tioner to be informed of these attendances, it would also be useful for the accident and emergency department to know more about the patients and perhaps even have details of previous consultations with their doctors. Communication between general practitioners and hospital accident units can be only in the best interests of patients. With advances in information technology there is good reason to believe that this will happen soon.[4]

GENERAL PRACTICE

We have identified failures in consultation in general practice and have shown that their distribution among general practitioners is not equal. We have also shown, within this group, an important amount of unreferred illness. Not every attendance or even admission necessarily reflected an error by the general practitioners (table VI), but if furnished with information about their patients' attendances to the department individual practitioners would be able to review their performances in consultations. In particular, they would be able (*a*) to review the consultation to identify the cause of any breakdown in communication, (*b*) to identify patients who might have unreasonable expectations, and (*c*) if patients were admitted, particularly for some serious reason, to review their clinical decision and criteria for referral.

Underreferral clearly exists and should be minimised in the best interests of patients. Information on underreferral, if supplied to general practitioners, could be a more useful indicator of failure to refer than is a low referral rate. The government's intention is to use referral rates as a means of identifying doctors who underrefer and overrefer[6] (though referral rates are difficult to calculate and even more difficult to interpret).[6,7] General practitioners with high referral rates will be asked to refer less and, presumably, those with low rates to refer more. We may, however, expect more emphasis on referring less for economic reasons. The problem with this approach is that there is an important distinction between underreferral and a low referral rate. Underreferral implies a possible error of management whereas there may be many good reasons for a low referral rate. A doctor with a low referral rate is not necessarily one who underrefers.

We suggest that it may be more useful for general practitioners to be informed of specific cases of possible underreferral than simply to be told that they have a low referral rate. At least in this way doctors with good reasons for having low referral rates will not be targeted unnecessarily by the authorities and those who may have average referral rates but whose patients are frequently admitted to hospital or referred on by the accident unit will be made aware of the fact and have some specific pointers from their own experience to help them prevent it. The data might be collected by computerised accident and emergency systems programmed to record this type of self referral. If this information was provided to general practitioners the number of referrals to consultants would probably increase.

This study has shown considerable unreferred illness. Any changes in practice arising from our recommendations would have economic and ethical repercussions, which would have to be resolved.

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Chemical inactivation of HIV on surfaces

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Abstract

To assess whether alcohol and glutaraldehyde are effective disinfectants against dried HIV the virucidal effects of 70% alcohol (ethanol and industrial methylated spirit) and 1% and 2% alkaline glutaraldehyde were tested against cell associated and cell free HIV dried on to a surface. Virus stock (100 μl) or 10 000 cultured C8166 T lymphocytes infected with HIV were dried onto sterile coverslips and immersed in 2% and 1% alkaline glutaraldehyde and 70% ethanol for 30 seconds and one, two, four, and 10 minutes, there being an additional time point of 20 minutes for cell free virus disinfected with 70% industrial methylated spirit. In addition, virus stock in neat serum was tested with 1% and 2% alkaline glutaraldehyde to see whether the fixative properties of glutaraldehyde impair its virucidal properties. Virus activity after disinfection was tested by incubating the coverslips (cell associated virus) or the coverslips and sonicated cell free virus with C8166 T lymphocytes. The lymphocytes were examined for the formation of syncytia and HIV antigens were assayed in the culture fluid. Both 2% and 1% alkaline glutaraldehyde inactivated cell free HIV within one minute; 2% alkaline glutaraldehyde also inactivated cell free virus in serum within two minutes, but a 1% solution was ineffective after 15 minutes' immersion. Cell associated HIV was inactivated by 2% alkaline glutaraldehyde within two minutes. Seventy per cent industrial methylated spirit failed to inactivate cell free and cell associated HIV within 20 and 15 minutes, respectively, and 70% ethanol did not inactivate cell free virus within 10 minutes.

Seventy per cent industrial methylated spirit and ethanol are not suitable for surface disinfection of HIV. Fresh 2% solutions of alkaline glutaraldehyde are effective, but care should be taken that they are not too dilute or have not become stale when used for disinfecting HIV associated with organic matter.

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Introduction

Alcohols and 2% alkaline glutaraldehyde are widely used for disinfection in laboratories, dental surgeries, and hospitals. Several investigators have reported successful inactivation of HIV in aqueous solutions by alcohols. In 1984 Spire *et al*, using a reverse transcriptase assay to detect surviving virus, found a 99%

reduction in enzyme activity after exposure of the virus to 19% alcohol for five minutes.[1] Martin *et al*, using a tissue culture infectivity and antigen capture assay, found that 35% isopropyl alcohol and 50% ethanol inactivated HIV within 10 minutes, the shortest time tested.[2] Resnick *et al* reported inactivation of a suspension of virus by 70% alcohol within one minute with a tissue culture infectivity and reverse transcriptase assay.[3] The efficacy of alkaline glutaraldehyde in inactivating HIV has been reported only once. Spire *et al* tested the effect of 0·0125% glutaraldehyde on HIV and found that reverse transcriptase activity was greatly reduced within five minutes.[1] Reverse transcriptase, however, is a comparatively insensitive indicator of virus activity,[3] and the assay itself is impaired by concentrations of alcohol above 4% and glutaraldehyde above 0·0125%.[1]

As a contaminant in clinical practice HIV is found only in the presence of organic protein. Both cell free and cell associated virus may be encountered either wet or dried to the surface of equipment such as fibreoptic endoscopes. We tested the effect of glutaraldehyde and alcohol on HIV dried on to surfaces by the method of Tyler and Ayliffe.[4] Inocula of cell free and cell associated virus were used and the effect of serum on the efficacy of glutaraldehyde was observed. Because reusing 2% glutaraldehyde may reduce its concentration to 1% concentrations of both 1% and 2% were studied.

Methods

VIRUS STOCK

HIV-I (RF variant) was boosted by cocultivation of infected and uninfected T cell lines in a ratio of 1:10. Culture fluid was harvested after seven days, clarified by centrifugation, and stored in liquid nitrogen. Infectivity was measured by inoculating 100 000 C8166 T lymphocytes with serial dilutions of prepared virus, which were then cultured at 37°C in 4 ml RPMI 1640 culture medium with 10% fetal calf serum and 1% penicillin, streptomycin, and 1% L-glutamine. The stock of virus was found to have an infectivity of four times the logarithm of the tissue culture infective dose (the tissue culture infective dose is the reciprocal of the highest dilution at which syncytia develop and HIV antigens are expressed).

CELL ASSOCIATED VIRUS

H9 cells chronically infected with HIV-I (RF variant) were washed in phosphate buffered saline and resuspended in RPMI 1640 culture medium with 10% fetal calf serum and 1% L-glutamine, penicillin, and streptomycin. Cells were checked for viability before use and more than 90% were found by membrane immunofluorescence to express HIV antigens on their surface.

TEST METHOD

Virus stock (100 µl) or 100 000 cells infected with HIV were dried on to sterile glass coverslips (13 mm) at room temperature. With silica gel to provide conditions of low humidity drying occurred within 90 minutes. The disinfectants tested were freshly activated 2% and 1% alkaline glutaraldehyde (Cidex, Surgikos Ltd) and 70% industrial methylated spirit (Alcohols Ltd; final concentrations: ethanol 63·8%, methanol 3·5%, and water 26·5% v/v). We also tested 70% ethanol (v/v) on cell free virus alone.

Coverslips were immersed in disinfectant for 30 seconds and one, two, four, and 10 minutes, with an additional time point of 20 minutes for cell free virus in industrial methylated spirit. All measurements were performed in triplicate. Disinfectant was eluted by sequential immersion of the coverslips in two flasks of buffered saline (10 ml). Cell free virus was removed from the coverslips by ultrasonication for one minute in RPMI culture medium (1 ml); cell associated inocula were not sonicated. Residual material fixed by glutaraldehyde was gently loosened from the coverslip with the tip of a pipette. Coverslips and medium were then incubated with 300 000 C8166 cells in RPMI culture medium at 37°C in 5% carbon dioxide for 21 days. Cultures were examined for the formation of syncytia and culture fluid harvested for the assay of HIV antigens (Abbott Diagnostic Products, Delkenheim, West Germany) roughly every seven days. Culture fluid from all cultures yielding positive results was passaged into fresh cultures of C8166 T lymphocytes to assess the residual infectivity.

To determine whether the fixative effect of glutaraldehyde impairs its virucidal activity in the presence of organic matter 100 µl fetal calf serum was dried over an inoculum of cell free virus. Triplicate samples were immersed in glutaraldehyde for up to 15 minutes and assayed for residual viral infectivity as described above.

CONTROLS

Triplicate samples were treated in the same way but with buffered saline substituted for disinfectant. The infectivity titre of cell free control samples was determined as previously described; infectivity of cell associated control samples was assessed by using the antigen capture assay but without titration in tissue culture.

To exclude disinfectant activity in the recovery medium as a result of carryover on the coverslips recovery medium was inoculated in triplicate with the virus suspension (100 µl). Viral growth was observed with extensive formation of syncytia in all samples.

Results

Infectious virus was considered to be present in cultures that were positive for HIV antigens; this sometimes occurred in the absence of visible cytopathic changes. The correlation of antigen positivity with the presence of infectious virus was confirmed by passaging culture fluid that was positive for HIV antigen into fresh cultures and observing cytopathic changes in recipient cultures.

The infectivity of the stock of virus was four times the logarithm of the tissue culture infective dose; after having been dried and then immersed in buffered saline control virus had a residual infectivity of the same value, indicating that loss of infectivity due to the test method was negligible.

Viral inocula without added serum remained infectious after 30 seconds' exposure to 2% and 1% glutaraldehyde but not after an exposure of one minute or more (table). In addition, 2% glutaraldehyde was effective against cell associated HIV in two minutes. Virus in neat serum was inactivated by 2% glutaraldehyde within two minutes; 1% glutaraldehyde, however, failed to inactivate virus in neat serum during the 15 minutes tested.

Seventy per cent industrial methylated spirit failed to inactivate cell free virus within 20 minutes and cell associated virus within the 10 minutes tested. When the experiments were repeated with a separate batch of industrial methylated spirit and with 70% ethanol (v/v) the same results were found.

Discussion

Tissue culture is the most appropriate technique for showing the survival of viruses in a virucidal assay. Disinfectants, however, are toxic to living cells and require dilution to minimise their toxicity. Dilution of

Concentrations of HIV antigen (pg/ml) recovered in tissue culture fluid after timed exposure of viral inoculum to alkaline glutaraldehyde, industrial methylated spirit, or ethanol

HIV inoculum	Disinfectant	Time in disinfectant (minutes) 0·5	1	2	4	10	15	20
Cell free	2% Alkaline glutaraldehyde	60	None	None	None	None		
Cell free + serum				None	None			
Cell free	1% Alkaline glutaraldehyde	60	None	None	None	None		
Cell free + serum					350	475	60	
Cell free	70% Industrial methylated spirit		>2000		>2000	>2000		>2000
	70% Ethanol	>2000	>2000	>2000	>2000	>2000		
Cell associated	2% Alkaline glutaraldehyde	155	15	None	None	None		
	70% Industrial methylated spirit	>2000	>2000	>2000	>2000	>2000		

None = HIV antigens not detected.
Blank spaces in columns indicate test not done.

any residual infectious virus occurs in parallel, possibly to a concentration lower than the sensitivity of the assay. In a suspension test, therefore, it is necessary to start with a viral inoculum of a comparatively high titre. In a surface test an inoculum of a low titre can be used because disinfectant can be eluted and undiluted virus recovered on tissue culture. There is no universally accepted criterion for virucidal efficacy, although several investigators recommended that there is a fourfold logarithmic reduction in infectious titre.[4,5] The different results produced by various experimental methods underline the need to establish an international standard for virucidal assays.

Dried HIV may present a similar risk to health workers as aqueous solutions of HIV. Resnick *et al* were able to recover infectious virus after it had been dried in 50% human plasma at room temperature for up to three days, with a reduction in the infectivity titre by the logarithm of the median tissue culture infective dose every nine hours.[3] Disinfectants that are effective against aqueous HIV cannot be assumed to be equally effective against dried virus, particularly if they also have a fixative effect. The presence of water is essential to the activity of alcohols, which are most effective in a 60-80% dilution.

Klein and Deforest have classified viruses according to their susceptibility to disinfectants.[6] Lipophilic viruses, including retroviruses, were reported to be susceptible to 40% ethanol and glutaraldehyde. Tyler and Ayliffe examined the susceptibility of dried herpes simplex virus to 70% ethanol and glutaraldehyde and found both to cause at least a fourfold logarithmic reduction in infectious titre within a minute.[4] We found that 70% industrial methylated spirit failed to inactivate an inoculum of HIV of four times the logarithm of the tissue culture infective dose within 20 minutes, a finding that disagrees with those from previous reports based on suspension tests.[2,3] This discrepancy may reflect different susceptibilities of dried and aqueous HIV to alcohol. In the presence of organic protein alcohols are likely to be less virucidal, although we did not test them in the presence of neat serum.

Glutaraldehyde has been considered to be effective against HIV, even though published experimental evidence is equivocal. Its activity is reduced by protein, which competes with the virus for binding sites[7]; furthermore, by denaturing protein a mechanical barrier is created that might impair penetration by the disinfectant. We found that serum dried on the viral inoculum did not present a great barrier to 2% glutaraldehyde, even though the serum was denatured into a hard plaque within one or two minutes. The efficacy of 1% glutaraldehyde, however, was considerably impaired by the presence of serum. Whole blood and body tissue may prove an even greater challenge to disinfectants than serum; the margin of safety in 2% glutaraldehyde is clearly much smaller than originally suggested by Spire *et al*.[1] Most glutaraldehyde preparations are considered to be reusable for 14 days: we have found that reuse for 20 endoscopy procedures for this period reduces the concentration of 2% glutaraldehyde to nearly 1%.

Our findings indicate that 70% industrial methylated spirit and ethanol are unsuitable for surface disinfection of HIV; given the volatility of 70% alcohol, its contact time with infected material on surfaces and in spillages will be less than 10 minutes. Organic material should be removed if possible by cleaning or washing before disinfectant is applied; the duration of reuse of disinfectants should take into account the likely extent of dilution in use and the purpose for which they are used.

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ONE HUNDRED YEARS AGO

With the view of deciding what form of ambulance to adopt for use throughout the metropolis, the Street Ambulance Committee of the Hospitals Association invited the Directors-General of the medical departments of the army and navy, and several of the principal surgeons of the London general hospitals, to inspect, in the great hall of St Bartholomew's Hospital on May 15th, twelve specimens of the best kind of ambulances, including that now in use by the metropolitan police. After a careful examination of the several ambulances, Sir Thomas Crawford and the other surgeons present expressed a strong opinion in favour of the Ashford litter. The Committee now propose to proceed at once to order a sufficient number of litters to enable them to place one within an area of 500 yards of every part of London within the four miles district. Contributions in aid may be sent to the Chairman of the Committee, Sir Sydney H Waterlow, St Bartholomew's Hospital, or to the Treasurer. (*British Medical Journal* 1889;i:1186)