Acrylic Microspheres *In Vivo* VIII: Distribution and Elimination of Polyacryldextran Particles in Mice

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Abstract □ The disposition of different ^{14}C-labeled, biodegradable polyacryldextran microparticles after intravenous injection has been studied in the mouse. The particles were rapidly cleared from the circulatory system by the reticuloendothelial system. They were predominantly (60–80%) found in the liver and spleen and to some extent in the bone marrow. Large particle aggregates were found in the lungs 6 hr postinjection. After redistribution, the particles were eliminated from the organs with an apparent $t_{1/2}$ of 12–30 weeks, depending on the composition of the particles. Highly cross-linked particles with 2% acrylic groups (DTC = 11-2-75) had a half-life similar to that of polyacrylamide particles (TC = 8-25). The metabolism rate was also correlated with the degradation *in vitro* with isolated rat lysosomes. After intravenous injection of small ^{14}C-labeled polyacrylamide particles (0.2–0.5 μm), significant amounts of radioactivity were detected in the bile and gallbladder.

Keyphrases □ Polyacryldextran—microparticles, *in vitro* degradation, *in vivo* metabolism and elimination in mice □ Microparticles—of polyacryldextran, *in vitro* degradation, *in vivo* metabolism and elimination in mice □ Metabolism—of polyacryldextran microparticles in mice following intravenous injection □ Elimination—of polyacryldextran microparticles in mice by the reticuloendothelial system following intravenous injection

Previous studies have revealed that proteins immobilized in microparticles of polyacrylamide can retain biological properties (1). The particles are small enough (0.2–1 μm in diameter) to be injected intravenously without respiratory problems (2). When such particles were injected parenterally, they were cleared rapidly from the circulatory system by cells of the reticuloendothelial system (RES), predominantly *via* the liver and spleen, where the particles were localized in the lysosomal vacuome (3). The spheres were eliminated from these organs with a half-life of ~20–25 weeks (2).

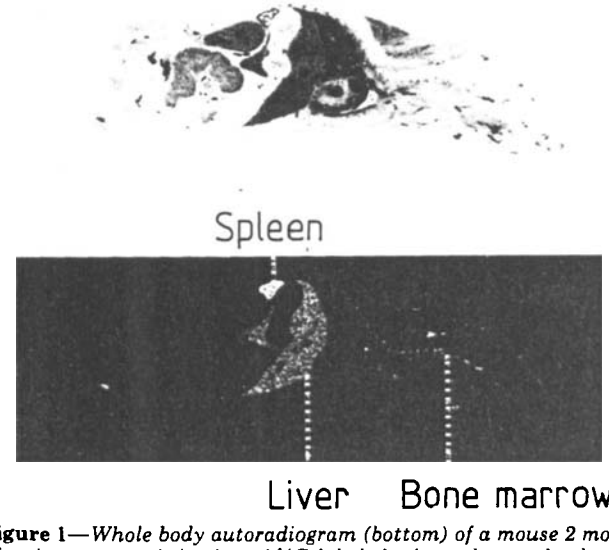

Figure 1—*Whole body autoradiogram (bottom) of a mouse 2 months after intravenous injection of ^{14}C-labeled microspheres of polyacryldextran, DTC = 11-1-75 (48,200 dpm in 2.6 mg). The top is the corresponding stained section, 20 μm.*

Derivatized natural polymers (*e.g.*, acryldextrans) recently have been introduced as a matrix for the preparation of microparticles, particularly for use *in vivo* (4), as substantially fewer synthetic polymer threads are then needed to form the microspheres. The total concentration of acrylic groups is only 0.5–2% in the water phase prior to the polymerization and formation of polyacryldextran. Moreover, the amount of protein immobilized in the microparticles is increased without any significant loss of the biological properties of the immobilized protein, with improved stability against heat denaturation (4). Such particles carrying immobilized dextranase have been used successfully to treat an artificially induced storage disease in mice (5). The present paper will describe quantitatively the distribution of polyacryldextran microparticles in mice and the influence of the polyacryl chains on the degradation of the microparticles *in vivo* and by dextranase *in vitro*.

EXPERIMENTAL

Materials—Acrylamide[1], *N,N'*-methylenebisacrylamide (BIS)[1], *N,N,N',N'*-tetramethylethylenediamine[2], dextran[3], 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside[2], 4-methylumbelliferone[2], and other chemicals were of analytical grade. Acrylic acid-glycidyl[4], dextranase[2] (E.C. 3.2.1.11), and [^{14}C]paraformaldehyde[5] were used without further purification. ^{14}C-Labeled *N,N'*-methylenebisacrylamide was prepared as described by Sjöholm and Edman (2).

Synthesis of Acrylic Dextran—Acrylic dextran was prepared using a reported method (4). Dextran (T-40, mol. wt. 40,000) was dissolved in 250 ml of 0.01 *M* phosphate buffer, pH 9.0. Acrylic acid-glycidyl (25 ml) was added, and the emulsion was stirred with a magnetic stirrer for 9–10 days at room temperature. The dextran was precipitated and washed with ethanol. The precipitated dextran was dissolved in phosphate buffer, precipitated again, and washed with ethanol. This procedure was repeated at least five times. The content of acrylic groups in the dextran was determined by halogenation of the double bonds, as described previously (4).

Preparation of Microparticles—^{14}C-Labeled microparticles (DTC = 11-1-75, DTC = 11-2-75, and TC = 8-25)[6] were prepared by the methods of Ekman *et al.* (1) and Edman *et al.* (4). In a typical example, the monomers (acrylamide, ^{14}C-labeled *N,N'*-methylenebisacrylamide, and acryldextran) were dissolved in 5 ml of 0.1 *M* phosphate buffer, pH 7.4. Oxygen was removed from the solution using nitrogen gas. At the same time, 200 ml of a mixture of toluene and chloroform (4:1), containing a detergent[7] (0.5 g), was flushed with nitrogen. After addition of the catalyst, ammonium peroxodisulfate (100 μl of a 0.5-g/ml solution), the

[1] Eastman Kodak Co.
[2] Sigma Chemical Co.
[3] Dextrans (T40 and T500) were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden.
[4] Fluka AG.
[5] Radiochemical Centre, Amersham, England.
[6] The particles prepared are characterized using the nomenclature suggested by Hjertén (6) and Edman *et al.* (4). The first numeral denotes the concentration of derivatized dextran (D) participating in the particle formation (g/100 ml of solvent). The second numeral denotes the total amount (T) of acrylic monomers (g/100 ml of solvent) and the third, the amount of cross-linking agent (C), *N,N'*-methylenebisacrylamide, expressed as the percentage (w/w) of the total amount of monomers.
[7] Pluronics F-68 (polyoxyethylene-derived polyoxypropylene) was obtained from Trebac AB, Stockholm, Sweden.

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Table I—Distribution of ^{14}C-Labeled Polyacryldextran Particles after Intravenous Administration to Mice [a]

Organ	Time Postdose	Organ Weight, g	Dpm	Percentage of Injected Dose
		DTC = 11-1-75		
Liver	6 hr	1.10 ± 0.05	18,750 ± 650	38.88 ± 1.4
Spleen	6 hr	0.07 ± 0.01	2550 ± 350	5.3 ± 1.7
Lung	6 hr	0.16 ± 0.01	10,460 ± 1340	21.69 ± 2.78
Kidneys	6 hr	0.16 ± 0.01	490 ± 70	1.02 ± 0.15
Liver	1 week	1.45 ± 0.08	25,970 ± 1440	53.85 ± 2.99
Spleen	1 week	0.16 ± 0.01	5940 ± 380	12.32 ± 0.79
Lung	1 week	0.17 ± 0.01	890 ± 220	1.85 ± 0.46
Kidneys	1 week	0.18 ± 0.02	440 ± 30	0.91 ± 0.06
Liver	2 weeks	1.50 ± 0.07	27,340 ± 1110	56.69 ± 2.30
Spleen	2 weeks	0.16 ± 0.01	5500 ± 180	11.40 ± 0.37
Liver	4 weeks	1.27 ± 0.04	21,800 ± 950	45.20 ± 1.97
Spleen	4 weeks	0.12 ± 0.01	4500 ± 200	9.33 ± 0.41
Liver	8 weeks	1.31 ± 0.07	18,480 ± 510	38.32 ± 1.06
Spleen	8 weeks	0.13 ± 0.01	4160 ± 200	8.63 ± 0.42
Liver	12 weeks	1.36 ± 0.06	13,500 ± 300	27.99 ± 0.64
Spleen	12 weeks	0.12 ± 0.01	2200 ± 160	4.55 ± 0.33
Liver	20 weeks	1.47 ± 0.08	11,350 ± 720	23.55 ± 1.50
Spleen	20 weeks	0.13 ± 0.01	1990 ± 90	4.13 ± 0.19
		DTC = 11-2-75		
Liver	1 day	1.33 ± 0.07	39,640 ± 1310	70.04 ± 2.31
Spleen	1 day	0.08 ± 0.01	3880 ± 1000	6.86 ± 0.18
Lung	1 day	0.14 ± 0.01	2120 ± 100	3.75 ± 0.18
Kidneys	1 day	0.13 ± 0.01	210 ± 10	0.37 ± 0.02
Liver	1 week	1.51 ± 0.03	46,240 ± 1620	81.70 ± 2.86
Spleen	1 week	0.15 ± 0.01	4040 ± 300	7.14 ± 0.53
Lung	1 week	0.16 ± 0.01	500 ± 10	0.88 ± 0.02
Kidneys	1 week	0.15 ± 0.01	190 ± 10	0.34 ± 0.02
Liver	2 weeks	1.46 ± 0.05	41,050 ± 410	72.53 ± 0.72
Spleen	2 weeks	0.12 ± 0.01	4260 ± 300	7.53 ± 0.53
Liver	4 weeks	1.33 ± 0.04	41,860 ± 610	73.96 ± 1.08
Spleen	4 weeks	0.13 ± 0.01	4300 ± 190	7.60 ± 0.34
Liver	8 weeks	1.23 ± 0.02	32,320 ± 1130	57.10 ± 2.00
Spleen	8 weeks	0.13 ± 0.01	3050 ± 130	5.39 ± 0.23
Liver	13 weeks	1.35 ± 0.03	35,900 ± 1490	63.43 ± 2.63
Spleen	13 weeks	0.12 ± 0.01	3330 ± 250	5.88 ± 0.44
Liver	27 weeks	1.42 ± 0.03	27,380 ± 350	48.38 ± 0.62
Spleen	27 weeks	0.12 ± 0.01	2570 ± 270	4.54 ± 0.65

[a] Distribution of ^{14}C-labeled polyacryldextran particles in the liver, spleen, lung, and kidneys after injection of 2.6 mg iv of polyacryldextran particles with compositions of DTC = 11-1-75 and DTC = 11-2-75 containing 48,300 and 56,600 dpm, respectively. Each value is the mean obtained from 4–5 animals ± *SE*.

water phase was homogenized in toluene–chloroform to produce a water-in-oil emulsion. The polymerization was started by adding 1 ml of the accelerator, *N,N,N′,N′*-tetramethylethylenediamine, to the emulsion. The suspension was stirred for 20 min, and the phases were separated by centrifugation. The organic phase was removed, and the microparticles located at the bottom of the water phase were washed several times with buffer and physiological saline. Under the conditions used, the polyacrylamide particles (TC = 8-25) had a mean diameter of 0.2–0.5 μm; the polyacryldextran particles (DTC = 11-1-75 and DTC = 11-2-75) had a mean diameter of 0.5–1.5 μm.

Determination of Particle Size—The size of the particles was determined from photographs taken by scanning electron microscopy, using a previously described method (2). With this method, 85.0% of the polyacryldextran particles with DTC values of 11-2-75 used in this study had a diameter of 0.2–1.2 μm, 1.7% were <0.2 μm, and 4.0% were >2.4 μm. Among the particles having DTC = 11-1-75, 72.8% had a diameter of 0.2–1.2 μm, 8.9% were <0.2 μm, 12.5% had a diameter of 1.2–2.1 μm, and 5.8% were >2.1 μm. More than 300 particles were measured from the photographs in each case.

Distribution and Elimination of Polyacryldextran Particles After Intravenous Injection—Male mice[8], weighing 18–20 g at the beginning of the experiments, were used. The radiolabeled particles were dispersed in physiological saline (0.1–0.2 ml) and then injected intravenously. The doses corresponded to 2.6 mg of lyophilized microparticles DTC = 11-1-75 and DTC = 11-2-75, containing 48,200 and 56,600 dpm, respectively. At different time intervals (6 hr, 1, 2, 4, 8, 12, and 20 or 27 weeks) animals were killed by cervical dislocation, and the liver, spleen, lung, and kidneys were removed and prepared for radioactivity measurements. Tissues (the spleen, both lungs, one kidney, or 0.2–0.3 g of the liver) were dissolved in 2 ml of a tissue solubilizer[9]. After digestion at 37° for 12–14 hr (yielding a clear solution), 2-propanol and hydrogen peroxide were added to bleach the samples and minimize color quenching. A scintillation cocktail was added, and the radioactivity was measured after appropriate equilibration. The counting efficiency, generally ~75–80%, was calculated with an external standard.

Determination of Dextranase Activity—The activity of the dextranase was determined photometrically by the method of Janson and Porath (7). The enzyme in free form (0.1 ml) was added to dextran in 0.1 *M* potassium phosphate buffer (1.9 ml). After incubation for 30 min at 37°, 1 ml of the incubation solution was mixed with 1.0 ml of a color reagent (5 g of 3,5-dinitrosalicylic acid, 1 g of phenol, 0.25 g of sodium sulfite, and 100 g of potassium sodium tartrate in 500 ml of 2% NaOH) and heated in a boiling water bath for 15 min. After dilution with 10 ml of water, the absorbance was measured at 540 nm. A standard curve was prepared with maltose: one unit of activity caused the release of 1.0 μmole of maltose/min under the aforementioned conditions.

Autoradiography—Whole-body autoradiography was performed using the method of Ullberg (8). ^{14}C-Labeled particles were injected intravenously in mice. After 2 months, the animals were killed with ether and frozen in a mixture of solid carbon dioxide and hexane (−78°). The mice were embedded in an aqueous carboxymethylcellulose gel, freeze-dried (−15°), and cut into 20- or 60-μm sagittal sections with a microtome. Autoradiograms were made by apposition of the freeze-dried sections to X-ray films, the time of exposure was 6 months.

Isolation of Lysosomes from Rat Liver Homogenate—The livers from untreated male adult Sprague-Dawley rats were removed immediately after sacrifice and immersed in a beaker containing ice-cold 0.25 *M* sucrose, pH 5.5. After weighing, the tissue was cut into pieces and dispersed in 3 volumes of the medium with a homogenizer[10]. The resulting mixture was centrifuged at 750×*g* and 4° for 10 min. The sediment was rehomogenized three times and centrifuged. The resulting supernatants were pooled and centrifuged at 12,000×*g* for 10 min. The supernatant was discarded, and the pellet was dissolved in 0.25 *M* sucrose and centrifuged for 10 min at 6500×*g*. The sediment was redispersed in approximately the same quantity of medium and centrifuged at 6500×*g*

[8] NMRI-mice, Anticimex, Stockholm, Sweden.
[9] Soluene 350, Packard.
[10] Potter-Elvehjelm, Kebo Grave, Stockholm, Sweden.

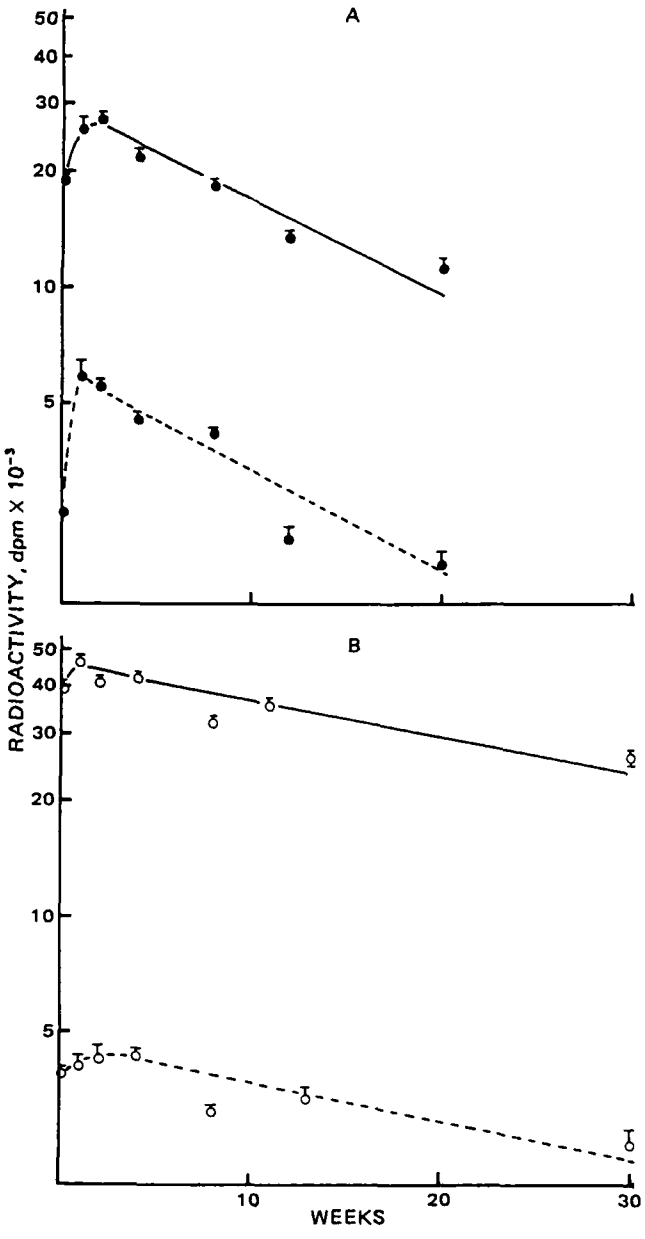

Figure 2—*Radioactivity content of the liver (—) and spleen (- - -) after a single 2.6-mg iv dose of ^{14}C-labeled polyacryldextran particles with composition of DTC = 11-1-75 (●) or DTC = 11-2-75 (○). Each point represents the mean ± SEM for 4–5 animals.*

for 10 min. This procedure was repeated three times. The supernatants from the washings were combined and centrifuged at 12,000×*g* for 15 min. The resulting pellet was redispersed in medium (pH 4.5) to a final volume equal to that of the original tissue. The isolated lysosomal fraction was usually preserved with benzylpenicillin and streptomycin. The described procedure follows that outlined by Beaufay *et al.* (9).

The concentration of lysosomes in the different supernatants and pellets during the preparation was determined by assay of a lysosomal enzyme, β-*N*-acetyl glucosaminidase (10). This enzyme was determined by using 1.5 m*M* 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside as substrate in 1 ml of 0.15 *M* acetate–acetic acid buffer, pH 4.2. The sample (0.5 ml) was added, and the mixture was incubated at 37° for 15 min. The reaction was terminated by adding 3 ml of 0.5 *M* Na_2CO_3, pH 10.5, to each tube, and the free 4-methylumbelliferone was estimated by spectrophotometry at 360 nm. Standards containing 4-methylumbelliferone in buffer were included in every assay. The protein contents in the different fractions were estimated according to the method of Lowry *et al.* (11).

Preparation of Liver and Spleen Samples for Microscopic Studies—Tissue samples were cut from the organs immediately after sacrifice and immersed in chilled 5% formaldehyde in 0.15 *M* phosphate buffer, pH 7.2. The specimens were embedded in paraffin in a routine manner, cut at 2–3 μm, stained with hematoxylin and eosin, and examined under a light microscope.

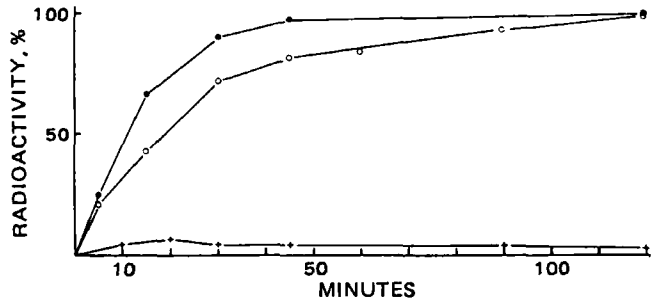

Figure 3—*Degradation of microparticles of polyacryldextran at 23° and pH 6.2 with 0.5 IU of dextranase/ml. Key: (●) DTC = 11-1-75; (○) DTC = 11-2-75; (+) TC = 8-25 (polyacrylamide).*

RESULTS

Distribution and Elimination of ^{14}C-Labeled Polyacryldextran Particles *In Vivo* After Intravenous Injection—On intravenous injection in mice, the microparticles were rapidly cleared from the circulatory system with a $t_{1/2}$ of ~60 min[11]. The distribution of the polyacryldextran particles (DTC = 11-1-75 and DTC = 11-2-75) was followed qualitatively and quantitatively in mice. The autoradiogram (Fig. 1) indicates, as expected, that the particles are phagocytosed by the macrophages belonging to the reticuloendothelial system (RES). The spheres were found in the liver, spleen, and bone marrow. Microparticles with 1% (DTC = 11-1-75) or 2% (DTC = 11-2-75) acrylic groups showed the same distribution pattern, which was also seen with particles containing no derivatized dextran (TC = 8-25) (2).

The quantitative distribution of the particles in the liver, spleen, kidneys, and lung was followed with time (Table I). Initially, some of the largest spheres and/or aggregates were entrapped in the lung capillaries, giving a higher radioactivity in the lungs than earlier noted with polyacrylamide particles (2). This phenomenon is due to the somewhat larger size of the polyacryldextran spheres. However, the aggregates were soon dissociated, and the radioactivity was <1% of the injected dose after 1–2 weeks. The values obtained from the lungs after 2 weeks, therefore, are not included in the table, nor are those from the kidneys which contained only very small amounts of radioactivity after 2 weeks.

The apparent half-life of the different microparticles in the liver and spleen was calculated from the curves in Fig. 2. As is evident, particles made of dextran containing a smaller number of acrylic groups (DTC = 11-1-75) were eliminated more rapidly than those containing more acrylic groups (DTC = 11-2-75). The terminal half-lives, as found by linear regression analysis, were 12.5 and 12 weeks and 33 and 29 weeks, respectively, in the liver and spleen. The figures also indicate that a redistribution took place after the first rapid elimination from the circulatory system, so that the maximal radioactivity was seen after 1–2 weeks in the liver and spleen, independent of the particle composition. About 70–80% of the total injected radioactivity was recovered in these tissues 2 weeks after injection. The uptake of the particles into the liver and spleen resulted in an enlargement of these organs. The weight increase compared with the control regressed after 4–8 weeks.

Ultrastructural Studies—Intravenous injection of a 2.6-mg dose of polyacryldextran particles (DTC = 11-1-75) produced pronounced changes in the liver and spleen. After 1–2 days, the sinusoids were dilated, and vacuolization of the liver parenchymal cells could be seen. The spleen showed an infiltration of inflammatory cells. Weight changes of the liver and spleen paralleled the ultrastructural changes seen microscopically. Formation of small abscesses and granulomas was observed after this period. After 4–8 weeks, the tissues appeared normal except for small granulomas. When a dose of <1 mg was given to the mice, only insignificant morphological changes could be detected in the liver and spleen.

Degradation of Polyacryldextran Particles *In Vitro* with Dextranase—To investigate the stability of the polymer polyacryldextran, ^{14}C-labeled particles of different polymer composition were incubated with dextranase (0.5 IU/ml of particle suspension) for up to 2 hr at 22° and pH 6.2. After centrifugation for 5 min at 4°, the supernatant was withdrawn and the dissolved radioactivity determined (Fig. 3). Micro-

[11] P. Artursson, T. Laakso, and P. Edman, unpublished observation, 1982.

particles of polyacrylamide (TC = 8-25) were not significantly affected. Polyacryldextran particles with composition DTC = 11-1-75 were rapidly hydrolyzed, and all the radioactivity was found in the supernatant after 45 min. Particles with a higher cross-linking degree, DTC = 11-2-75, were more stable, but were dissolved after digestion for 120 min.

Degradation of Different Polyacryldextran Particles *In Vitro* with Isolated Lysosomes from Rat Liver—^{14}C-Labeled particles (dry weight, 0.5 mg) of different gel-composition, (TC = 8-25, DTC = 11-1-75 and 11-2-75) were mixed with 1.0 ml of isolated lysosomes from rat liver homogenate in small plastic tubes. They were incubated at 37° for 1, 3, 6, 24, and 48 hr and then centrifuged for 10 min at 4°. The resulting supernatant was filtered through a filter[12] with 0.45-μm pore size. The radioactivity content in 300 μl of the filtrate was determined in the scintillation counter. Polyacrylamide particles (TC = 8-25) were not significantly affected during 48 hr: only background values were obtained. Polyacryldextran particles with DTC = 11-2-75 composition behaved in the same manner as the polyacrylamide particles. However, polyacryldextran particles with DTC = 11-1-75 were metabolized to some extent, and 0.56 ± 0.003% (mean ± *SD*, n = 4) of the radioactivity in the particles was recovered from the filtrate after incubation for 48 hr.

Excretion of Microparticles in Bile—The biliary excretion of microparticles was studied after a 4-mg iv injection of ^{14}C-labeled polyacrylamide microparticles (containing 400,000 dpm) in mice. The particles had a TC value of 8-25 and a diameter of 0.1–0.4 μm (92.6% of the particles had a diameter within this range, 2.2% were <0.1 μm, and 5.2% had a diameter of 0.4–0.5 μm). The particles were dispersed in physiological saline prior to injection to avoid aggregation. At different time intervals (3, 9, and 27 weeks), animals were killed, and the gallbladder with remaining bile was taken out and digested in 1 ml of 0.5 *M* quaternary ammonium hydroxide. Irrespective of the time elapsed after the injection of particles, the bile contained radioactivity (38, 91, and 34 dpm after 3, 9, and 27 weeks, respectively) after subtraction of background values obtained from untreated mice. The counting times used were long enough to minimize the statistical error to 2–3% for samples and blanks.

DISCUSSION

The most obvious conclusion which can be drawn from the results presented herein is the similarity of the disposition of polyacryldextran and polyacrylamide particles in mice. The half-life of the microparticles in the circulatory system and the general distribution of the microparticles in the mice are similar. They are predominantly taken up by the reticuloendothelial system (*i.e.*, liver, spleen, and bone marrow) as shown by autoradiography and quantitative meaurements in the tissues. Moreover, the morphological changes detected microscopically after intravenous administration of large doses (>50–100 mg dry weight/kg body weight) are similar (3) and parallel the weight changes observed. It is obvious that incorporation of cross-linked dextran in the microparticles does not change the distribution *in vivo*, and it is highly likely that the observations are representative for any uncharged particulates of the same size (*i.e.*, 0.2–1.0 μm in diameter) injected intravenously or intraperitoneally.

Similar effects—morphological changes in the liver—have also been detected with liposomes. A study with mice (12) on the effects of negatively charged liposomes given intravenously revealed that liposomes caused an enlargement of the Kupffer cells with vacuolization observed 7 hr postinjection.

The polyacryldextran particles exhibited a somewhat larger initial uptake in the lungs than seen with polyacrylamide particles. The uptake was reversible, and was detected only in the sample taken 6 hr after the injection. After a week, insignificant amounts of the radioactively labeled microparticles remained. This phenomenon may be due to the larger size of the polyacryldextran particles, with ~15% exceeding 1.2 μm in diameter; the polyacrylamide particles had diameters between 0.2 and 0.5 μm. The reason for the difference in size between the two different particle preparations is that the higher viscosity of the dextran aqueous solution makes it more difficult to prepare small polyacryldextran particles (<0.5 μm). It is important to stress that only small-sized particles should be used *in vivo* to avoid respiratory distress. This point was clearly illustrated in studies with dogs (13, 14), in which polystyrene particles (mean diameter 3 μm) injected intravenously resulted in death for three of four dogs, probably due to lung emboli.

The most significant advantages of the polyacryldextran particles over the polyacrylamide particles is the faster metabolism. Both the experiments *in vitro* with lysosomal enzymes or dextranase and those performed in the mice clearly showed that the degradation and elimination is related to the hydrocarbon content of the microparticles. The lysosomal preparations were able to release >0.5% of the radioactivity within the first 48 hr from particles containing 1% acryl groups. As expected, these particles were also degraded much faster with dextranase than those containing 2% acryl groups. The terminal half-lives *in vivo* in the liver and spleen were also significantly shorter for the polyacryldextran particles with 1% acryl groups (~12 weeks) than for the other particles. The results suggest that it is possible to control elimination by changing the composition of the microparticles, which is of great value when the particles are used as enzyme carriers. In such cases, the composition of the carrier should be chosen such that the degradation of the particles agrees with the denaturating rate of the enzyme. It is, however, also obvious that the size of the particles is a decisive factor for the rate of elimination, and it should be remembered that the polyacryldextran particles used in this study were larger than the polyacrylamide particles used previously (2).

An earlier report from this laboratory indicated that microparticles after intravenous injection were identified in two layers of the gut lumen, the microvilli region and the basal membrane, as judged by the whole-body autoradiograms (2). The reason for such a specific localization was unclear, but long-term studies have now shown that small but significant amounts of radioactivity—originating from the polyacrylamide particles—were found in the gallbladder. Thus the radioactivity in or at the microvilli may be radioactive metabolites excreted from the liver.

[12] Millipore Corp.

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