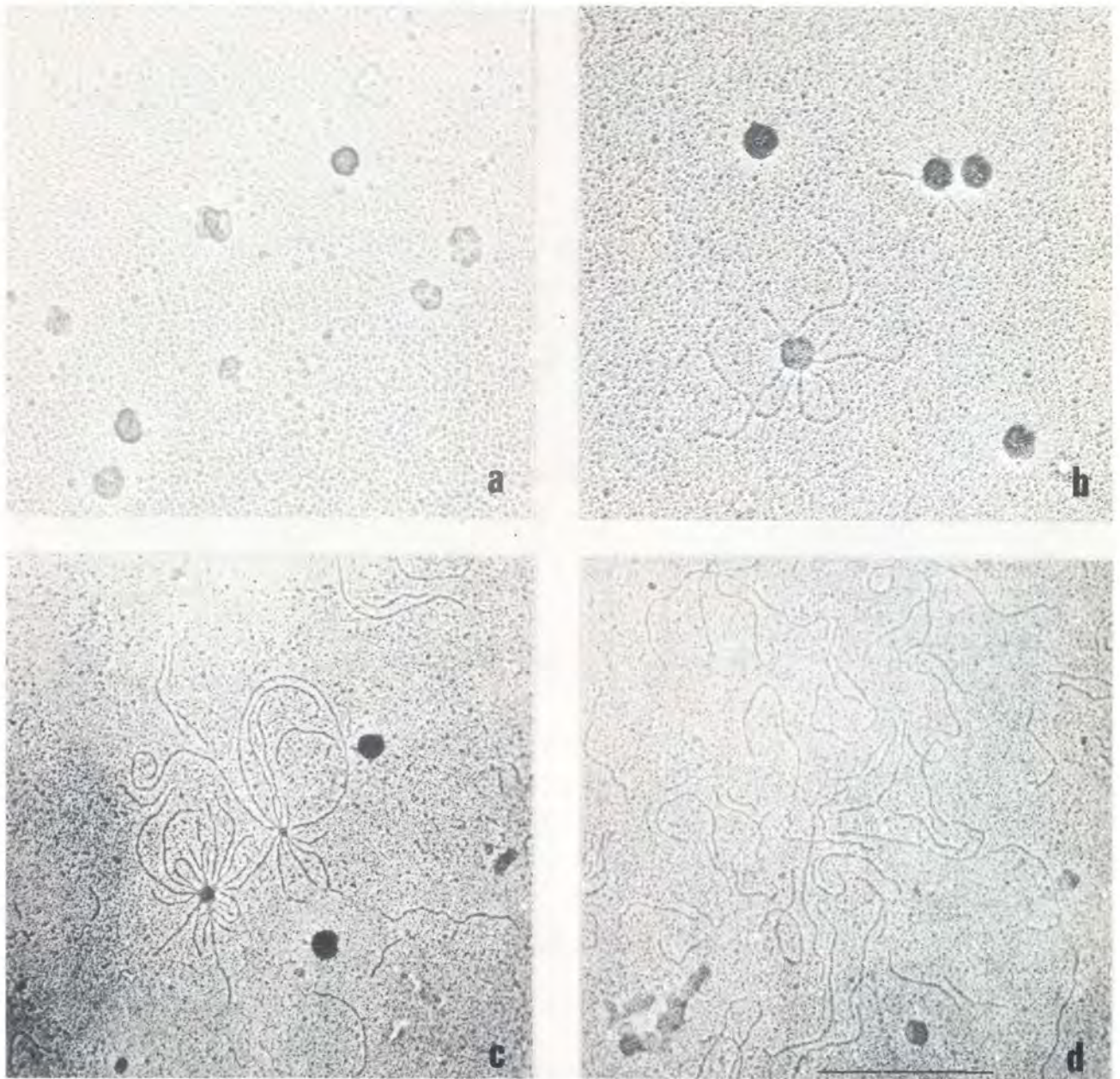


FIG. 1 Electron micrographs of bluetongue virus particles at different stages of disruption. Following the spreading the virus-protein monolayer was transferred to grids (a) after 1 min - destruction of capsid; (b) after 3 min (incomplete "spider"); (c) after 8 min (complete "spider"); and (d) after 12 min ("splash"). The bar equals 0,5 μm .

a maximum of about 6,5 μm and only 10 single fragments as long as 5 to 7 μm have been found in approximately 200 experiments. Analysis of the "spiders" indicated an average of 6 fragments released per virion. In some "spiders" 10 fragments could be discerned. However, due to the granular background of the monolayer, the presence of more than 10 fragments could not be identified with certainty. The average total length of all the released filaments attached to "spiders" in the interval between 5 and 10 min was about 3,5 μm which gives an estimated RNA molecular mass of about $7,4 \times 10^6$ per virion. Since the scattered filaments could not be clearly designated to a particular "spider" or "splash" they were not taken into account and therefore the estimated values given above are likely to be underestimates. The value for the molecular mass is considerably lower than the reported molecular mass of $11,84 \times 10^6$ obtained from chemical analysis (Verwoerd, *et al.*,

1972). If the value of 6,5 μm is considered as a better approximation of the real length this would result in an estimated molecular mass of $13,6 \times 10^6$ which agrees well with the expected value.

The micrographs in the plate demonstrate typical examples of the type of RNA release pattern obtained in BTV particles. The pattern is very similar to that found for reovirus particles. A more elaborate investigation should lead to a closer agreement of results for both viruses particularly the number of fragments per virion and their total length and consequently the molecular mass of the RNA.

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