Infections caused by hepatitis A virus (HAV) are endemic throughout the world (6, 21, 41). Apart from community-based epidemics (7, 15, 16, 26), outbreaks of the disease are frequently associated with hospitals (3, 13, 25, 45), day-care centers (12, 15-17, 40), schools and institutions for mentally ill people (27, 31, 37, 41), and eating establishments (19, 25, 30). Whereas fecally contaminated food (e.g., shellfish) and potable water are well recognized as vehicles for HAV infection (6, 8, 36, 41), in nearly 50% of the cases the vehicle(s) responsible for virus spread remains unidentified (15). Strong circumstantial evidence indicates that virus-contaminated hands play a major role in the spread of the virus, particularly in institutional settings (7, 19, 25, 26, 28, 30, 35).

For hands to be important HAV vehicles, the virus must survive on hard surfaces for prolonged periods (22) and also that it is relatively resistant to many of the commonly available hard-surface disinfectants (19). Infectious virus transfer from contaminated to clean surfaces through contact has been reported (2, 14, 18, 20). Therefore, this study was designed to determine HAV survival on human hands and to assess how routine interactions of hands with other contaminated surfaces could further promote the spread of HAV. The study design also included determination of the roles that inoculum age, pressure, and friction may play in the transfer of infectious HAV from contaminated to clean surfaces.

**MATERIALS AND METHODS**

**Cells.** A seed culture of FRhK-4 cells was kindly provided to us by M. D. Sobsey, University of North Carolina, Chapel Hill. The methods for the cultivation and maintenance of these cells have been described previously (32, 33, 47).

**Virus.** The HM-175 strain of HAV was also received from M. D. Sobsey. Stock virus was prepared by infecting FRhK-4 monolayers at a multiplicity of infection of 0.01. The virus was allowed to adsorb for 90 min at 37°C before the addition of maintenance medium and a further incubation at 37°C until 60-80% of each virus-infected monolayer was affected by virus cytopathology (4 to 5 days of incubation). The cultures were frozen (-20°C) and thawed three times, and the culture fluid was centrifuged for 10 min at 1,000 x g. The virus was concentrated 10-fold by polyethylene glycol hydroxyethylation as described by Ramia and Sattar (42). Briefly, Eagle minimum essential medium (GIBCO, Grand Island, N.Y.) with 10% fetal bovine serum (GIBCO), 2 mM glutamine (GIBCO), 0.1 mM nonessential amino acids (GIBCO), 50 μg of gentamicin sulfate (Gidomycin; Roussel, Montreal, Quebec, Canada) per ml, 100 μg of kanamycin (GIBCO) per ml, 0.015 M HEPES (N-2-hydroxyethylpiperazone-N-2'-ethanesulfonic acid; GIBCO), and 0.113% sodium bicarbonate (BDH Chemicals, Toronto, Ontario, Canada) was used for growing the cells. The cells were maintained in the same medium, but the maintenance medium contained only 2% fetal bovine serum.

**Virus plaque assay.** Determinations of HAV PFU were carried out in FRhK-4 monolayers in 12-well plastic plates (Costar, Cambridge, Mass.) as described previously (32, 33).

Briefly, three wells were used for each virus dilution tested, and each well received 0.1 ml of the inoculum. HAV was then allowed to adsorb for 90 min at 37°C. The overlay
consisted of Eagle minimum essential medium, 2% fetal bovine serum, 2 mM glutamine, 0.1 mM nonessential amino acids, 50 μg of gentamicin sulfate per ml, 100 μg of kanamycin sulfate per ml, 2 μg of amphotericin B (Fungizone; Gibco) per ml, 0.015 M HEPES, 0.113% sodium bicarbonate, 0.75% agarose (type II; Sigma Chemical Co., St. Louis, Mo.), and 26 mM magnesium chloride (BDH). The plates were sealed in laminated plastic bags (Philips, Toronto, Ontario, Canada) and incubated for 8 days at 37°C. The procedure for fixing and staining the monolayers prior to counting plaques has been described previously (29).

Virus-suspending medium. The fecal sample used in this study was obtained from a healthy 5-month-old baby. A 10% (wt/vol) suspension of it was prepared in normal saline; it was clarified of gross particulate matter by centrifugation for 10 min at 1,000 × g and passed through a 0.2-μm-pore-size membrane filter (Nalge Co., Rochester, N.Y.) to remove bacteria and fungi. The filtrate was found to be free of cytopathic and endogenous viruses when it was tested in FRhK-4 cells by the procedure described before (29). When HAV was diluted 1:10 in this fecal suspension and held at room temperature for 4 h, no loss in virus infectivity was observed.

Disks. Stainless-steel disks (diameter, 1 cm) were punched out of locally purchased no. 4 finish polished sheets (thickness, 0.75 mm) and were used to represent environmental hard surfaces. The procedure for their decontamination, cleaning, and sterilization before reuse has been described in detail before (44).

Volunteers. Permission to place the virus on the hands of adult volunteers was first obtained from our university's Ethics Committee. Any individual with cuts or abrasions on the hands was automatically excluded from the study. Each volunteer was then thoroughly briefed on the experimental protocol and the risks involved before being asked to sign a consent form. The age of the participants ranged from 26 to 45 years.

At the end of the experiment, the volunteer was asked to gently press the experimentally contaminated fingerpads on a piece of paper towel soaked in 0.5% solution of sodium hypochlorite (Javex Canada Inc., Toronto, Ontario, Canada) for 3 min for their decontamination. The hands were then washed thoroughly with ordinary soap and running tap water and dried with a paper towel.

HAV survival on human hands. Virus survival on the hands of volunteers was tested by the procedure of Ansari et al. (2). The hands were first washed in lukewarm running tap water for 30 s, disinfected by thoroughly rubbing them with 70% ethanol (BDH), and then air dried. To demarcate the areas for virus deposition, each fingerpad was pressed hard over the mouth (8-mm inside diameter) of an empty plastic vial (no. 72,694.006; Sarstedt Inc., St. Laurent, Quebec, Canada).

Before each experiment, the stock virus was diluted 10-fold in the fecal suspension. Ten microliters of the fecally suspended virus was immediately placed, using a positive-displacement pipette (Gilson Medical Instruments, Villiers-le-Bel, France), into each of two glass vials containing 990 μl of Earle balanced salt solution; these acted as the input virus controls.

Ten microliters of the virus suspension was also deposited at the center of the demarcated area on each fingerpad. In each experiment, the amount of virus in the inoculum on the fingerpads (0-min control) was determined by immediately recovering the deposited virus separately from four fingerpads of the left hand, as follows. The contaminated area was placed over the mouth of a vial identical to that used for fingerpad demarcation but containing 990 μl of Earle balanced salt solution. The vial was inverted, with the fingerpad still over it, and held in position for 5 s. This was followed by 20 full inversions of the vial with the vial still in place and then an additional 5 s of soaking and 20 more full inversions. The surface of the fingerpad was then scraped on the inside rim of the vial to recover as much of the fluid as possible. This technique was found to recover approximately 6.0 × 10^7 PFU or 70% of the input infectious virus. To determine HAV survival on hands, virus was randomly eluted from separate fingerpads at 20, 60, 120, 180, and 240 min after their inoculation.

Five volunteers participated in the experiments on virus survival on hands, and each experiment was performed three times on each volunteer.

Contamination of disks and virus elution. Each clean and sterile disk received 10 μl of fecally suspended HAV. In every experiment, the amount of infectious virus deposited on the disks (0-min control) was determined by placing two inoculated disks immediately into two separate glass vials containing 990 μl of Earle balanced salt solution. The vials were sonicated to elute the virus from the disks. This procedure recovered approximately 6.25 × 10^7 PFU, which represented nearly all of the infectious virus placed on each disk (33).

Pressure and friction. The effect of a low (approximately 0.2 kg/cm²) and a high (approximately 1.0 kg/cm²) level of pressure was tested in the virus transfer experiments. The low level of pressure, with or without friction, was taken to represent ordinary touching of environmental surfaces. Animate-animate interaction through handshaking was represented by a pressure of 1.0 kg/cm² without friction, while the same pressure with friction simulates opening of doors and other, equivalent actions.

Depending on the type of experiment, a clean or HAV-contaminated disk was placed at the center of the pan of a scale with a digital readout (model no. 1206 MP; Sartorius, Göttingen, Germany), and a virus donor or recipient fingerpad was pressed on it until the desired pressure was achieved and was held there for 10 s; pressure was computed from the balance reading and the area of the contaminated surface. For virus transfer from fingerpad to fingerpad, the recipient fingerpad was placed on the scale and placed into contact with the donor.

In an attempt to standardize the friction to be applied during virus transfer, each volunteer was first thoroughly briefed on and given the opportunity to properly practice the following procedure. Once contact was made between the disk and the fingerpad and the desired pressure level was attained, the finger was rotated in half circles 10 times over the 10-s period of contact; in the case of fingerpad-to-fingerpad transfer, the donor was required to apply friction.

HAV transfer experiments. The extent of infectious HAV transfer upon fingerpad-fingerpad interaction and fingerpad-environmental surface contact was examined as follows. After letting the virus inoculum dry on the donor surface for the desired length of time, contact was made at the required pressure, with or without friction, with the recipient surface for 10 s. Virus was then eluted from the recipient as well as the donor surfaces. The two values thus obtained were added together to represent the total amount of infectious virus detectable on the donor before transfer, and the amount of virus transferred was determined as a percentage of this total. The disk-to-fingerpad and the fingerpad-to-disk experiments were conducted on each of two volunteers.
whereas the fingerpad-to-fingerpad mode involved volunteer F as the donor and volunteer A as the recipient.

Statistical analyses. HAV survival on the fingerpad of each volunteer at each time point (x value) was expressed as a fraction of HAV eluted at 0 min. These observations were then normalized to the 0-min value as 100%. Such data were used to generate log-linear plots (log,,) in a computer program (SigmaPlot version 4.0; Jandel Corp., Corte Madera, Calif.). First-order linear regression lines were then fitted to the plots in the same program. HAV decay rates on the fingerpad were obtained from the gradient of the fitted regression lines as loss of virus infectivity titer in log,, PFU per minute. The half-lives were calculated from the decay rate constants (K), as discussed by Segel (46). To analyze the data obtained from HAV survival and transfer between the three models (fingerpad-fingerpad, fingerpad-disk, disk-fingerpad) over 4 h by using two volunteers, the data were normalized as described above. Bar chart plots were then produced in the SigmaPlot computer program.

In order to assess the effects of pressure, friction, and the transfer mode used on the amount of HAV transferred, the raw data were entered into an SAS program (Statistical Analysis Systems, University of North Carolina, Chapel Hill) and a three-way analysis of variance was performed. The possible interactions between the variables (pressure, friction, and type of model) were also analyzed. The Tukey test was performed post hoc in the same program. To compensate for differences in virus transfer because of time, an average of the values obtained with the 20- and 60-min HAV transfers was computed and used in the three-way analysis of variance.

RESULTS

HAV survival on human hands. The mean virus titer at 0 min on fingerpads in the HAV survival experiments was approximately 6.0 x 10^4 (range, 5.3 x 10^4 to 7.2 x 10^4) PFU in the 10-μl inoculum. The results of these experiments are presented in Fig. 1. The pattern of HAV decay on fingerpads appeared to be biphasic. Nearly 68% of the input infectious virus became undetectable within the first 60 min after contamination of the fingerpads. Even though this early loss in virus infectivity was particularly pronounced in volunteer E, the rate of decay was essentially similar during the 60- to 240-min period for all five volunteers. The amount of HAV detectable at the end of 4 h ranged from 16 to 30% of the initially detectable amount; the actual PFU recovered at the end of 4 h were 15,740 and 8,350 for volunteers D and E, respectively.

In view of the apparent biphasic nature of the loss in HAV infectivity on fingerpads, infectious virus half-lives and K values (expressed as the rate of loss in virus infectivity in log,, per minute) were determined separately for the 0- to 240-min and the 60- to 240-min periods. The half-lives of the virus for both the periods were the shortest in volunteer E (5.50 and 14.44 h, respectively) and the longest in volunteer D (7.70 and 23.10 h, respectively) (Table 1). However, the differences in virus half-lives and K values for the five volunteers were not found to be statistically significant either for the 0- to 240-min period or the 60- to 240-min period.

HAV transfer after 20 and 60 min of drying. The mean amounts of virus on the fingerpads and disks at 0 min were 3.1 x 10^8 and 2.9 x 10^8 PFU/ml, respectively. There was always virus transfer. A three-way analysis of variance revealed the significant main effects because of the mode of transfer, pressure, and friction (F = 3.35, P < 0.05; F = 133.51, P < 0.05; and F = 81.29, P < 0.05, respectively). A post hoc analysis (Tukey tests) of the main effect of the transfer mode showed a significant difference in virus transfer between the fingerpad-fingerpad (mean, 634.08 PFU) and the disk-fingerpad (mean, 454.72) modes. Similarly, there was a significant difference between the fingerpad-disk (mean, 607.21) and disk-fingerpad modes in the amount of virus transferred. The amount of virus transferred was not significantly different between the fingerpad-fingerpad and the fingerpad disk modes.

The analysis also revealed significant differences in virus transfer because of pressure and friction. The increase in pressure from 0.2 kg/cm² resulted in a significant increase in the amount of virus transferred (F = 133.51, P < 0.05); the mean transfer rate went from 212.82 PFU at 0.2 kg/cm² to 917.85 PFU at 1 kg/cm². Similarly, the incorporation of friction resulted in a significant elevation in the amount of virus transferred (F = 81.29, P < 0.05). The mean HAV transfer rate with no friction was 290.27 PFU, whereas with friction, the mean value was 840.40 PFU. The combination (interaction) of pressure and friction was also found to statistically significant.

| TABLE 1. HAV survival on the fingerpads of five volunteers |
|----------------|----|---|---|----|---|
| Volunteer      | 0-240 | 0-240 | 0-240 | 0-240 | 0-240 |
|                | (min) | (PFU/ml) | (min) | (PFU/ml) | (min) | (PFU/ml) | (min) | (PFU/ml) | (min) | (PFU/ml) |
| A               | 6.10 | 16.50 | 0.0019 | 0.0007 | 0.7936 | 0.9811 |
| B               | 7.20 | 19.25 | 0.0018 | 0.0006 | 0.6471 | 0.8164 |
| C               | 6.10 | 16.50 | 0.0019 | 0.0007 | 0.7365 | 0.7225 |
| D               | 7.70 | 23.10 | 0.0015 | 0.0005 | 0.6700 | 0.8738 |
| E               | 5.50 | 14.44 | 0.0021 | 0.0008 | 0.6852 | 0.9453 |

* The differences between K values for the five volunteers were not statistically significant.

* Coefficient of determination (a measure of closeness of fit of the scatter graphs to their regressions).
significantly affect the amount of HAV transferred ($F = 33.98, P < 0.05$). The two-way effect of mixing pressure and friction is illustrated in Fig. 2. The combination of friction and the higher pressure (1 kg/cm$^2$) resulted in a profound increase in HAV transfer (mean, 1,370.76). No statistically significant interactions were observed between mode of transfer and pressure and friction, mode of transfer and friction, or mode of transfer and pressure.

HAV transfer over a period of 4 h. The ability of HAV to be transferred over a 4-h period was assessed by using two volunteers (volunteers A and E). The three modes of virus transfer were tested by using only the low level of pressure without any friction. The mean amounts of virus at 0 min in the 10-μl inoculum on fingerpads and disks in these experiments were $1.1 \times 10^4$ PFU ($1 \times 10^4$ to $1.2 \times 10^4$ PFU) and $1.08 \times 10^4$ PFU ($1.07 \times 10^4$ to $1.09 \times 10^4$ PFU), respectively.

(i) Virus transfer from disks to fingerpads. Nearly 33% of the input virus PFU remained detectable on the disks at the end of 4 h under ambient conditions of temperature (22 ± 2°C) and relative humidity (45 ± 5%) (Fig. 3). When a clean fingerpad was pressed against a disk with an inoculum that was dried for 20 min, about 22% of the PFU (2,667 PFU) could be transferred, whereas no detectable virus was transferred to fingerpads when the inoculum was left to dry on the disks for 4 h.

(ii) Virus transfer from fingerpads to disks. Nearly 25% of the input virus PFU remained detectable on the fingerpads even after 4 h (Fig. 4). The amount of virus transferred from contaminated fingerpads to clean disks on contact was nearly 27% of the virus (3,484 PFU) remaining after 20 min of drying; when the inoculum was dried for 4 h, 1.6% of the surviving virus (50 PFU) was transferred.

(iii) Virus transfer between fingerpads. About 10,433 PFU of HAV was deposited on the fingerpad of volunteer E, but only 1,967 PFU (19%) remained at the end of 4 h (Fig. 5). The survival pattern was similar to that observed earlier (Fig. 1). The amount of HAV transferred from the fingerpads of volunteer E to the fingerpads of volunteer A was 2,800 PFU after 20 min of drying (23% of the available PFU) but only 17 PFU (0.9% of the available PFU) at the end of 4 h.

DISCUSSION

Gwaltney et al. (14) demonstrated that as much as 70% of the infectious rhinovirus on hands can be transferred to a recipient finger after a contact of only 10 s. Similarly,
Hendley et al. (20) have shown that dried rhinoviruses picked up from environmental surfaces may persist on human hands long enough to permit self-inoculation and that virus transmission may proceed by transfer of the virus from the hands of the infected person to an intermediary surface or directly to the fingers of a susceptible recipient. Respiratory syncytial virus has also been shown to survive in the environment, allowing its transfer to human hands (18), and studies on the transfer of human rotavirus between hands and inanimate surfaces also implicate hands and inanimate surfaces as potential rotavirus vehicles (2). The results of all of these studies suggest that human hands and environmental surfaces may be potential routes of virus transmission.

The results presented here show that considerable amounts of HAV remained infectious on the fingerpads after 4 h, even though 68% of virus infectivity was lost in the first 1 h. Whereas the reasons for the biphasic pattern of virus decay and the relatively rapid drop in virus titer during the first 60 min are not clear, it may be related, at least in part, to the rate of moisture loss from the inoculum. Although HAV inocula appeared dry after about 20 min, the decay curve shown in Fig. 1 leveled off at around 60 min. This suggests that 60 min was the end of the drying period.

Other factors, such as the physiology and chemistry of the skin surface, may also play a role in HAV survival and inactivation on hands. Both immunoglobulins and serum proteins contained in normal human sweat have been shown to inhibit bacterial growth (38). It is not clear that these substances behave in a similar way with viruses. Surface immunoglobulin A has also been shown to contribute to the immunological defense of the skin (23). The presence of two HAV subpopulations in the inocula may also be responsible for the biphasic pattern in virus decay, so that the more susceptible virus population is inactivated faster during the drying period and the more resistant virus population persists over the whole study period (4 h).

Because a fecal suspension of HAV was used to simulate natural conditions in which HAV contaminates both animate and inanimate surfaces, it is expected that the results may be an indication of what may occur in the field. Residual HAV on human hands at the end of 4 h could also be transferred to others and may be enough to initiate infection upon self-inoculation. High levels of infectious HAV were exchanged between the fingerpads and the disks over the 4-h period. These results suggest that human hands and fomites are potential ways in which self-inoculation and communication of the virus can occur over an extended time. The significance of pressure and friction in determining the amount of HAV transferred in the three models examined in this study was shown. We are not aware of any previous quantitative studies in which such an interaction in the transfer of infectious virus has been documented.

Virus transfer between contaminated and clean surfaces is greatest when the inoculum is wet (2, 14, 18, 20) and is observed to decrease as the virus dries. Also, during drying some virus is usually inactivated. In the observed transfer of infectious virus at specific times, the effects of drying on virus inactivation as well as the physical transfer of virus particles and their recovery are confounded. However, in order to assess the virus loss because of drying, we used a reference point, which was the indication that the virus inocula were visibly dry on the contaminated surfaces. An inoculum of 10 μl of HAV in fecal suspension appeared to be dry after 18 to 20 min on the metal disks (33) and 11 to 17 min on fingerpads. For this reason, 20 min was taken as the minimum time that HAV inocula would require to initially become dry. The first reading was therefore taken at 20 min and indicates virus loss because of drying.

The period of experimentation used in this study is within the limits for human hands to stay contaminated before a hand wash or interaction with other animate and inanimate environments. The metal disks used in this study were selected to represent environmental surfaces. In our previous studies (33), stainless steel disks allowed the virus to persist for several days; glass and plastic surfaces may behave similarly (44).

HAV outbreaks are often associated with hospitals (3, 13, 25, 45). The mode of transmission in those studies was unclear, but the source was usually an asymptomatic child or adult. The observation that the spread of the infection is associated with nurses and other medical personnel suggests that human hands may play a vehicular role in virus transmission.

An assessment of HAV outbreaks in the community shows that diaper-changing personnel in many day-care centers also participate in food-handling activities (9, 10). HAV may be acquired from children who are excreting HAV; the majority of these children are asymptomatic (10). Although no such study has been done for HAV, the day-care center environment has often been found to be contaminated with enteric organisms which have modes of transmission similar to those of HAV (9, 24, 39).

HAV that has contaminated toys and other play items may persist for prolonged periods of time (33); toys are shared items in day-care centers, and children are known to suck them frequently (4, 9). Such behavior tends to encourage virus transmission through the fecal-oral route among the children. The children also associate closely with the environment around them as well as with adults assigned to them in the day-care center (4). Interactions between such a contaminated environment and the institutionalized population, especially children, may recycle the virus until susceptible hosts, usually adults, are infected. Such a secondary
spread of HAV often results in communitywide outbreaks (17).

Restaurant-associated HAV outbreaks are frequently reported (7, 26, 28, 35). In these instances, the index case was frequently a food handler who was subclinically infected with HAV. Secondary spread of the disease is a prominent feature of the outbreaks (7, 26, 28) and may occur over an extended period of time (30), indicating a continuing source of infection. Refrigerated foodstuffs as well as foods stored at room temperature after handling are known to permit the survival of HAV for several days (11) and, together with the index case, furnish a continued source of the virus. It has also been suggested that HAV which persisted on a sandwich board contaminated by hands moistened with oropharyngeal secretions from an anicteric food handler was responsible for contaminating many sandwiches (28).

The potential of human hands to spread viral agents has serious implications in the food industry and eating establishments, and it may be particularly important when foodstuffs do not require cooking after handling. HAV contamination of foodstuffs by an institutional food handler can culminate in communitywide HAV outbreaks (6, 7, 19, 26, 28, 35).

The persistence of HAV on surfaces and the ability of the virus to be interchanged in animate and inanimate environments suggests that human hands and environmental surfaces constitute important epidemiological factors in the spread of HAV. Approximately 50% of HAV cases reported to the Centers for Disease Control every year show no identifiable source of the virus (15). Previously, we have shown that HAV on environmental surfaces can remain infectious for several days (33). Taken together with the results of this study, this suggests that human hands and environmental surfaces may serve as sources of HAV dissemination, especially in institutionalized populations.

Hand washing is probably the single most important measure for controlling and preventing the spread of infections (5, 22, 43). However, compliance with hand washing guidelines among medical personnel is frequently suboptimal (1, 10) and is sometimes ignored altogether (9). Moreover, many commonly available surface disinfectants have been shown to be ineffective against HAV (20), and Mboth et al. have shown that commonly available antisepsics are inefficient in removing or inactivating HAV. Use of antisepsics has previously failed to contain nosocomial HAV thus permitting secondary spread of the virus in hospital wards.

The findings of this study show that the control and prevention of HAV outbreaks in settings such as day-care centers, hospitals, eating establishments, and other, similar facilities must place a greater emphasis on both human hands and environmental surfaces as virus vehicles.

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