VALENTINE, R. C. & ISAACS, A. (1957). *J. gen. Microbiol.* 16, 195-204

The Structure of Influenza Virus Filaments and Spheres

By R. C. VALENTINE AND A. ISAACS

National Institute for Medical Research, Mill Hill, London, N.W. 7

SUMMARY: Influenza virus filaments treated with acid on an electron microscope film developed along their length rows of spheres which were completely digested by trypsin. Influenza virus spheres similarly treated revealed trypsin-resistant polygonal rings which by their behaviour with enzymes have been identified as ribonucleoprotein.

The demonstration by Mosley & Wyckoff (1946) and by Chu, Dawson & Elford (1949) that influenza virus could occur in long filaments as well as in the more usual spherical forms was followed by many investigations into, and theories about, the nature of the two forms and their relation to one another. Theories have been based on study of the conditions under which filaments are formed and on the biological properties of filaments and spheres. In the present investigation, we have compared the morphology of filaments and spheres, after different chemical treatments, as shown by the electron microscope. The results emphasize striking structural differences between filaments and spheres, and at the same time allowed us to examine the gross structure of the influenza virus nucleoprotein.

METHODS

Viruses. Two strains of influenza virus A were investigated. The MEL (1935) strain contains many spheres and only a very small proportion of filamentous forms. The A/Persia/2/52 virus which has had only a few passages in hen eggs produces roughly equal numbers of filaments and spheres. Both strains were propagated by passage in the allantoic cavity of 10-day chick embryos. The virus was prepared for electron microscopy by adsorbing on to chick red cells at 0° and eluting into saline at 37°; it was usually concentrated about fivefold by this technique.

Trypsin. Crystalline trypsin (Armour) was kept as a 4% (w/v) solution in 0.01 N-HCl at -10°. Before use it was diluted in phosphate buffer (pH 8.0) to give a 0.1% solution and centrifuged lightly.

Ribonuclease. Crystalline ribonuclease prepared from beef pancreas by the method of Kunitz (1940) was kindly supplied by our colleague Dr R. R. Porter. In one experiment a preparation of three times crystallized ribonuclease was used. The ribonuclease was made up in phosphate buffer (pH 8.0) at a concentration of 0.1% (w/v).

Electron microscope techniques. The film supports used were made of platinum. A very thin film of nitrocellulose was used to cover them, stabilized by evaporating on to it the thinnest possible film of carbon, an adaptation of the method described by Bradley (1954).

A drop of the virus suspension in saline was placed on each film for 1 min. and then washed away by immersing it in distilled water for 1 min. Many of the virus particles that impinge on the film are held there by short range inter-molecular forces and do not then wash off. The platinum supports with the virus thus adsorbed on the films were placed in the various reagents to be described, with a brief wash in distilled water between treatments. The structures on the films were then fixed with osmium tetroxide vapour for 5 min., stained by immersion for 5 min. in a 5% (w/v) solution of phosphotungstic acid (Hall, 1955) and then finally washed in distilled water. After lightly blotting at the edge with filter-paper, the preparations were allowed to dry. From the moment the virus suspension had been placed on the film until after this final washing no drying had occurred; this formed an important part of the technique. The phosphotungstic staining was used to replace the more usual metal shadowing in all experiments but one. The superiority of this staining technique for revealing underlying structures was at once apparent.

The preparations were viewed in a Siemens ÜM 100 electron microscope using an anode potential of 60 kV. Plates were taken at a magnification of either 14,000 or 55,000 times.

RESULTS

Morphology of the virus after chemical treatments

Treatment of filaments and spheres with the virus adsorbed on the film allowed us to carry out a series of consecutive treatments with different reagents while the virus remained *in situ* and in a good condition for electron microscopic observation. A mixture of partially purified MEL and A/Persia/2/52 viruses was used in order to have a high concentration of spheres and filaments on the same electron microscope film, and therefore treated under identical conditions. This filament+sphere mixture was examined after the following consecutive treatments:

- (1) Untreated control.
- (2) 0.1 N-HCl. Treatment was carried out at room temperature.
- (3) 0.1% trypsin at 37°.
- (4) 0.1% ribonuclease at 37°.
- (5) A second treatment with 0.1 N-HCl and 0.1% trypsin.

In the usual experiment, five specimens were prepared, along with the additional controls which are considered later, and at the end of each stage in the treatment one was washed, fixed and stained with phosphotungstic acid. The results to be described summarize the observations on many experiments of this kind in all of which the appearances at each stage were quite consistent.

(1) *The untreated suspension.* Untreated suspensions were examined after fixation in osmium tetroxide vapour and impregnation with phosphotungstic acid as described under Methods. Electron micrographs showed the virus elementary bodies as roughly spherical with a diameter of about 800 Å. and the filaments with a more or less uniform width of about 650 Å. and a very

variable length ranging from 2,000 Å. up to 50 μ . The spheres were normally more electron dense than the filaments (Pl. 1, fig. 1). Running along the length of the filaments could be seen structures which on close examination appeared as dense threads about 50 Å. thick (Pl. 1, fig. 2). The spheres seemed to have a similar structure but the threads were denser and rather thicker than in the filaments and packed together more tightly. For this reason they were seen less clearly, many of the spheres appearing almost homogeneously dense (Pl. 1, fig. 3). Examination of the filaments never revealed spheres within them but spheres were often seen attached to their sides and tips (Pl. 1, fig. 1).

(2) *Treatment with hydrochloric acid.* While 30 min. in 0.1 N-HCl did not obviously alter the appearance of the virus spheres, 30 sec. in this acid produced striking changes in the filaments (Pl. 1, figs. 4, 5). They characteristically broke up into strings of spherical bodies, some swollen to several times the diameter of the original filament, while others bore a very remarkable resemblance to virus spheres. It is not easy to believe that this resemblance was purely coincidental, but the results described in the next section point to a fundamental difference between the true virus spheres and those produced in filaments by acid treatment.

(3) *Treatment with trypsin.* By itself trypsin produced little change in either filaments or spheres (2 hr. treatment with 0.1 % trypsin, pH 8.0, at 37°). But when the preparation of virus particles on the film was first treated with 0.1 N-HCl for 5 min. and then transferred to the trypsin, considerable digestion rapidly took place, 5 min. in the enzyme being sufficient to remove almost all traces of the filaments. The spheres were left frequently as irregular ring-like forms and after about 1 hr., the digestion had gone about as far as possible (Pl. 3, fig. 11), since the appearances remained the same throughout a further 4 hr. in trypsin. Not all the trypsin-treated spheres showed ring forms, but in those which did not, appearances were often consistent with those of intact or broken rings viewed at different angles.

Examination of the structures left after digestion with trypsin showed no continuous resistant structure of resolvable size running along the length of the filaments. A considerable part of the structure of the virus spheres resisted digestion but after treatment of the mixture of filaments and spheres these remains were never obviously arranged in chains showing that trypsin-resistant structures do not occur periodically along the filaments. This does not rule out the possibility that an occasional point along the filaments might have resisted digestion but unlike the spheres, the filament structure was mostly trypsin-sensitive.

The two most notable features of the spheres after treatment with acid and trypsin were first that the resistant structures appeared typically as rings (often suggestively polygonal in outline) with no evidence of any resistant central portion (Pl. 2, figs. 6-8), and secondly that the structures sometimes expanded up to several times the diameter of the original virus sphere (Pl. 2, fig. 9). A double line of staining separated by a clear space about 40 Å. wide could be seen in some places running round the ring of the resistant structure. It is well shown in Pl. 2, fig. 9, where the structure had opened right out

and the ring broken, but it was also commonly observed in the smaller rings.

When, instead of the treatment with phosphotungstic acid, the preparations were shadowed with platinum, the trypsin-resistant structures cast very definite shadows and in some cases they must have represented up to 30 % by volume of the original particles (Pl. 2, fig. 10).

(4) *Treatment with ribonuclease.* Preparations treated with acid and trypsin were then rinsed briefly in distilled water and transferred to a solution of ribonuclease (0.1 % crystalline ribonuclease, pH 8.0, for 80 min. at 37°). Examination after fixing and staining showed that digestion of the virus structure had proceeded no further as judged by morphological appearance after this further treatment (Pl. 3, fig. 12). The ribonucleic acid content of the virus spheres has been determined as about 0.8 % by weight of their total mass (Ada & Perry, 1956) and this nucleic acid would be expected to form a part of the trypsin-resistant structure. Evidence that the enzyme had really acted on the resistant structures despite the lack of any marked morphological change was provided by the treatment now described.

(5) *Second trypsin treatment.* Following the treatment with acid, trypsin and ribonuclease, films were then further treated with acid for 5 min. and trypsin for 80 min. under the same conditions as before. When these films were fixed and stained, only a very occasional trace of any structures was to be found (Pl. 3, fig. 13). It was therefore concluded that the structures resistant to the first treatment with trypsin were composed of protein closely linked with ribonucleic acid and only susceptible to proteolytic digestion after the nucleic acid had been attacked by the nuclease.

Control observations. Virus particles were removed from the suspension by adding fowl red blood cells on to which the virus was adsorbed, followed by light centrifugation to spin out the cells. The haemagglutination titre of the virus suspension used was reduced in this way by 99.6 % and when examined in the microscope only a very few particles could be found. These preparations treated with acid and trypsin showed no structures similar to those seen when the unadsorbed suspensions were treated in this way.

When the virus particles were immersed first in trypsin and then in acid, the result was exactly like that produced by acid alone, confirming that trypsin had little effect on the spheres or filaments unless they were first treated with acid.

Virus particles were treated with ribonuclease and then with acid followed by trypsin, but the result was the same as without ribonuclease; this enzyme thus acted only after the particles had first been attacked with trypsin. By itself, ribonuclease had no effect on the appearance of the virus particles.

As described above, the virus was entirely digested by the sequence of treatments: 0.1 N-HCl (5 min.), 0.1 % trypsin (80 min.), 0.1 % ribonuclease (80 min.), 0.1 N-HCl (5 min.), 0.1 % trypsin (80 min.). The same preparation was treated in parallel but with omission of the ribonuclease and extension of the trypsin treatment, viz. 0.1 N-HCl (5 min.), 0.1 % trypsin (160 min.), 0.1 N-HCl (5 min.), 0.1 % trypsin (80 min.). The result was that usually

observed after acid and trypsin, the typical resistant structures of the spheres still being present.

A preparation of 'incomplete' (von Magnus, 1946) MEL virus was treated with acid and trypsin as described above, but the ring structures were similar to those seen after treating standard MEL virus. 'Incomplete' virus has a lower ribonucleic acid content than standard virus (Ada & Perry, 1956), but unfortunately we have had difficulty with the MEL strain of virus in preparing 'incomplete' virus with less than one-hundredth of the relative infectivity of standard virus. According to Ada & Perry (1956) this virus should have half the concentration of ribonucleic acid of standard virus. It is possible that 'incomplete virus' with an even lower ribonucleic acid content than this might show structural differences from standard virus after treatment with acid and trypsin, and further experiments along this line are contemplated.

Pepsin. Treatment with pepsin (0.1 % pepsin in 0.1 N-HCl for 1 hr. at 37°) showed less digestion than treatment with trypsin, the most obvious difference being the effect on the filaments. These had ballooned at points, presumably because of the hydrochloric acid, but much of their structure remained clearly identifiable, in striking contrast with the results after treatment with trypsin. After pepsin treatment, many of the spheres showed resistant rings of material similar in appearance to the structures resistant to trypsin, but again the digestion had gone less far.

Effect of water on the virus

Burnet (1956) showed that influenza filaments became beaded and then disintegrated in distilled water without, however, any significant fall occurring in the infectivity of the preparation. We confirmed that filaments of the Persia strain adsorbed on the film showed a marked change in appearance after immersion in distilled water for 1 hr. (Pl. 3, fig. 14). The filaments were mostly ballooned into a series of disk-like forms. The spheres appeared unaffected by this treatment but they too were not always able to withstand completely salt-free distilled water for long. Pl. 3, fig. 15, shows the striking appearance of a suspension of the MEL strain, initially in saline, after overnight dialysis against 300 times its volume of distilled water followed by 4 hr. further dialysis against a similar volume of fresh distilled water. The spheres had then obviously started to break down and showed thread-like structures often forming small polygonal shapes, the threads extending outwards from the spheres.

Further observations on the occurrence and behaviour of the filaments

Occurrence. Short filaments occur in a number of viruses of the mumps-influenza (*Myxovirus*) group, e.g. influenza B and C and fowl plague, but abundant long filaments are characteristic of recently isolated strains of influenza virus A. We have found that filament production is a property which is independent of the O-D phase behaviour of the virus (Burnet & Bull, 1948) and of the 'incompleteness' of the virus (von Magnus, 1946). A strain of influenza virus A was isolated in monkey kidney in the 'O' (or original) phase (Burnet & Bull, 1948); when examined in the electron microscope it showed

typical virus filaments which could be adsorbed on guinea-pig red cells. Also, 'incomplete virus' prepared from the A/Persia/2/52 virus by the technique of von Magnus (1951) had an infectivity/agglutinin titre ratio 10^{-3} that of standard virus; the appearance of this virus in the electron microscope was indistinguishable from that of standard virus.

Infectivity. The experiments of Donald & Isaacs (1954) suggested that influenza virus filaments were about as infectious as spheres, but since a pure preparation of filaments had not been obtained it was difficult to exclude with certainty the possibility that filaments were not infectious. We have recently found that the extremely long filaments present in infected allantoic fluid tended to break when adsorbed on red cells; this was evident from the fact that although the very long filaments were removed from a virus preparation by absorption with red cells, no filaments as long were to be seen on these cells. Presumably they broke during the pipetting needed to redisperse the centrifuged red cells. This means that the counts of filaments made by Donald & Isaacs (1954) were probably over-estimates of the true number of filaments present, and hence that there is an increased probability (which unfortunately cannot be expressed quantitatively) that the filaments are infectious. In an attempt to prepare a pure preparation of filaments we tried to filter the spheres through collodion membranes of average pore diameter 260 and 990 $m\mu$. in order to see whether the filaments would be retained above the membrane. Repeated washings were carried out in this way, but the 260 $m\mu$. membrane kept back the same proportions of filaments and spheres, while the 990 $m\mu$. membrane adsorbed all the filaments in the filter pores.

The finding of Chu *et al.* (1949) that filaments can be agglutinated by a convalescent ferret antiserum was confirmed by electron microscopic examination (Bang & Isaacs, 1956). This is further evidence that the filament has virus-specific antigen along its surface.

DISCUSSION

The structure of an influenza virus filament suggested by this technique is a row of spheres which are acid-resistant but trypsin-sensitive after acid treatment, linked by some acid-soluble material. The spherical structures produced in the filaments by acid treatment are presumably protein containing the viral haemagglutinin. The alternative is that the filament contains haemagglutinin distributed uniformly along its surface and that on treatment with acid this becomes bunched into spherical bodies. The need for acid treatment before digestion occurs with trypsin may be due to the greatly increased rate of digestion of globular proteins that follows their denaturation (Haurowitz, Tunca, Schwerin & Goksu, 1945), the acid exposing the vulnerable links in the protein chain to the action of the enzyme. Another possibility is that the acid may be necessary to remove a protective coating from around the virus particle.

It appears that intact virus spheres are made of an outer protein coat, which includes the viral haemagglutinin, around a ribonucleoprotein structure; the

idea that the ribonucleoprotein is enclosed within a protein coat is based on the fact that ribonuclease will not act until the spheres have been first treated with acid and trypsin. If the infectivity of the virus is closely bound up with its nucleic acid, as is suggested by the results of Ada & Perry (1956), the ribonucleoprotein structure is probably the essential replicating part of the virus, and it seems that filaments do not have these structures occurring along their length. Possibly each filament has a single ribonucleoprotein structure at one end, but since filaments are very rapidly digested by trypsin, and since it has not been possible to obtain a pure preparation of filaments we have been unable to verify this possibility. The suggestion, however, would be compatible with the findings (Donald & Isaacs, 1954) that on treatment with ultrasonic vibrations, influenza virus filaments broke up into numerous rods and there was a big rise in the haemagglutinin titre but no change in the virus infectivity; influenza virus spheres similarly treated showed no change in viral haemagglutinin or infectivity. Studies of the nucleic acid content of filaments, which are now in progress, may help to clarify this picture.

The ribonucleoprotein structure found within the sphere has been of great interest. From shadowed preparations it appears to make up a substantial proportion of the intact sphere, possibly up to about 30 %. The ribonucleoprotein structure did not disappear following treatment by ribonuclease, but only when ribonuclease treatment was followed by a second trypsin treatment. If the ribonucleoprotein is about 30 % of the mass of the viral sphere, then the ribonucleic acid, which forms about 0.8 % of the intact sphere is only about 2.5 % of the ribonucleoprotein structure. The ribonucleic acid is intimately bound up with the protein in such a way that the protein is resistant to tryptic digestion until this comparatively small amount of nucleic acid has been first attacked by ribonuclease, but there is no evidence of how the nucleic acid may be arranged in the ribonucleoprotein. The double line of staining seen very clearly in Pl. 2, fig. 9, is apparently not an artefact since in preliminary experiments a corresponding nucleoprotein ring found in Newcastle disease virus of fowls showed a similar but treble line of staining, but the experiments do not indicate whether those lines are due to nucleic acid or to protein.

A striking feature of the ribonucleoprotein structure has been the frequency with which it has shown a polygonal outline. Many of the polygons seem to be pentagons viewed from different angles, but it is difficult to be sure that they are not hexagons with one side foreshortened. The question arises, what is the structure of the nucleoprotein in the intact virus sphere? Three possibilities may be considered. It may occur as a polygonal ring lying near the circumference of the virus sphere, or as a long thread coiled up within the sphere which uncoils to form a ring when the surrounding protein is digested away, or the structure may be a hollow sphere collapsed in its centre to give a ring-like appearance. The last suggestion seems the least likely from an examination of many pictures, and it does not account for the frequent finding of polygons. The first suggestion, that a polygonal ring occurs within each virus sphere would seem the most probable, but intact virus spheres have given little indication of any such internal structure. It is still necessary to account for the

fact that some of the ribonucleoprotein structures are very much larger than the original virus sphere. Possibly the nucleoprotein has some sort of folded, coiled or helical substructure which is capable of uncoiling under certain conditions, and the way in which it is attached to the film might decide whether uncoiling could occur.

Preliminary work on fowl plague virus and the virus of Newcastle disease of fowls has shown that they, too, are digested to give trypsin-resistant rings. Schafer & Zillig (1954) have shown that fowl plague virus can be fractionated by ether into two components. One, termed the 'haemagglutinin', consists of spherical bodies 300 Å. in diameter and the other, the 'bound antigen', of rods 150 Å. wide and up to 1,000 Å. long. This bound antigen was found by chemical analysis to be a ribonucleoprotein and the electron micrographs published by these authors suggest that their rods might well be broken sides of the trypsin-resistant rings we have just described. Frisch-Niggemeyer & Hoyle (1956) have similarly fractionated influenza A virus and found that the entire ribonucleic acid content of the virus is combined with protein in their 'soluble antigen' fraction. It would be worth while to see whether the 'haemagglutinin' fraction of these viruses was sensitive to treatment with acid and trypsin, and the ribonucleoprotein to treatment with ribonuclease and trypsin.

In contrast with this picture of the nucleoprotein of the influenza virus is the picture now well established for the pox group of viruses (Dawson & McFarlane, 1948; Peters & Stoeckenius, 1954). These viruses contain deoxyribonucleic acid and when digested with pepsin show a central resistant 'nucleus' cuboid in form. The central body can be digested away only when treated with deoxyribonuclease followed by treatment with further pepsin (Peters & Stoeckenius, 1954)—a procedure analogous to that described here using ribonuclease and trypsin for the influenza nucleoprotein. It would be of great interest to know whether the animal viruses which contain ribonucleic acid differ in the gross structure of their nucleoprotein from those which contain deoxyribonucleic acid and this possibility is now under study.

Liu (1955) found by means of fluorescent-labelled antibody that influenza virus 'soluble' antigen, presumably the ribonucleoprotein, was first detectable in the nucleus and later in the cytoplasm of the infected cells of ferret nasal turbinates, whereas the virus specific antigen, i.e. the haemagglutinin, was only found close to the cell surface. We may speculate that the nucleoprotein structures which we have demonstrated are formed in the cell nucleus and pass into the cytoplasm towards the cell surface where they induce the formation of viral haemagglutinin. If the infecting virus is poorly adapted to growth in the particular cells, it may not interfere with the formation of microvilli by these cells. As a result virus may be excreted from the cells to a large extent in the form of long filamentous processes representing microvilli which have been converted into virus filaments (Bang, 1955). If the infecting virus is well adapted to the cells it may so upset the cell surface activity as to inhibit the formation of microvilli with the result that the virus is excreted mainly as ribonucleoprotein rings incorporated in spheres of haemagglutinin. This hypothesis is based on scanty evidence but it may stimulate experiments to see

whether by suitable treatment cells can be encouraged to produce increased numbers of filaments or spheres.

REFERENCES

- ADA, G. L. & PERRY, B. T. (1956). Influenza virus nucleic acid: relationship between biological characteristics of the virus particle and properties of the nucleic acid. *J. gen. Microbiol.* **14**, 623.
- BANG, F. B. (1955). Morphology of viruses. *Annu. Rev. Microbiol.* **9**, 21.
- BANG, F. B. & ISAACS, A. (1956). Morphological aspects of virus cell relationships in influenza, mumps and Newcastle disease. *Ciba foundation symposium*. London. (In the Press.)
- BRADLEY, D. E. (1954). Evaporated carbon films for use in electron microscopy. *Brit. J. appl. Phys.* **5**, 65.
- BURNET, F. M. (1956). Filamentous forms of influenza virus. *Nature, Lond.* **177**, 130.
- BURNET, F. M. & BULL, D. R. (1943). Changes in influenza virus associated with adaptation to passage in chick embryos. *Aust. J. exp. Biol. med. Sci.* **21**, 55.
- CHU, C. M., DAWSON, I. M. & ELFORD, W. J. (1949). Filamentous forms associated with newly isolated influenza virus. *Lancet*, *i*, 602.
- DAWSON, I. M. & MCFARLANE, A. S. (1948). Structure of an animal virus. *Nature, Lond.* **161**, 464.
- DONALD, H. & ISAACS, A. (1954). Some properties of influenza virus filaments shown by electron microscope particle counts. *J. gen. Microbiol.* **11**, 325.
- FRISCH-NIGGEMEYER, W. & HOYLE, L. (1956). The nucleic acid and carbohydrate content of influenza virus A and of virus fractions produced by ether disintegration. *J. Hyg., Camb.* **54**, 201.
- HALL, C. E. (1955). Electron densitometry of stained virus particles. *J. biophys. biochem. Cytol.* **1**, 1.
- HAUROWITZ, F., TUNCA, M., SCHWERIN, P. & GOKSU, V. (1945). The action of trypsin on native and denatured proteins. *J. biol. Chem.* **157**, 621.
- KUNITZ, M. (1940). Crystalline ribonuclease. *J. gen. Physiol.* **24**, 15.
- LIU, C. (1955). Studies in influenza infection in ferrets by means of fluorescein-labelled antibody. 11. The role of 'soluble antigen' in nuclear fluorescence and cross reactions. *J. exp. Med.* **101**, 677.
- MAGNUS, P. VON (1946). Studies on interference in experimental influenza. 1. Biological observations. *Ark. Kemi Min. Geol.* **24b**, 1.
- MAGNUS, P. VON (1951). Propagation of the strain of influenza A virus in chick embryos. 11. The formation of 'incomplete' virus following inoculation of large doses of seed virus. *Acta path. microbiol. scand.* **28**, 278.
- MOSLEY, V. H. & WYCKOFF, R. W. G. (1946). Electron micrography of the virus of influenza. *Nature, Lond.* **157**, 263.
- PETERS, D. & STOECKENIUS, W. (1954). Structural analogies of pox viruses and bacteria. *Nature, Lond.* **174**, 224.
- SCHÄFER, W. & ZILLIG, W. (1954). Über den Aufbau des virus—elementarteilchens der klassischen Geflügelpest. *Z. Naturf.* **9b**, 779.

EXPLANATION OF PLATES

PLATE 1

Preparations fixed in osmium tetroxide vapour and then treated with phosphotungstic acid.

Fig. 1. Untreated preparation of influenza virus filaments and spheres. $\times 20,000$.

Fig. 2. Part of a filament. $\times 120,000$.

Fig. 3. Influenza virus spheres. $\times 120,000$.

Fig. 4. Filaments and spheres after treatment with 0.1 N-HCl. $\times 20,000$.

Fig. 5. Part of a filament after treatment with 0.1 N-HCl. $\times 120,000$.

PLATE 2

Preparations fixed in osmium tetroxide vapour. Figs. 6-9 treated with phosphotungstic acid; fig. 10 shadowed with platinum.

Figs. 6-10. Influenza virus spheres after treatment with 0.1 N-HCl and 0.1 % trypsin. $\times 120,000$.

PLATE 3

Preparations fixed in osmium tetroxide vapour and then treated with phosphotungstic acid.

Fig. 11. Influenza virus filaments and spheres after treatment with 0.1 N-HCl and 0.1 % trypsin. $\times 20,000$.

Fig. 12. Influenza virus filaments and spheres after treatment with 0.1 N-HCl, 0.1 % trypsin and 0.1 % ribonuclease. $\times 20,000$.

Fig. 13. Influenza virus filaments and spheres after treatment with 0.1 N-HCl, 0.1 % trypsin, 0.1 % ribonuclease and a second treatment with HCl and trypsin. $\times 20,000$.

Fig. 14. Influenza virus filaments and spheres after immersing in water for 1 hr. $\times 20,000$.

Fig. 15. Influenza virus spheres after dialysis against water. $\times 50,000$.

(Received 11 August 1956)

R. C. VALENTINE AND A. ISAACS STRUCTURE OF INFLUENZA VIRUS. PLATE 1

(Facing p. 204)

R. C. VALENTINE AND A. ISAACS -STRUCTURE OF INFLUENZA VIRUS. PLATE 2