Intestinal Uptake of Fluorescent Microspheres in Young and Aged Mice[1] (42825)

M. E. LeFevre, A. M. Boccio, and D. D. Joel
Medical Research Center, Brookhaven National Laboratory, Upton, New York 11973

Abstract. **Rhodamine B-labeled synthetic latex particles (microspheres), 1.8 μm in diameter, were administered by gavage 5 days per week to young (24 days) and aged (18 months) mice. After 25 days (19 gavages), the particles were assayed in solubilized tissues by depositing them on filters and counting under fluorescence microscopy. Aged mice exhibited significantly more fluorescent particle accumulation in Peyer's patches but significantly less in lungs than young mice. Mesenteric lymph nodes and Peyer's patch-free intestinal segments contained measurable latex, but differences between young and aged animals were not significant. Liver contained only trace amounts of latex, and spleen and kidney were latex free in both young and aged animals. Nonquantitative observations on KOH-glycerol-cleared whole Peyer's patches and slices of liver, lung, and mesenteric lymph node were similar.** [P.S.E.B.M. 1989, Vol 190]

We previously reported the accumulation of polymeric latex particles (microspheres) in Peyer's patches of mice that chronically ingested the particles (1–4). Some particles were demonstrable in intact or solubilized mesenteric lymph nodes (1, 3), but attempts to determine whether such particles reached the blood circulation were inconclusive. Latex particles were not found in tissues distant from mesenteric nodes except for lungs and possibly liver (3). The present work describes a new method, more sensitive than those used previously, to test for latex particle penetration into Peyer's patches and migration to extraintestinal tissues. The study involved the chronic administration of fluorescent, rhodamine B-labeled particles, 1.8 μm in diameter, to young and aged mice. Alkali-solubilized lungs, liver, spleen, mesenteric lymph nodes, kidneys, intestinal segments, and intestinal Peyer's patches were passed through filters which retained the particles. The filters were examined by fluorescence microscopy and the rhodamine-labeled particles quantitated.

Differences between young and aged mice with respect to particle accumulation were sought. Solubilized Peyer's patches of aged mice contained more latex than those of young mice. Latex microspheres were extremely infrequent in tissues other than Peyer's patches, mesenteric lymph nodes, and lungs in both young and aged mice.

Materials and Methods

Fluorescent Microspheres. Carboxylated rhodamine B-conjugated polystyrene latex particles (lot 33838; mean diameter ± SD, 1.8 ± 0.13 μm) were obtained from Polysciences Co. (Warren, PA).

Mice. Female Swiss albino mice, Charles River CD-1 strain, were used for all experiments. Young mice were 24 days old. Aged mice were retired breeders, approximately 18 months at the time of the experiments. The mice were numbered by ear clipping and kept in groups of five.

Experimental Plan. Five days a week the mice were lightly anesthetized with ether and gavaged with 0.2 ml of latex suspension containing approximately 1.3×10^9 particles. The stock suspension was quantitated weekly and no significant variation was noted. A ball-tipped mouse-feeding needle of the appropriate size was used. The gavaging was continued for 25 days; the total number of gavages was 19. The estimated number of particles delivered into the stomach in the 25-day experimental period was 2.47×10^{10}. Control groups of young and aged mice were gavaged with water; additional control groups were untreated.

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Following the last gavage, the mice were kept for 4 days in a latex-free environment to rid the intestinal lumen of the majority of free particles. The mice were killed by ether overdose. To reduce the possibility of contamination of tissue samples by latex particles remaining in fur, the skin was removed prior to opening the abdominal cavity. With the alimentary tract left intact, liver, lungs, kidneys, mesentery, and spleen were removed and placed in a vial. The gastrointestinal tract was then removed and put in a separate vial. Tissues were fixed in 70% alcohol.

In agreement with previous reports (4, 5), the number of Peyer's patches visible to the naked eye was approximately 10 per intestine for both young and aged mice. Six Peyer's patches were cut from the mid-intestine with scissors and trimmed of ordinary mucosa under a dissecting microscope. Those from a single mouse were thoroughly washed and pooled. A 1-cm segment of Peyer's patch-free mid-intestine was opened and cleaned of digestive contents. The spleen and one kidney were trimmed of fat. The mesenteric lymph node (sometimes in two parts) was dissected free of fat and mesentery. To avoid excessive sediment only one third of the liver from each mouse was analyzed. Both lungs were used. All of these tissues were cut into small pieces with razor blades, placed in 4 ml of 15% KOH in individual plastic test tubes, and rotated for 4 days. At the end of that time the tubes were stored in a cold room until ready for assay. The tubes of solubilized tissue could be kept indefinitely and reassayed after weeks or months.

Fluorescent Particle Assay. Fluorescent particles were deposited on filters as follows. A 13-mm Millipore filter holder was fitted with a black gridded filter of pore size 0.8 μm (Millipore AABG01300). Grid size was 9 mm^2 as stated by Millipore and confirmed by direct measurement. The filter area available for particle deposition was 86.6 mm^2. Solubilized tissues and other samples were thoroughly vortexed and an aliquot, usually 0.5 ml, was added to water to a total volume of 4.5 ml. The entire 4.5 ml was mixed and drawn into a disposable, vertically held 5-ml plastic syringe. The filter assembly was placed on the syringe and the contents were gently forced through the filter. The filter was placed grid side up on a microscope slide and examined by a Zeiss fluorescence microscope equipped with epi-illumination and optical filters for detecting rhodamine B fluorescence. The particles appeared as bright red dots scattered uniformly over a black background interrupted by the lines of the grid.

The latex particles were counted at 250× using an eyepiece reticule to progress systematically over the squares of the Millipore grid. The number of squares counted depended on the abundance of particles. If the particles were in excess of approximately 150 per square, additional dilutions were made. All determinations were made in duplicate or triplicate. Drying of the filter as counting proceeded did not quench the fluorescence nor affect the results.

The alkali treatment used for tissue solubilization did not destroy the fluorescence of the particles even after weeks of exposure. Slight leaching of the dye from the microspheres and diffuse background fluorescence were sometimes observed, but the intensity of particle fluorescence under the microscope was such that even leached particles stood out brilliantly from the background.

Protection against Contamination by Ambient Fluorescent Particles. Throughout the procedures, disposable plastic tubes, razor blades, syringes, filter holders, etc., were used. Care was taken to keep samples covered. All work with stock latex suspensions was carried out in a different room from that used for procedures involving experimental samples. To avoid unnecessary handling and potential contamination, tissue weights were not determined.

Preliminary Test of Particles Added to Tissue. To test the accuracy of the assay procedure, two known concentrations of particles were added to alcohol-fixed tissues from four untreated mice. For the higher particle concentration, a serial three-step dilution of the stock suspension was made, and 0.1 ml containing an estimated 6740 particles was added to 4 ml of alkali plus tissue. A lower concentration of the stock suspension was made by an analogous procedure, and 0.1 ml containing an estimated 337 particles was added to similar samples. The samples were carried through the solubilization procedure and 0.5-ml aliquots were deposited on filters as described above. Four squares of the gridded Millipore filter were counted in the assay for the higher particle concentration. For the lower concentration, two filters were prepared and eight squares counted for each determination.

Clearing. Whole Peyer's patches, in addition to the six taken for solubilization from the mid-intestine, were dissected from alcohol-fixed intestinal segments and gently cleaned with a jet from a syringe. These, along with slices (approximately 0.5-mm thick) of lung, liver, and mesenteric lymph nodes were cleared by KOH-glycerol mixtures as described previously (2). Cleared tissue was placed in glycerol in a depression slide and examined with a fluorescence microscope at 250×.

Results

Determinations of latex particle concentration in the stock suspension were made by the filter method. Counts were made in triplicate on four separate $1:10^7$ dilutions and the results were averaged. Calculated mean value ± SD for the stock suspension was $6.74 \pm 0.95 \times 10^9$ particles/ml. This value was taken as the standard for the stock suspension. By Coulter count the particle concentration (particles/ml) in the stock suspension was $8.12 \pm 0.68 \times 10^9$ (SD, four determina-

tions), 20.5% higher than the value found by the filter method. The reason for the discrepancy is unknown.

Table I gives results of a preliminary test of recovery of fluorescent latex particles added to three types of alcohol-fixed tissue (liver, spleen, and Peyer's patches) from untreated mice. Tissue and particles were carried through the alkali solubilization procedure. Particle recovery ranged from 64.4 to 102.9%.

Young and aged mice were gavaged with a suspension of rhodamine-labeled latex particles or water as described in the Experimental Plan. Weight changes after 25 days are shown in Table II. Gavaged young mice did not gain as well as the controls, but the differences were not significant. Gavaged aged mice showed slight weight losses of borderline significance ($P < 0.05$). Weight changes of mice gavaged with water did not differ significantly from those gavaged with latex.

Table III summarizes the findings of fluorescent latex in solubilized tissues. Peyer's patches of aged mice contained significantly more particles than those of young animals. Mesenteric nodes showed the same trend, but the difference was not significant because of the large variation. Lungs exhibited the opposite pattern; those of aged mice contained significantly fewer particles than those of young animals. Although particles were abundant in Peyer's patches, they were relatively infrequent in segments of central intestine devoid of patches. A few fluorescent microspheres were present in liver residue of both young and aged animals, but not enough for accurate counts. Kidney and spleen were negative for particles. Except for an occasional fluorescent particle in lungs, tissues of control mice were latex free.

Table I. Assay for Particles Added to Control Tissue

	Particles added	Count[a]	% Recovery
Liver[b]	6740	5731.1 ± 981.1	85.0
Liver[b]	337	217.1 ± 45.2	64.4
Liver[b]	0	0	—
Spleen[c]	6740	6933.6 ± 1324.7	102.9
Spleen[c]	337	305.4 ± 79.6	90.6
Spleen[c]	0	0	—
Peyer's patches[d]	6740	6192.9 ± 446.8	91.9
Peyer's patches[d]	337	279.0 ± 58.0	82.8
Peyer's patches[d]	0	0	—

[a] Values are mean ± SD for four determinations.
[b] One third of liver.
[c] Whole spleen.
[d] Six Peyer's patches.

Table II. Weight Change of Mice during Experiment

	Gavage with latex	Gavage with water	Control (No gavage)
	g	g	g
Young[a] (initial weight)	17.53 ± 0.65[b]	17.11 ± 0.96	17.45 ± 0.86
Weight change (25 days)	5.33 ± 1.22	4.95 ± 0.98	6.26 ± 0.84
Aged[a] (initial weight)	38.88 ± 1.81	38.96 ± 2.04	37.12 ± 1.94
Weight change (25 days)	−0.60 ± 1.11*	−0.72 ± 1.56*	+1.26 ± 0.94

[a] Age of mice at beginning of experiment: young, 24 days; aged, 18 months. Mice were gavaged once a day, 5 days per week.
[b] Values are mean ± SD for five mice.
* $P < 0.05$, mean weight change vs mean of control mice.

Table III. Latex Particle Abundance in Tissue Samples

Tissue	Latex-gavaged		Water-gavaged	
	Young	Aged	Young	Aged
Peyer's patches[a]	27,359 ± 8,681[b]	121,635 ± 45,799**	0	0
Intestine piece[c]	88.5 ± 107.0	257.8 ± 361.7	0	0
Lungs[d]	2,862.9 ± 664.2	1039.0 ± 552.6**	Trace	Trace
MLN[e]	238.6 ± 252.4	1189.0 ± 992.0	0	0
Liver[f]	Trace	Trace	0	0
Spleen[g]	0	0	0	0
Kidney[h]	0	0	0	0

[a] Six Peyer's patches.
[b] Values are mean ± SD for five mice.
[c] 1-cm segment.
[d] Both lungs.
[e] Whole mesenteric lymph node.
[f] One third of liver.
[g] Whole spleen.
[h] One kidney.
** $P < 0.01$, young vs aged.

Whole Peyer's patches and slices of cleared liver, lung, and mesenteric node tissue from experimental mice were examined microscopically for the presence of fluorescent latex particles. In all Peyer's patches many fluorescent latex particles could be seen in the apex of the dome; Peyer's patches from aged mice contained markedly more particles than those from young mice. A few fluorescent particles could be seen in lung and mesenteric node tissue. Mesenteric nodes from aged mice appeared to contain more fluorescent particles than those from young animals, but no age difference was detectable in the amount of latex in lung tissue. In lungs the particles were widely scattered with none appearing in airways. No particles were visible in cleared liver slices. No fluorescent latex particles were found in cleared tissues from control mice.

Discussion

The filter assay for fluorescent latex particles (microspheres) described here is a simple and useful procedure for detecting rhodamine-labeled latex in solubilized tissue samples. It can be applied to any dilute suspension for the quantitation of fluorescent particles and can be used where automated counting of fluorescent particles is inapplicable. Because epi-illumination (involved in the procedure) utilizes illumination and detection of light from above, the opaqueness of the Millipore filter on which the particles are deposited is an asset rather than a liability.

Like nonfluorescent microspheres, rhodamine-labeled particles are inert, discrete, and resistant to the actions of digestive and lysosomal enzymes, but they have the additional advantage of being quickly and definitively recognized in tissues and sediments. They are more readily traced and more easily quantitated than the nonfluorescent microspheres used in previous intestinal uptake studies in this laboratory (1–4). In addition, the absorption and emission wavelengths of rhodamine B are in the visible light range; they traverse glass, glycerol, and cleared tissue. Thus, rhodamine-labeled particles can be detected in glycerol-cleared tissue slices despite the relative thickness of the slices and the presence of a glass coverslip.

Previous studies from this laboratory have demonstrated uptake of ingested particles, ranging from 27-nm carbon (5–8) to 5.6-μm latex (3), into murine Peyer's patches. Quantitation of amount of particulate matter ingested or recovered was by weight (2–4, 6), radioactive labeling of particles (5, 9), or optical counting (3, 4). In tests with latex, the particles were mixed with drinking water and the number ingested was estimated (1, 3). In the present experiments the particles were administered by gavage for more certainty in dose size. However, although certainty in dose size was accomplished, the gavaged mice did not thrive as well as ungavaged ones (Table II). Nevertheless, the differences were not extreme and valid information was obtained. The experimental plan utilized chronic administration rather than short-term or single-dose administration because particle uptake from the mouse intestine, when it occurs (8, 10), appears to be cumulative, and is most easily demonstrated by long-term experiments.

The results (Table III) confirm previous findings of uptake of synthetic latex particles from the intestinal lumen into Peyer's patches and migration of a few particles to mesenteric lymph nodes (2, 3). Since only a trace amount of latex appeared in liver, it is unlikely that large numbers of particles were transported from intestine to portal blood. Furthermore, the absence of particles in kidney and spleen indicates that particles probably did not reach peripheral blood. The source of particles in lungs is unknown, but may have resulted from inhalation of fecal debris. Direct introduction of stock latex suspension into the airways during gavage is unlikely because counts that were orders of magnitude greater than those actually observed would have been produced.

Although they were nonquantitative, observations on cleared tissues agreed in general with results found by the filter assay method. Tissues with high particle content on filter assay also had high particle content on examination of cleared fragments.

The interesting finding that Peyer's patches of young mice accumulated fewer particles than those of aged animals (Table III) is unexpected in view of the generally rapid absorption of nutrients and small molecules by the intestines of young animals. The finding may be accounted for by slower transit of digesta through the small intestine of aged animals. Particle uptake involves intimate contact of particles with lymphoid tissue surfaces, and is a slow process; thus, longer exposure would be expected to lead to greater uptake. In contrast, nutrient uptake involves much larger regions of the small intestine and, with a few exceptions, is rapidly completed; thus, transit time is less important. We previously reported slower transit of labeled carbon particles through the whole gut of aged compared with young mice (5), but determinations were not made for the small intestine alone. Another explanation for the presence of fewer particles in the Peyer's patches of young mice is that clearance of particles from Peyer's patches (2) is accelerated in these mice.

The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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