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VIRUS-LIKE PARTICLES WITH $T=19$ ICOSAHEDRAL SYMMETRY IN A HUMAN GASTROENTERITIS STOOL

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Abstract—Virus-like particles not previously described were observed in a human gastroenteritis stool using negative-stain TEM. The stool was among a number of acute-phase illness stools which had been collected in Egypt during 1980. The particles measured 65–70 nm in diameter, and it was possible to detect individual capsomeres on many of these particles. The capsomeric pattern identified on the particles corresponded to an icosahedrally symmetric $T=19$ capsid. Distinctive five-fold vertices, usually appearing as darker spots on the capsid, were an additional feature of these particles. The capsid structure, which is skew, could readily be distinguished from the $T=25$ capsid of adenovirus and the holey capsids of rotavirus and reovirus. Antibody to the particles was detected in both the shedding individual's acute- and convalescent-phase serum specimens using IEM, although an antibody increase was not demonstrated.

Index key words: New virus-like particles, $T=19$ symmetry, human gastroenteritis stool, Egypt, negative-stain TEM.

INTRODUCTION

Transmission electron microscopy (TEM) has been of fundamental importance in the detection of gastroenteritis viruses, such as rotavirus, which eluded detection by routine cell-culture methods. In the examination of illness stool specimens, TEM permits the detection and differentiation of a number of virus types based on the characteristic virus morphologies observed after negative-staining. Although other detection methods have been developed for certain viruses (Yolken *et al.*, 1977; Greenberg *et al.*, 1978; Nakata *et al.*, 1983), negative-stain TEM retains the advantage of being able to detect a range of antigenically unrelated viruses during a single examination and includes the possibility of observing new viruses.

This method was employed in an examination of the stools of individuals suffering respiratory or gastrointestinal illness after swimming at a polluted beach near Alexandria, Egypt in 1980. Expected rotavirus and adenovirus particles were observed in some of the stools; however, in one stool a new type of virus-like particle was observed. These particles, measuring 65–70 nm in diameter, are described in this report.

MATERIALS AND METHODS

Direct TEM examination of the stool material was carried out using the following procedure. For each specimen, a moderately turbid suspension of stool was made in distilled water and a drop placed on a 200-mesh copper grid with carbon support film. After 1 min, excess material was removed with a piece of filter paper and the grid rinsed with one or two drops of distilled water. The grid was then stained with 2% phosphotungstic acid (PTA), pH 7. Excess stain was removed with the filter paper. After drying, the grid was examined at 80 kV on a JEOL JEM-100CX electron microscope for the presence of virus. One hundred and twenty-eight stool specimens were examined in this manner. The specimens had been stored at $-70°C$ since collection. Poliovirus was used as the reference standard for virus-particle size determination. In addition to rotavirus and adenovirus particles observed in some of the stools, cell-culture propagated reovirus (types 1–3), adenovirus (type 7), and simian rotavirus (SA11) particles were examined for morphological comparison to the newly observed particles.

Immune electron microscopy (IEM) was used

to detect antibody to the new virus-like particles in the shedding individual's serum specimens. A distilled water suspension of the particle-containing stool was carefully examined to assure the absence of antibody-coated or aggregated particles. Aliquots of 0.04 ml were delivered by pipet into six wells of a microtiter plate. The individual's acute- and convalescent-phase serum specimens were diluted 1:10 in phosphate-buffered saline (PBS), pH 7.4, and 0.02 ml volumes were added to two of the wells. Acute- and convalescent-phase serum specimens (1:10 dilutions in PBS) from a rotavirus-shedding individual in the U.S. were also added to two wells in 0.02 ml volumes. This individual's serum pair was known to demonstrate a seroresponse to rotavirus by both IEM and radioimmunoassay (RIA). To the remaining two wells were added 0.02 ml volumes of PBS without serum. The microtiter plate was briefly shaken, left at ambient temperature (24°C) for 15 min, and then placed at 4°C overnight. The next morning, the contents of the wells were examined with the electron microscope. A serum–stool mixture was considered positive if antibody-coated or aggregated particles were observed. Each of the mixtures was coded prior to examination and their identities not established until after the results had been recorded.

Throughout the study, the grids were always inserted into the electron microscope with the specimen side nearest the electron source. For printing, the EM plates were inserted into the enlarger with the emulsion side away from the photographic paper. Image enhancement of specific electron micrographs was performed using the rotation technique of Markham *et al.* (1963).

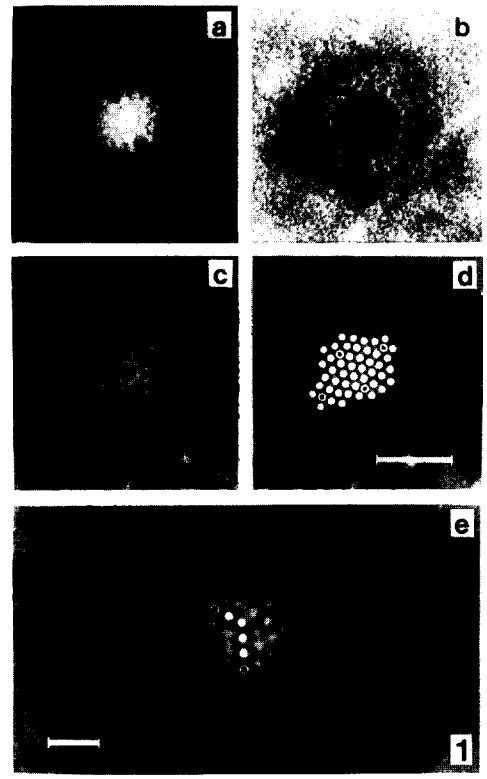

Fig. 1. Characteristic negatively-stained particles observed in the stool specimen. (a) Particle with five-fold vertices darkly stained. (b) Particle with stain-penetrated core. (c) Particle revealing large area of capsomeric detail. (d) Same as (c) with the locations of the 6-coordinated capsomeres (white dots) and the 5-coordinated capsomeres (white circles) indicated. The more darkly-stained areas at the five-fold vertices suggest that the 5-coordinated (vertex) capsomeres are either more stain-penetrable, or more readily lost than the 6-coordinated capsomeres. Bar = 50 nm for (a d). (e) Image enhanced micrograph (from Fig. 2b, $n = 3$) with one capsomeric path between five-fold vertices indicated. Bar = 25 nm.

RESULTS

The morphologically distinct particles which were observed in the acute-phase gastroenteritis stool of a 5-year-old girl are shown in Fig. 1. Capsomeric detail was observable on many of the particles. It was relatively easy to detect the icosahedral nature of the capsid structure as the five-fold vertices appeared as darker spots on the capsid (Fig. 1a). The capsomeres at these positions appeared to be more stain-penetrable or they were more readily lost than the other capsomeres. The mean diameter of 35 particles $\pm$ SD was 67 ± 2 nm. Frequently, the particles were seen with stain-penetrated cores (Fig. 1b). These particles were visually striking and more easily observable against stool background material. The mean core diameter of these stain-penetrated particles was 42 ± 1 nm. The particles resembled stain-penetrated particles of rotavirus, although the distinctive smooth rim of rotavirus was not observed.

On certain particles, virtually the entire capsomeric pattern of one side could be identified (Fig. 1c and d). One capsomeric path between neighbouring five-fold vertices is indicated on the image enhanced micrograph in Fig. 1e. All corresponding paths observed on other particles were the same. The observed structure corresponds to the icosahedral structure proposed by Caspar and Klug (1962) with a *T*

number = 19. The structure is of skew class $P=h^2+hk+k^2=19$ $(h=3,\ k=2)$ with $f=1$. Virus particles possessing this structure have not previously been reported. Although the apparent hand indicated by the electron micrographs is levo, the absolute hand was not established.

Figure 2 shows three particles viewed along axes of two-fold (a), three-fold (b) and five-fold (c) rotational symmetry. Markham rotations for different values of n are shown below the original micrographs. Noted image enhancement was observed at the expected values of $n=2$ for Fig. 2a, $n=3$ for Fig. 2b and $n=5$ for Fig. 2c. Rotations using other values of n resulted in blurring of detail.

Comparison of the $T=19$ particles with rotavirus and adenovirus particles observed in other stools, and with reovirus from cell culture, is shown in Fig. 3. Although stain-penetrated $T=19$ particles might be confused with stain-penetrated rotavirus particles, the more intact $T=19$ particles are quite distinct from all three virus types. The holey capsids observed with rotavirus and reovirus particles (Fig. 4a and b)

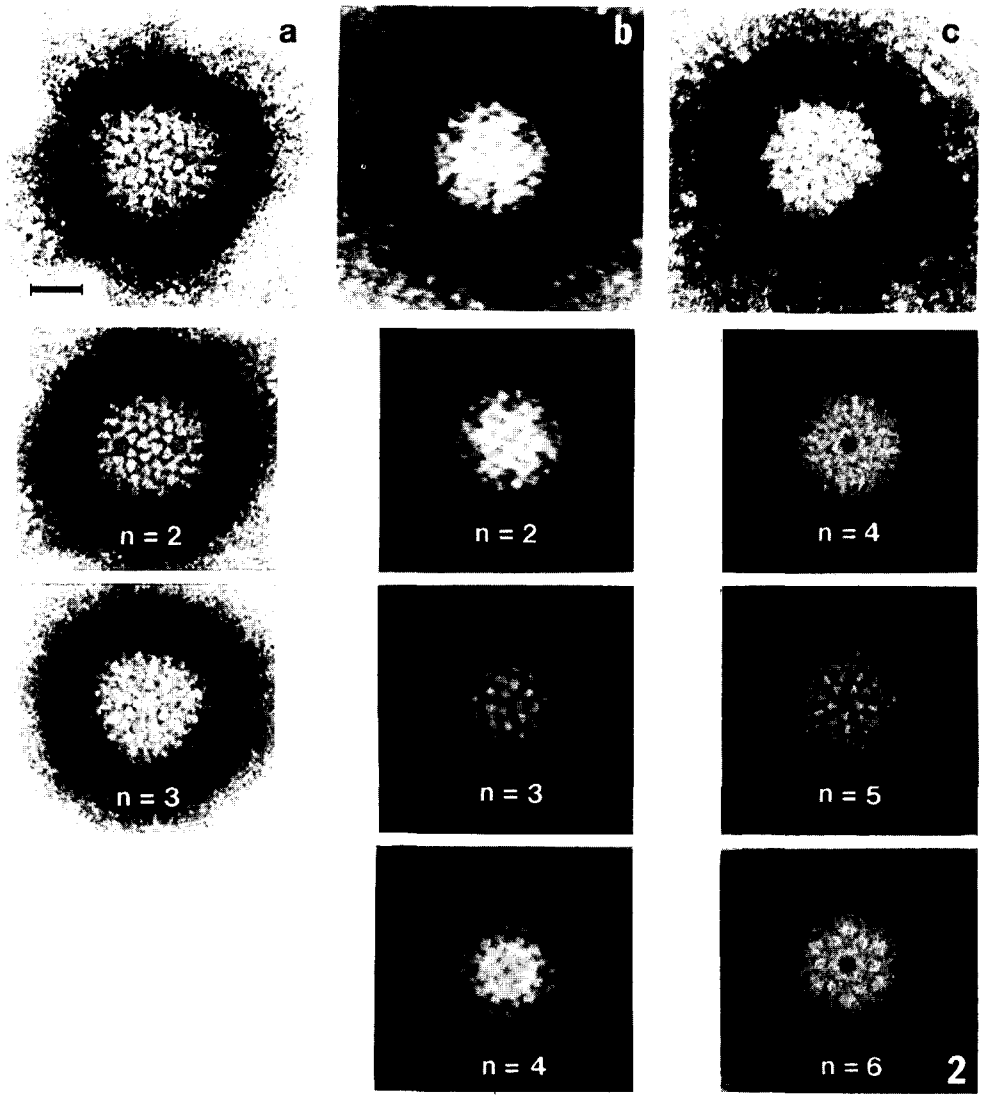

Fig. 2. The $T=19$ particles viewed along axes of (a) two-fold, (b) three-fold and (c) five-fold rotational symmetry. Markham rotations for different values of n appear below the original micrographs. Slight image enhancement of (a) can be seen with $n=2$, but note the enhancement of (b) at $n=3$ and (c) at $n=5$. Bar = 25 nm.

can be seen to be fundamentally different from the capsid structure of the $T = 19$ particles. It is the holes which are the vertex points which have been used to determine the T number for these two viruses (Palmer and Martin, 1982). Although T number determination for rotavirus and reovirus has not been clear-cut, recent studies suggest $T = 13$ icosahedral symmetry for both viruses (Roseto *et al.*, 1979; Metcalf, 1982; Khaustov *et al.*, 1984).

The capsid structure of adenovirus (Fig. 4c) has been well-established for some time (Horne *et al.*, 1959) and the $T = 25$ icosahedral symmetry of this virus is easily distinguished from the skew symmetry of the $T = 19$ particles.

The coded serum analysis by IEM revealed the presence of antibody activity against the $T = 19$ particles, only in the shedding individual's serum specimens. Antibody-coated and aggregated particles (Fig. 5) were observed in the reaction mixtures of both this individual's acute- and convalescent-phase serum specimens, although an antibody increase was not demonstrated. No serological relationship to rotavirus was indicated as the rotavirus-shedding individual's serum specimens were both negative.

It was not clear what role the $T = 19$ particles may have had in the shedding individual's gastrointestinal illness. Although no Norwalk-like particles were seen in the stool, a significant rise in antibody titer to Norwalk virus, an established gastroenteritis agent, was detected in the individual's serum pair when examined by RIA at the University of Massachusetts Medical School (Dr. N. R. Blacklow, personal communication). Extremely pleomorphic coronavirus-like particles were additionally present in this stool (Fig. 6); however, such particles remain to be clearly associated with human gastroenteritis (Macnaughton and Davies, 1981).

DISCUSSION

The 65–70 nm virus-like particles described in this report were distinct from all stool viruses previously observed in this laboratory. The particles were readily distinguished from rotavirus, reovirus and adenovirus, and did not appear to be an altered or degraded form of these viruses. The $T = 19$ icosahedral symmetry of these particles has not been reported for other known viruses, and published micrographs of pararotavirus (Espejo *et al.*, 1984), Hantaan virus (Lee and Cho, 1981; McCormick *et al.*, 1982), and novel virus-like particles in canine enteritis stools (Hill and Yang, 1984) also suggest particles different from those described here.

The limited amount of specimen material available has prevented extensive IEM and other testing. Although the $T = 19$ particles would appear to represent a new virus to be recognized in stools, confirmation must await successful propagation of the agent. Unfortunately, attempts to propagate the particles in MA-104, LLC-Mk_2, BGM and Vero cells have thus far not succeeded.

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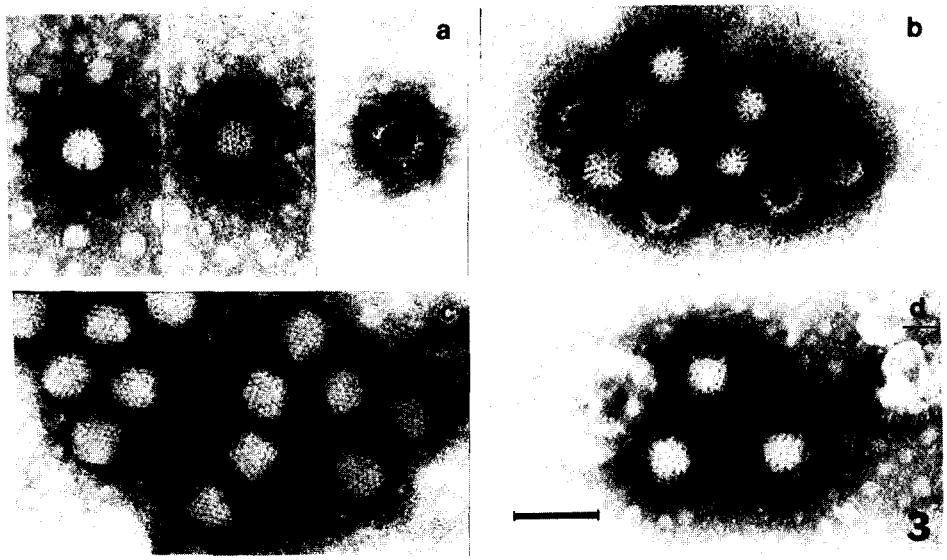
Fig. 3. Comparison of the $T = 19$ particles (a), with rotavirus particles (b) and adenovirus particles (c) observed in other stool specimens. The reovirus particles (d) are from cell culture as these particles were not observed in other stools. Bar = 100 nm.

Fig. 4. The capsid structure of (a) rotavirus (SA11), (b) reovirus (type 1) and (c) adenovirus (type 7). Bar = 50 nm.

Fig. 5. Small group of three antibody-coated $T = 19$ particles after incubation with the shedding-individual's convalescent serum. Bar = 100 nm.

Fig. 6. Coronavirus-like particles additionally present in the stool containing the $T = 19$ particles. Bar = 100 nm.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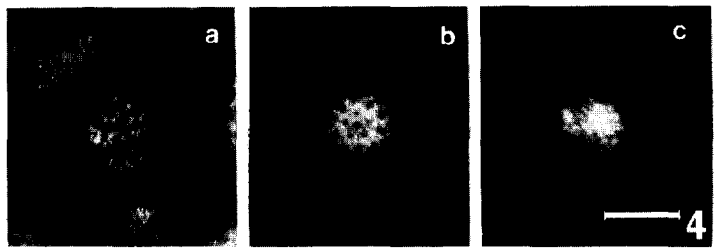
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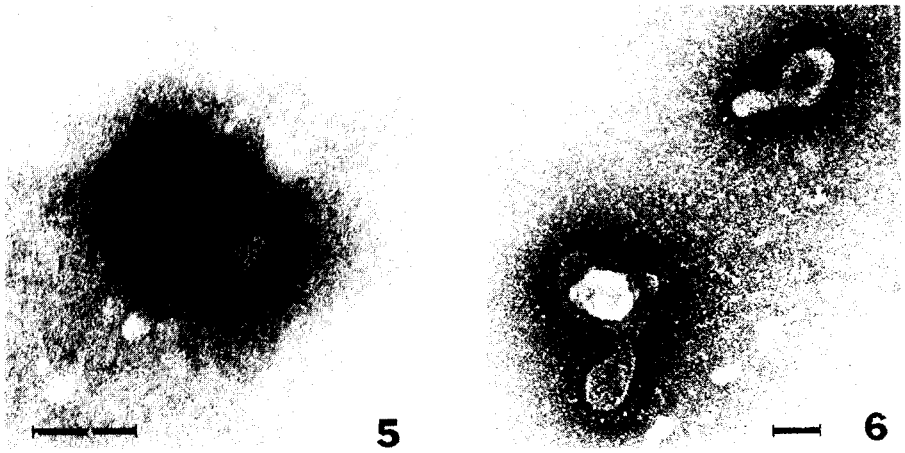
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