MORPHOLOGICAL RESPONSE OF HUMAN ROTAVIRUS TO ULTRA-VIOLET RADIATION, HEAT AND DISINFECTANTS

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SUMMARY. The morphological damage induced in human rotavirus particles by exposure to ultraviolet (UV) radiation at a wavelength of 254 nm increased progressively with length of treatment. Exposure of the virus in suspension to 9000 ergs/cm²/s was sufficient to remove the smooth capsid layer from 50% of particles after 1 min and from all the virions within 10 min. By this time, the number of stain-penetrated or empty particles increased markedly, along with the appearance of virus-derived debris in the form of disrupted and isolated capsomeres. After treatment for 120 min no intact virus particles were observed. The action of wet (100°C) or dry (60°C) heat resulted in changes similar to those effected by UV radiation, with a rapid loss of viral outer capsid shell from the virions followed by stain penetration and disintegration of particles. Sodium hypochlorite, cetrимide and 70% ethanol induced a rapid loss of the outer capsid layer, but, compared with UV radiation or heat, a slower increase in the number of stain-penetrated particles was noted. This was particularly evident with cetrимide. Chlorhexidine and phenol had effects on virus structure only after extended periods of exposure, whilst glutaraldehyde treatment had little influence on virus morphology. Glutaraldehyde 2% v/v would appear to be most suitable for the disinfection of rotavirus-containing electronmicroscope grids before their examination.

INTRODUCTION

Human rotaviruses are a major cause of infantile gastroenteritis, and are readily detected in the stools of children suffering from diarrhoea. By negative stain electronmicroscopy, the virus particles are 65–70 nm in diameter, double shelled with an outer, smooth capsid layer, essential for infectivity, and an innermost 38 nm core (Flewett and Woode, 1978).

The action of disinfectants on bacterial viability and structure has been established (Hugo, 1980). However, there have been fewer reports of the action of such agents on

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viruses and, in particular, of their effect on viral structure (Taylor, 1982). Rodgers *et al.* (1982) reported the effects of ultra-violet (UV) radiation on poliovirus infectivity and morphology in the presence and absence of faecal material. Thraenhart *et al.* (1978) outlined, by electronmicroscopy, a morphological alteration and disintegration test (MADT) for the disinfection of human serum containing hepatitis B virus (HBV). These workers correlated the time of exposure to disinfectants with sequential changes in virus morphology. Such alterations, which were equated with virucidal activity, were seen initially at the outer shell of the HBV and terminated with disintegration of the virus particles. The virucidal activity of such agents as sodium hypochlorite, lysol and formalin on lamb rotavirus (Snodgrass and Herring, 1977) and of various alcohols on bovine rotavirus (Kurtz *et al.*, 1980) has been shown. The disinfecting potential of commercially available biocides on simian rotavirus SA11 has also been studied (Tan and Schnagl, 1981; Sattar *et al.*, 1983). Butler (1981) and Harakeh and Butler (1984) also described the action of selected disinfectants on the infectivity of many human enteric viruses, including rotavirus, commonly found in wastewater effluents.

Several virucidal procedures have been used to reduce the virus population in hospital environments, one such being in the form of hand scrubs to prevent cross-infection in wards, in clinical virology laboratories handling patient-derived specimens and in water treatment plants. Moreover, in diagnostic electronmicroscopy laboratories, various protocols have been used for inactivation of stool material from patients with diarrhoea before examination of grids for viruses such as rotavirus. Until now there have been no reports on the morphological responses of human rotavirus to such disinfecting procedures. The purpose of this investigation was to establish the changes induced in the electronmicroscopic appearances of rotavirus suspended in faecal material after exposure to UV radiation, dry and wet heat, or a selection of commonly used disinfecting agents.

MATERIALS AND METHODS

Rotavirus. A stool specimen from a child with acute gastroenteritis had been shown to contain a high concentration of smooth rotavirus particles without copric antibodies when examined by negative stain diagnostic electronmicroscopy (Brenner and Horne, 1959; Whitby and Rodgers, 1980). A fresh sample was prepared as a 10% w/v suspension of faeces in buffer, clarified by centrifugation at 1000 *g* for 15 min, and the supernate containing the virus was stored at -148°C in 0.2-ml portions.

Treatment of virus

(1) *Ultraviolet irradiation.* The rotavirus suspension was treated with ultraviolet radiation (UV) at a wavelength of 254 nm with a Mineralight U.V. lamp model R52 (U.V. Products Inc., San Gabriel, CA, USA) with a current rating of 60 amp. After an initial warm-up period of 10 min, the lamp achieved an incident energy of 9000 ergs/cm²/s at a target to source distance of 4.8 cm. This output was maintained throughout the experiment and was monitored with a Blak-ray UV intensity meter, model J225 (U.V. Products Inc.). There was no recordable contribution in the infra-red to the illuminating source. To maintain homogeneous treatment, the virus suspensions were exposed as 55- μl volumes in the round bottomed wells (surface area 0.28 cm²) of microtitration plates. Exposure to the incident energy studied was for 1, 10, 30, 60 and 120 min with mixing at 30-s intervals. These times corresponded to energies delivered to the target of 0.54×10^6 , 5.4×10^6 , 1.62×10^7 , 3.24×10^7 , 6.48×10^7 and 1.38×10^8 ergs/cm², respectively. Unexposed samples were used as controls. After each period of treatment, the contents of four exposed wells were pooled and prepared for electronmicroscopy.

(2) *Heat*. Portions of virus suspension were added to formvar-carbon-coated electronmicroscope grids, and the excess fluid removed with filter paper. Grids were allowed to dry in air at room temperature before removing them to a 60°C oven. Duplicate grids were removed after exposure to the dry heat for periods of 1, 2, 4, 6, 8, 10 and 20 min. In addition, 55- μ l volumes of the virus suspension were placed in sealed glass vials and exposed to wet heat, monitored at 100°C, in a boiling water bath. Samples were removed at timed intervals similar to those for dry heat, and grids prepared for electronmicroscopy. Samples not heat-treated were used as controls.

(3) *Disinfectants*. The virus suspension was applied to grids which were then exposed to selected commercially available disinfecting agents. Those examined were: glutaraldehyde 2% v/v, sodium hypochlorite at 10–14% available chlorine, ethanol 70% v/v, cetrimide (a quaternary ammonium compound coupled with a cationic surfactant) 1% w/v, chlorhexidine (a diguanide) 1% w/v and phenol 2% w/v. Virus coated grids were added to 30- μ l portions of the disinfectants at room temperature. The solutions were placed in the wells of PTFE-coated multispot slides held in moisture boxes to prevent changes in disinfectant concentration. Duplicate grids were removed at each time interval as indicated for dry heat, and the disinfectant removed by rinsing the grids in running distilled water. Similarly treated control grids were exposed to phosphate buffered saline in place of the disinfectants. Washings from all grids were inactivated by storage in hypochlorite for 24 h.

Electronmicroscopy. Treated samples and unexposed controls were negatively stained with 3% w/v phosphotungstic acid pH 6.8 and examined immediately in either a Jeol 100C or AEI Corinth 500 electronmicroscope at instrumental magnifications of $\times 30\,000$. A minimum of 500 virus particles from each sample was counted and assayed for structural properties, including; size, shape, structure of the inner and outer capsid layers, penetration of the core by the negative stain and particle disintegration.

RESULTS

The virus particles seen by negative stain electronmicroscopy in the faecal suspension used as untreated control specimens had the characteristic appearances of rotaviruses.

UV radiation at 9000 ergs/cm²/s induced a rapid modification in the virus morphology, which after exposure for 1 min presented as a loss of the outer capsid coat in 50% of the virus particles (fig. 1). Destruction of the smooth layer on all virions was complete after treatment for 10 min. Concomitantly, the number of virus particles penetrated by negative stain increased with period of exposure. The cores of such particles appeared electron-dense whilst the number of disrupted virus particles increased with length of treatment. After exposure to UV radiation for 60 min, 50% of particles appeared as groups of isolated capsomeres, and by 120 min this degradation was complete (fig. 1).

Heating the virus at 60°C, as dried preparations on electronmicroscope grids, or as faecal suspensions in vials at 100°C, effected changes to the virus morphology, similar in appearance to those caused by UV radiation. The loss of the smooth capsid layer, penetration of virions by negative stain and conversion of particles to isolated capsomeres and debris was more rapid at 100°C in fluid suspension than at the lower, dry temperature (fig. 2a and b).

The response of the virus to the disinfectants varied. Treatment with glutaraldehyde for up to 20 min had little effect on the morphology of the virus (fig. 3a), whereas ethanol, cetrimide and chlorine (in the form of sodium hypochlorite) rapidly stripped the smooth outer capsid layer from the virus particles. In the case of hypochlorite, this was complete within 1 min (fig. 3b). However, unlike UV radiation or heat, the action

FIG. 1.—Morphological response of rotavirus to 254-nm wavelength UV radiation delivered at 9000 ergs/cm²/s. The number of smooth particles with intact outer capsids (●), full particles not penetrated by negative stain (■) and viral debris in the form of disrupted capsids and capsomeres (▲) are expressed as percentages of the total counts of particle types detected after each treatment time and for control, untreated preparations.

FIG. 2.—Morphological response of rotavirus to (a) dry heat at 60°C and (b) wet heat at 100°C. (see fig. 1 for explanation).

of the agents on either the viral genome, seen as penetration of virion cores or particle integrity, was slower with ethanol (fig. 3c), minimal for cetrимide (fig. 3d), and required prolonged treatment with hypochlorite (fig. 3b). With the last agent, no virus particles or capsomeres were found in any of the preparations treated for 20 min. By comparison, chlorhexidine (fig. 3e) and phenol (fig. 3f) required protracted treatment to induce damage to virus morphology.

The morphological response of structurally normal rotavirus (fig. 4a) to these treatments was shown as a loss of the outer capsid layer (fig. 4b), penetration of the core by negative stain (fig. 4c) and collapse of particles (fig. 4d). In addition to removal of sections of the smooth layer (fig. 4e), partial stain penetration (fig. 4f) and the initial stages of disruption of smooth empty (fig. 4g) and rough empty (fig. 4h) particles, complete dissociation of virus particles (fig. 4i) was evident. Treatment with glutaraldehyde for up to 20 min had no effect on virus structure (fig. 4j).

FIG. 3.—Morphological response of rotavirus to: (a) glutaraldehyde 2% v/v; (b) sodium hypochlorite at 10–14% available chlorine; (c) ethanol 70% v/v; (d) cetrimide 1% w/v; (e) chlorhexidine 1% w/v; and (f) phenol 2% w/v. (see fig. 1 for explanation of symbols).

DISCUSSION

Although many disinfectants inactivate human and animal rotaviruses (Snodgrass and Herring, 1977; Kurtz *et al.*, 1980; Tan and Schnagl, 1981; Sattar *et al.*, 1983; Harakeh and Butler, 1984), the morphological response of these viruses to disinfecting agents has not been characterised. In a previous, unpublished study, UV radiation or glutaraldehyde treatment were shown to inactivate adenovirus and vaccinia virus particles present on electronmicroscope grids but had little effect on the structure of the two viruses. The morphological response of rotavirus to UV was surprising, in that unlike the response of adenovirus or vaccinia virus, the loss of the outer, smooth layer of capsomeres was rapid, occurring during the initial 10 min of exposure, and was

FIG. 4.—Electronmicrographs of rotavirus particles showing the morphological damage effected by the treatments. (a) Two particles from a control preparation showing the typical structure of rotavirus. Note the intact smooth outer capsid layer and the core not penetrated by stain. (b) Single rough, unpenetrated (full) particle after ethanol treatment for 10 min. Note removal of outer layer. (c) Single smooth penetrated (empty) particle after chlorhexidine treatment for 10 min. (d) Four rough particles, one full and three empty, after 6 min at 100°C. Note partial collapse and breakup of particles (arrow). (e) Single full particle after ethanol treatment for 4 min. Partial removal of outer capsid layer (arrow) is evident. (f) Single rough, partially penetrated particle after cetrimide treatment for 4 min. (g) Single smooth, empty particle undergoing disruption (arrow) after phenol treatment for 10 min. (h) Single rough, empty disrupting particle after sodium hypochlorite treatment for 10 min. (i) Disrupted particle presenting as dissociated capsomeres after UV treatment for 60 min. (j) Group of smooth, full rotavirus particles after glutaraldehyde treatment for 20 min. Note unaffected virus morphology. All magnifications $\times 150\,000$. Bar = 100 nm.

followed by an increasing number of empty and disrupted virus particles. Earlier reports on the action of UV radiation on poliovirus indicated that viral infectivity was lost before modifications to the virus morphology occurred (De Sena and Jarvis, 1981; Rodgers *et al.*, 1982). Prolonged treatment resulted in the sequential loss of the viral polypeptides VP4 and VP2 and led to capsid damage. The conversion of poliovirus particles to partially penetrated (P) and empty (E) particles was described by Katagiri *et al.* (1967) and such modifications were correlated with the dose of UV radiation applied (Rodgers *et al.*, 1982). A similar sequence would appear to apply to the damage induced in rotavirus by UV treatment.

With the exception of glutaraldehyde, the initial response of the virus to treatment with disinfectants was similar to that with UV; removal of the outer smooth capsomeres was followed by penetration of the core by negative stain and disintegration of the particles. Tan and Schnagl (1981) showed that the infectivity of SA11 rotavirus was abolished by treatment with ethanol, phenol or high concentrations of formaldehyde, whilst dilute sodium hypochlorite was ineffective. Sattar *et al.* (1983) demonstrated that despite the presence of organic material, glutaraldehyde inactivated rotaviruses, whilst chlorhexidine and cetrimide were of little value. As part of the preparation of clinical specimens for diagnostic electronmicroscopy, most laboratories use an inactivation procedure to prevent the introduction of infectious material into electronmicroscopes, parts of which would otherwise be difficult to sterilise before servicing. UV radiation, heat, sodium hypochlorite, phenol and ethanol rapidly removed the virus outer coat which would suggest virus inactivation. However, the morphological damage induced in the virus by these agents makes them unsuitable for routine use in diagnostic electronmicroscopy. Damage to the outer coat of the virus was minimal with glutaraldehyde, whilst glutaraldehyde ethanol, centrimide or chlorhexidine induced least particle disruption.

Glutaraldehyde is a well established histological fixative, preserving the fine structure of tissues as well as virus-infected cells. In addition to its neutralising potential for rotavirus infectivity (Sattar *et al.*, 1983), glutaraldehyde preserved the morphology of this virus for negative stain electronmicroscopy. Studies are in progress to characterise further the mode of action of selected disinfectants in relation to the ultrastructural changes they induce in human rotaviruses.

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