The Structure of Fowl Plague Virus and Virus N

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Fowl plague (KP) virus and virus N have been examined in the electron microscope as both metal shadowed and negatively stained preparations. The particles of KP virus are very similar to those of influenza A. The products of ether splitting are (1) the G antigen, a ribonucleoprotein whose helically arranged protein subunits can be resolved and (2) the haemagglutinin, a star-shaped structure about 350 Å diameter made up of the spikes which project from the surface of the intact particle. Incomplete forms have been prepared by serial undiluted passage, and these show great pleomorphism, but the same outer coat as complete virus. Ether splitting of incomplete forms yields a haemagglutinin like that from complete virus, but only traces of G antigen. Virus N is more pleomorphic than KP virus, but the products of ether splitting are very similar.

The work of Schäfer, Munk and Armbruster
1 (1952) established that the particles of fowl plague (KP) virus were approximately spherical, with a diameter of about 700 Å. Schäfer and Zillig
2 (1954) showed that by treatment with ether, as described by Hoyle
3 (1952) for influenza, the virus could be split, with release of two well-characterised components, an inner rod-shaped ribonucleoprotein (G antigen) and a spherical haemagglutinating structure about 340 Å in diameter (haemagglutinin). These preparations were examined in the electron microscope after metal shadowing. By this method of preparation the G antigen could be seen to consist of short rods, some of which had a periodicity along their length, but it was not possible to resolve details of the fine structure of the haemagglutinin, nor of the intact particles. It was not possible, either, to determine exactly the way in which the two components might be incorporated into the intact particles.

Schäfer and Rott
4 (1959) reported that ether treatment of mumps and Newcastle disease viruses also yielded rod-shaped, but much longer, components probably of ribonucleoprotein. By the technique of negative staining, using phosphotungstic acid, Horne and Waterson
5 (1960) showed that a corresponding structure is located inside the virus particle and has subunits helically arranged about a central hollow. The products of ether splitting of influenza virus have also been examined using this technique (Hoyle, Horne and Waterson
6, 1961) and the G antigen (soluble antigen) resolved as a structure somewhat similar to, though smaller than, the corresponding component of mumps and Newcastle disease viruses. The haemagglutinin component of influenza presented a less uniform picture than that of KP virus but its smallest and most constant component had the appearance of a star, with the spikes which normally project from the outside of the particle radiating from its centre. The authors supposed that this component was analogous to the haemagglutinin of KP virus described by Schäfer and Zillig
2. This paper reports the examination of various preparations of KP virus and of the related virus N (Rott and Schäfer
7, 1960) prepared for electron microscopy both by metal shadowing and by negative staining.

Materials and Methods

KP virus. The strain “Rostock” was used throughout. For examination of intact virus particles the virus was concentrated from infected allantoic fluid by differential centrifugation, as previously described (Horne, Waterson, Wildy and Farnham
8, 1960). The pellet of virus was resuspended in 1% ammonium acetate. For examination of the products of splitting, the virus was prepared, and the ether treatment performed, as described by Schäfer and

3 L. Hoyle, J. Hgy., 50, 229 [1952].
Zillig. The haemagglutinin was resuspended, after final centrifugation, in 1% ammonium acetate. The G antigen was dialysed against 1% ammonium acetate. For preparation of von Magnus incomplete forms allantoic fluid of the third undiluted passage was used. The virus was concentrated and purified by adsorption to, and elution from, fowl red cells, and finally fractional centrifugation (Rott and Schäfer, 1960) with resuspension in 1% ammonium acetate. The ratio of plaque-forming units to haemagglutinin titre of the concentrate was $10^{3.2}$. The haemagglutinin titre was $2^{−14}$. Ether treatment was performed as with standard virus.

**Virus N.** Virus N was grown by allantoic inoculation of hen's eggs as described by Rott and Schäfer. The concentrate for electron microscopy was prepared by adsorption to, and elution from, fowl red cells, followed by differential centrifugation, the pellet being suspended finally in 1% ammonium acetate. Ether treatment was performed as with KP virus.

**Electron microscopy.** (a) Metal shadowed preparations were fixed in osmium vapour, washed three times with distilled water and shadowed with platinum-rhodium at an angle of about 25°. They were examined in the A.E.G.-Zeiss EM 8 microscope.

(b) Negatively stained preparations (Brenner and Horne, 1959) were mixed with an equal volume or double volume of 2% phosphotungstic acid neutralised to pH 7 with potassium hydroxide. They were sprayed onto carbon-coated or Formvar grids and examined at an instrumental magnification of 40,000 in the Siemens Elmiskop, using double condenser illumination.

**Results**

**KP Virus.** (a) **Intact particles** (fig. 1*, 4). The particles of KP virus are very similar to those of influenza. No details of the inner structure could be resolved. Some particles joined in pairs like dumbbells were seen, as reported by Rott and Schäfer.

(b) **G antigen** (fig. 2, 9, 10). This is an elongated structure appearing lengths of up to 1000 Å. Protein subunits of diameter 25–30 Å could be resolved along its length, probably in a helix or double helix with a periodicity of 60–75 Å. This structure is similar to that of the soluble antigen (G antigen) of influenza, except that it appears to be a little more flexible than that of influenza.

(c) **Haemagglutinin** (fig. 3, 5, 6, 7, 8). This gave a uniform appearance of star-shaped rosettes with radially arranged spikes of about 30–40 Å width. The diameter of these rosettes is about 350 Å. In some of them the centre appears coiled.

(d) **von Magnus incomplete forms** (fig. 11, 14, 15, 16). These resembled normal complete virus in having a similar outer layer. They differed markedly in having a much greater variety of size and shape. There were forms smaller than the normal spherical forms, joined in pairs or short chains, some quite irregular particles, and some long forms quite similar to filamentous forms. The phosphotungstate had penetrated many of the incomplete forms, showing as a dark centre to the particle. Actual ring forms were also seen. On ether splitting the haemagglutinin (fig. 12, 13) was found to be identical with that of complete virus. The G antigen was present in quantities to small for electron microscopy.

**Virus N** (fig. 17, 20). The particles showed considerable variation in shape and size (1000 Å to 2000 Å). No details of internal structure could be resolved. The outer layer of projections was very well seen. The larger particles are very pleomorphic, and some show two or more phosphotungstate-filled depressions on their surface. The products of ether treatment (fig. 18, 19, 21, 22) are practically indistinguishable from those of KP virus.

**Discussion**

These findings show that KP virus is basically very similar to influenza A virus. The intact particles, the G antigen and the haemagglutinin are all very similar in the two viruses. There were none of the large forms of haemagglutinin, as seen in the preparations of Hoyle, Horne and Watson. This difference is probably to be explained, not by the different nature of the two viruses, but by differences in the technique of ether treatment. The KP was prepared by shaking with ether for 12 hours, which evidently causes complete destruction of the coat into small pieces, and the rolling of these into the rosette forms, whereas the influenza was split by treatment for only two hours. It seems, then, that

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* Fig. 1–22 s. Tafel S. 156 a u. b.

the haemagglutinin, in the form seen after ether treatment, does not represent a structure which is present as such in the virus particle before splitting, but a modification of a portion of the outer coat.

The G antigen, on the contrary, is almost certainly present in the form observed after ether splitting. The only question in doubt is the total length of G antigen in the particle, and whether it is in the form of several short pieces, or of one long piece which is disrupted by the ether treatment. The most significant outcome of these findings is that they confirm the prediction of Schäfer \(^{11}\) (1957) that the G antigen would be found to correspond, as a structure, to the whole particle of the smaller RNA viruses. A typical small "spherical" virus (mouse encephalomyelitis (ME) virus) has been sprayed with some G antigen of KP virus, and the two together exemplify the two common forms of arrangement of subunits, i.e. the helical and the polyhedral, e.g. icosahedral or dodecahedral. It is the G antigen which contains the RNA, and which presumably alone carries the genetic information of the particle, and which has protein subunits symmetrically arranged (a symmetry of form which is not seen in the intact particle).

As far as the von Magnus incomplete forms are concerned, it is clear that the similarity of KP to influenza extends also to the phenomenon of production of incomplete virus by passage of large inocula and that there are similarities between the incomplete forms of the two viruses (Waterson and Horne \(^{12}\), 1961; Rott and Schäfer \(^9\), 1960). The particles of virus N show much more variation in shape and size than do those of KP, although it is fair to point out that in some passages of KP virus almost as much variation may be found.

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Reindarstellung und Charakterisierung des für die Lyse T2-infizierter Zellen verantwortlichen Enzmys

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T2-infected cells of E. coli B synthesize an enzyme whose role it is eventually to lyse the cell from within, whereby the new crop of T2-phage is released from it. Procedures for isolating this enzyme in pure form are described. The enzyme is characterized as a lysozyme. It has a molecular weight of 15 200.

Die erste Beobachtung über ein anscheinend nur von phageninfizierten Zellen synthetisiertes, lytisches Enzym stammt von Sertic \(^1\). Er beschreibt einen "Lysinzonen bildenden" Phagen Fc2 (Wirtsbakterium ist der mucoide E. coli-Stamm Fb) und führt die Erscheinung der Zonenbildung, d. h. das Auftreten eines Hofes rings um einen Phagen-Plaque, auf ein lytisches, im Agar diffundibles Agens zurück, das die infizierte Zelle zusammen mit den neugebildeten Phagenteilchen verläßt. Es kann von diesen abgetrennt werden.

\(^1\) V. Sertic, Zbl. Bakteriol., Parasitenkunde Infektionskrankh., Abt. I, Orig. 110, 125 [1929].


Zu ähnlichen Resultaten kam Gratia \(^2\), der ebenfalls mit einem E. coli-Phagen-System arbeitete.

Humphries \(^3\) sowie Park \(^4\), Adams und Park \(^5\) beschreiben ein entsprechendes Enzym in Lysaten verschiedener Stämme von Klebsiella pneumoniae, die mit dem Phagen Kp infiziert waren. Dieses Enzym liegt dabei sowohl an das Phagenteilchen gebunden wie auch in freier Form vor.

Sämtliche bisher aufgeführten Bakterienstämmen besitzen eine Polysaccharidkapsel; die Hofbildung ist auf ein Aufflösen dieser Kapsel durch das betref-