Electron Microscopy of Stool-Shed Viruses: Retention of Characteristic Morphologies After Long-Term Storage at Ultralow Temperatures

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Storage of stool specimens at \(-70^\circ\text{C}\) has been reported to destroy the characteristic morphology of calicivirus. To determine if other stool-shed viruses are similarly affected, stool specimens previously examined by electron microscopy and observed to contain virus particles were reexamined after 6–10 years of storage at \(-70^\circ\text{C}\) to \(-85^\circ\text{C}\). The stools contained virus particles of different morphological types, including astrovirus, small round structured virus, adenovirus, and rotavirus as well as calicivirus. Also reexamined were stools containing coronavirus-like particles and \(T = 19\) virus-like particles. Characteristic virus particles, including calicivirus particles, were recognized in all the stools reexamined. The results indicate that long-term storage of stools at ultralow temperatures does not present a significant problem for the morphological identification of stool-shed viruses.

KEY WORDS: astrovirus, calicivirus, SRSV, adenovirus, rotavirus, virus-like particles

INTRODUCTION

Unlike other methods used to examine stools for viruses, electron microscopy (EM) permits the visualization and morphological characterization of individual virus particles. With EM, a stool specimen can be scanned for a number of morphologically distinct viruses in a single examination. EM does not require cell culture and is not confined, as are other methods, to detecting viruses with common antigens or similar genomes. Specimens must, however, be examined one at a time with EM. This can be time-consuming if many specimens are to be examined. When large numbers of specimens are involved, as frequently happens during outbreaks of illness, proper storage of the specimens is essential.

Commonly, specimens awaiting viral analyses are stored at \(-70^\circ\text{C}\) [Lennette et al., 1980]. However, it has been reported that such frozen storage destroys the characteristic morphology of at least one virus found in stools, calicivirus [Humphrey et al., 1984; Cubitt, 1987]. To determine if other stool-shed viruses are similarly affected by such storage, fecal specimens previously examined in this laboratory by EM and observed to contain virus particles were reexamined after 6–10 years of storage at \(-70^\circ\) to \(-85^\circ\text{C}\).

MATERIALS AND METHODS

Fecal Specimens

The specimens that were reexamined were part of a collection of EM-positive (for virus particles) fecal specimens that had been acquired from waterborne gastroenteritis investigations [Hopkins et al., 1984; Williams, 1985], from a multiyear infection surveillance study [Camann et al., 1985], and through other virus-related research activities. Most of the specimens were collected within the United States, but several came from Egypt.

The specimens contained virus particles of five distinct morphological types, including astrovirus, calicivirus, small round structured virus (SRSV) [Caul and Appleton, 1982], adenovirus, and rotavirus. Morphologically featureless viruses, such as those of the enterovirus and parvovirus groups, were not included in this study. However, stored specimens containing two morphologically distinct virus-like particles, coronavirus-like particles (CVLPs) and \(T = 19\) virus-like particles, were included in the reexamination.

The SRSV-containing stools included three specimens that had been identified as Norwalk-positive with a Norwalk virus radioimmunoassay (RIA) devel-

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EM of Stool Viruses After Storage

TABLE I. EM Examination of Stool Specimens After Long-Term Storage at −70°C to −85°C.

| Virus                        | Size (nm)  | Morphological characteristics                  | No. of specimens examined | Years in storage (No. of specimens) | Number of specimens with characteristics still visible |
|------------------------------|------------|------------------------------------------------|---------------------------|------------------------------------|------------------------------------------------------|
| Astrovirus                   | 28-30      | Starlike surface configurations                | 2                         | 6 (1), 8 (1)                       | 2                                                    |
| Calicivirus                  | 30-40      | Dark-staining cup-shaped depressions           | 1                         | 6 (1)                             | 1                                                    |
| Small round structured virus | 27-40      | Ragged-edged with poorly defined surface structure | 4                         | 7 (2), 8 (1), 9 (1)               | 4                                                    |
| Rotavirus                    | 65-75      | Wheel-like appearance when stain penetrated    | 4                         | 8 (3), 10 (1)                     | 4                                                    |
| Adenovirus                   | 70-90      | Large icosahedral shape, well defined capsomers | 5                         | 6 (1), 8 (4)                      | 5                                                    |
| Coronavirus-like particles*  | Variable   | Highly pleomorphic distinctive fringe          | 5                         | 6 (1), 7 (2), 8 (1), 9 (1)        | 5                                                    |
| T = 19 virus-like particles* | 65-70      | Capsomers in T = 19 arrangement, darker staining vertices | 1                         | 8 (1)                             | 1                                                    |

*It remains to be determined whether these virus-like particles are human viruses.

RESULTS

The results of the reexamination of fecal specimens are shown in Table I. Virus particles were visualized in each of the specimens reexamined. In each specimen, the particles retained sufficient structural integrity to permit morphological identification. This identification was consistent with the original identification made 6–10 years earlier.

Astrovirus particles were identified by the starlike configurations exhibited on suitably oriented particles (Fig. 1A). Characteristic particles were apparent in each of the two specimens reexamined. However, recognition of the starlike configurations depended greatly on a favorable negative-stain environment.

In the single calicivirus-containing stool reexamined, the observed virus particles were striking in their definition (Fig. 1B). They exhibited well defined cup-like depressions and at times revealed a characteristic Star of David (see arrowhead, Fig. 1B), a configuration that is not exhibited by astroviruses.

Much less well defined than the calicivirus particles, but still morphologically distinct, were the SRSV particles observed in the reexamined stools (Fig. 1C). These particles possessed a visible surface structure. They were ragged-edged and often exhibited a lacy or lattice-like appearance. No morphological differences were noted between the SRSV particles present in the three Norwalk RIA-positive and single Norwalk RIA-negative specimens.

Rotavirus, a larger virus, was easily distinguished by the wheel-like appearance of stain-penetrated particles. Rotavirus particles were recognized in four reexamined specimens. The pictured particles (Fig. 1D) were from the stool specimen that had been stored the longest; it had been stored for 10 years.

Adenovirus, another large virus, was identified by its large icosahedral shape and well visualized capso-
Fig. 1. Electron micrographs of the virus particles observed in stool specimens after long-term storage at −70°C to −85°C. A: Astrovirus after 6 years. B: Calicivirus, 6 years. C: SRSV (Norwalk RIA-positive), 7 years. D: Rotavirus, 10 years. E: Adenovirus, 6 years. F: CVLPs, 7 years. G: T = 19 virus-like particles, 8 years. Particularly characteristic particles are indicated with arrowheads. Bar = 100 nm for all micrographs.
meric construction (Fig. 1E). Characteristic adenovirus particles were recognized in five specimens. The two virus-like particles were also identified in the reexamined stools. Characteristic CVLPs were highly pleomorphic and exhibited a fringe of projections surrounding the particles (Fig. 1F). CVLPs were recognized in five reexamined specimens. Although the true nature and significance of CVLPs have not been determined [Macnaughton and Davies, 1981; Schnagl et al., 1987], they are among the most common virus-like particles encountered in stools by EM.

T = 19 virus-like particles (Fig. 1G) were visualized after 8 years of storage. During a study of swimming-related illness in Egypt [Williams, 1985], these particles were observed in the stool of a young girl afflicted with gastroenteritis. Characteristic particles exhibited capsomeric construction with T = 19 icosahedral symmetry and displayed vertices (at the fivefold axes of rotational symmetry) that usually stained darker than other areas. As with the CVLPs, the significance of the T = 19 virus-like particles remains to be determined.

DISCUSSION

Of the five morphological types of stool-shed viruses and two types of virus-like particles reexamined in this study, all proved recognizable in specimens that had been stored a minimum of 6 years. The characteristic morphology of rotavirus was observed to be retained even after 10 years of storage at −70°C to −85°C. A similar observation has been reported by Albrey and Murphy [1976], who found that rotavirus particles present in pooled stool specimens retained their characteristic features after storage at −20°C for at least 9 years.

We did not expect to find remarkably distinct calicivirus particles present after 6 years of storage. Humphrey et al. [1984] have reported that the calicivirus particles they observed in stools lost their characteristic morphology after only a few weeks of storage at −70°C. Cubitt [1987] recommended holding stools to be examined for calicivirus at 4°C. The present study does not support these findings, although it must be noted that only one calicivirus-containing stool was reexamined in this study.

Although some degradation of virus particles may have occurred during storage, or through freezing and thawing, it was not significant enough to prevent the recognition of characteristic virus particles in any of the stools reexamined in this study. The results of this study indicate that storage of stool specimens at ultralow temperatures presents no significant problems for the morphological identification of viruses.

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