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Cause of Cessation of Viral Passage through Artificially-induced Holes in Latex Condoms

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Laboratory experiments have demonstrated that viral passage under pressure through small, artificially-induced holes in latex condoms ceased almost completely within minutes upon commencement of testing. Three explanatory hypotheses have been put forward: hydration might cause the latex to swell, thereby shrinking the hole; loose particles could block the hole; or the restrainer used to limit condom expansion under pressure might cause blockage of the hole. Experiments which measured the passage of virus under pressure through artificial, laser-drilled holes as a function of time were performed, using a low-pressure condom test apparatus without a restrainer. The initial rate of viral passage exhibited the theoretically-predicted fourth power dependence on hole radius. However, within a few minutes viral flow ceased. Microscopic examination of the holes before (dry condom) and after (wet) the experiment indicated that the holes did not shrink. Particles (donning powder, etc.) *were found inside condoms that were large enough to clog holes. The kinetics of viral passage were consistent with sudden, complete closure of the holes. Thus, these data suggest that hole blockage be considered the primary cause of cessation of viral passage through small holes during laboratory experiments with these latex condoms. Further, it is unlikely that such hole blockage would be relevant in real-life use of condoms, with rare, naturally-occurring small holes.*

In laboratory experiments designed to quantitate virus passage through latex condoms with holes, evidence has been reported indicating that the virus penetration stopped or decreased substantially after a few minutes[1,2]. This was true for holes produced with laser radiation[2] and for punctures (small tears) produced with small acupuncture needles[1]. The test was done by collecting and assaying the virus particles that pass into a collection buffer through a condom that had been filled with virus-containing buffer, with the condom pressurised and prevented from expansion by a restrainer[2,3].

Three hypotheses have been put forth to explain this phenomenon: (i) exposure to water could induce hydration-caused expansion of latex, thereby shrinking (and possibly closing) the holes[1,2]; (ii) the holes could clog from the collection of particles naturally found in condoms such as dusting powder[2]; (iii) the

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restrainer covering the pressurised condom might directly or indirectly cause blockage of holes[1,2].

It is important to consider the ramifications of each of the hypotheses when considering the use of condoms in real life. Hypothesis 1, hole shrinkage by hydration, would be likely to take place in actual use if it were found to occur in the laboratory. Hypothesis 2, hole blockage by particles, might be less likely to take place in actual usage because of motion during sexual intercourse. Hypothesis 3, hole blockage by the restrainer, would hold no significance in real life (since restrainers are used solely to control experimental conditions) and therefore, cessation of viral passage would be merely a laboratory artifact.

The purpose of this investigation was to test the three hypotheses. The approach was (i) to visually inspect and photograph holes before and after experiments for evidence of change in hole dimensions or of clogging, and (ii) to determine any changes in the kinetic nature of virus penetration at low pressure, such as the time of hole closure (if any) and any change in rate of penetration (slope). Low pressure was used to eliminate the need for a restrainer (Hypothesis 3) and to provide virus passage that was slow enough to allow accurate determinations of passage rate.

MATERIALS AND METHODS

Virus

Bacteriophage PRD1 was used as the challenge virus in this study. This approximately spherical virus has a diameter of about 65 nm. Its bacterial host is *Salmonella typhimurium* LT2. Descriptions of the growth and plaque assay of this virus have been published[4]. For experiments, these viruses were suspended in Dulbecco's phosphate-buffered saline without Ca^{++} or Mg^{++} ($DPBS^-$).

Condoms

Non-lubricated latex condoms of a single brand with reservoir tips were used. Artificially-induced holes of approximately cylindrical shape were created through the reservoir tips by means of an excimer laser (Resonetics, Inc., Nashua, NH). The holes ranged in diameter from 1.7 to 28.5 microns. The thickness of each condom at the location of the hole was measured and varied from 74 to 145 microns.

Condom Test at Low-pressure

A special apparatus was constructed to allow quantitation of virus passage through the hole in the condom reservoir tip under controlled, low hydrostatic pressure (about 8 cm of water). In this manner the use of a restrainer was avoided, and kinetics of virus passage could be determined. The test apparatus is shown in *Figure 1*. A condom was secured on the cylindrical mandril by means of the centering sleeve. The condom was then filled with 6 mL of virus suspension. Immediately, the reservoir tip (containing the hole) was submerged in a collection cup containing 1.0 mL to 2.2 mL of $DPBS^-$. Hydrostatic pressures (see *Figure 1*) were recorded and varied slightly from experiment to experiment because of variation in the condoms. Twenty-μL aliquots were removed from the collection cup as a function of time, diluted and assayed by plaque formation. Viral penetration, or the volume of challenge virus which accounted for the amount of virus found in the collection cup, was calculated by dividing the amount of virus in the collection fluid (which is the product of

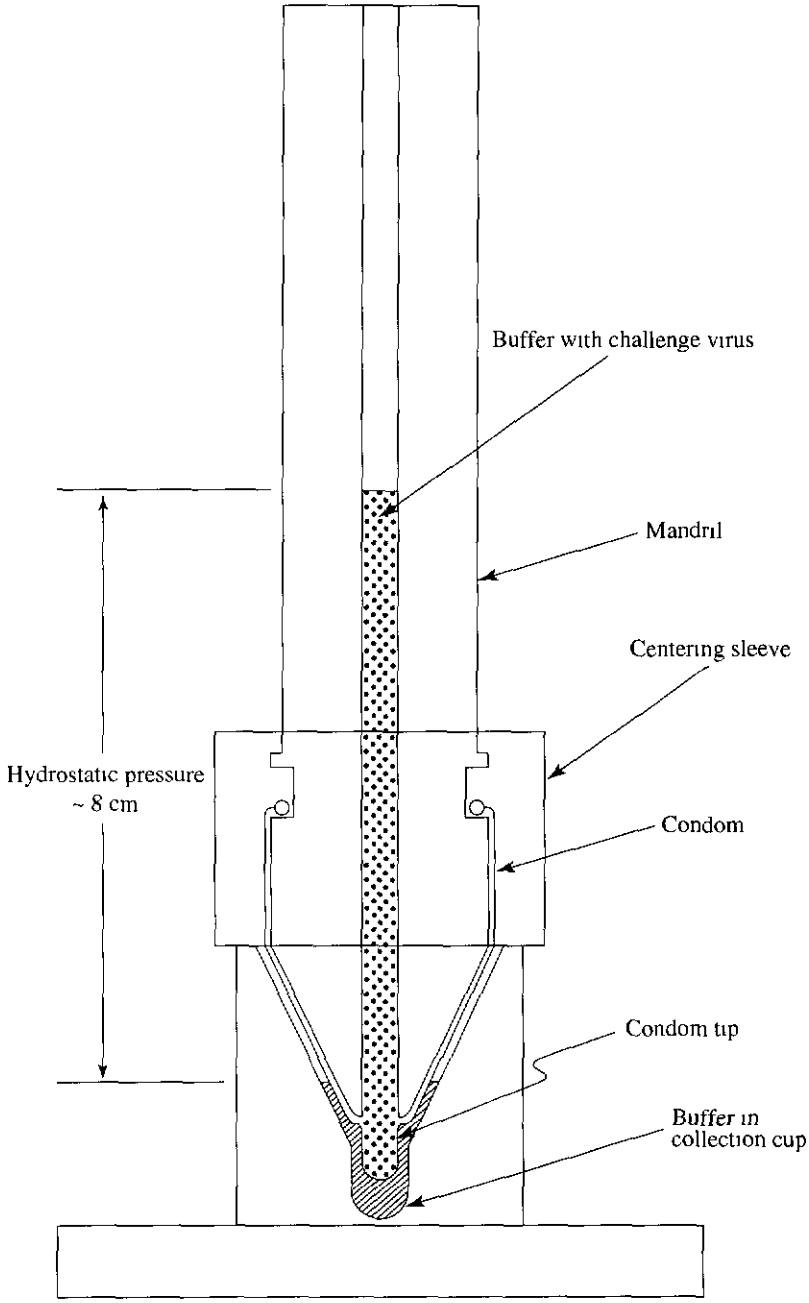


Figure 1 Schematic diagram of low-pressure condom test apparatus Essentially only the condom tip was immersed in collection buffer

the titer in the collection cup and the volume of collection buffer) by the titer inside the condom[3]

Condom Test at High-pressure

To look for additional evidence of hole blockage, some experiments were performed at high pressure (81 cm of water) where a restrainer was necessary to prevent undesired condom expansion[2,3]. The descriptions of the high-pressure apparatus and test procedure have been described elsewhere[2].

Photomicroscopy

Holes in the condoms were examined at a magnification of 160× on a Carl Zeiss Photomicroscope #3. Photographs were taken before (dry condoms) and after (wet condoms) experiments, and the sizes of holes were compared The holes were examined at the inside surface of the condom, since the hole diameter was better defined where the laser beam exited the material. The sizes of the holes were calibrated from photomicrographs taken at the same magnification of an acupuncture needle measured to be 120 microns in diameter.

RESULTS

Kinetics of Viral Penetration

The kinetics, or amount of virus penetration through a hole as a function of time, obtained from these tests at low pressure are represented by three examples in *Figure 2* and indicate that i) the initial viral passage increased linearly with time; ii) viral penetration usually ceased; iii) cessation appeared to be abrupt and complete; and iv) occasionally viral passage resumed

The kinetics of all 18 experiments were characterised by initial linear increases, although occasionally there was a lag (up to 90 sec) before virus penetration was detectable In most cases (14 of 18 experiments), cessation of viral passage occurred, as shown in the graphs by plateaus (*Figures 2b* and *2c*) The cessation times fell between 2.0 min and 6.5 min, with a slight tendency for passage through larger holes to cease earlier (*Table 1*). The cessation appeared as a sudden change from a linear increase to no increase at all (*i e* , complete cessation). In a few cases (4 of the 14 experiments), resumption of viral passage occurred after a clearly-defined cessation (*Figure 2c*)

Agreement with the Poiseuille Equation

Fluid flow through a cylindrical hole is given by the Poiseuille Equation

$$Q = P\pi r^4/8\eta l \qquad 1$$

where Q is flow rate, P is pressure difference across the length of hole, r is hole radius, η is viscosity of the liquid, and l is hole length (*i e* , thickness of condom)[5]. To verify that amount of viral passage was consistent with fluid-flow predictions, the initial rates of viral passage (rates before cessation occurred) were plotted against the diameters of the laser-drilled holes (*Figure 3*) The line of best fit through the graph of normalised flow rates *versus* hole diameters (on a log-log scale) had a slope of 4 055 and a correlation coefficient of 0 984. This consistency of virus penetration with the Poiseuille Equation confirms the previously-assumed relationship between virus passage and fluid flow[1,6], at least through these nearly cylindrical, laser-drilled holes in latex condoms

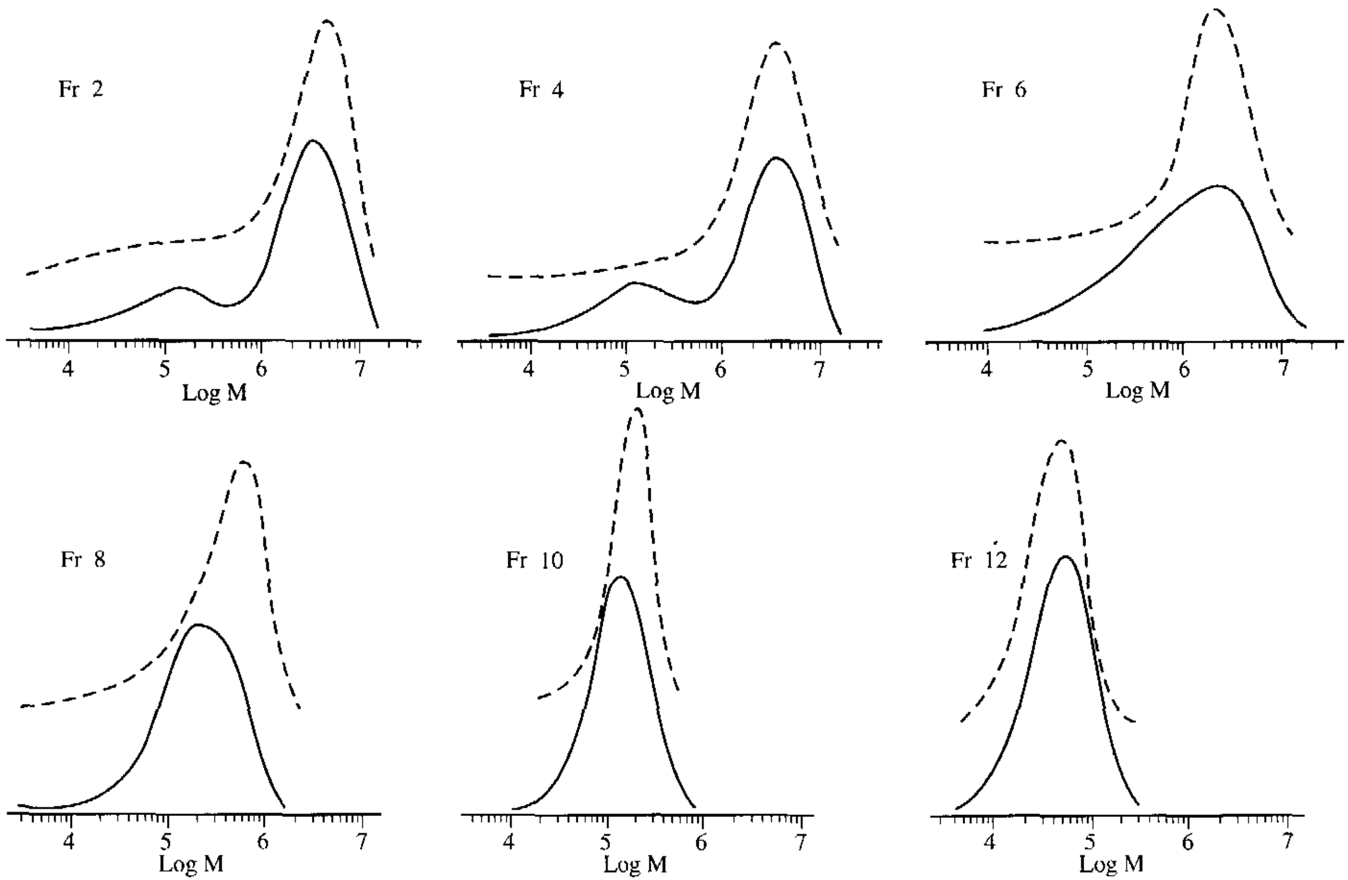


Figure 1 Changes in molecular weight distributions (MWDs) of fractionated rubber fractions from FL-latex upon transesterification Broken and solid lines show the MWDs before and after transesterification respectively

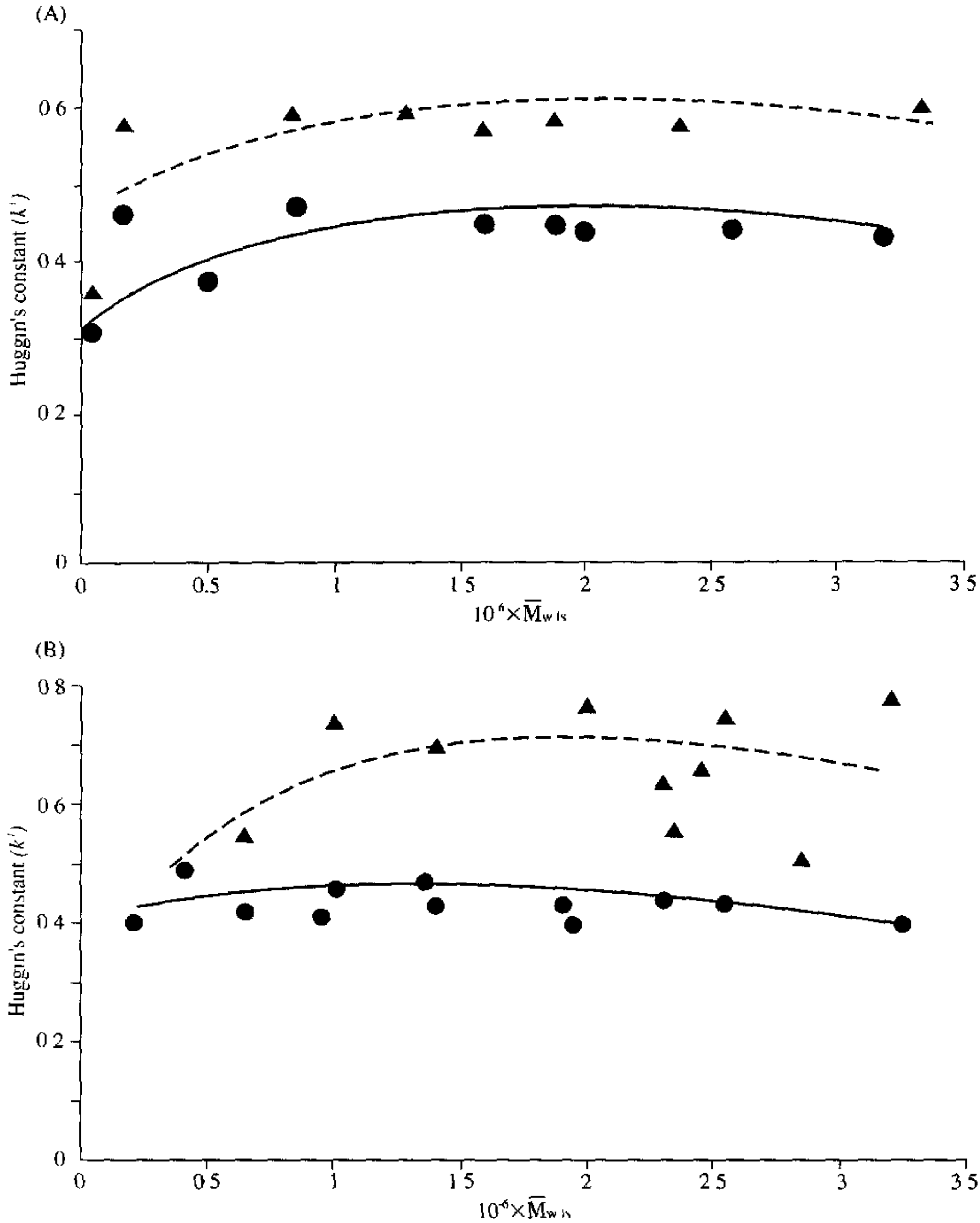


Figure 2 Relationship between Huggins' k' constant and $\overline{M}_{w\,LS}$ of fractionated deproteinised rubber fractions from (A) FL-latices and (B) HA-latices, before and after transesterification. Broken and solid lines correspond of those before transesterification, respectively

TABLE 1. TIME OF VIRUS PASSAGE CESSATION FOR DIFFERENT SIZE HOLES IN LATEX CONDOMS

Hole diameter[a] (microns)	Number of condoms in which cessation occurred during these time periods (minutes) 1.0 – 3.9	4.0 – 6.9	7.0 – 10.0	>10.0
1.0 – 7.9	1	3	0	2
8.0 – 14.9	1	0	0	1
15.0 – 21.9	2	1	0	0
22.0 – 28.9	4	2	0	1

[a]Hole diameters provided by Resonetics, Inc.

Microscopic Examination

Microscopic examination of the condom surface showed that there were many particles, presumably consisting primarily of dusting powder (*Figure 4*). Most of the particles were loose (*i.e.*, they could easily be moved by liquid); they varied in size and shape; and some particles appeared to be larger than the diameters of holes being investigated. Most of the larger particles were flat; some appeared to be crystalline (*Figure 4a*), possibly talc or silica, and some appeared to be non-crystalline and flexible, even folded (*Figure 4b*) (possibly aggregated latex particles?).

Examination of holes before and after experiments tested at low pressure indicated that hole diameter did not change significantly over the course of the experiments (*Table 2*). Typical holes are shown in *Figure 5*, demonstrating the different appearances between dry and wet conditions. Further, nine of ten holes that were microscopically examined after the 10-minute virus-passage experiments showed no visual signs of blockage (complete data not shown).

Since no visual signs of blockage were usually found at low pressure, even in the presence of the particles, hole blockage was examined after experiments at high pressure (which provided greater liquid flow through the holes, and perhaps a greater chance that a particle might be pushed further into the hole). Microscopic examination of holes after the high-pressure procedure indicated that blockage of holes did occur (*Figure 6*). Eight of 10 holes were found to be blocked by particles that either accumulated in the holes or obstructed the entrances to the holes.

DISCUSSION

The hypotheses put forward to explain the cessation of virus penetration (*i.e.*, flow of carrier fluid) through holes in latex condoms were (i) shrinkage of holes; (ii) blockage of holes by particles; and (iii) blockage of holes by the restrainer. It was found that cessation of viral passage occurred in the absence of a restrainer, thus eliminating the third hypothesis. This is consistent with the finding that sometimes viral passage decreased from punctured latex gloves where no restrainer was used[7].

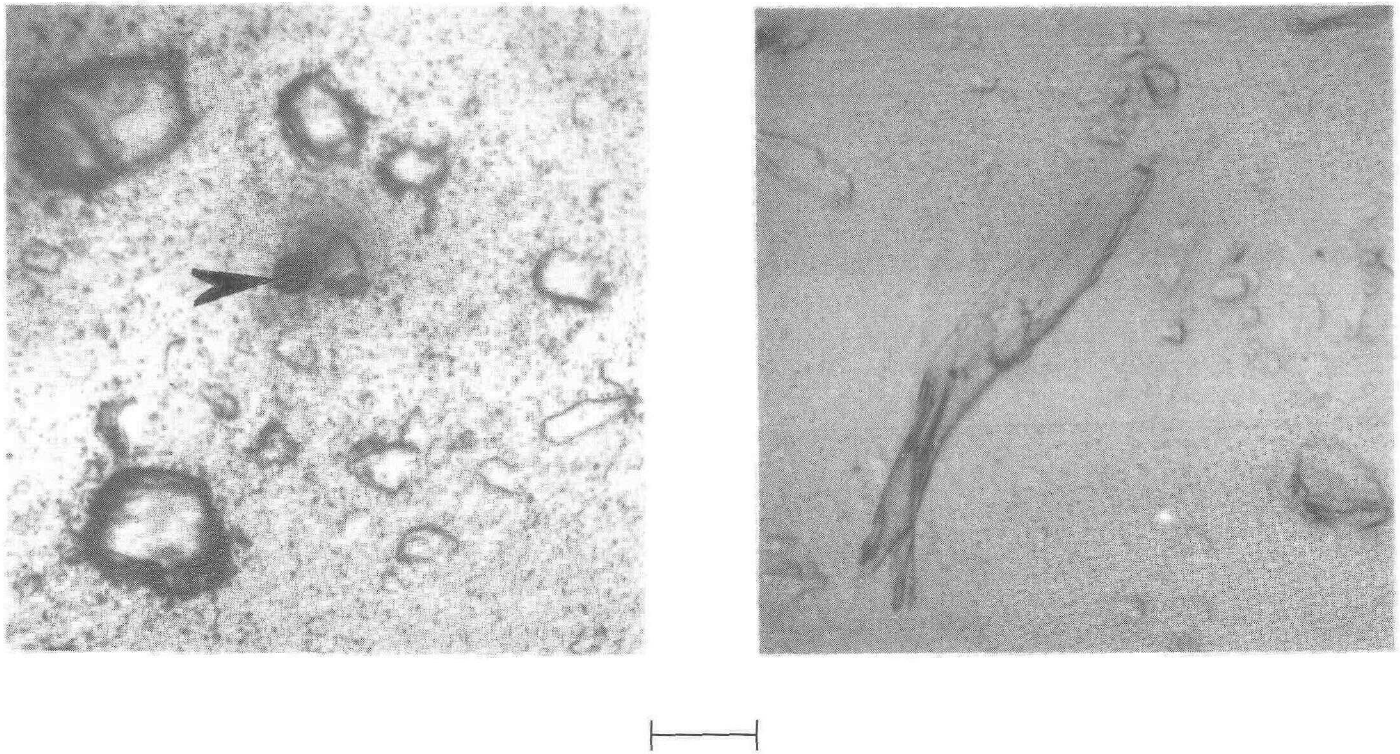
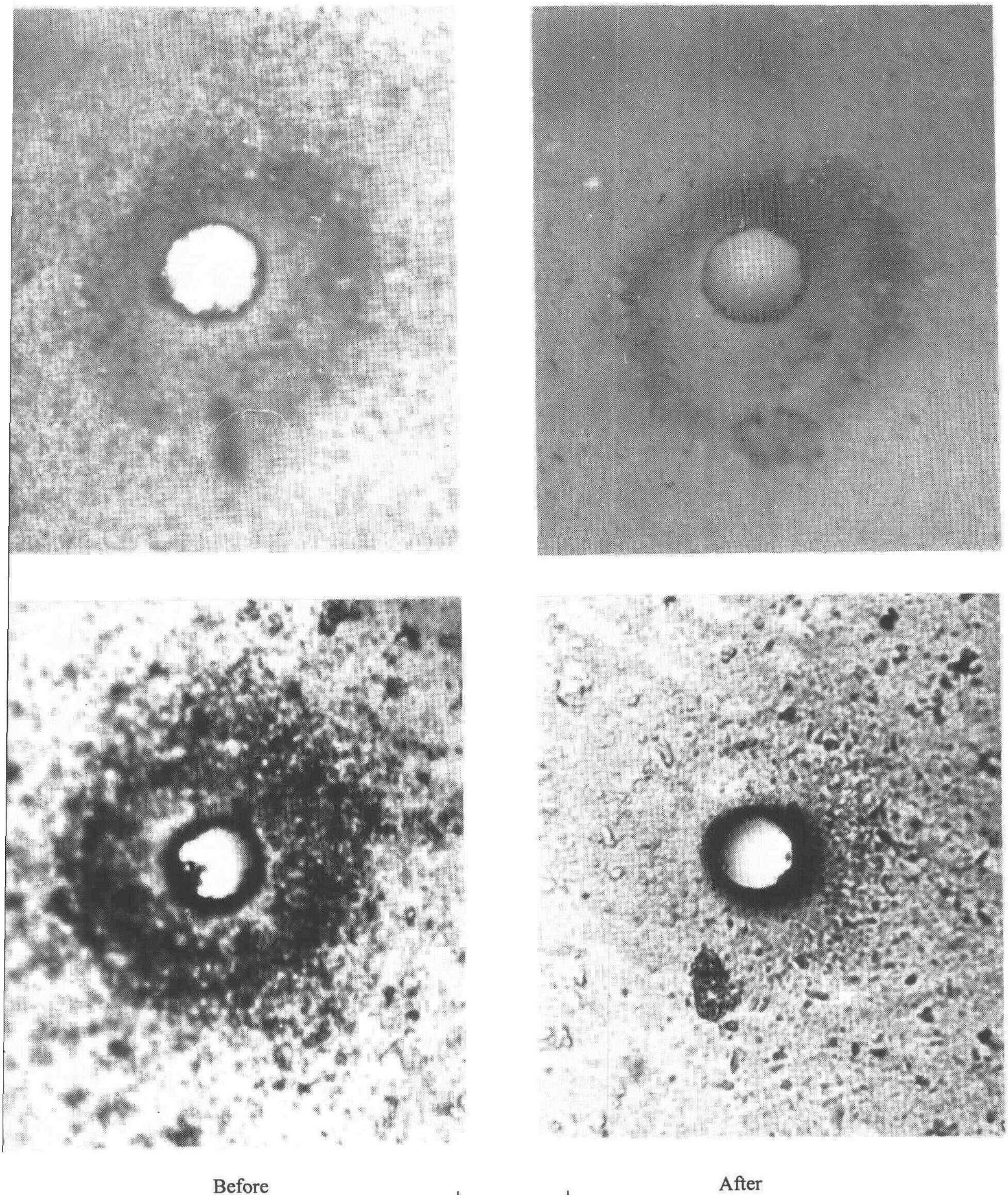
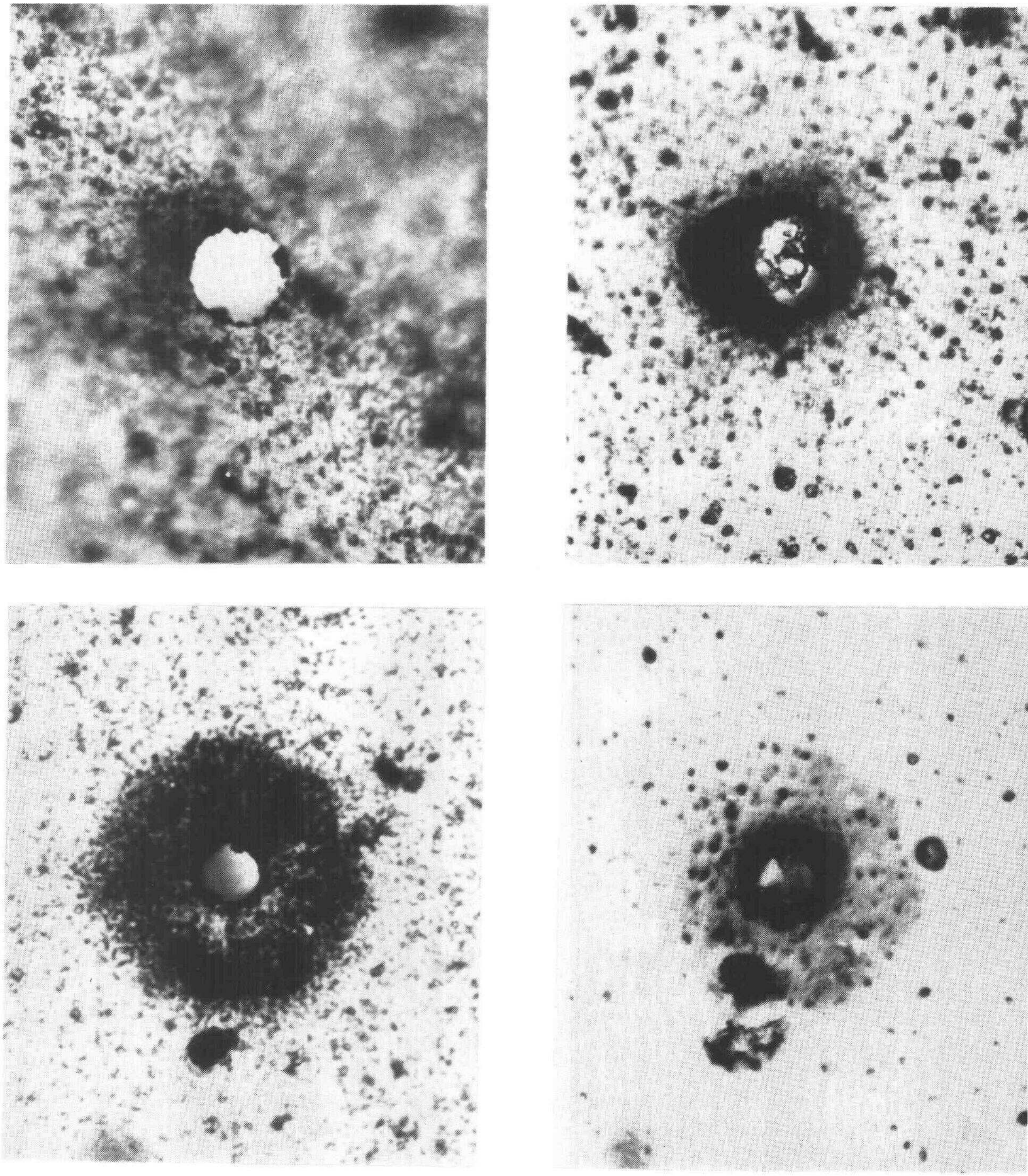


Figure 4. Photomicrographs of a hole and particles found on a latex condom, showing the variety of sizes and shapes of the particles, particularly compared to the size of the hole (10 micron diameter; indicated by arrow): (a) typical large crystalline flat pieces, and (b) an example of a non-crystalline, flexible sheet. The scale bar represents 30 microns.



Before　　　　　　　　After

Figure 5. Photomicrographs of two holes taken before (dry; left) and after (wet; right) an experiment at low pressure, showing that hole shrinkage did not occur. Note that no blockage particles were visible in the hole at the end of the experiment. The scale bar represents 30 microns.



Before

After

Figure 6. Photomicrographs of two holes taken before (dry; left) and after (wet; right) experiments at high pressure, indicating that blockage of holes occurred. Blockage particles were readily visible in the holes. The scale bar represents 30 microns.

TABLE 2. COMPARISON OF HOLE DIAMETERS IN CONDOMS BEFORE VIRUS PASSAGE EXPERIMENTS (DRY) AND AFTER EXPERIMENTS (35 – 45 MINUTES OF EXPOSURE TO DPBS)[a]

Hole diameter before (microns)	Hole diameter after (microns)	Ratio (after/before)
11.7	11.5	0.98
17.0	19.0	1.12
19.5	19.4	0.99
20.3	21.2	1.04
23.5	23.8	1.01
23.8	23.9	1.00
30.1	29.7	0.99
30.9	32.4	1.05
31.6	32.4	1.03
Mean ratio (standard error)		1.02 ± 0.01

[a]Holes photographed on inside surfaces of condoms (exit hole of laser beam that created hole). Uncertainties in hole diameters were less than 0.5 micron.

Microscopic examination indicated that holes were not reduced significantly in size (*Table 2*), although virus passage ceased, essentially eliminating the first hypothesis. In addition to this finding, the broad range of times at which cessation occurred and the resumption of flow preclude the possibility of hole shrinkage since shrinkage would be expected to be a gradual, monotonic process.

Cessation of virus passage, however, can readily be explained by hole blockage caused by particles being carried to, and perhaps into, the hole by the challenge buffer. Hole blockage by large particles would be expected to take place suddenly, consistent with the abrupt kinetics (*Figure 2*). Although time of blockage may be somewhat dependent on flow rate, the times of cessation would primarily rely on probability and the number of particles that were present inside the condoms. Microscopic examination of condoms tested at high pressure clearly indicated hole blockage. The higher pressure could cause longer-lasting clogging, since the higher pressure would result in higher fluid flow through the holes thereby increasing the chances that particles would actually be drawn into the holes. Permanent hole blockage would be a rational interpretation of permanent cessation or reduction of flow.

The use of low pressure allowed for rates of initial viral passage to be calculated since cessation did not occur as quickly as at high pressure. Although viral passage did cease at low pressure, clogged holes were rarely found upon subsequent microscopic examination. The inability to find blocked holes can be explained: particles which move with liquid flow could cover the openings of holes, but would only remain there under pressure. Transient or long-term release of pressure would allow the particle to move away from the hole, such as during the process of taking aliquots when the condom might be bumped or during manipulations after the experiment The 'stuck' particles might be released in much the same manner as a piece of paper held by a fan would be released if the fan were turned off Manipulation of the condoms for photography following the experiment could quite possibly dislodge loosely-bound particles These proposed mechanisms would account for temporary cessation followed by a resumption of flow and the failure to find many clogged holes after low pressure.

The real-life implications of this investigation could be significant the question arises as to whether hole blockage is merely a laboratory artifact or a real-life occurrence. Although hole blockage in latex condoms is important in a static laboratory setting, it may have little bearing to the real-life application of condoms. We believe that such blockage may not be significant in real-life usage of condoms, because the pressure exerted on holes (if at all present) would not be sustained long enough to result in long-term hole blockage and because short-term blockage seems unlikely with the unrestrained real-life motion that was not simulated in the laboratory However, since hole blockage during laboratory testing hinders the passage of viruses, these data suggest that static laboratory tests may underestimate the amount of virus able to penetrate holes during actual use, at least for this brand of condom. It is not yet known whether the hole blockage process occurs for condoms of other brands Lastly, it would be logistically quite difficult to determine whether hole blockage could occur with the rare, naturally-occurring small holes in condoms

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