The Structure of Viruses of the Newcastle Disease-Mumps-Influenza (Myxovirus) Group

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SUMMARY: Particles of influenza, mumps, fowl plague, Newcastle disease and Sendai viruses were adsorbed on electron microscope films and treated with acid, trypsin and ribonuclease. All of these viruses contained trypsin-resistant rings of ribonucleoprotein and with some strains these rings showed lines of staining which may indicate the arrangement of the nucleic acid.

We recently described an investigation of the structure of influenza A virus by a new technique (Valentine & Isaacs, 1957). The virus particles were adsorbed on to the nitrocellulose film covering an electron microscope specimen support, treated with proteolytic enzymes and other reagents *in situ* and examined directly in the microscope. By this method it was shown that influenza virus spheres contain a ring of ribonucleoprotein enclosed within a protein coat; the arrangement of the carbohydrate and lipid in the particles is not known. This result contrasts with the structure of the pox viruses shown in the work of Dawson & McFarlane (1948), Peters & Stoeckenius (1954) and others where a central solid nucleus-like structure of deoxyribonucleoprotein is enclosed within a protein coat. We have now investigated other members of the *Myxovirus* group, and all those which we have studied have contained a trypsin-resistant ring of ribonucleoprotein. In some cases these rings themselves have shown remarkable structural details.

METHODS

Virus strains. Newcastle disease virus: 'Herts' strain; Vaccine 'B' strain supplied by Dr F. B. Bang. Sendai virus (Kuroya, Ishida & Shiratori, 1953) supplied by Dr W. W. Ackermann. Mumps virus: Enders strain, EMA 41. Fowl plague: Brescia strain. Influenza B: LEE, 1940 and Crawley, 1946 strains. Influenza C: the original 1233 strain of Taylor (1949).

Preparation of viruses. Newcastle disease and fowl plague viruses were inoculated into the allantoic cavity of 10-day chick embryos and harvested after 18-24 hr. at 37°. Sendai and influenza B viruses were similarly inoculated, but harvested after 2-3 days of incubation. Mumps virus was inoculated into the allantoic cavity of 8-day eggs and the fluids harvested 5 days later. Influenza C virus was inoculated amniotically into 10-day eggs, and the allantoic fluids harvested after 2-3 days of incubation. In some experiments virus was adsorbed on to chick red cells and eluted into saline, but in most experiments virus in the allantoic fluid was used directly for electron microscopy after light centrifugation only.

Other techniques. Other materials and the techniques of electron microscopy were described in detail in our previous communication (Valentine & Isaacs, 1957); the following is a brief description of the methods used. A drop of the virus preparation to be examined was placed on a nitrocellulose film on a platinum support for 1 min. and then washed away by immersing it in distilled water. The specimen support with many virus particles adsorbed on its film was then placed in 0.1 N-HCl for 2 min., washed and incubated in 0.1% (w/v) crystalline trypsin at pH 8.0 for 1 hr. at 37°. The pre-treatment with acid was essential for digestion and probably served to denature the protein. In some experiments the digested particles were examined after the trypsin treatment, but in most experiments the films were subsequently immersed in 0.1% (w/v) crystalline ribonuclease at pH 8.0 for 1 hr. at 37°. After treatment with these enzymes, the structures on the films were fixed in osmium tetroxide vapour, treated in 5% (w/v) phosphotungstic acid and washed in distilled water. No drying of the films took place until after this final wash. Control preparations of the same virus material were examined in the electron microscope without treatment with enzymes but with the same fixation with osmium tetroxide and subsequent treatment with phosphotungstic acid.

The regions where the phosphotungstic acid is taken up by the specimen scatter electrons and appear electron-dense; they are reproduced dark in the Plates. Such treatment has usually been referred to by electron microscopists as 'staining' by analogy with the use of the word in light microscopy to describe treatment with a coloured pigment. The word 'staining' will be used here to mean the effect of treatment with phosphotungstic acid in rendering parts of the specimen specifically electron-dense.

RESULTS

After treatment with acid and trypsin, the viruses of the Myxovirus group all showed rings of trypsin-resistant material similar to those already described for influenza A (Valentine & Isaacs, 1957). The rings had often expanded during the digestion and were, as a rule, slightly larger than the particles from which they had come. These rings took up very little phosphotungstic acid and were rather vague and ill-defined. Pl. 1, fig. 1, shows a Newcastle disease virus particle after HCl and trypsin treatment. However, when this treatment was followed by further treating the films with ribonuclease, the rings, still undigested, now 'stained' more intensely, often showing striking structural detail (Pl. 1, fig. 2). The ring structures were completely digested by following the ribonuclease with further HCl and trypsin treatment. But this complete digestion of the particles was only obtained by using the full sequence of treatments; HCl, trypsin, ribonuclease, HCl and trypsin, carried out in that order. Thus, as in the case of influenza A (Valentine & Isaacs, 1957), the rings can reasonably be identified as ribonucleoprotein structures contained within the intact virus particles.

Ring structures were prepared from the different viruses by treating virus

particles adsorbed on the supporting film with acid, trypsin and ribonuclease, followed by fixing and 'staining'. Pl. 1, figs. 2-4, shows rings obtained from two strains of Newcastle disease, and Pl. 1, fig. 5, one from Sendai virus. The general appearances of the rings from these three viruses were very similar. In many places 3 to 6 evenly spaced dark lines of 'staining' could be seen running along the rings. These lines were broken at points but they seldom appeared to cross one another. When instead of being treated with phosphotungstic acid, the rings were shadowed with platinum (Pl. 2, fig. 6) the length of the shadows suggested that the rings were about as high as they were thick, i.e. that they might well have been circular in cross-section.

Plate 2, fig. 7, shows a ring typical of those obtained from mumps virus; lines of 'staining' were not clearly seen. Fowl plague virus, on the other hand, showed rings with 3 or more lines (Pl. 2, fig. 8). Some of the rings from the LEE strain of influenza B were similar but with less distinct lines (Pl. 2, fig. 9); others showed no detail. No detail was seen in the rings of the Crawley strain of influenza B nor in those of influenza C (Pl. 2, fig. 10).

DISCUSSION

Two facts of interest have emerged from this investigation. First, all the viruses of the Myxovirus group which we examined showed trypsin-resistant structures in the form of rings apparently composed of ribonucleoprotein. Secondly, after treatment with ribonuclease the ring structures frequently showed characteristic dark lines of 'staining' most strikingly seen with Newcastle disease virus. This 'staining' effect can be explained in one of two ways. The ribonuclease might have removed threads of ribonucleic acid from the surface of the rings and exposed basic groups with a strong affinity for phosphotungstic acid in the underlying protein. Alternatively, the ribonuclease might have remained combined with the nucleic acid and itself taken up phosphotungstic acid. Though the first explanation seems the more probable, there is no conclusive evidence in favour of either. However, whichever is correct, it would appear that the dark lines of 'staining' may well indicate the arrangement of the ribonucleic acid in the ring structures.

It was earlier suggested (Valentine & Isaacs, 1957) that the treatment with HCl necessary before the trypsin had any marked effect on the virus particle served to denature the protein and thus render it susceptible to the enzyme. If this is so, the fact that a second treatment with HCl was needed before trypsin could digest away the ribonuclease-treated ring has some significance. It suggests that the first treatment with HCl denatured all the protein in the virus particle except that in the nucleoprotein ring. This could only be denatured after the nucleic acid in the ring had been attacked by ribonuclease. The nucleic acid must thus confer considerable structural stability on the protein that forms the bulk of the ring. This stability is apparently often combined with some rigidity, for in the intact particle the rings would have been orientated at random with respect to the supporting film and become adsorbed on to it as the rest of the particle was digested away.

Nevertheless, at least with Newcastle disease and Sendai viruses, the rings normally appeared as regular structures despite the many distorting forces which must have acted on them.

The actual arrangement of the dark lines of 'staining' is of particular interest if they can in fact be identified with the position of the nucleic acid in the virus. It seems clear that the lines must represent threads rather than lamellae for it would be extremely unlikely that lamellae so closely spaced would so often lie parallel to the electron beam. If threads, then it is probable

Fig. 1. Proposed arrangement of ribonucleic acid (RNA) and protein in ribonucleoprotein ring of Newcastle disease virus.

that they lie on the surface of the ring since one line at least often seems to run along an edge. Some of the dark lines are denser and thicker than others and in places can be seen to be double (e.g. Pl. 1, fig. 4). The most likely interpretation is that here two lines are being viewed almost superimposed, one being on the top and one on the bottom of the ring.

In those viruses that have shown well-defined lines of 'staining', in particular those of Newcastle disease and Sendai, we therefore picture the nucleoprotein as a ring consisting of a protein core stabilized by threads of ribonucleic acid running along its surface as shown in Fig. 1. This has been drawn with 6 threads but the number that would be seen on an electron micrograph of such a structure could vary between 3 and 6 depending on whether they were superimposed in the image. The essential feature of our model is the general arrangement of the nucleic acid and protein and not the actual number of nucleic acid chains which cannot be deduced with any certainty from the pictures. In the intact spherical particle we suppose the ring of nucleoprotein to lie just within the surface, embedded in the other components of the virus.

The influenza and mumps viruses whose rings have not clearly shown similarly arranged dark lines of 'staining' may have a different arrangement of their nucleoprotein. On the other hand, these lines may for some reason merely be more difficult to reveal.

Frisch-Niggemeyer (1956) pointed out that, despite their varying sizes, all the viruses which contain ribonucleic acid and for which reliable chemical analyses are available appear to contain roughly the same mass of nucleic acid per particle, corresponding to about 6000 nucleotides. Little is yet known about the detailed structure of ribonucleic acid, particularly as it exists within the virus. If the molecules were fully extended structures the distance between the nucleotides would be about 7.5 Å. and thus the mean length of ribonucleic acid in each virus about 45,000 Å. However, if the structure is not fully extended but coiled in some way as is deoxyribonucleic acid (Crick & Watson, 1954) the length of the nucleic acid would, of course, be considerably less than 45,000 Å.; in the double helix of deoxyribonucleic acid, for example, 20 nucleotides correspond to a length of 34 Å. and so 6000 nucleotides would be contained along a structure only 10,000 Å. long. In fact, the actual total length of the lines of 'staining' seen in the Newcastle disease virus rings (Pl. 1, figs. 2-4) has varied from *c.* 10,000 to 30,000 Å. per ring. Thus these rough estimates at least serve to show that there is no obvious inconsistency between the suggested structure as deduced from the electron micrographs and what is at present known chemically about the nucleic acid content of the virus particles.

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R. C. VALENTINE & A. ISAACS—VIRUS STRUCTURE. PLATE 1

(Facing p. 684)

EXPLANATION OF PLATES

PLATE 1

The effect of treating the virus particles with 0.1N-HCl and 0.1 % trypsin (fig. 1) and 0.1N-HCl, 0.1 % trypsin and 0.1 % ribonuclease (figs. 2-5). The treated preparations were fixed with osmium tetroxide vapour and then treated with phosphotungstic acid. All magnifications $\times 210,000$.

- Figs. 1, 2. Newcastle disease virus ('Herts' strain).
- Fig. 3. Newcastle disease virus (Vaccine B strain).
- Fig. 4. Newcastle disease virus ('Herts' strain).
- Fig. 5. Sendai virus.

PLATE 2

The effect of treating the virus particles with 0.1N-HCl, 0.1 % trypsin and 0.1 % ribonuclease. The treated preparations were fixed with osmium tetroxide vapour and either shadowed with platinum at 30° (fig. 6) or treated with phosphotungstic acid (figs. 7-10). All magnifications $\times 210,000$.

- Fig. 6. Newcastle disease virus ('Herts' strain).
- Fig. 7. Mumps virus.
- Fig. 8. Fowl plague virus.
- Fig. 9. Influenza B virus.
- Fig. 10. Influenza C virus.

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