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Degradation of influenza virus by non-ionic detergent

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SUMMARY

Preparations of influenza virus A0 PR8/34 and A2 Malaysia/68 have been studied in the electron microscope. They were similar in appearance to preparations made by others. Each preparation was degraded by Triton N 101. The process of degradation appeared to be different from that observed using ether and, by inference, a number of other agents.

INTRODUCTION

Corbel & Rondle (1970) described the biological properties of soluble materials obtained by treatment of influenza virus with Triton N 101 (Nonylphenoxypolyethoxyethanol). The physical process of virus disruption was followed using an electron microscope. As far as is known to the authors the probable sequence of events was unique and is therefore reported in this paper.

MATERIALS AND METHODS

Virus strains and virus purification

The work was done with A0 PR8/34 and A2 Malaysia/68. The history of the strains and full details of purification are described by Corbel & Rondle (1970). Most work was done with virus purified by centrifugation in sugar density gradients. Experiments using ether as degrading agent were done with virus purified only by differential centrifugation.

Virus degradation

Purified virus was removed from suspension by centrifugation at 100,000 *g* for 1 hr. The supernatant fluid was discarded. In most experiments virus pellets were resuspended in phosphate-buffered saline containing 1% Triton N 101 and stored 1 hr. at 4° C.; in one experiment a pellet of A0 PR8/34 was resuspended in phosphate-buffered saline and an equal volume of ether was added and the mixture shaken for 1 hr. at 4° C. After treatment the preparations were centrifuged at 100,000 *g* for 1 hr. and the supernatant fluids discarded.

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Electron microscopy

Drained pellets of purified virus and drained pellets of degraded material were kept at 4° C. and processed within 1 hr. of preparation. Pellets were resuspended in distilled water (0·1 ml.). A drop of each suspension was mixed with one drop of 3 % (w/v) neutralized phosphotungstic acid on a clean microscope slide and the suspension applied immediately to carbon grids. Excess fluid was removed after 15 sec. and the grids allowed to dry. They were examined in a GEC/AEI EM 6 electron microscope and photographs were taken on Ilford N 50 film.

RESULTS

A representative specimen of A 0 PR 8/34 is shown in Pl. 1, fig. 1. Virus particles reasonably regular in size and each possessing external projections occupy most of the field. There is little debris to be seen.

A probable early effect of treatment with Triton N 101 is shown in Pl. 1, fig. 2. The external projections are stripping off the virus particle but remaining attached to each other. Plate 2, fig. 1 shows a probable later stage in this process. The field is littered with strings of projections and contains a few uncoated particles of the size of influenza virus. At a later stage, as shown in Pl. 2, fig. 2, the strings of projections cannot be found and only 'ghost' particles, some penetrated by stain, can be seen. Finally, as stated by Corbel & Rondle (1970), more drastic treatment with Triton N 101 leads to complete disruption of the virus with production of soluble material not sedimented by centrifugation at 100,000 *g* for 1 hr.

This probable series of events differs from that occurring after treatment with ether. As shown in Pl. 3, fig. 1, ether treatment of A 0 PR 8/34 caused 'rounding-up' of the external projections followed by complete disruption of virus particles and release of internal components (Pl. 3, fig. 2). The observed effects of ether are in agreement with the results of Hoyle, Horne & Waterson (1961).

The effect of treating influenza virus with Triton N 101 was studied also using A 2 Malaysia/68. A typical purified virus preparation is shown in Pl. 4, fig. 1. It was not possible to obtain preparations of this virus as uniform or as free from debris as the preparations of A 0 PR 8/34. This was possibly due to its relatively recent isolation and poor degree of adaptation to the chick chorio-allantois. However, as shown in Pl. 4, fig. 2, individual particles appeared as typical influenza virus and when treated with Triton N 101 (Pl. 5, fig. 1) the external projections were removed and ghost particles finally produced (Pl. 5, fig. 2).

DISCUSSION

Examination of purified influenza virus preparations in the electron microscope gave pictures similar to those published previously (Hoyle *et al.* 1961). The virus strain A 2 Malaysia/68 was more pleomorphic than A 0 PR 8/34 which is well adapted to the chick chorio-allantois. Both viruses, however, were degraded in a similar way by Triton N 101. The external projections were removed probably

at first as a continuous string, and virus ghosts were formed. The strings of external projections were apparently degraded in turn to smaller subunits morphologically similar to the monovalent haemagglutinin preparations obtained by Choppin & Stoeckenius (1964). However, the effects of Triton N 101 and ether differ considerably in the early and intermediate stages of treatment.

Other methods have been used to disrupt influenza virus. Thus Valentine & Isaacs (1957) used hydrochloric acid and trypsin; Laver (1963) used sodium deoxycholate and sodium dodecyl sulphate; Waterson, Hurrell & Jensen (1963) used formaldehyde and chloroform; and Choppin & Stoeckenius (1964) used ultrasonic vibrations. The effect of these reagents ranged from destruction of external projections to complete fragmentation of virus. Blough (1963*a*, *b*) studied the effect of ionic and non-ionic surface-active agents on influenza virus but did not report effects similar to those seen with Triton N 101.

Again, the effect of enzymes on influenza virus has been studied. Thus Simpson & Hauser (1965, 1966) used phospholipase C. This enzyme caused progressive disruption of virus without release of discrete surface structure. Finally, the effect of a number of proteolytic enzymes on influenza virus has been studied; Reginster (1965) used pronase, and Biddle (1968) used a bacterial protease. Under certain conditions these enzymes destroyed the external projections.

The results suggest that Triton N 101 differs from ether and other agents in its effect on influenza virus. Mild treatment with Triton N 101 leads initially to removal only of the outer coat of the virus. This mild degradation leaves a particle still bounded by a membrane. The finding supports the view of Kates, Allison, Tyrrell & James (1962) who suggested that influenza virus has two outer membranes. They stated that one membrane might be derived from the host nuclear membrane and the second manufactured in host-cell cytoplasm or derived from host-cell membrane. It is possible that isolation and further investigation of the early products of Triton N 101 degradation might lead to a better understanding of the structure of influenza virus.

REFERENCES

Biddle, F. (1968). The action of protease on influenza A 2 virus. *Journal of General Virology* **2**, 19–28.

Blough, H. A. (1963*a*). The role of the surface state in the morphogenesis of influenza virus filaments. *Virology* **19**, 112–14.

Blough, H. A. (1963*b*). The effect of vitamin A alcohol on the morphology of myxoviruses. I. The production and comparison of artificially produced filamentous virus. *Virology* **19**, 349–58.

Choppin, P. W. & Stoeckenius, W. (1964). Interactions of ether-disrupted influenza A 2 virus with erythrocytes, inhibitors and antibodies. *Virology* **22**, 482–92.

Corbel, M. J. & Rondle, C. J. M. (1970). Soluble antigens obtained from influenza virus by degradation with non-ionic detergent. *Journal of Hygiene* **68**, 81–96.

Hoyle, L., Horne, R. W. & Waterson, A. P. (1961). The structure and composition of the myxoviruses. II. Components released from the influenza virus particle by ether. *Virology* **13**, 448–59.

Kates, M., Allison, A. C., Tyrrell, D. A. J. & James, A. T. (1962). Lipids of influenza virus and their relation to those of the host cell. *Biochimica et Biophysica Acta* **52**, 455–66.

LAVER, W. G. (1963). The structure of influenza viruses. 3. Disruption of the virus particle and separation of neuraminidase activity. *Virology* **20**, 251–62.

REGINSTER, M. (1965). Inactivation of influenza virus by caseinase C from *Streptomyces albus* G culture filtrate. *Journal of General Microbiology* **40**, 157–69.

SIMPSON, R. W. & HAUSER, R. E. (1965). Structures associated with influenza virus suspensions treated with phospholipase C. *Virology* **27**, 642–6.

SIMPSON, R. W. & HAUSER, R. E. (1966). Influence of lipids on the viral phenotype. I. Interaction of myxoviruses and their lipid constituents with phospholipases. *Virology* **30**, 684–97.

VALENTINE, R. C. & ISAACS, A. (1957). The structure of viruses of the Newcastle disease–mumps–influenza (Myxovirus) group. *Journal of General Microbiology* **16**, 680–85.

WATERSON, A. P., HURRELL, J. M. W. & JENSEN, K. E. (1963). The fine structure of influenza A, B and C viruses. *Archiv für die Gesamte Virusforschung* **12**, 487–95.

EXPLANATION OF PLATES

Magnification *ca.* 200,000 except for Pl. 4, fig. 2 which was *ca.* 400,000. All preparations were negatively stained with phosphotungstic acid.

PLATE 1

Fig. 1. Influenza virus A0 PR 8/34 purified by centrifugation in a sugar density gradient.
Fig. 2. Preparation shown in Fig. 1 treated with Triton N 101.

PLATE 2

Fig. 1. Preparation shown in Pl. 1, fig. 1 treated with Triton N 101. A probable further stage in degradation.

Fig. 2. Preparation shown in Pl. 1, fig. 1 treated with Triton N 101. Probably a late stage in degradation.

PLATE 3

Fig. 1. Influenza virus A0 PR 8/34 treated with ether.
Fig. 2. Probably a further stage of degradation from Fig. 1.

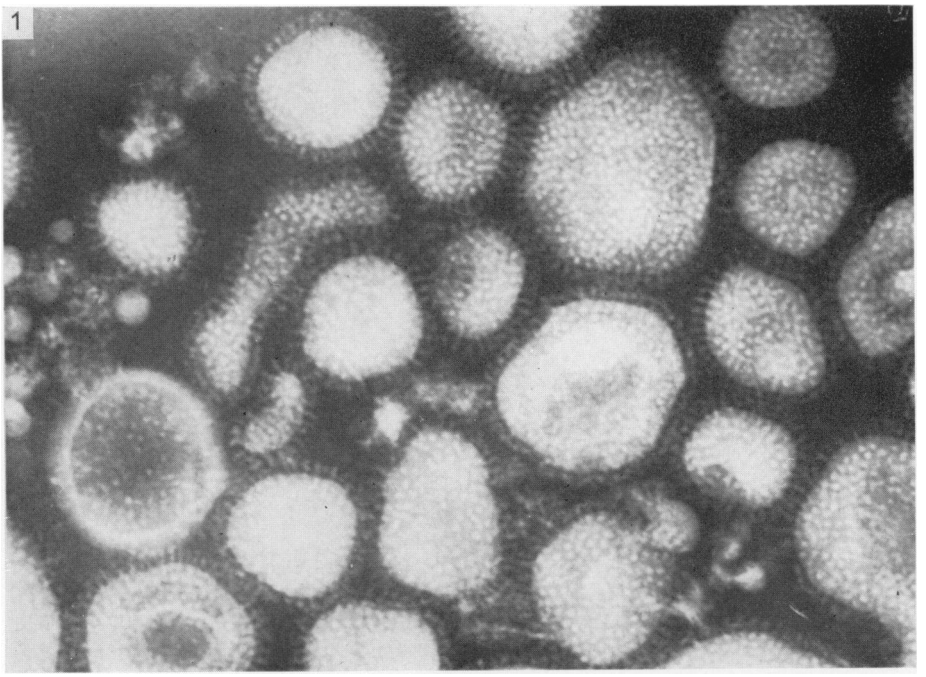
PLATE 4

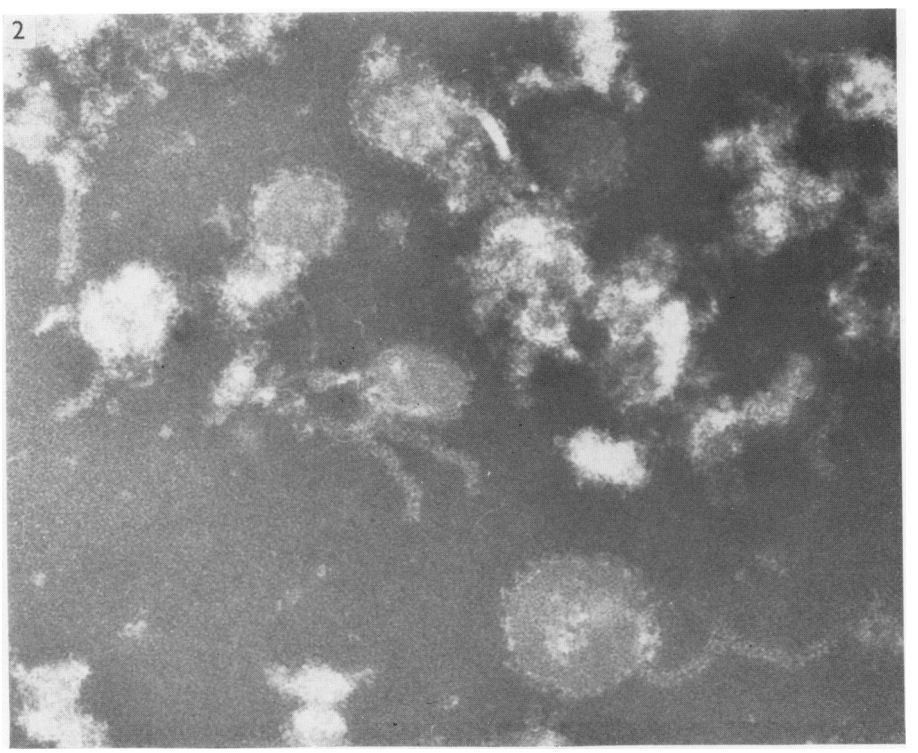
Fig. 1. Influenza virus A2 Malaysia/68 purified by centrifugation in a sugar density gradient.
Fig. 2. Single particle showing myxovirus morphology.

PLATE 5

Fig. 1. Particle from preparation shown in Pl. 4, fig. 1 after treatment with Triton N 101. External projections mostly removed.

Fig. 2. Preparation shown in Pl. 4, fig. 2 after treatment with Triton N 101. Only 'ghost' particles remain.





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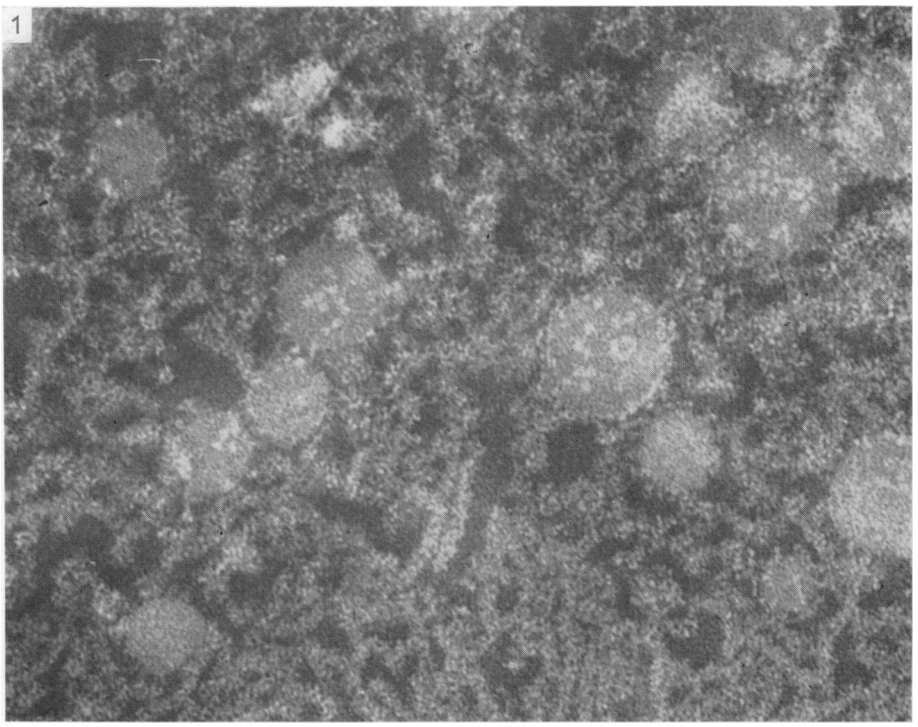
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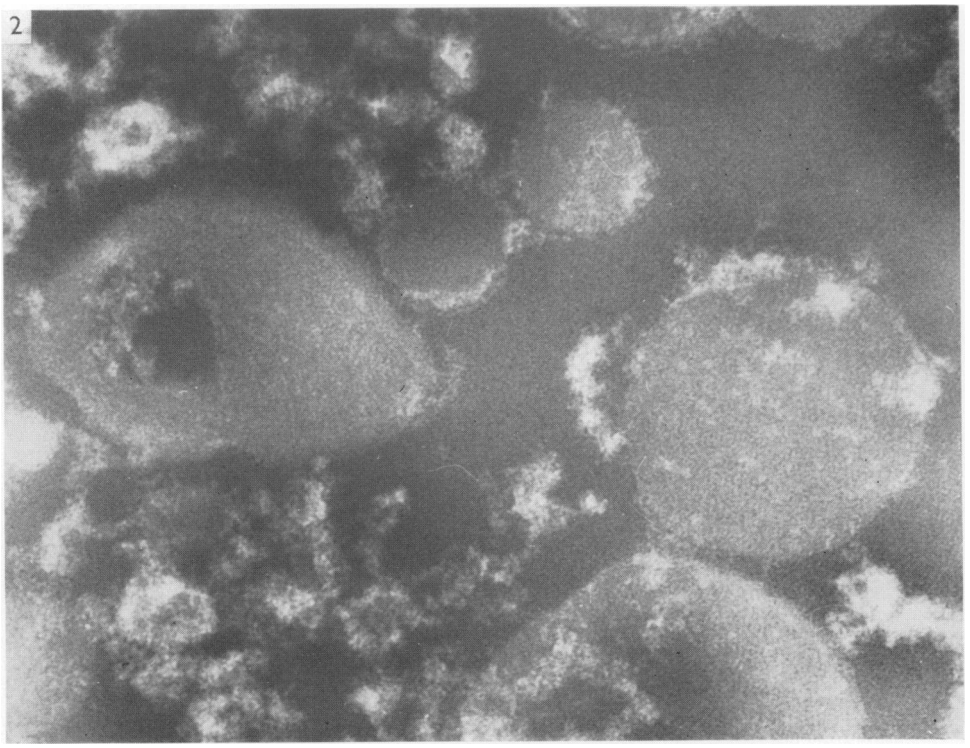
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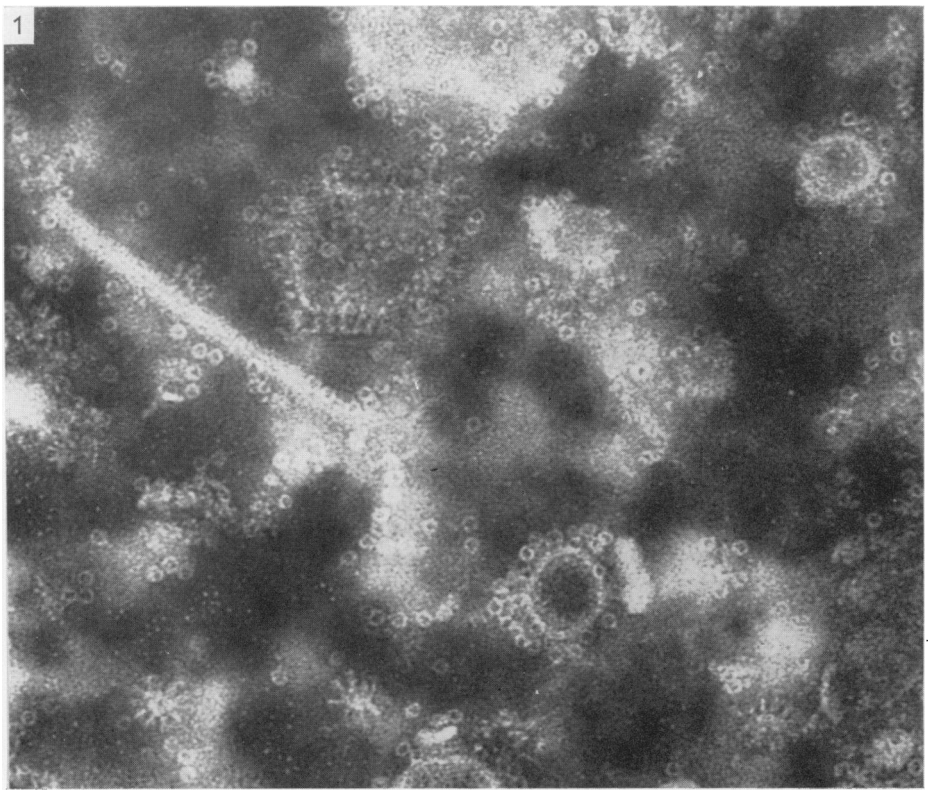
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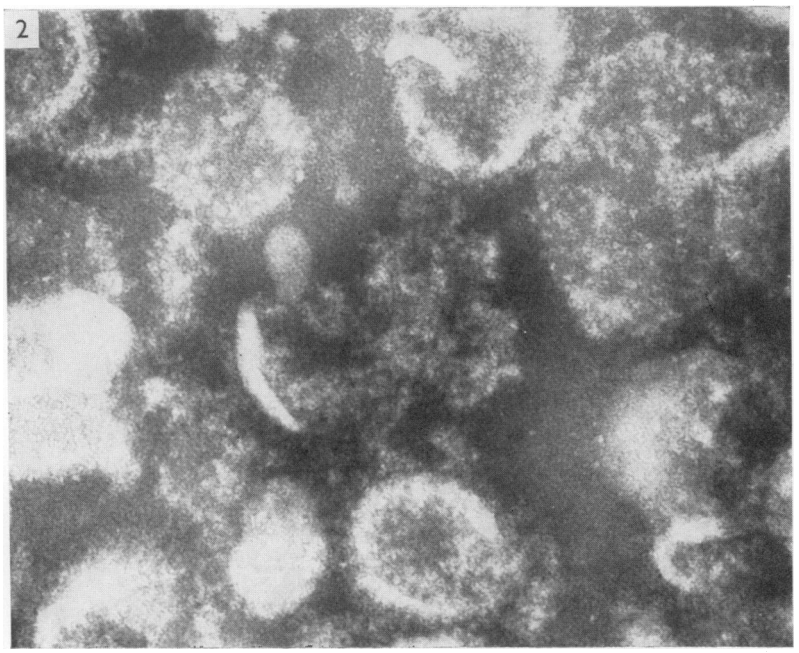
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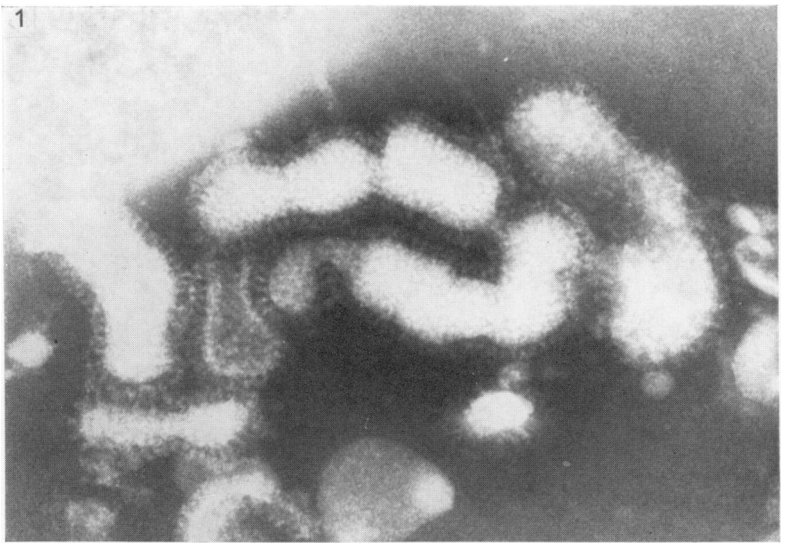
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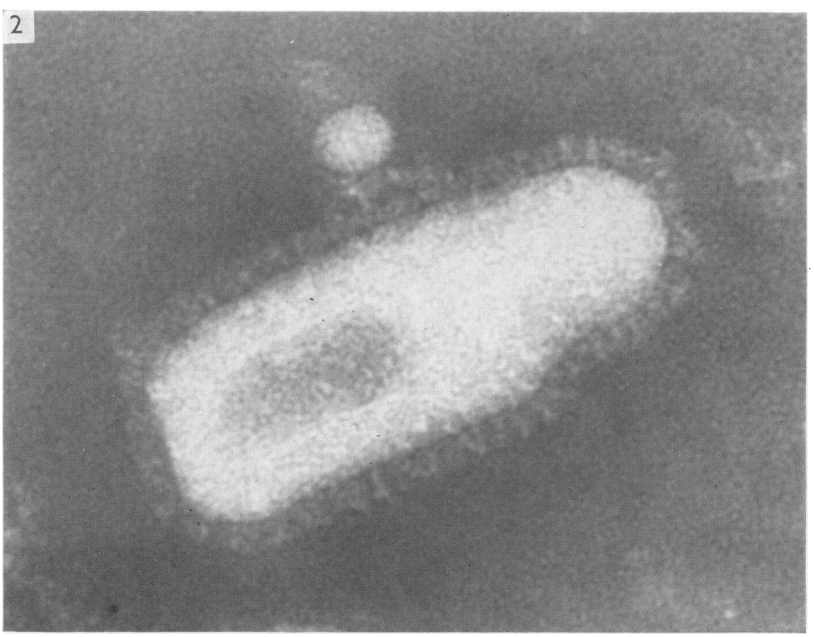
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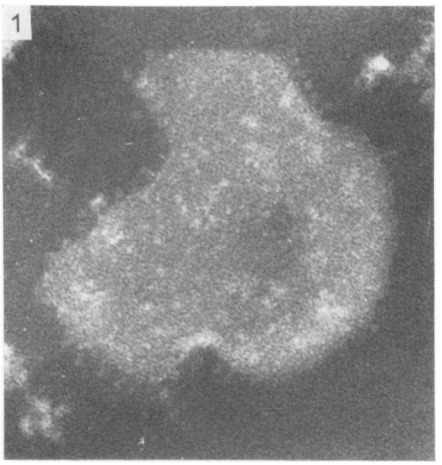
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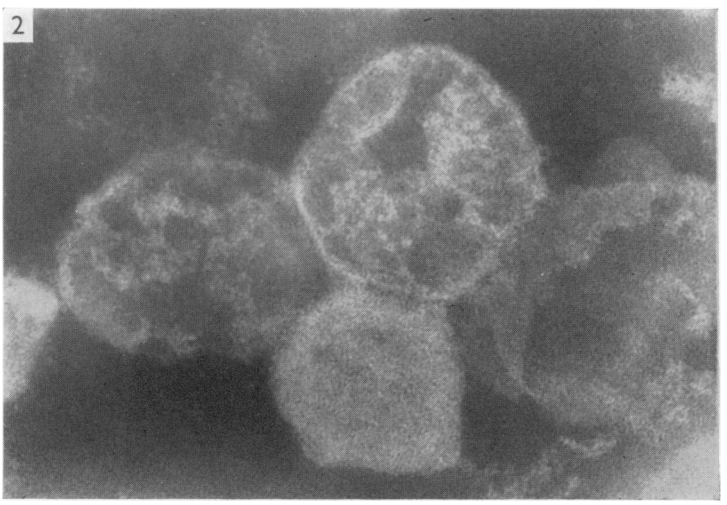
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