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Important Factors for Testing Barrier Materials with Surrogate Viruses

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This study evaluated bacteriophages ΦX174, T7, PRD1, and Φ6 as possible surrogates for pathogenic human viruses to challenge barrier materials and demonstrated some important factors for their use. Chemical incompatibility with test material was demonstrated when lipid-enveloped Φ6 was inactivated by an aqueous eluate of vinyl gloves, but 0.5% calf serum protected Φ6 from the eluate. Low concentrations (2%) of calf serum also prevented the exaggerated binding of the bacteriophages to filters. Recovery of viruses from surfaces decreased with increasing time before recovery. Penetration through punctures displayed different types of kinetics. The combined data indicate that (i) some bacteriophages may serve as surrogate viruses, (ii) experimental conditions determine whether a particular virus is appropriate as a challenge, and (iii) ΦX174 is an excellent choice as a surrogate virus to test barrier materials. The data further indicate that before barrier materials are challenged with viruses, adequate tests should be performed to ensure that the virus is compatible with the test material and test conditions, so that meaningful data will result.

Concern over transmission of infectious agents through barrier materials, such as those used in medical gloves and condoms, has prompted experimentation designed to evaluate penetration of different viruses (1–3, 9–13, 16, 18, 20–22). While human pathogenic viruses, e.g., the human immunodeficiency viruses (types 1 and 2), the herpes simplex viruses (types 1 and 2), and hepatitis B virus, are of primary interest, there is a need to substitute surrogate viruses for evaluating barrier materials and devices. Appropriately selected surrogate viruses should be used to make testing faster, less expensive, and, most of all, safer (16). Some bacteriophages should serve well as such surrogates. In this article, we evaluate and discuss some properties of importance in the selection and use of a surrogate virus.

The properties of virus particles which are expected to most influence penetration through barrier materials are shape, hydrodynamic size, composition, and stability. Most human viruses are approximately spherical (17); thus, only bacteriophages which are nearly spherical need be considered. The size ranges for human viruses and spherical bacteriophages are similar (4) (Table 1). Thus, smaller bacteriophages would serve as surrogates for the smaller human viruses, such as the hepatitis viruses (7, 8, 17), and should provide a safety margin for the larger human viruses, such as the herpesviruses and the poxviruses (17).

The composition of the virus particle, especially the outermost layer, determines whether there is any interaction between the barrier material and the virus. This interaction could be accomplished either by binding of the particle to the material or by chemical reactions between the virus and the material or chemicals eluted from the material. Such interactions could prevent the virus from being a useful probe for detecting virus penetration. Thus, compatibility of the challenge virus with the test material is important. The stability of the virus particle depends on lack of interaction with the test material and may depend on other test conditions, e.g., chemical concentrations and pH of test fluids, temperature, etc.

The purpose of this article is to evaluate four bacteriophages, ΦX174, T7, PRD1, and Φ6, under the following conditions which we found may confound virus penetration tests of barrier materials: (i) binding of virus to test material, (ii) virus inactivation by material components, and (iii) desiccation. Finally, the extent of penetration through very-small, artificially induced punctures in latex gloves was measured for the four bacteriophages.

MATERIALS AND METHODS

Viruses. The bacteriophages, their host cells, and the composition and size of the virus particles are listed in Table 1. Φ6, an enveloped bacteriophage, was chosen as a possible surrogate for the human immunodeficiency viruses (HIV-1, HIV-2). Standard growth and assay procedures were used (15). For most procedures, a virus cocktail containing approximately equal concentrations of the four bacteriophages in Dulbecco phosphate-buffered saline (DPBS) was used. Independent assay of the individual bacteriophages was possible because the bacteriophages did not cross-infect the other host strains over the range of titers used in this study. (Wild-type revertants of T7am28, which can also infect *Escherichia coli* C, occurred at a frequency of 6×10^{-6}; no other cross-infectivity was found.)

Chemicals. DPBS (Advanced Biotechnologies, Inc., Columbia, Md.) was used for virus suspension because it provided a pH (7.0) and salinity which maintained virus particle integrity.

Calf serum (M.A. Bioproducts, Walkersville, Md.) normally used for cell culture experiments was used to provide serum in designated experiments.

Virus binding. A 3-ml volume of the virus cocktail at about 10^3 PFU/ml was slowly filtered through 25-mm-diameter Millipore filters (high protein binding; 0.22-μm pore size).

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TABLE 1. Details on candidate surrogate bacteriophages and some important human viruses[a]

Surrogate or human virus	Bacterial host	Virus details	
		Composition[b]	Diam (nm)
Surrogate			
ΦX174	*Escherichia coli* C	ssDNA, protein	27
T7am28	*E. coli* O11	dsDNA, protein, 17-nm tail	65
PRD1	*Salmonella typhimurium* LT2	dsDNA, protein, internal lipid	65
Φ6	*Pseudomonas phaseolicola*	dsRNA, protein, external lipid	80
Human			
Hepatitis B		dsDNA, protein, external lipid	42
Hepatitis C		ssRNA, protein, external lipid	30–60
Hepatitis delta virus		ssRNA, protein	30
Human papilloma virus		dsDNA, protein	55
Human immunodeficiency virus types 1 and 2		ssRNA, protein, external lipid	90–130
Herpes simplex virus types 1 and 2		dsDNA, protein, external lipid	120–150
Cytomegalovirus		dsDNA, protein, external lipid	120–150

[a] Data from Fraenkel-Conrat (4), except for hepatitis C virus (7) (hepatitis non-A, non-B virus, strain H, identified as hepatitis C virus [19]), hepatitis delta virus (8), and human immunodeficiency virus (5, 6).
[b] ss, single stranded; ds, double stranded.

Titers of the four viruses were determined in the original cocktail and in the filtrate. The effect of the presence of calf serum at different concentrations was determined by including it in the virus cocktail at the time of the experiment.

Virucidal activity. A glove (surgical latex glove, size $6\frac{1}{2}$, or vinyl examination glove, size small) was hung in the same manner as for a previously described 1,000-ml water leak test (14) and filled with deionized water.

For direct exposure of the viruses to the glove, a 50-ml plastic centrifuge tube containing 40 ml of the virus cocktail at 10^3 PFU/ml was prepared. A finger of the glove (one of the three central fingers) was dipped into the virus cocktail (Fig. 1). Aliquots of the fluid in the centrifuge tube were assayed for the four viruses as a function of time of contact with the glove finger.

For indirect exposure of the viruses to the glove (exposure to an eluate from the glove), a similar procedure as described above was performed except that the glove finger was dipped into DPBS alone for different periods of time and then the virus cocktail was added to the DPBS and assayed 2 and 5 min later. Thus, the virus cocktail was indirectly exposed to whatever eluted from the glove finger into the DPBS.

Recovery of viruses from surfaces. The glove was hung as described above and filled with deionized water. Small beads (2 μl) of virus cocktail (at 10^7 PFU/ml) were carefully placed on the outside of one or more fingers approximately 2 cm from the tips. The virus-containing bead was recovered by dipping the finger into a 50-ml centrifuge tube containing 40 ml of DPBS. The extent of virus recovery was determined by plaque assay. As a control, glass slides with a "nonreactive" surface were used with 2-μl beads of virus cocktail. The virus titer controls were accomplished by directly spiking 2 μl of virus cocktail into 40 ml of DPBS in a centrifuge tube.

Test of surrogate viruses leaking through punctured glove fingers. Latex glove fingers were punctured with a 30-gauge (0.30-mm diameter) acupuncture needle (from China National Medicines and Health Products Corp., Shanghai); the glove was punctured while lying flat, providing punctures through both sides of the finger about 2 cm from the tip. The glove was hung as described above (Fig. 1) and filled with DPBS containing approximately 10^7 PFU of each of the surrogate viruses per ml. The glove was observed for visual leaks for 2 min as in the Food and Drug Administration water leak test (14). Any virus leaking from a punctured finger was collected by dipping the finger in 40 ml of DPBS in a 50-ml

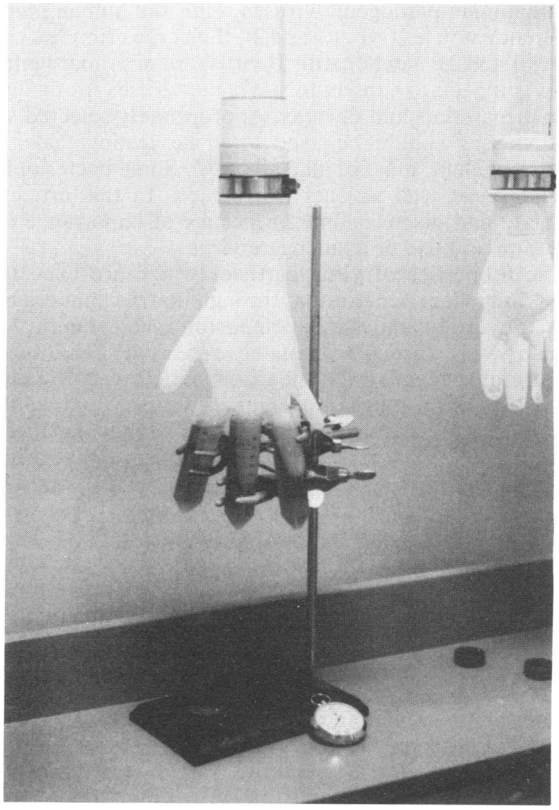

FIG. 1. Photograph of a latex glove filled with 1,000 ml of DPBS (or deionized water) and method of dipping the middle fingers into 50-ml centrifuge tubes containing 40 ml of DPBS.

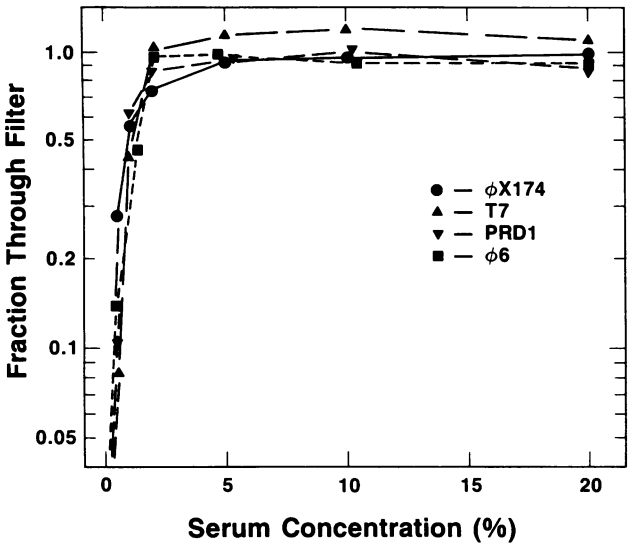

FIG. 2. Fractions of viruses in DPBS which pass through protein-binding filters in the presence of different concentrations of calf serum. Data represent mean values (± standard errors) from two to four experiments.

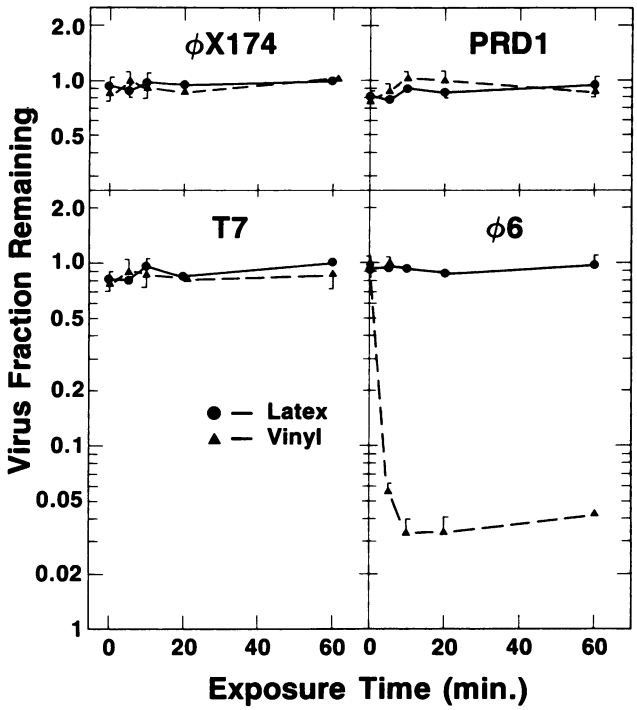

FIG. 3. Virus survival (in DPBS) after direct contact with latex or vinyl gloves for different periods of time. Fingers of gloves were immersed in 40 ml of DPBS containing the four viruses noted in the graph. Data represent mean values (± standard errors) from two experiments.

centrifuge tube and assayed at 15-min intervals. The amount of virus in the 40 mL of collection fluid was used to calculate the penetration (in microliters), i.e. the equivalent volume of the original challenge virus suspension which penetrated. The penetration was calculated by dividing the amount of virus in the collection fluid (titer × 40 ml) by the titer inside the glove.

RESULTS

Virus binding. Binding of virus to materials can be demonstrated by using an exaggerated case. When the virus cocktail in DPBS was passed through a protein-binding filter, each virus type was completely (>95%) removed from the DPBS which passed through the filter. The presence of serum competed sufficiently well with the virus for the binding sites in that 2% serum protected the viruses from binding (Fig. 2). The virus binding and the protection by the serum were essentially the same for each of the cocktail viruses.

Virucidal activity. Reduction of viable virus by two commonly used barrier materials was also readily demonstrated for one of the challenge viruses. Direct contact of the virus cocktail (in DPBS) with fingers of vinyl gloves resulted in partial inactivation of Φ6 within minutes (Fig. 3). Latex gloves had no effect under the experimental conditions used here. However, if the ratio of glove surface to fluid volume was increased by placing a whole latex glove in a centrifuge tube with only 10 ml of virus cocktail suspension for 60 min, the viable Φ6 titer fell below 10%, with no effect on the other viruses (data not shown). Thus, direct contact reduced the titer of one virus but not those of the other viruses. From these data it cannot be ascertained whether the titer reduction resulted from binding of virus to the glove surface or to chemical inactivation.

Indirect contact of the four challenge viruses with latex or vinyl gloves was accomplished by dipping the glove fingers into DPBS for different elution times and by subsequently adding the virus cocktail to the DPBS with the eluate. There was no opportunity for the viruses to directly contact the glove. Again, Φ6 was partially inactivated within minutes (Fig. 4). The trends of the data presented in Fig. 4 suggest the following two observations: (i) the virucidal activity was less potent for the longer elution times, perhaps reflecting a lack of stability of the eluted activity, and (ii) the inactivation was nearly as great after 2 min of reaction with the virus as after 5 min for each of the eluates.

The effect of different serum concentrations on inactivation of Φ6 by direct contact with the vinyl glove or by eluted sample (indirect contact) is shown in Fig. 5. Serum at 0.5% in DPBS was sufficient to completely protect Φ6 from inactivation caused by either direct or indirect contact.

Recovery of viruses from surfaces. If a small amount of virus suspension penetrated through to the air-exposed side of a material, could the virus be recovered and how well would the virus survive as a function of time? This was tested by spotting beads of the virus cocktail in DPBS on latex glove fingers or glass surfaces (ΦX174 was used alone with vinyl glove fingers) and by then recovering the virus. The data in Table 2 show that virus recovery was nearly complete if attempted very soon after the bead was spotted. However, all four viruses partially lost recoverability as the time before recovery was lengthened, with ΦX174 being the most easily recovered and Φ6 being the least. Presumably, structure of the virus particles was important to recoverability, since the simplest structure (ΦX174) was the most stable. Further, the external membrane of Φ6 may have rendered it the least stable for these conditions.

Penetration through punctured latex gloves. As a demonstration, the actual penetration of viruses in the virus cock-

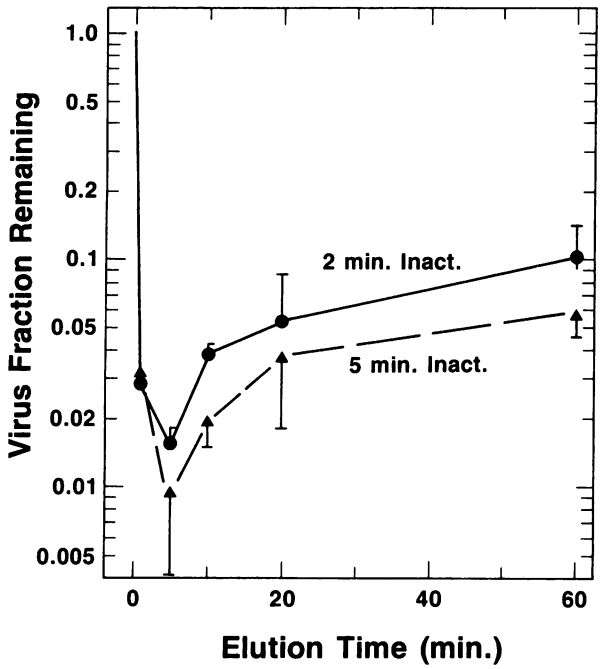

FIG. 4. Elution of virucidal activity into DPBS from fingers of a vinyl glove as a function of elution time at room temperature. Virucidal activity was determined by adding Φ6 to the eluate (in DPBS) and assaying 2 and 5 min later. Data represent mean values (± standard errors) from two experiments.

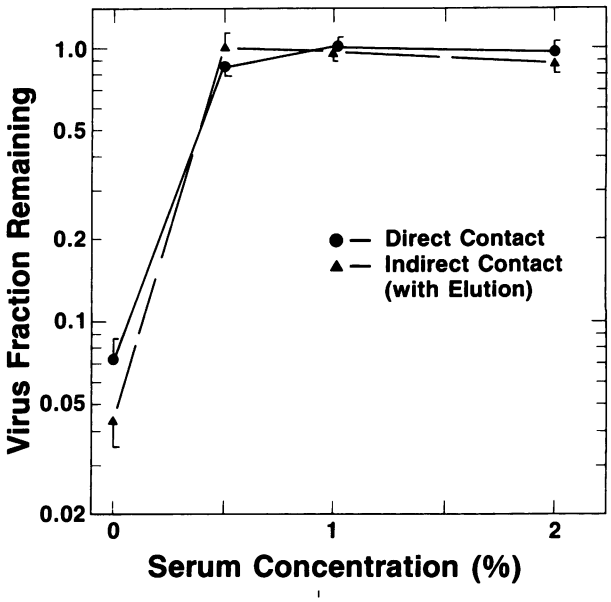

FIG. 5. Effect of different serum concentrations on inactivation of Φ6 by direct contact with vinyl glove or by eluate (indirect contact). The eluted sample was obtained by 5 min of contact of DPBS with a finger of the glove. The virus inactivation time was 5 min for either the direct or indirect contact method. Data represent mean values (± standard errors) from three to six experiments.

tail was determined through a compromised barrier, a latex glove finger containing a small puncture. Examples of two different leaks are shown in Fig. 6. Figure 6A shows that penetration through one puncture did not increase after 15 min, suggesting that the puncture hole apparently closed within the first 15 min after the test started. The different viruses in the cocktail penetrated different amounts, with ΦX174 apparently penetrating the most readily and Φ6 apparently penetrating the least readily. Figure 6B shows greater penetration through a different puncture, the rate of which stayed approximately constant over the 60-min test, indicating that the puncture hole size remained constant. In this case, three of the viruses penetrated at nearly equal rates, with only Φ6 apparently penetrating less.

DISCUSSION

This series of studies demonstrates some different factors which must be considered when viruses are used to challenge the integrity of barrier materials. Four bacteriophages were used as possible surrogate viruses for human virus pathogens. The results clearly indicate that some factors may be important for nearly all virus challenges, while other factors may affect only certain viruses.

The first factor was binding of the virus particles to test material. Even with the exaggerated binding to the protein-binding filters, 2% serum was enough to protect the viruses from being bound. Thus, the presence of extra protein such as that found in serum or in bacteriological broths may usually suffice to negate binding.

While direct contact of the virus challenge with the test material may result in binding, chemical inactivation may take place through either direct or indirect contact. With the specific vinyl gloves used in this protocol, the enveloped virus Φ6 was inactivated under conditions which did not affect the other three viruses. Further, 0.5% serum was sufficient to protect Φ6 from such inactivation.

Test methods for virus penetration may provide for collection of virus which penetrates to the air-contact side of a test material by rinsing after a specified test period. The data shown in this study demonstrate that part of the penetrated virus may not be recoverable under those conditions. Furthermore, one virus, ΦX174, appeared to be more stable on latex and glass surfaces than the other candidate viruses.

The use of the virus challenge to evaluate punctured latex gloves gave some information on different types of kinetics of penetration through individual punctures. Use of the virus cocktail as the challenge also showed similarities and differences among the different viruses. With the lesser level of penetration (implying a smaller hole) where the amount of penetration plateaued (Fig. 6A), it appeared that two viruses, ΦX174 and PRD1, penetrated better than the other viruses. With the greater level of penetration (Fig. 6B), three of the viruses showed similar levels of penetration. In 13 of 14 punctures which allowed virus penetration, ΦX174 penetrated as well as or better than the other viruses. On the other hand, the enveloped virus, Φ6, penetrated least well in 13 of 14 punctures. At present, there is no definitive explanation for different penetration rates by the different surrogate viruses.

An overall implication of our data is that some barrier materials may be incompatible with the use of certain viruses as challenge particles. Barrier materials may be capable of removing viable virus from suspension by binding the virus particles or by inactivating them. Such removal would confound the use of virus particles as a challenge for testing penetration through barrier materials. Components in biological fluids, such as serum, may be capable of protecting

TABLE 2. Recovery of different bacteriophages from surface of latex or vinyl gloves or glass slides

Surface material	Time of exposure[a] (min)	Virus recovered[b]			
		ΦX174	T7am28	PRD1	Φ6
Latex glove	0	1.04 ± 0.08	0.70 ± 0.06	1.05 ± 0.18	0.59 ± 0.03
	10			<0.02	<0.01
	15	0.74 ± 0.05	0.03 ± 0.01	<0.03	<0.01
	30	0.72 ± 0.08	0.03 ± 0.01	0.04 ± 0.01	<0.01
	60	0.39 ± 0.16			
Vinyl glove	0	0.94 ± 0.31			
	15	0.63 ± 0.02			
	30	0.69 ± 0.12			
Glass	0	0.95 ± 0.11		0.85 ± 0.12	0.54 ± 0.20
	10			0.09 ± 0.08	0.02 ± 0.01
	60	0.36 ± 0.08			

[a] Time of exposure of virus cocktail in DPBS to test surface before attempting virus recovery.
[b] Fraction of virus recovered after exposure to test surface, compared with corresponding preexposure virus titer. Mean values from two experiments presented (± standard errors).

the virus particles from being removed from the challenge suspension. Therefore, before any virus is used as a challenge particle, it should be determined whether the virus, the material, and the experimental solutions are compatible and could yield meaningful test results.

One virus, ΦX174, of the four tested has the following properties which make it an excellent candidate as a surrogate virus to test barrier materials. (i) It is smaller than the human viruses of concern and therefore represents a conservative challenge virus. (ii) It was stable under most test conditions and was compatible with the barrier materials tested. (iii) It is easy, fast, and inexpensive to use. (iv) Because the biological assay time is short (4 h), the test material need not be sterilized before the biological challenge. The enveloped virus, Φ6, a possible surrogate for human immunodeficiency virus, was least useful since it was inactivated under conditions in which the other viruses were stable.

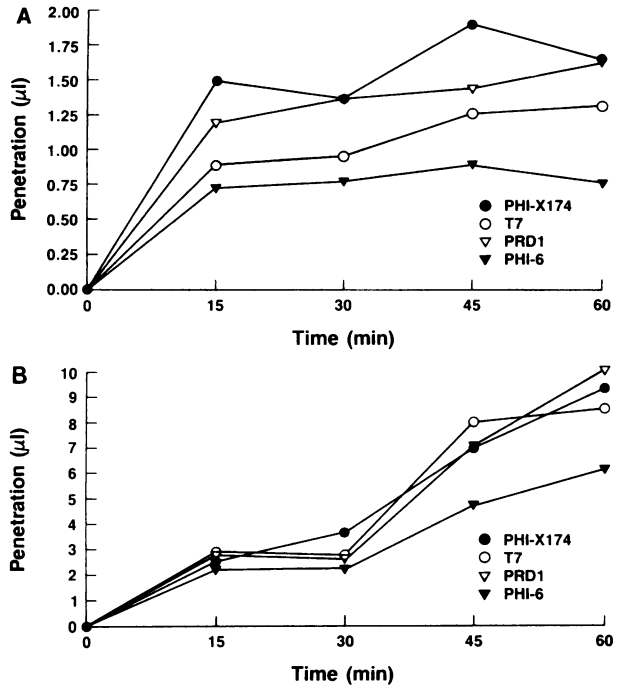

FIG. 6. Kinetics of penetration by four viruses through punctures in two individual fingers of different latex surgical gloves. Penetration refers to the equivalent volume of virus suspension that has passed from the glove interior to the collection fluid (see Materials and Methods). (A) Data for an instance where the penetration rate decreased as the test proceeded; (B) data for an instance where the penetration rate stayed constant. Data from representative single experiments are shown, since the extent of virus penetration varied greatly among punctured glove fingers.

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