Royal Victoria Infirmary, Newcastle-upon-Tyne for their cooperation with histological and histochemical analysis of rectal biopsy specimens.

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CORONAVIRUS PROPAGATED FROM PATIENT WITH NON-BACTERIAL GASTROENTERITIS

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Summary A fæcal suspension from a patient with gastroenteritis, which contained large numbers of coronavirus particles when examined by electron microscopy, was inoculated into human embryo intestinal-organ cultures and primary human embryo-kidney monolayers. In the organ cultures, the villous epithelium became detached and large numbers of coronavirus particles were seen by electron microscopy both in the medium and in thin sections of the intestinal epithelial cells, where the virus appeared to be multiplying. In organ cultures and in human embryo-kidney cells, intracytoplasmic virus was demonstrated by indirect immunofluorescence with convalescent serum from the patient.

MEMBERS of the coronavirus family cause gastroenteritis in pigs (transmissible gastroenteritis [TGE] virus[1]) and calves,[2] both of which have now been propagated in cell culture.[3 4] Mathan et al.[5] have seen coronavirus-like particles in the fæces of patients with sprue.

Coronavirus particles have been seen in samples from three outbreaks of non-bacterial gastroenteritis in humans. The first (Weston) was among Service apprentices in 1965;[6] the second (Bristol) was among hospital nurses in November, 1971; and the third (Somerset) outbreak affected nursery nurses in July, 1975. Fæcal samples from the first two outbreaks were inoculated into organ and cell cultures and the coronavirus seen in a fæcal sample from the second outbreak is believed to have been propagated.

Electron Microscopy

An extract of fæces from one Bristol patient (case X) collected the day after the onset of symptoms was examined by electron microscopy and large numbers of coronavirus particles with typical projections were seen (fig. 1a). The particles were pleomorphic with an overall diameter ranging from 120 to 230 nm with fringes about 20 nm wide. No internal structure was seen in any of the particles, but some showed the collapsed appearance previously observed with coronaviruses.[7]

The particles described here were very similar in appearance to some of those described by Mathan et al.[5] in fæces of both normal people and patients with sprue in India, including the occasional bizarre-shaped particle. The morphology of the particles is distinct from that of the oncornaviruses, arenaviruses, and orthomyxoviruses, but identical with that of the coronaviruses.[8]

Attempted Propagation

0·05 ml of a 10% fæcal emulsion from patient X was inoculated into human embryo intestinal-organ culture. After 2 days' incubation, the columnar epithelium of the villi of the inoculated cultures could be seen macroscopically to have become detached. Electron microscopy of the fluids from duodenal, jejunal, and ileal organ cultures all showed very large numbers of coronavirus particles (fig. 1b) similar to those seen in the fæces.

The detachment of villous epithelium was thought to result from the multiplication of virus, as it did not occur in uninoculated cultures, nor in those inoculated with fæcal suspension heated to 56°C for 30 minutes, and there was no bacterial contamination. The villous epithelium was not shed nor were particles seen in fluids from organ cultures inoculated with fæces from the earlier Weston outbreak which contained as many coronavirus particles as the fæces from patient X. Fluids collected 2 days after inoculation with fæcal emulsion from patient X were passed into further organ cultures, and again the villous epithelium became detached, although to a lesser extent, and coronavirus particles in smaller numbers were seen on electron microscopy.

Sections of organ cultures 18 hours after the inoculation of fæcal emulsion from patient X showed virus particles with a mean diameter of about 62 nm within cytoplasmic vesicles of the columnar epithelial cells (fig. 2a). Evidence of multiplication was provided by the presence of crescents of thickened vesicle membrane, which had underlying densely staining material, bulging into the vesicles (fig. 2a). Furthermore, inclusion bodies containing tubular structures with a mean diameter of 32 nm were seen (fig. 2b). All these appearances have been previously described in sections of cells infected with coronaviruses.[9 10]

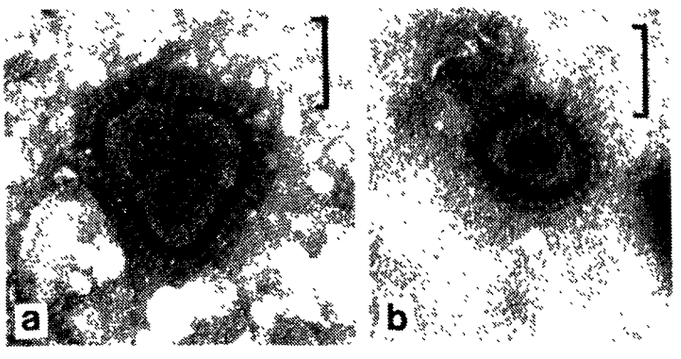

Fig. 1—Negatively stained electron micrograph of particles from (a) fæces of patient X; (b) medium from human embryo intestinal-organ culture inoculated with fæces from patient X. (×105 000. Bar=100 nm)

The nuclei were normal, and budding from the cell surface which is described for many other viruses, but not for coronaviruses, was not seen. Control cells showed none of these changes.

Fæcal emulsion from patient X was also inoculated into primary human embryo-kidney monolayers. Both these cells and sections of inoculated organ cultures showed immunofluorescence with convalescent serum from patient X and fluorescein-conjugated anti-human globulin. In the monolayers, after 2 days' incubation, small particulate areas of fluorescence were seen in the cytoplasm of some of the cells (fig. 3a), which progressed after a further 4 days' incubation to large inclusion-like masses (fig. 3b). This appearance has been described in coronavirus-infected cells.[11] No fluorescence was seen in uninoculated cultures. In the organ cultures 12 hours after inoculation, fluorescence was seen only in the epithelium of the villi and not in other cells—similar to the appearance of pig intestine infected with TGE.[11] The increase in the amount of fluorescence between the 2nd and 4th days in human embryo-kidney cells suggests multiplication and not phagocytosis.

More work is required to elucidate the relationship of this virus to gastroenteritis, and to the animal intestinal coronaviruses. Sharpel and Mebus[12] have found antibodies to calf coronavirus in human sera. These particles cannot be identified as coronaviruses on morphology alone, but the typical electron microscopic appearance in cell sections is very suggestive. Definite characterisation of the particles as coronaviruses must await further work.

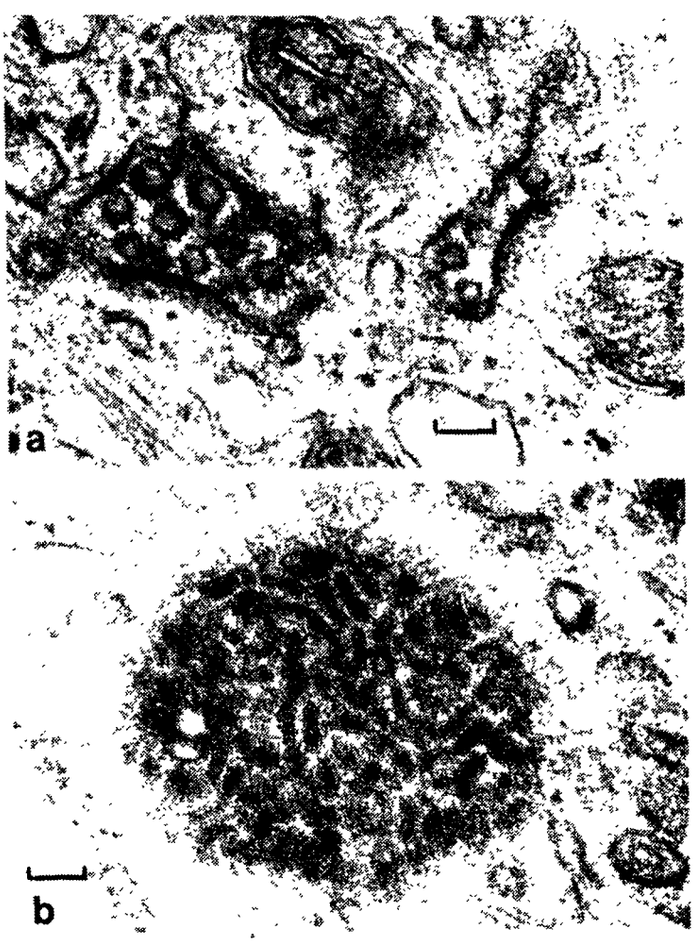

Fig. 2—Thin section of columnar epithelial cell from human embryo intestinal-organ culture inoculated with fæces from patient X: (a) showing crescents of thickened membrane lining the cystoplasmic vesicle (virus particles are in the vesicle); (b) inclusion containing tubular structures. (x 66 000. Bar=nm.)

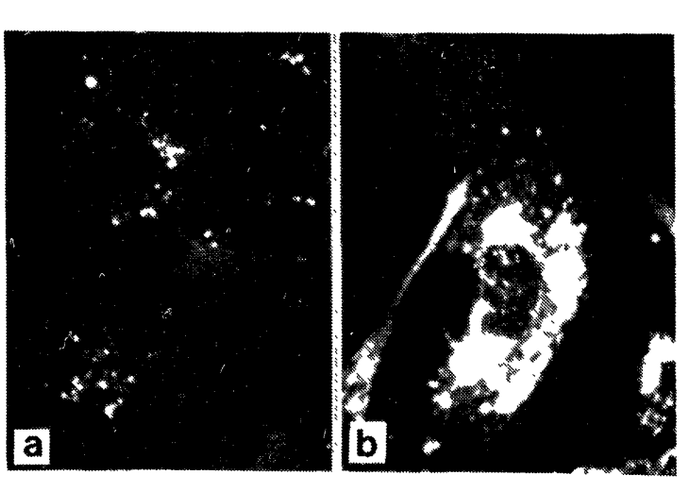

Fig. 3—Human embryo-kidney monolayer inoculated with fæces from patient X (indirect immunofluorescence with convalescent serum from patient X); (a) 2 days after inoculation; (b) 6 days after inoculation.

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CLINICAL SIGN OF OBSTRUCTED AXOPLASMIC TRANSPORT

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Summary Orthograde and retrograde axoplasmic transport in retinal ganglion-cell axons can be interrupted by axonal ischæmia. This report is believed to be the first to illustrate how this phenomenon can be observed clinically in man in cases of retinal vascular disease. The intense retinal "whiteness" of small cottonwool spots and at the periphery of larger areas of retinal ischæmia represents gross localised axonal distension secondary to the cessation axoplasmic flow.

INTRODUCTION

A CONSTANT flow of subcellular particles and molecules takes place within the axons of nerve-cells. This flow is called "axoplasmic transport", and the visual pathway provides physiologists with a convenient experimental system for its investigation. Using enzyme markers and autoradiography, slow and rapid phases of