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The Morphology of Three Previously Uncharacterized Human Respiratory Viruses that Grow in Organ Culture

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SUMMARY

A simple method is described for examining organ cultures by electron microscopy for the presence of virus particles. The method was used to detect the presence of three hitherto uncharacterized viruses. Two of these have particles resembling those of infectious bronchitis of chickens and the third morphologically resembles the parainfluenza group of viruses.

INTRODUCTION

Organ cultures of respiratory epithelium provide a practical and sensitive means of propagating human respiratory viruses. Some viruses can at present only be grown in this way (Tyrrell & Bynoe, 1965, 1966; Hoorn & Tyrrell, 1966). Unfortunately, several such viruses can only be detected by inoculating volunteers. It was therefore decided to try to detect these viruses and characterize them morphologically by the electron-microscope technique of negative staining. Originally negative staining was applied to purified preparations of virus particles (Brenner & Horne, 1959), but it was later shown that crude preparations of whole cells could also be used to study cell-associated viruses (Horne & Nagington, 1959; Almeida & Howatson, 1963; Parsons, 1963).

In the present instance, suspensions obtained by mild treatment of the organ cultures in a glass homogenizer have been used for microscopy and three uncharacterized respiratory viruses have been seen. Two of these were of a morphological type not previously associated with human disease.

METHODS

Organ culture. Nasal epithelium and trachea were dissected from human embryos of 14 to 24 weeks gestation and planted on scratched areas of plastic Petri dishes 60 mm. diameter AA grade (Esco Rubber Co.). The nasal epithelium was supplied with 2 ml. of Eagle's medium containing 0·2% (w/v) bovine plasma albumin; the trachea received 1·25 ml. The dishes were incubated at 33° in sealed humidified plastic boxes in an atmosphere of 5% (v/v) carbon dioxide in air. After 2 days' incubation, the medium was changed and the cultures inoculated by dropping 0·2 ml.

of virus suspension on to the tissue. The cultures were then incubated for a further 4 days, when the medium was removed and, where possible, titrated for virus in roller tube tissue cultures incubated at 33°.

Electron microscopy. The tissue fragments were treated very lightly in a loosely fitting glass homogenizer of the TenBroeck type with a few drops of distilled water. Most of the tissue fragment remained intact and mainly the superficial, virus-infected cells were detached. A drop of this cell suspension was mixed with an equal quantity of 3% (w/v) phosphotungstic acid adjusted to pH 6·0 with potassium hydroxide. A small amount of this mixture was then placed on a carbon+formvar coated grid, excess fluid removed with filter paper and the specimen examined immediately in a Philips 200 electron microscope. If this procedure could not be carried out within a few hours of harvesting the tissue fragments, they were frozen at −70° until convenient.

Several known viruses were examined to establish the practicability of the method which was used then on three uncharacterized viruses that cause human upper respiratory disease. These are (*a*) strain 229 E of Hamre & Procknow (1966); (*b*) strain B 814 (Tyrrell & Bynoe, 1965); (*c*) strain LAKEY (Tyrrell & Bynoe, 1966).

RESULTS

Virus particles or viral components were detected in almost all the cultures inoculated with known viruses, and in no instance in an uninoculated control. An additional control was provided by examining cultures that had been inoculated with herpes simplex and vaccinia viruses and then not incubated but held at 4°. No virus particles were seen in these preparations.

Each of the three uncharacterized human viruses revealed virus particles or viral components associated with the negatively stained cellular fragments. Strain 229 E contained particles (Pl. 1, figs. 1, 2) resembling closely the particles of avian infectious bronchitis (Berry *et al.* 1964). The particles are pleomorphic in form and although varying somewhat in size have an average diameter of about 800 to 1200 Å. The surface of the particles is covered with a distinct layer of projections roughly 200 Å, long. These projections seem to have a narrow stalk just within the limit of resolution of the microscope and a 'head' roughly 100 Å across.

Similar particles were found in organ cultures infected with the second uncharacterized strain, B 814, which cannot at present be grown in tissue cultures (Tyrrell & Bynoe, 1965). The particles from this strain (Pl. 1, figs. 3, 4) were indistinguishable both from those of 229 E (Pl. 1, figs. 1, 2) and of avian infectious bronchitis.

The third unknown strain, LAKEY, had been obtained from the nasal washings from a patient with a cold (Tyrrell & Bynoe, 1966). It had been passed twice in organ cultures and the medium from these cultures produced colds in volunteers. On one occasion a very poor haemadsorption had been seen in a few outlying cells of a roller tube culture of rhesus monkey kidney inoculated 10 days previously with culture medium. Although this observation could not be repeated it was a clue suggesting that this might be some type of myxovirus. The electron-microscope preparation showed a great deal of helical material with a diameter of 180 Å which was indistinguishable from the internal component of viruses such as the parainfluenza

group (Pl. 2, figs. 5, 6). Until adsorption to and elution from red cells has been definitely established it is not possible to say that it is a myxovirus, but the morphology found does establish that the virus belongs to the subgroup of compound viruses having a morphology like that of Newcastle disease virus (Waterson & Almeida, 1966).

DISCUSSION

The procedure that we have used for identifying viruses grown in organ culture is both simple and speedy. Any attempt at a conventional purification procedure from the tissue fragments would be difficult and inefficient since the amount of material available is so small and the proportion of infected cells is so low. When the culture is handled in the way described, we believe that the cells that go into suspension and are used for electron microscopy are mainly those that are infected with virus. Virus particles were identified even when the titre in the supernatant was low.

Probably the most interesting finding from these experiments was that two human respiratory viruses, 229 E and B 814, are morphologically identical with avian infectious bronchitis. Their biological properties, as far as they are known, are consistent with this. Both the human viruses are ether sensitive as is avian infectious bronchitis 229 E, have a similar size by filtration and multiply in the presence of an inhibitor of DNA synthesis. It will be interesting to compare the serology of the morphologically similar human and chicken viruses.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1 *a*, *b*. This type of particle was seen when organ cultures infected with strain 229E were examined by the present technique. The particles are pleomorphic, in the size range 800 to 1200 Å, and are surrounded by a distinct 200 Å long fringe. They are indistinguishable from the particles of avian infectious bronchitis, the only virus previously known to have this morphology.

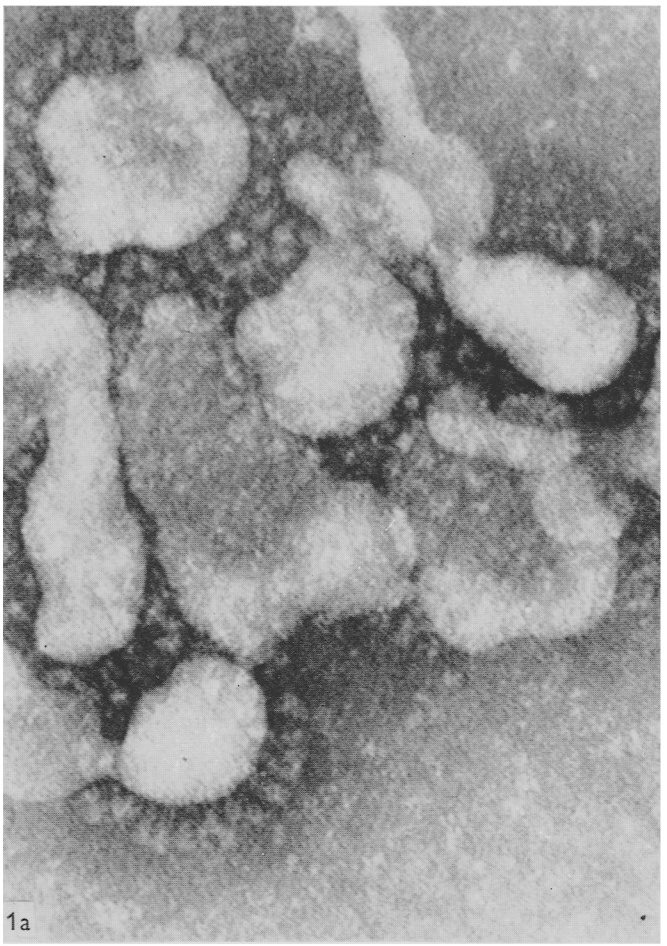
Fig. 2. An additional single particle of strain 229E (for comparison with figs. 3 and 4).

Figs. 3 and 4. Human respiratory virus strain B814, which also revealed particles indistinguishable from avian infectious bronchitis. (Magnification see Pl. 2).

PLATE 2

Fig. 5. Part of the cytoplasm of a disrupted cell containing a large quantity of helical component similar to that present in viruses such as parainfluenza (see fig. 6). In this case the virus was an uncharacterized human respiratory virus (LAKEY). Infected specimens of trachea and nasal epithelium both contained abundant viral material.

Fig. 6. Length of internal helical component from parainfluenza virus type 3 grown in organ culture of nasal epithelium for comparison with fig. 5. The bar represents 1000 Å.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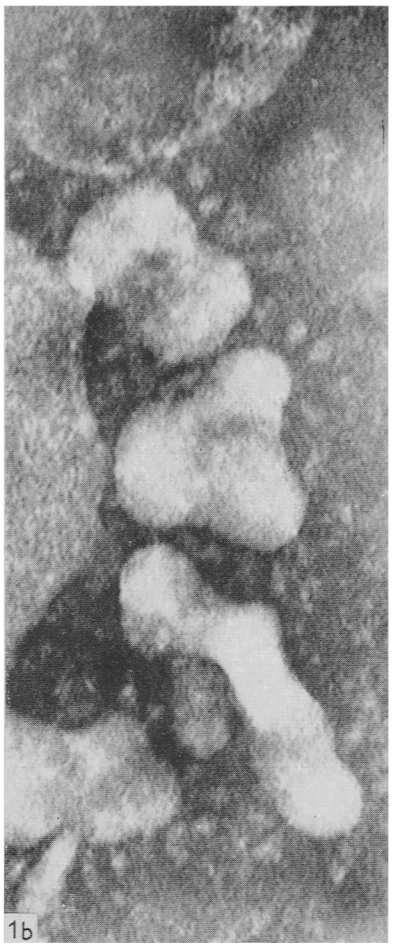
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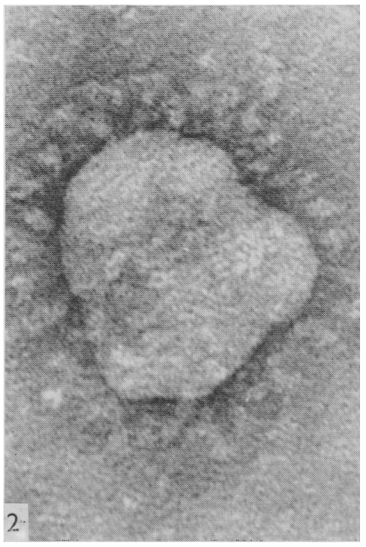
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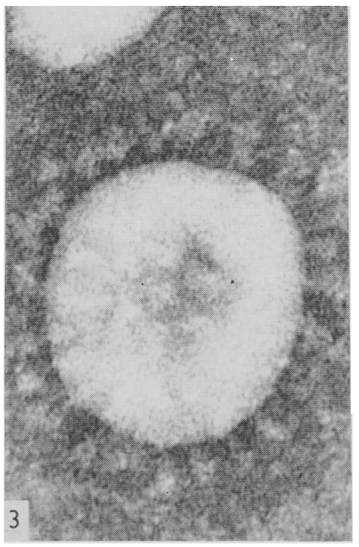
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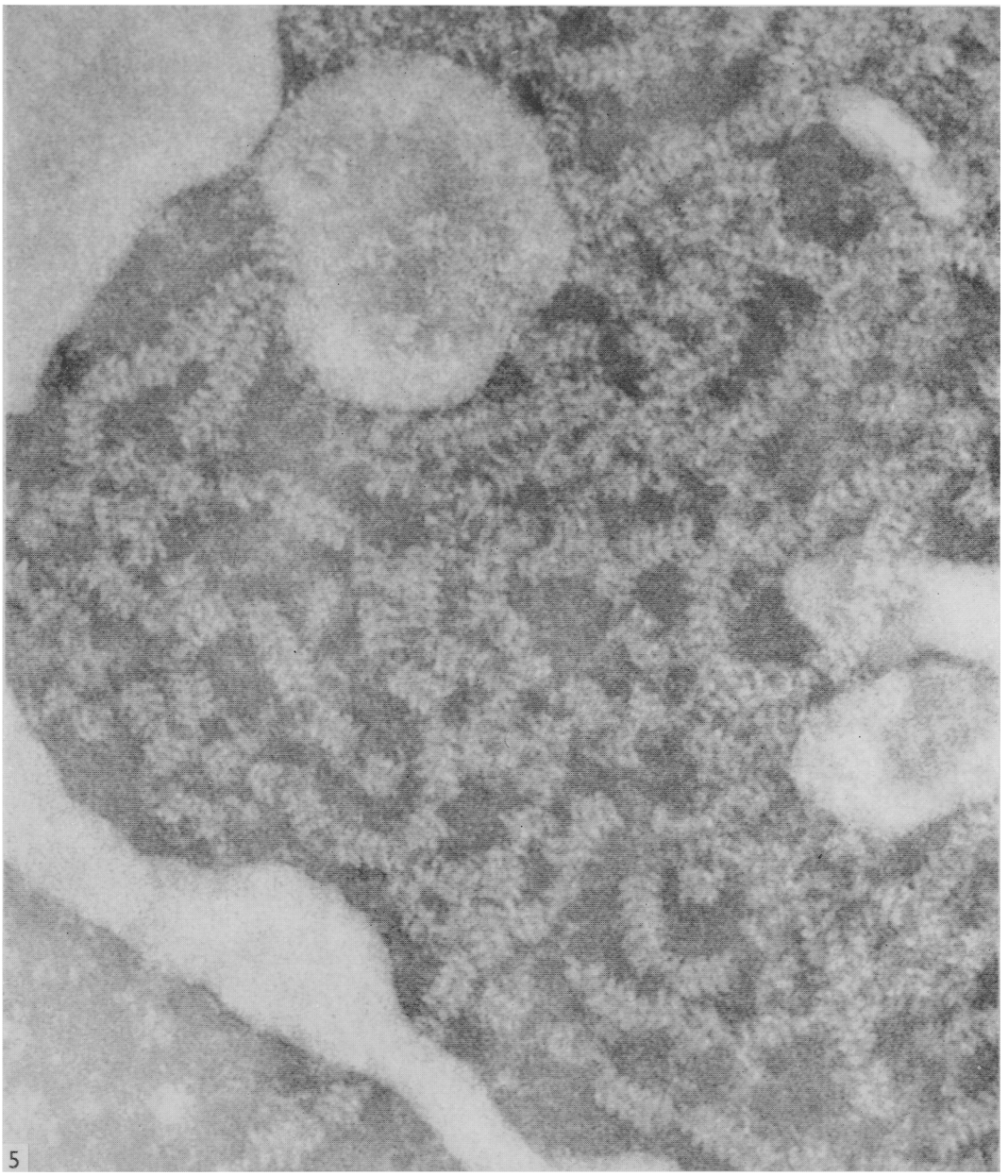

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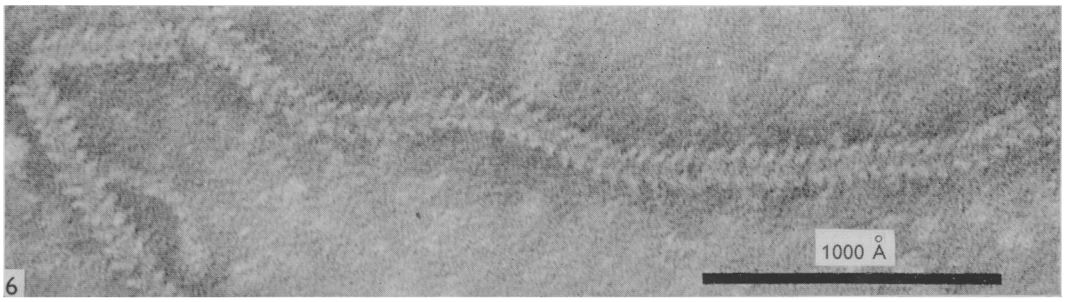

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