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Distribution of Label after Intragastric Administration of ^{7}Be-Labeled Carbon to Weanling and Aged Mice[1] (42318)

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Abstract. Single doses of ^{7}Be-labeled carbon particles prepared by proton bombardment of carbon black were administered by gavage to weanling (4 weeks) and aged (18 months) mice. Body distribution of ^{7}Be radioactivity was determined 4 hr and 1, 2, 5, and 14 days later. The results were compared with those obtained after administration of a solution of $^{7}BeCl_2$. ^{7}Be radioactivity in nonintestinal tissues was 17–58 times greater in mice gavaged with the soluble salt than in those gavaged with the particles, indicating that particle-bound ^{7}Be was well confined to the gastrointestinal tract. Upper limits of the amount of labeled carbon remaining in intestinal Peyer's patches were as follows: 1 day: weanling, 1.8×10^{-3}%, old, 5.5×10^{-3}%; 15 days: weanling, 3.3×10^{-5}%, old, 8.4×10^{-5}%. The particles are potentially useful as models for diesel emissions or other particulate pollutants.

Studies on the biological properties and fate of ingested particulate pollutants would be markedly enhanced by the availability of stably labeled particles, particularly small, submicron varieties. We report the results of a study utilizing ^{7}Be-labeled particulate carbon, a product recently developed at Brookhaven National Laboratory. The study assessed the stability of the label after intragastric administration of the particles to mice and compared the results with those obtained after intragastric administration of a solution of $^{7}BeCl_2$. Estimates were made of the upper limit of the amount of particulate carbon taken up and retained in murine intestinal Peyer's patches of weanling and aged mice after a single dose of a suspension of the labeled particles.

Materials and Methods. *^{7}Be-labeled carbon.* Details of the preparation of ^{7}Be-labeled carbon from nonradioactive carbon black have been described in a separate publication (1). In brief, γ-emitting ^{7}Be (half-life, 53 days; energy, 0.478 MeV) was produced from the ^{12}C nucleus by a spallation reaction during high-energy-proton bombardment of dry carbon black (Elftex 8, Cabot Corp.; diameter, 27 nm). Figure 1 shows a γ spectrum of the isotopically labeled carbon and illustrates the relative absence of contaminating radionuclides.

Two weeks after the termination of bombardment the particles were washed by dilution and centrifugation in a 1:1 ethanol water mixture and subsequently in 1% Tween 80. Physical tests (dialysis and centrifugation) indicated that the label remaining after washing was firmly bound (1). Sonication of a suspension of the labeled particles before centrifugation did not result in release of radioactivity into the supernatant. Immediately before use in animal studies, the carbon suspension was washed in distilled water (15× vol) and centrifuged at 12,000*g* for 1 hr. The supernatant was aspirated, and the particles were resuspended in distilled water by stirring and sonication. Activity of the washed ^{7}Be-labeled carbon suspension was 2.1×10^{7} cpm/ml as calculated from counts of 0.5-ml aliquots of a 1:50 dilution. Counting was carried out in a gamma scintillation counter (Nuclear Chicago, 1185 series). The suspension contained 28 mg carbon/ml. Particle size in the final suspension was too small to be measured by optical microscopy. Drops of suspension were placed on Nuclepore filters, dried, and examined by scanning electron microscopy (SEM). The particles were roughly spherical in shape and ranged from 0.01 to 0.1 μm in diameter, which was considerably larger than the size of the starting material. Aggregation may have taken place during preparative drying for SEM, and the observed size range can be considered to be an upper limit.

$^{7}BeCl_2$. Carrier-free $^{7}BeCl_2$ (activity con-

[1] 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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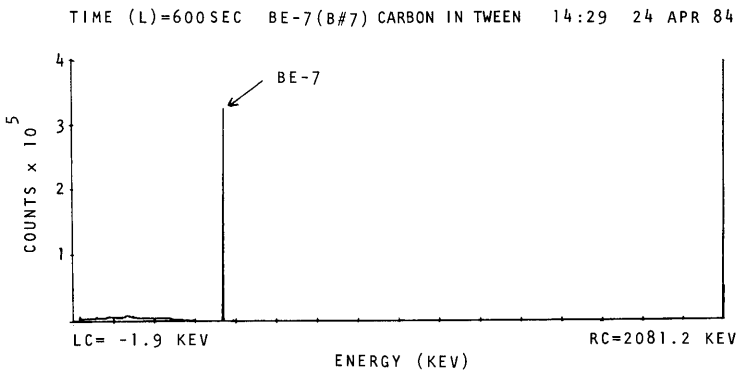

FIG. 1. γ-Ray spectrum of an aliquot of ^{7}Be-labeled carbon taken from a carbon black target bombarded with 192 MeV protons for 28 days. The spectrum was obtained 11 days after the end of bombardment using an intrinsic germanium γ-ray detector (Ortec, Inc.). Range is from −1.9 KeV at left to 2081.2 KeV at right, and is divided into 16 segments of 130.2 KeV each. ^{7}Be peak is at 478 KeV. (Courtesy of L. F. Mausner)

centration 0.36 mCi/ml) in 0.1 *M* HCl was obtained from Amersham/Searle Corp. Immediately before use, 4.5 ml of the stock solution was diluted with 18 ml of 0.01 *M* NaOH containing 0.04% Tween 80. The final solution was slightly acidic and contained Tween 80 at approximately the same concentration as the carbon suspension used for gavage. Activity of the final solution was 7.0×10^{6} cpm/ml, determined as described for the particle suspension.

Mice. Female Swiss albino mice (Charles River CD-1 strain) were used for all experiments. Weanlings were 4 weeks old. Aged mice were retired breeders, approximately 18 months old at the time of the experiments. Some mice were kept in individual metabolic cages (changed daily) which permitted the collection of urine and feces separately. The other mice were kept in groups of four in ordinary cages. To minimize the effects of coprophagy and the contamination of fur with fecal isotope, group cages were changed every day during the first week and every second day during the second week of the experiments.

The isotope was administered by gavage to lightly etherized, nonfasting mice using a syringe fitted with a ball-tipped needle of the appropriate size (Popper and Sons, Inc.). The outside of the needle was wiped thoroughly to prevent contamination of the larynx during gavage.

In experiments determining total body distribution of isotope, groups of eight mice were killed by ether overdose at 4 hr and 1, 2, 5, and 14 days after intragastric administration of 0.25 ml of ^{7}Be-labeled carbon suspension or $^{7}BeCl_2$ solution. The thorax and abdomen were opened widely, and gauze was placed around the incision to prevent radioactive particles on skin and hair from contaminating internal organs. Additional measures used to

TABLE I. WHOLE BODY AND ORGAN WEIGHTS OF WEANLING AND AGED MICE

	Weanling (g)	Aged (g)	*P*
Whole body	19.62 ± 1.97	42.92 ± 5.01	<0.001
Whole GI tract[a]	1.462 ± 0.172	2.016 ± 0.191	<0.001
Liver	1.305 ± 0.117	1.806 ± 0.150	<0.001
Lungs	0.155 ± 0.011	0.199 ± 0.015	<0.001
Small intestine	0.846 ± 0.116	1.042 ± 0.156	<0.01
Peyer's patches[b]	0.0473 ± 0.0134	0.0442 ± 0.0145	NS

Note. Values are means ± SD. Number of animals: 10 weanling, 10 old.
[a] Tissue cleaned of contents.
[b] Mean patch number ± SD per intestine: weanling, 8.3 ± 1.9; old, 8.4 ± 1.7.

TABLE II. DISTRIBUTION OF RADIOACTIVITY AFTER ADMINISTRATION OF A SINGLE DOSE[a] OF ^{7}BE-LABELED CARBON PARTICLES TO WEANLING AND AGED MICE

	cpm									
	4 hr		1 day		2 days		5 days		14 days	
	Weanling	Aged	Weanling	Aged	Weanling	Aged	Weanling	Aged	Weanling	Aged
Whole gut[b]	4,124,000 ± 470,000	4,295,000 ± 430,000	12,830 ± 7,280	64,940 ± 54,050*	414.0 ± 111.5	1,045.3 ± 375.2***	32.83 ± 22.72	54.89 ± 16.58*	14.55 ± 10.82	35.28 ± 22.52*
Liver	19.32 ± 15.72	21.89 ± 15.12	9.68 ± 4.18	12.97 ± 7.75	8.35 ± 4.86	13.40 ± 9.92	5.44 ± 2.21	8.28 ± 3.36	3.65 ± 3.64	6.21 ± 5.23
Lungs	3.45 ± 3.01	1.67 ± 1.68	1.09 ± 1.36	1.76 ± 1.20	0.63 ± 0.60	1.47 ± 2.46	ND	ND	ND	ND
Kidneys	11.25 ± 7.12	10.75 ± 4.69	4.13 ± 1.51	6.27 ± 2.16*	2.92 ± 1.67	6.22 ± 3.74*	0.21 ± 0.16	0.51 ± 0.44	ND	ND
MLN[c]	8.05 ± 7.22	13.39 ± 12.59	0.74 ± 1.11	1.17 ± 1.06	ND	0.68 ± 1.18	ND	0.62 ± 0.84	ND	ND
Blood[d]	0.25 ± 0.22	0.25 ± 0.19	0.26 ± 0.13	0.20 ± 0.13	0.17 ± 0.24	0.16 ± 0.06	ND	ND	ND	ND
Carcass[e]	668.9 ± 551.3	247.8 ± 118.4	171.4 ± 68.4	432.0 ± 373.6	114.2 ± 53.1	156.4 ± 82.1	75.41 ± 17.67	52.23 ± 11.58**	25.23 ± 7.70	20.60 ± 8.36
Feces[f]	—	—	4,701,000 ± 1,443,000	4,566,000 ± 2,036,000	14,414 ± 11,650	36,120 ± 28,950	257.2 ± 272.6	650.0 ± 742.3	—	—
Urine[f]	—	—	154.1 ± 109.4	364.6 ± 220.1*	19.25 ± 17.02	22.79 ± 17.61	14.99 ± 20.70	24.56 ± 31.88	—	—

Note. Values are means ± SD. ND = not detectable (mean count <0.1 cpm). $N = 8$ for each group.

[a] 0.25 ml of particle suspension containing 5.25×10^{6} cpm.

[b] Including contents.

[c] MLN = mesentery with lymph nodes.

[d] 0.5 ml from right ventricle.

[e] Eviscerated.

[f] Samples not taken at 4 hr and 14 days.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; weanling vs aged.

TABLE III. RADIOACTIVITY IN SKIN AFTER ADMINISTRATION OF A SINGLE DOSE[a] OF ^{7}BE-LABELED CARBON PARTICLES TO WEANLING AND AGED MICE

	cpm			
	1 day		14 days	
	Weanling	Aged	Weanling	Aged
Skin	67.3 ± 25.8	127.7 ± 34.4*	6.39 ± 3.12	2.25 ± 0.72*
Skinned carcass[b]	122.8 ± 16.4	182.2 ± 61.7	15.26 ± 5.34	9.20 ± 2.79

Note. Values are means ± SD. $N = 4$ for each group.
[a] Dose = 0.25 ml of particle suspension containing 4.36×10^{6} cpm.
[b] Eviscerated.
* $P < 0.05$, weanling vs old.

prevent cross-contamination included the use of all-new plastic and glassware and the acid washing of instruments. A 0.5-ml blood sample was taken from the right ventricle of the heart. With the alimentary tract kept intact, liver, lungs, and kidneys were removed. The gastrointestinal (GI) tract from stomach to anus (including contents) and mesentery (including mesenteric lymph nodes) were then removed. All samples were placed in vials and preserved in 10% Formalin containing 0.5 *M* sodium acetate. Feces and urine from mice in metabolic cages were transferred to vials and kept at 4°C.

Radioactivity of the samples was determined in a well-type gamma scintillation counter (Nuclear Chicago, 1185 series). Base and window settings were 0.37 and 0.18 MeV, respectively, for a full-scale range of 1 MeV. When activity was low, counting periods of 50 to 100 min were used. The data were corrected for background and isotopic decay.

After determination of radioactivity in the whole GI tract was completed, the small intestine was cleaned by dividing it into 4-cm segments and gently expressing the contents. Each segment was slit, the inside was wiped with a cotton-tipped swab, and inside and outside were rinsed with a jet from a syringe. All visible Peyer's patches were removed and trimmed of ordinary mucosa under a dissecting microscope.

To determine the contribution of the skin to carcass radioactivity, groups of four weanling and old mice were maintained 1 to 14 days after gavage with 0.25 ml ^{7}Be-labeled carbon suspension. Mice maintained 1 day were kept in individual metabolic cages; mice maintained 14 days were kept in ordinary cages which were changed every day for the first week after gavage and every other day thereafter. The mice were killed by ether overdose and skin was removed except for head and tail. Blood was taken as described above and the carcass was completely eviscerated. The skin and carcass were counted separately.

Statistics. Comparisons were made between weanling and aged mice with regard to tissue weights and radioactivity. The significance of the difference between means was calculated by Student's *t* test for unpaired values.

Results. Table I summarizes whole body and fresh organ weights for 10 mice randomly selected from the two experimental age groups. With the exception of Peyer's patches, organ weights were significantly greater in aged mice as compared to weanlings.

Table II summarizes the distribution of ^{7}Be radioactivity (expressed as cpm) in weanling and aged mice after a single dose of ^{7}Be-labeled carbon particles containing 5.25×10^{6} cpm of ^{7}Be radioactivity. At 4 hr most of the radioactivity was associated with the GI tract in both weanling and aged mice; counts in extraintestinal viscera and blood were extremely low. In GI tracts the measured activity was less than the calculated administered dose due to the coincidence error associated with high count rates. Counts in the carcass were appreciable, but still only a very small fraction of the administered dose. The low activity in lungs indicates that the suspension was successfully introduced into the stomach without accidental contamination of the airways.

Counts in whole guts from weanlings were lower than those from aged mice, particularly

TABLE IV. DISTRIBUTION OF RADIOACTIVITY AFTER ADMINISTRATION OF A SINGLE DOSE[a] OF $^{7}BeCl_2$ TO WEANLING AND AGED MICE

	cpm									
	4 hr		1 day		2 days		5 days		14 days	
	Weanling	Aged	Weanling	Aged	Weanling	Aged	Weanling	Aged	Weanling	Aged
Whole gut[b]	1,438,000 ± 351,900	1,582,000 ± 347,400	3319 ± 2293	10,974 ± 4,484***	387.9 ± 104.6	817.1 ± 248.0***	253.8 ± 53.3	523.2 ± 148.0***	112.4 ± 37.1	321.3 ± 133.7***
Liver	834.6 ± 404.2	1,364 ± 564*	213.3 ± 57.9	261.3 ± 122.2	170.2 ± 21.2	362.5 ± 99.8***	124.4 ± 31.8	134.5 ± 25.7	22.74 ± 4.32	38.34 ± 23.72
Lungs	30.22 ± 9.09	11.80 ± 4.59***	12.51 ± 2.97	10.63 ± 4.56	10.41 ± 2.39	10.19 ± 4.22	7.22 ± 2.71	8.43 ± 3.70	1.09 ± 0.91	6.36 ± 3.49**
Kidneys	131.5 ± 115.0	122.5 ± 45.5	44.46 ± 6.10	91.13 ± 28.05***	20.07 ± 2.95	47.00 ± 19.01**	4.25 ± 0.86	6.94 ± 1.01***	0.53 ± 0.38	0.98 ± 0.67
MLN[c]	33.42 ± 15.42	62.79 ± 38.39	24.80 ± 10.46	39.56 ± 12.62*	10.59 ± 6.41	28.87 ± 14.32**	3.85 ± 1.45	19.72 ± 10.85**	ND	3.33 ± 2.29
Blood[d]	16.67 ± 15.81	11.71 ± 11.02	4.25 ± 2.47	2.85 ± 2.62	3.35 ± 1.75	2.04 ± 1.13	1.12 ± 1.72	0.73 ± 0.98	ND	ND
Carcass[e]	4,830 ± 3,272	3,870 ± 2,604	1728 ± 1227	2,760 ± 1,029	965.3 ± 200.9	1614 ± 825*	762.3 ± 165.0	787.3 ± 480.8	235.1 ± 42.1	104.6 ± 60.2***

Note. Values are means ± SD. ND = not detectable (mean count < 0.1 cpm). $N = 8$ for each group.

[a] Dose = 0.25 ml of solution containing 1.75×10^6 cpm.

[b] Including contents.

[c] MLN = mesentery with lymph nodes.

[d] 0.5 ml from right ventricle.

[e] Eviscerated.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; weanling vs old.

at 1 and 2 days. Practically all of the label was excreted in the feces although the relative amount in urine increased with time. Since complete separation of urine and feces was not possible, some urine activity may reflect fecal contamination. At 14 days a small amount of radioactivity persisted in carcass, liver, and GI tract of both weanling and aged mice, but was almost completely absent from the other samples.

Table III summarizes findings on radioactivity in skin of mice after gavage with 0.25 ml of carbon suspension having 4.36×10^6 cpm. The results indicate that a large proportion of carcass radioactivity (20 to 41%) was present in the skin.

^{7}Be radioactivity in tissues represents either intact labeled particles or $^{7}Be^{2+}$ leached from the particles. To obtain information on absorption of non-particle-bound ^{7}Be, a soluble beryllium salt ($^{7}BeCl_2$) was administered by gavage. The dose was 0.25 ml of $^{7}BeCl_2$ solution having 1.75×10^6 cpm. The distribution of radioactivity at different time periods for weanling and old mice is given in Table IV. At all time periods, radioactivity in visceral organs, blood, and carcasses of mice gavaged with $^{7}BeCl_2$ was considerably higher than that in comparable organs of mice gavaged with ^{7}Be-labeled carbon particles. As in the previous experiments (Table II), disappearance of radioactivity from the GI tract was slower in aged mice than in weanling mice. Table V compares radioactivity in pooled extraintestinal tissues of mice gavaged with $^{7}BeCl_2$ with that in comparable pooled tissues of mice gavaged with ^{7}Be-labeled carbon.

A purpose of the experiments was to obtain quantitative information on the amount of particulate carbon taken into intestinal Peyer's patches after an intragastric dose. To accomplish this, the small intestines of mice from the 1, 2, 5, and 14-day experiments summarized in Table II were carefully cleaned of contents and the Peyer's patches removed. Detectable ^{7}Be radioactivity was found in Peyer's patches of all groups tested (Table VI). Furthermore, a large proportion (21–52%) of the activity of small intestinal tissue was accounted for by that of Peyer's patches, although the latter made up only a small fraction of the total weight of the small intestine (Table I). After 1 day 0.0018% of the administered dose of ^{7}Be-labeled carbon was retained in Peyer's patches of weanling mice and 0.0055% of the dose in Peyer's patches of aged mice. This is the upper limit of the amount of particulate carbon that might have been absorbed. Radioactivity in Peyer's patches diminished with time, but not as precipitously as in the remainder of the small intestine (Table VI). Peyer's patches contained a significantly greater proportion of the total radioactivity of the small intestinal wall at 5 and 14 days than at 1 and 2 days.

Discussion. ^{7}Be-labeled carbon particles prepared by high-energy-proton bombardment of carbon black are potentially useful as a model for diesel emissions or other particulate pollutants. Although diesel exhaust particles (2, 3) are more variable than the experimental particles utilized in the experiments described here (1), both are carbonaceous in nature and submicron in size. ^{7}Be (formed by the action of cosmic rays on atmospheric components) is present in the environment only in minuscule amounts (4); thus the danger of contamination by ^{7}Be from natural

TABLE V. PERCENTAGE $\times 10^{-4}$ OF ADMINISTERED RADIOACTIVITY IN NONINTESTINAL TISSUES[a]

Isotope	Mice	% of dose[b] ($\times 10^{-4}$)				
		4 hr	1 day	2 days	5 days	14 days
^{7}Be-carbon	Weanling	136	35.9	24.2	16.2	5.49
^{7}Be-carbon	Aged	56.5	86.7	34.1	11.7	5.11
$^{7}BeCl_2$	Weanling	3400	1170	682	518	148
$^{7}BeCl_2$	Aged	3230	1810	1180	549	87.7

Note. $N = 8$ mice for each group.

[a] Liver, lungs, kidneys, mesentery, blood, and eviscerated carcass.

[b] Dose ^{7}Be-labeled carbon = 0.25 ml of particle suspension containing 5.25×10^6 cpm.

[c] Dose $^{7}BeCl_2$ = 0.25 ml of solution containing 1.75×10^6 cpm.

TABLE VI. RADIOACTIVITY IN PEYER'S PATCHES AND SMALL INTESTINE AFTER ADMINISTRATION OF A SINGLE DOSE[a] OF ^{7}BE-LABELED CARBON PARTICLES TO WEANLING AND AGED MICE

	cpm							
	1 day		2 days		5 days		14 days	
	Weanling	Aged	Weanling	Aged	Weanling	Aged	Weanling	Aged
Peyer's patches	93.5 ± 56.1	287.2 ± 173.1**	6.10 ± 2.34	12.58 ± 5.93*	2.40 ± 0.99	3.29 ± 1.77	1.71 ± 0.97	4.43 ± 3.13*
Remainder of small intestine	220.7 ± 95.2	1180.8 ± 662.7*	18.79 ± 5.97	45.92 ± 17.31***	2.73 ± 0.92	5.85 ± 2.67*	1.56 ± 0.81	4.67 ± 3.61*
% of small intestinal radioactivity in Peyer's patches	29.8	21.2	24.5	21.5	46.8	36.0	52.3	48.7
% of administered dose in Peyer's patches ($\times 10^{-5}$)	180	550	12	24	4.6	6.3	3.3	8.4

Note. Values are means ± SD. $N = 8$ for each group.
[a] Dose = 0.25 ml of particle suspension containing 5.25×10^6 cpm.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; weanling vs aged.

sources is minimal in experiments involving ^{7}Be-labeled carbon. The stable Be isotope is also uncommon in the ordinary laboratory environment (5).

Assessment of the usefulness of ^{7}Be-labeled particles for whole-animal experimentation requires knowledge of their stability *in vivo.* The results reported here show that little ^{7}Be was found in nonintestinal tissues after gavaging mice with ^{7}Be-labeled carbon particles, indicating that the label was basically confined to the GI tract. At 1 day less than 0.01% of the administered radioactivity was present in nonintestinal tissues; the amount present in similar tissues of mice gavaged with $^7BeCl_2$ was 20 to 60 times greater (Tables IV and V). Thus ^{7}Be-labeled carbon particles appear to be appropriate for experiments which involve exposure of particles to the GI milieu. Preliminary reports (6) suggest that the particles are also suitable for pulmonary studies.

Although absorption of ^{7}Be was greater after an intragastric dose of $^7BeCl_2$ than after a comparable dose of ^{7}Be-labeled carbon, total uptake was nonetheless small. This is in accordance with previous reports of poor absorption of ^{7}Be from the gut, due in part to the rapid formation of insoluble beryllium phosphates and protein complexes (5).

Our experiments showed slow but definite clearance of radioactivity from tissues after the initial rapid passage of unabsorbed ^{7}Be-labeled carbon or $^7BeCl_2$ through the GI tract (Tables II and IV). Considerable radioactivity remained associated with the GI tract, and carcass counts represented an increasingly larger fraction of non-GI activity during the 14-day follow-up period. Much carcass activity probably resulted from deposition of ^{7}Be in bone (5), particularly in weanling mice where bone formation is taking place rapidly. However, some external contamination of skin by fecal ^{7}Be was suspected in view of the surprisingly high radioactivity found in the skin of mice given ^{7}Be-labeled carbon (Table III). Radioactivity disappeared more rapidly from the guts of weanling than from the guts of aged mice suggesting more rapid transit of particles through the GI tracts of weanlings. Another possibility which cannot be assessed is greater coprophagy in aged mice.

We previously reported the accumulation

of small carbon particles in Peyer's patches (a gut-associated lymphoid tissue) of mice that ingested carbon suspensions for several weeks (7). The accumulation was demonstrated histologically, carbon being seen as black deposits in Peyer's patch macrophages. No estimation of the magnitude of uptake was made. The use of ^{7}Be-labeled carbon permits an estimate of the upper limit for absorption of ingested carbon particles into Peyer's patches. The results suggest that Peyer's patches of older mice take up more carbon than those of young mice. At 1 day 0.0018% of the administered dose was retained in Peyer's patches of weanling mice, whereas 0.0058% of the administered dose was retained in Peyer's patches of aged mice. At 14 days the values were 3.3×10^{-5}% and 8.4×10^{-5}% for weanling and aged mice, respectively. Longer exposure of the Peyer's patch surface to particles in the older animals may be the reason for the difference. Peyer's patch weights of aged mice did not differ significantly from those of young mice, although the whole intestines of the former weighed significantly more (Table I). This is in agreement with the observations of Chin (8) and Chin and Hudson (9) that the number, shape, and size of Peyer's patches were similar in young (2 and 5 weeks) and aged (15 months) mice, although differences in ultrastructure were found. An interesting finding was that with time radioactivity in Peyer's patches constituted a greater proportion of total intestinal activity (Table VI). This suggests continued retention of residual ^{7}Be-labeled carbon particles in Peyer's patches. Luminal label was probably lost gradually, whereas ^{7}Be-labeled carbon within Peyer's patches was phagocytosed and stored in macrophages (10). ^{7}Be radioactivity in Peyer's patches at 5 and 14 days probably represents a truer estimate of the amount of particulate carbon which actually penetrated the follicle-associated epithelium than the corresponding radioactivity at 1 and 2 days.

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