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Accumulation of Latex in Peyer's Patches and Its Subsequent Appearance in Villi and Mesenteric Lymph Nodes[1, 2] (40336)

M. E. LEFEVRE, R. OLIVO,[3] J. W. VANDERHOFF,[4] AND D. D. JOEL

Medical Research Center, Brookhaven National Laboratory, Upton, New York 11973

Recent interest in the Peyer's patches of the small intestine has centered on the ability of these lymphoid structures to take in (sample) antigenic material from the intestinal lumen (1–4). Little attention has been paid to Peyer's patch uptake of inert particulates in the micron size range, in part because of the belief that large particles do not readily pass the intestinal epithelial border. We recently reported, however, that 2-μm latex particles accumulate in Peyer's patch macrophages during chronic feeding of latex to mice (5). The present communication extends this finding and presents additional observations on the transport of particles from Peyer's patches to adjacent villi and the mesenteric lymph node.

Materials and methods. Latex feedings. Ten-week-old female Swiss mice (Hale–Stoner strain) were used for all experiments. Table I gives information on latex feeding to six groups of mice. A water suspension of latex (mean particle diameter ± SD, 2.02 ± 0.014 μm; identification No. LS-1078-B, Dow Chemical Co.), was given *ad libitum* as drinking fluid. Periodic shaking of the bottles and the mixing action of air bubbles as the mice drank kept the latex suspensions relatively uniform and monodisperse. Examination of fresh intestinal contents indicated that the latex was distributed as single particles in the small intestine. All mice gained weight normally and appeared healthy.

Tissue preparation. To permit the examination of large amounts of tissue, clearing procedures were applied to whole Peyer's patches and to 0.5-mm-thick slices of mesenteric lymph node. The use of xylene-based solvents, which dissolve latex, was avoided. *Peyer's patches:* Intestinal segments of ether-killed mice were fixed in 70% alcohol for several days. Peyer's patches together with small adjacent areas of intestine were excised, gently cleaned with a jet of 70% alcohol from a syringe, and rinsed in water. The tissue was treated as follows: 2% KOH, 2 hr; clearing solution I (150 ml of 2% KOH, 150 ml of glycerol, 150 ml of 0.2% formalin), 2 days; clearing solution II (100 ml of 2% KOH, 400 ml of glycerol), 2 days. Cleared Peyer's patches were stored in 100% glycerol containing a crystal of thymol. *Mesenteric lymph node:* Whole alcohol-fixed mesenteric lymph nodes were too thick and slices were too fragile for successful clearing. Slices of formalin-fixed lymph node remained intact during the clearing process but did not become as transparent as alcohol-fixed material. They were, however, sufficiently cleared by lengthening the time of exposure to 2% KOH to 2 days. Cross sections for the present study were taken from the anterior and posterior regions of the major mesenteric node.

For observation, the cleared tissue was placed on a depression slide in glycerol, coverslipped, and examined with a Zeiss inverted microscope.

Results. Peyer's patches. Five ileal Peyer's patches from each mouse were examined after clearing. The major structures such as crypts and villi around the patch and reticular fibers within the patch could be discerned despite the transparency of the specimens. Each patch consisted of two to eight lymphoid follicles. In mice fed high concentrations of latex (Groups A, B, D, and E), the center of each follicle on the mucosal side (the dome) was characterized by an accumulation of particles. Figure 1 shows a low-power view of such an accumulation in the dome of a Group D mouse. Under high power the par-

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[3] Supported by Brookhaven National Laboratory Summer Student Program.

[4] Center for Surface and Coatings Research, Lehigh University, Bethlehem, Pa. 18015.

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TABLE I. LATEX-FEEDING REGIMENS.

	Short-term			Long-term		
	A	B	C	D	E	F
Number of animals	3	3	3	6	6	6
Percentage latex (w/v)	1.0	0.1	0.01	1.0	0.1	0.01
Latex fed (days)	3	3	3	61	61	61
Latex withheld[a] (days)	1.5	1.5	1.5	14–74	14–74	14–74

[a] Time between termination of latex feeding and sacrifice.

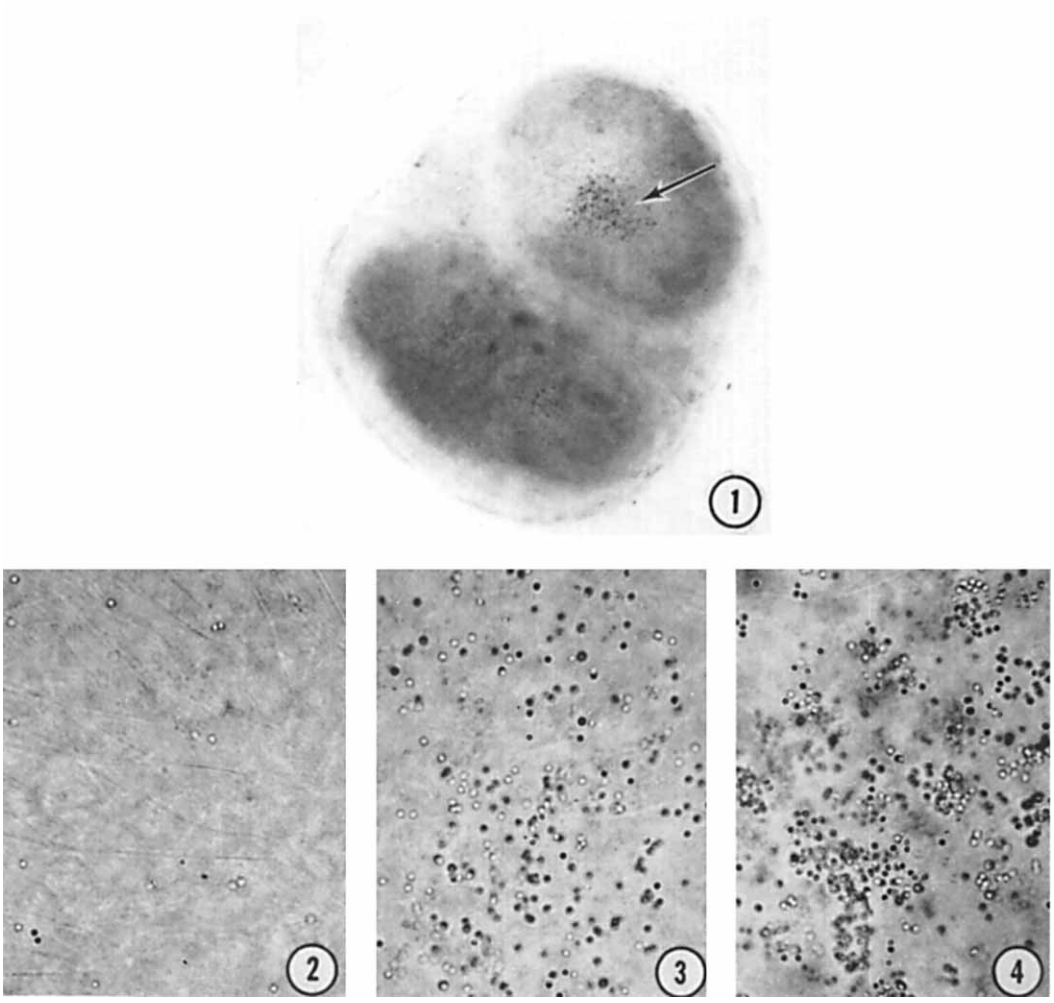


FIG. 1. Mucosal aspect of a cleared Peyer's patch from a young adult mouse given drinking water containing 1.0% latex for 61 days followed by 14 days without latex. Patch contains a single follicle (upper right) and two small indistinct follicles (below). Arrow points to an accumulation of latex particles in the center of the dome of the large follicle. Crypts and villi are not visible. × 35.

FIGS. 2–4. Representative latex accumulations in the domes of cleared Peyer's patch follicles from mice given 0.01% (Fig. 2), 0.1% (Fig. 3), and 1.0% (Fig. 4) suspensions of 2-μm latex as drinking fluid for 61 days. Latex feeding was terminated 14 days before sacrifice of the mice. Plane of focus is near the mucosal surface. Black circles are latex particles above the plane of focus. × 340.

ticles were refractile, uniform spheres, identical to the latex with which the mice had been fed. The amount of latex in large patches was not uniform; central domes usually contained more latex than peripheral ones. Nevertheless, the total amount of latex

present was related to the amount fed in both short-term and long-term experiments. Figures 2 through 4 show representative latex accumulations in Peyer's patch domes. Latex could also be seen near the serosal surface of Peyer's patches from mice of Groups D and E, often in aggregates of 15 to 25 particles. Individual latex-containing macrophages could be discerned in some but not all of the specimens (Fig. 5).

In mice fed the low concentration of latex, the particles were rare (Group F) or not discernible (Group C) in Peyer's patches. Cleared Peyer's patches of control mice given tapwater to drink contained no particles that resembled 2-μm latex although many small rod-shaped and crystalline-appearing particulates were present.

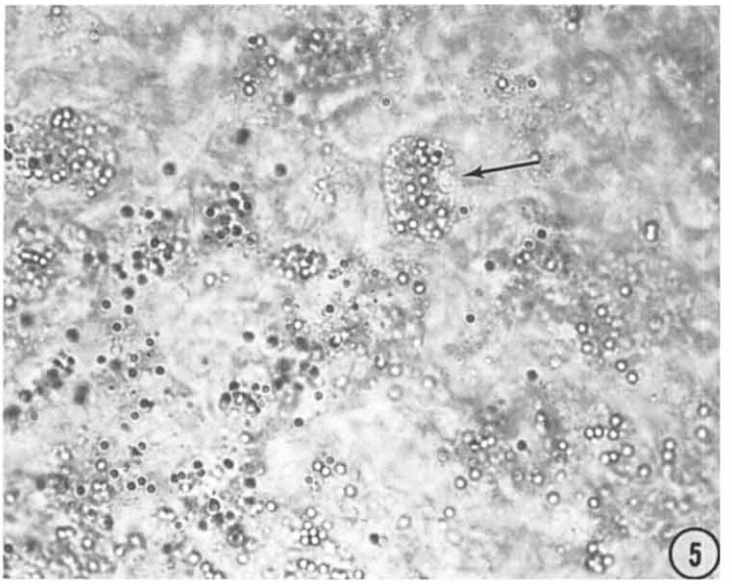
Latex particles were still present in Peyer's patches 74 days after the cessation of latex feeding in Groups D through F, but the number had declined to approximately 10% of the number seen in patches of comparably fed mice sacrificed 60 days earlier.

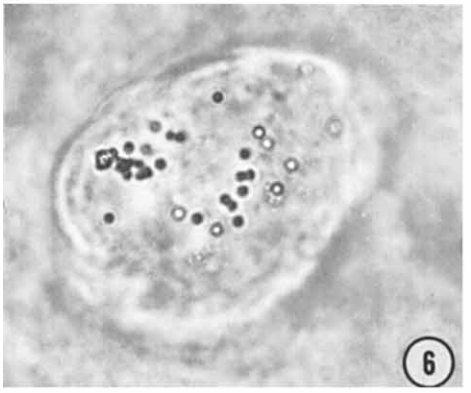
Villi. Latex frequently appeared in a few villi adjacent to follicles in long-term, but not short-term, experiments. In Group D mice, if a villus contained latex it almost always contained more than one particle; villi from Groups E and F usually contained only one particle. The total number of latex-containing villi was small, e.g., only 3 or 4 of the 10 to 20 villi surrounding a typical follicle might contain latex. Latex-containing villi were readily seen in a scan of the mucosal surface of a cleared Peyer's patch because of the location of particles in the villous tips (Fig. 6). The particles were usually isolated, in contrast to the close aggregates sometimes seen on the serosal side of Peyer's patches. The latex in villi was contained in granular structures, probably macrophages (Fig. 7). Latex was never observed in villi distant from Peyer's patches. No particles resembling latex were seen in villi of control mice although macrophages containing smaller particles were present. After the cessation of latex feeding, latex particles were still present in juxtafollicular villi of Groups D and E at 74 days, but in smaller numbers than at 14 days.

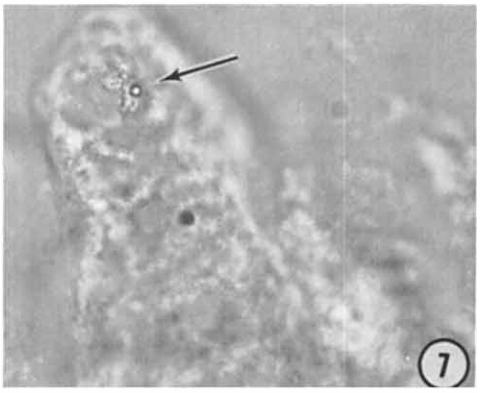
Mesenteric lymph node. Latex particles were present in cleared mesenteric lymph node tissue from latex-fed mice although they were extremely rare in short-term latex-fed mice. All subsequent observations apply to long-term experiments. The particles were seen around germinal centers and in the central region of mesenteric lymph nodes; no particles were observed in the germinal centers themselves. Particles were more abundant in the anterior than in the posterior region of the node, and they appeared singly, never in aggregates. The total number of particles observed in lymph-node tissue was generally related to the latex concentration fed. The number of particles in mesenteric lymph-node tissue 74 days after the cessation of latex feeding was the same or larger than in comparably fed mice 14 days after the cessation of feeding. Figure 8 illustrates an area of maximum accumulation of latex in mesenteric lymph-node tissue.

Discussion. The present report and a previous communication from this laboratory (5) describe the accumulation and retention of 2-μm latex particles of intestinal origin in mouse Peyer's patches. These observations support the contention that the Peyer's patch epithelium is continuously taking in (sampling) intestinal contents (1–4, 6, 7). Although latex particles remained in Peyer's patches for weeks, they were slowly eliminated after the cessation of latex feeding. The results also demonstrate the important finding that appreciable numbers of latex particles reached mesenteric lymph nodes (Fig. 8). The possibility of direct entry of latex particles into villi (8) as an explanation for the finding of latex in juxtafollicular villi (Figs. 6 and 7) cannot be totally ruled out, but our findings suggest that latex does not appear in villi until after its accumulation in Peyer's patches. The functional implications of these observations should be considered.

Peyer's patches produce immunoglobulin A (IgA) precursor cells which enter the circulation and eventually home to the mucous membranes of the gastrointestinal tract (3, 9–12). This production of IgA precursor cells is probably stimulated by the intake and transport of antigenic material through special cells in the Peyer's patch epithelium and its delivery to lymphocytes within the patch (4). The sampling of intestinal contents, however, is potentially dangerous in that it may permit entry of living pathogens and toxic materials. This risk can be minimized by delivery of sampled materials to a region rich







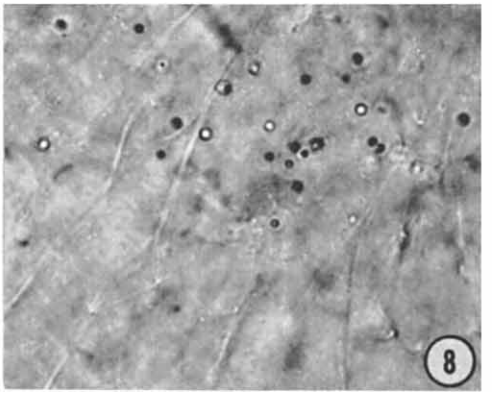


FIG. 5. Portion of a cleared Peyer's patch from a mouse treated as described for Fig. 1. Macrophages (one is designated by arrow) appear as granular bodies containing latex. × 510.

FIG. 6. Latex particles in the tip of a villus which adjoins a Peyer's patch from a mouse treated as described for Fig. 1. × 510.

FIG. 7. A latex particle (arrow) in a villus which adjoins a Peyer's patch from a mouse treated as described for Fig. 3. The particle is contained within a macrophage, a portion of which is visible as a stellate granular body. × 510.

FIG. 8. Latex particles among reticular fibers in cleared mesenteric lymph-node tissue. Mouse was given 1.0% latex for 61 days followed by 74 days without latex. Maximum accumulation of latex in this tissue is illustrated. × 510.

in macrophages which can phagocytize and inactivate some of the toxic material; macrophages are outstandingly abundant in Peyer's patch tissue (15, 16), particularly in the immediate subepithelial zone. Thus, in overall function, the Peyer's patches may constitute a specialized system for processing intestinal antigens and particulates with little risk to the rest of the body.

Phagocytized particulate matter cannot accumulate in Peyer's patches indefinitely, and mechanisms for its elimination must be sought. Our findings suggest the existence of a population of macrophages in Peyer's patches that ingest particulate material and then migrate to neighboring villi, mesenteric lymph nodes, and possibly other locations. Since large latex aggregates were not seen in mesenteric nodes or villi, the migratory population, if it exists, has either a limited capacity to engulf particulates or a relatively short residence time in areas containing free particulates. Latex-containing macrophages that migrate from Peyer's patches to the tips of neighboring villi are probably shed into the lumen of the gut. The finding of latex in some, but not all, villi adjacent to Peyer's patches is unexplained although this may simply reflect favorable lymphatic channeling.

An alternative explanation for the finding of latex in villi and mesenteric lymph nodes after its accumulation in Peyer's patches is the movement of free particles via open lymphatic channels connected to Peyer's patches. Carter and Collins (15) have described such lymphatic connecions in the mouse intestine.

The two suggested mechanisms for latex movement away from Peyer's patches (as free particles or within macrophages) are, of course, not mutally exclusive. Whether or not some particles are also shed directly from the Peyer's patch dome in the reverse of their route of entry is not known, but the finding by Bockman and Stevens (16) that the follicle-associated epithelium of appendix and Peyer's patches appears to conduct bidirectional transport of horseradish peroxidase suggests that direct elimination of particles from the dome may occur.

Summary. Latex particles (2 μm in diameter) accumulated in intestinal Peyer's patches and mesenteric lymph nodes of mice given latex suspensions as drinking fluid. After a 61-day period of latex feeding, the particles were also present in villi adjacent to Peyer's patches; they were not seen, however, after only 3 days of latex feeding. The amount of latex in Peyer's patches 74 days after the termination of latex feeding was much less than the amount present 14 days after the termination of feeding. It is suggested that migratory macrophages take up latex particles within Peyer's patches and subsequently move out of the patch to mesenteric nodes and villi. Some free particles may also be transported out of Peyer's patches to mesenteric nodes and villi through open lymphatic channels. The observations support the contention that Peyer's patches "sample" intestinal contents and they suggest a mechanism for the elimination of accumulated inert particulate matter from these lymphoid structures.

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