No other correlation was found between water solubilities and other observed or derived parameter values.

CONCLUSIONS

Within the chain length series of C_2–C_6, the decrease of elimination rates and disposition rate constants with increasing chain length was demonstrated. This observation is consistent with a dissolution rate-limited elimination model. Such a model was derived and successfully NONLIN computer fitted to the observed elimination data. The model-derived parameter of clearance from the cerebrospinal fluid through the lipid blood–brain barrier correlated well with the compound's water solubilities and projected octanol–water partition coefficients. Additional compounds need to be tested to evaluate the postulated model system.

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Distribution and Elimination of Poly(methyl-2-^{14}C-methacrylate) Nanoparticle Radioactivity after Injection in Rats and Mice

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Received December 22, 1978, from the **School of Pharmacy, Swiss Federal Institute of Technology, CH-8092 Zürich, Clausiusstr. 25, Switzerland,* and the ‡*Research Laboratories of Schering AG, Berlin/Bergkamen, Germany.* Accepted for publication June 5, 1979.

Abstract □ The organ distribution of poly(methyl-2-^{14}C-methacrylate) nanoparticles after 0.5, 1, 2, 6, and 24 hr and after 7 days, as well as the elimination of degradation products in urine, feces, and breath, was measured for 7 days after intravenous administration to rats. The radioactivity was determined quantitatively after preparation of the organs and qualitatively by macroautoradiography. In addition, nanoparticle distribution after intramuscular administration to mice was determined by macroautoradiography after 7, 35, and 70 days. Thirty minutes after intravenous administration, the nanoparticles were found in the lungs in high concentrations (758 μg/g fresh weight ≅ 22% of the administered dose); 60% (261 μg/g) of the dose was found in the liver. During the first 7 days, the concentration in the lungs decreased from 758 to 284 μg/g while the concentration in the liver increased from 261 to 372 μg/g (≅ 68% of the administered dose), the concentration in the spleen increased from 33 to 131 μg/g (≅ 4%), and the concentration in the bones increased from 3 to 6 μg/g. In all other organs and tissues, the radioactivity decreased significantly. During the first 7 days after intravenous administration, 1% of the administered dose was eliminated in the urine, 3.5% in the feces, and 1% in the breath. After intramuscular administration, all of the ^{14}C-radioactivity still present in the body persisted at the injection site for 70 days.

Keyphrases □ Poly(methyl methacrylate) nanoparticles—distribution and elimination, intravenous and intramuscular administration, radioactive tracer study, rats, mice □ Nanoparticles—poly(methyl methacrylate), elimination and distribution, intravenous and intramuscular administration, rats, mice □ Methyl methacrylate—polymers, distribution and elimination, intravenous and intramuscular administration, rats, mice

During the past few years, nanoparticles and nanocapsules were introduced as new drug delivery systems (1–4). Poly(methyl methacrylate) nanoparticles with incorporated or adsorbed antigens seem to be especially promising as adjuvants for immunology (1, 2, 5, 6). Incorporation into, as well as adsorption onto, these particles yielded much higher antibody titers than aluminum hydroxide and fluid vaccines and gave better protection (1, 5, 6).

Poly(methyl methacrylate) has been used in surgery for over 30 years as a material for artificial bones (7). Implanted poly(methyl methacrylate) seemed to be well tolerated if the implants were monomer-free and under a certain threshold size (8–11).

However, little is known about the biodegradability and the elimination of poly(methyl methacrylate) from the body. Oppenheimer *et al.* (12) implanted small pieces of poly(^{14}C-methyl methacrylate) films [—$CH_2C(CH_3)$-$COO^{14}CH_3$], having 3.6×10^4 cpm/mg, into rats. The rats began to excrete radioactive material after 54 weeks. When the film was removed, the urinary radioactivity disappeared. These investigators concluded that the radioactivity could not be due to any residual monomer in the films since no radioactive material appeared in the urine immediately upon embedding but only after an extended interval. Tomatis (13) implanted poly(methyl methacrylate) films, with a diameter of 15 mm^2, subcutaneously in mice. The urinary excretion of the label was initially low but slowly increased between 2 and 6 weeks after implantation. It fell suddenly to a minimal amount during the 9th week.

Particles in the nanometer range [nanoparticles and nanocapsules (2, 4)] exhibit a much larger surface area than the implants used by previous investigators. In addition, due to the minute size of the nanoparticles, transport from the site of application might occur even after intramuscular injection or implantation. This study was aimed at gaining information about the fate of these nanoparticles after intravenous and intramuscular administrations.

EXPERIMENTAL

Synthesis of Methyl-2-^{14}C-methacrylate [$CH_2{}^{14}C(CH_3)COOCH_3$] (I)—Compound I was synthesized from 2-^{14}C-acetone[1] by the cyanohydrin procedure (14).

[1] 2-^{14}C-Acetone was prepared by conventional methods starting from barium ^{14}C-carbonate *via* Grignard carboxylation and pyrolysis of lithium 1-^{14}C-acetate (20).

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Table I—Distribution of ^{14}C-Radioactivity after Intravenous Administration of Poly(methyl-2-^{14}C-methacrylate) Nanoparticles to Rats (n = 4; Two Males and Two Females)[a]

Sample	30 min		1 hr		2 hr		6 hr		24 hr		7 days	
	Percent Dose	Micrograms per Gram	Percent Dose	Micrograms per Gram	Percent Dose	Micrograms per Gram	Percent Dose	Micrograms per Gram	Percent Dose	Micrograms per Gram	Percent Dose	Micrograms per Gram
Blood (1 ml)	0.253 ± 0.102	20.2 ± 8.1	0.134 ± 0.059	10.7 ± 4.7	0.077 ± 0.024	6.16 ± 1.89	0.117 ± 0.069	7.77 ± 4.17	0.127 ± 0.093	10.1 ± 7.4	0.020 ± 0.000	1.60 ± 0.00
Fatty tissue abdominal[b]	0.032 ± 0.011	1.29 ± 0.42	0.020 ± 0.006	0.817 ± 0.225	0.019 ± 0.006	0.771 ± 0.259	0.032 ± 0.012	1.28 ± 0.46	0.014 ± 0.005	0.563 ± 0.189	0.006 ± 0.003	0.237 ± 0.103
Fatty tissue subcutaneous[b]	0.023 ± 0.006	0.904 ± 0.240	0.018 ± 0.004	0.700 ± 0.152	0.014 ± 0.003	0.549 ± 0.107	0.025 ± 0.011	1.00 ± 0.43	0.016 ± 0.006	0.648 ± 0.221	0.007 ± 0.001	0.289 ± 0.044
Skin[b]	0.026 ± 0.001	1.03 ± 0.04	0.030 ± 0.012	1.20 ± 0.49	0.023 ± 0.003	0.922 ± 0.121	0.104 ± 0.079	4.17 ± 3.18	0.046 ± 0.026	1.82 ± 1.05	0.005 ± 0.001	0.190 ± 0.037
Bones[b]	0.079 ± 0.026	3.17 ± 1.05	0.071 ± 0.024	2.85 ± 0.96	—	—	0.072 ± 0.029	2.87 ± 1.15	0.097 ± 0.043	3.89 ± 1.71	0.158 ± 0.061	6.34 ± 2.45
Spinal cord[b]	0.029 ± 0.010	1.14 ± 0.39	0.030 ± 0.010	1.21 ± 0.42	—	—	0.023 ± 0.007	0.908 ± 0.299	0.028 ± 0.022	1.11 ± 0.89	0.003 ± 0.001	0.133 ± 0.051
Skeleton muscles[b]	0.225 ± 0.177	8.99 ± 7.08	0.045 ± 0.028	1.81 ± 1.13	0.092 ± 0.038	3.66 ± 1.54	0.072 ± 0.037	2.90 ± 1.47	0.021 ± 0.004	0.855 ± 0.172	0.009 ± 0.002	0.353 ± 0.061
Cerebrum	0.048 ± 0.019	2.48 ± 1.10	0.058 ± 0.014	2.76 ± 0.71	—	—	0.042 ± 0.010	1.94 ± 0.48	0.009 ± 0.001	0.465 ± 0.029	0.008 ± 0.000	0.324 ± 0.050
Cerebellum	0.027 ± 0.003	1.86 ± 0.23	0.023 ± 0.010	1.81 ± 0.73	—	—	0.016 ± 0.008	1.17 ± 0.42	0.004 ± 0.002	0.754 ± 0.392	0.003 ± 0.000	0.258 ± 0.067
Heart	0.101 ± 0.034	7.22 ± 2.31	0.083 ± 0.029	5.64 ± 2.54	0.062 ± 0.015	4.12 ± 1.02	0.045 ± 0.008	3.06 ± 0.79	0.023 ± 0.004	1.75 ± 0.31	0.018 ± 0.002	1.27 ± 0.01
Liver	59.1 ± 1.7	261.2 ± 10.6	57.2 ± 1.7	276.5 ± 28.4	52.4 ± 3.9	268.1 ± 33.1	52.3 ± 2.5	281.9 ± 52.6	63.0 ± 2.5	304.7 ± 28.5	67.7 ± 5.4	371.8 ± 55.2
Lungs	21.8 ± 2.5	758.1 ± 50.5	19.1 ± 2.2	660.2 ± 79.1	19.2 ± 2.2	758.2 ± 149.0	17.4 ± 0.8	483.5 ± 155.9	16.3 ± 1.6	568.9 ± 158.3	13.2 ± 2.8	284.3 ± 89.3
Lymph nodes[b]	0.065 ± 0.029	2.62 ± 1.15	0.064 ± 0.015	2.58 ± 0.61	—	—	0.138 ± 0.131	5.51 ± 5.22	0.024 ± 0.018	0.947 ± 0.715	0.013 ± 0.002	0.538 ± 0.074
Spleen	1.23 ± 0.44	33.2 ± 11.8	1.17 ± 0.38	42.1 ± 13.4	1.01 ± 0.41	39.7 ± 5.3	1.33 ± 0.35	54.8 ± 24.3	2.98 ± 0.25	89.6 ± 12.5	3.92 ± 1.60	131.0 ± 66.4
Suprarenal gland	0.007 ± 0.001	8.06 ± 2.80	0.010 ± 0.007	8.82 ± 7.11	—	—	0.009 ± 0.003	6.85 ± 2.17	0.004 ± 0.001	5.20 ± 1.05	0.002 ± 0.000	1.52 ± 0.42
Kidneys	0.439 ± 0.054	13.3 ± 2.3	0.374 ± 0.026	10.6 ± 1.3	0.333 ± 0.065	10.3 ± 1.8	0.286 ± 0.079	8.42 ± 2.52	0.156 ± 0.024	4.69 ± 0.75	0.104 ± 0.002	2.82 ± 0.08
Thyroid gland	0.009 ± 0.003	4.28 ± 1.32	0.006 ± 0.002	2.48 ± 0.64	—	—	0.006 ± 0.004	2.40 ± 1.08	0.003 ± 0.001	1.30 ± 0.54	0.004 ± 0.001	1.35 ± 0.24
Eyes	0.005 ± 0.001	0.858 ± 0.143	0.003 ± 0.000	0.533 ± 0.085	—	—	0.006 ± 0.002	0.892 ± 0.354	0.002 ± 0.000	0.347 ± 0.126	0.001 ± 0.000	0.151 ± 0.012
Testicles	0.609 ± 0.369	11.4 ± 8.4	0.035 ± 0.001	0.595 ± 0.000	0.009 ± 0.002	0.171 ± 0.046	0.020 ± 0.004	0.348 ± 0.052	0.008 ± 0.001	0.164 ± 0.047	0.003 ± 0.002	0.030 ± 0.022
Ovary	0.023 ± 0.004	8.88 ± 0.60	0.010 ± 0.002	3.44 ± 0.55	—	—	0.008 ± 0.000	3.54 ± 0.19	0.005 ± 0.001	1.68 ± 0.11	—	—
Uterus	0.004 ± 0.003	0.596 ± 0.578	0.004 ± 0.000	0.471 ± 0.160	—	—	0.005 ± 0.001	0.622 ± 0.137	0.002 ± 0.000	0.343 ± 0.118	—	—
Pancreas	0.040 ± 0.009	2.83 ± 0.33	0.033 ± 0.003	2.37 ± 0.35	—	—	0.027 ± 0.002	1.94 ± 0.41	0.011 ± 0.006	1.50 ± 0.44	0.023 ± 0.003	1.85 ± 0.46
GI tract	1.74 ± 0.46	9.45 ± 2.02	1.84 ± 0.44	10.4 ± 1.8	1.07 ± 0.38	6.10 ± 1.31	0.631 ± 0.040	3.57 ± 0.20	0.203 ± 0.033	1.27 ± 0.27	0.173 ± 0.023	0.867 ± 0.126
Gastric wall	0.162 ± 0.050	7.33 ± 0.95	0.157 ± 0.032	5.88 ± 1.28	0.058 ± 0.011	2.33 ± 0.43	0.063 ± 0.017	2.44 ± 0.66	0.156 ± 0.124	6.56 ± 4.47	0.138 ± 0.125	5.77 ± 5.01
Residual body	6.18 ± 1.52	2.27 ± 0.58	10.5 ± 8.2	3.73 ± 2.77	5.41 ± 1.91	1.95 ± 0.66	5.60 ± 2.07	2.03 ± 0.75	5.23 ± 0.92	1.91 ± 0.46	3.81 ± 0.37	1.35 ± 0.02
GI tract contents	1.48 ± 0.76	—	1.40 ± 0.49	—	2.81 ± 0.53	—	3.07 ± 0.35	—	0.159 ± 0.036	—	0.030 ± 0.005	—
Percent of applied dose in rat body	93.7 ± 2.4	—	92.4 ± 6.9	—	82.6 ± 2.1	—	81.4 ± 5.1	—	88.6 ± 3.5	—	89.4 ± 6.9	—
Percent of applied dose in urine	0.076 ± 0.086	—	0.031 ± 0.001	—	0.593 ± 0.212	—	1.05 ± 0.02	—	1.03 ± 0.35	—	0.950 ± 0.980	—
Percent of applied dose in feces	—	—	—	—	—	—	—	—	—	—	3.53 ± 0.88	—
Total, %	93.8 ± 2.4	—	92.4 ± 6.9	—	83.2 ± 2.4	—	82.4 ± 5.1	—	89.6 ± 3.6	—	93.9 ± 7.4	—

[a] All values are given in percent of the administered dose (mean ± SD) and in micrograms of nanoparticles per gram of organ fresh weight (mean ± SD). [b] Refers to 1 g of organ fresh weight.

Table II—Elimination of ^{14}C-Radioactivity after Intravenous Administration of Poly(methyl-2-^{14}C-methacrylate) Nanoparticles to Rats (n = 4; Two Males and Two Females)[a]

Time	Feces	Urine	Breath
2 hr			0.0394 ± 0.0071
1st day	2.59 ± 0.73	0.850 ± 0.981	0.0186 ± 0.0064
2nd day	0.397 ± 0.096	0.042 ± 0.022	0.0114 ± 0.0076
3rd day	0.192 ± 0.075	0.017 ± 0.005	—[b]
4th day	0.103 ± 0.010	0.012 ± 0.005	—[b]
5th day	0.099 ± 0.022	0.010 ± 0.000	—[b]
6th day	0.066 ± 0.028	0.010[b] ± 0.000	—[b]
7th day	0.076 ± 0.015	0.008[b] ± 0.005	—[b]
Total	3.53 ± 0.88	0.949 ± 0.980	0.0694 ± 0.0157[c] 0.8328 ± 0.188[d]

[a] All values are in percent of the administered dose (mean ± SD). [b] Not significantly different from blank value. [c] Refers to 2-hr daily determination time. [d] Extrapolated to 24 hr.

2-^{14}C-Acetone Cyanohydrin (II)—Sulfuric acid (40%, 7.35 ml) was added to a well-stirred solution of potassium cyanide (2.2 g, 33.8 mmoles) and 2-^{14}C-acetone (2.5 ml, 34 mmoles, 34 mCi[1]) in 3.2 ml of water at 15° for 15 min. After another 15 min, the solution was filtered, extracted with ether, and dried over anhydrous sodium sulfate. Chloroacetic acid (5 mg) was added for stabilization, and the solvent was removed *in vacuo*. Distillation of the residue yielded 1.8 g (26 mCi) of II, bp 74–76°/15 mm.

Methyl-2-^{14}C-methacrylate (I)—The yield of cyanohydrin was added drop by drop to a stirred mixture of 98% H_2SO_4 (2.25 ml) and copper powder (20 mg) at 70–80°. After 15 min at 160° (bath temperature), the solution was cooled in ice water. Hydrochinone (20 mg), water (0.3 ml), and methanol (2.4 ml) were added and refluxed for 4.5 hr at 120°. The product was distilled using a copper spire in the distillation head. The distillate was extracted with 3 × 3 ml of water and dried over molecular sieves (4 Å). Distillation yielded 458 mg (5.2 mCi) of I, bp 100°.

The sample was analyzed using a gas chromatograph[2] equipped with a flame detector and a column (2 m, 10% SE 30, 80–100 mesh) at 80–210°, 10°/min, with helium as the carrier gas.

TLC was carried out on silica gel plates[3] in chloroform–acetone (9:1) and chloroform–methanol (9:1) with radioactivity[4] scanning.

Phosphate-Buffered Saline—To prepare the buffered saline, 7.60 g of $Na_2HPO_4 \cdot 2H_2O$, 1.45 g of KH_2PO_4, and 4.80 g of NaCl were dissolved in double-distilled water to give 1000.0 ml of phosphate-buffered saline.

Production of Nanoparticles—Methyl-2-^{14}C-methacrylate, 0.95 ml, was dissolved in phosphate-buffered saline to give 95 ml of a 1% solution. This solution was irradiated with 500 krad at a rate of 2.2 krad/min in a ^{60}Co-source (15). The resulting polymer was freeze dried. A powder consisting of 40% poly(methyl-2-^{14}C-methacrylate) nanoparticles and 60% salt (dibasic sodium phosphate dihydrate–monobasic potassium phosphate–sodium chloride, 7.6:1.45:4.8 w/w) was obtained. The specific radioactivity of this nanoparticle powder mixture was 1.74 μCi/mg of mixture.

Macroautoradiography—Four Wistar rats[5], ~100 g, received 0.5 ml of nanoparticle suspension intravenously. One-half milliliter of suspension contained 10 mg of nanoparticle powder mixture corresponding to 4 mg of polymer with a radioactivity of 17.4 μCi. The rats were sacrificed after 0.5, 6, and 24 hr and after 7 days.

Three male mice, ~35 g, received 50 μl of nanoparticle suspension intramuscularly. Fifty microliters of suspension contained 5.7 mg of the nanoparticle powder mixture corresponding to 2.28 mg of polymer with a radioactivity of 10 μCi. The mice were sacrificed after 7, 35, and 70 days.

Rats and mice were sacrificed with ether, sheared, and frozen for 2 min in an acetone–dry ice mixture. After 1 day in the freezer, the animals were imbedded in ground meat and cut into 40-μm sections with a cryomicrotome[6]. The slices were applied to adhesive tape, dried in a freezer for 2 days, and placed on an X-ray film for 10–40 days.

Organ Distribution and Elimination of Radioactivity—Ten milligrams of the nanoparticle powder mixture was suspended in water to give 0.5 ml of suspension containing 4 mg of polymer with a radioactivity of 17.4 μCi. Twenty Wistar rats[5], ~170 g, received 0.5 ml of suspension/rat iv. After 0.5, 1, 2, 6, and 24 hr and after 7 days, four animals (two males and two females) were sacrificed, and the organs were examined for radioactivity.

Radioactivity elimination in feces and urine was followed for 7 days in the four rats that were sacrificed after this time.

Organs—The organ fresh weight was determined, the organs were homogenized, and an aliquot (50 mg) was combusted in an oxidizer[7]. Smaller organs were combusted whole. The resulting $^{14}CO_2$ was absorbed in 5 ml of absorption medium[8] and analyzed after being mixed with 15 ml of scintillation cocktail[9] in the scintillation counter[10].

Urine—Urine, 0.5 ml, was mixed with 15 ml of scintillation cocktail A and analyzed in the scintillation counter[9].

Feces—The feces were freeze dried, homogenized, and weighed, and a 50-mg aliquot was combusted in the oxidizer[7]. The resulting $^{14}CO_2$ was absorbed in 5 ml of absorption medium[8], mixed with 15 ml of scintillation cocktail[9], and analyzed in the scintillation counter[10].

Breath—The animals were placed in special metabolism cages for 2 hr/day for 7 days to determine the $^{14}CO_2$ eliminated by exhalation. The air in the cages was pumped in succession through three wash bottles. The wash bottles were filled with a mixture of 50 ml of ethanolamine and 50 ml of methanol. The ^{14}C-activity was determined daily in a scintillation counter[10] after mixing with 15 ml of scintillation cocktail B–methanol (3:1).

Standardization—One-half milliliter of the nanoparticle suspension (≅4 mg of polymer) was dissolved in 5 ml of acetone. Methanol, 45 ml, was added; 0.5 ml of this standard solution was mixed with 0.5 ml of inactive urine or 50 mg of inactive, freeze-dried feces. The urine–standard solution mixture was analyzed in the scintillation counter[10] after addition of 15 ml of scintillation cocktail A. The feces–standard solution mixture was combusted in the oxidizer[7]. The resulting $^{14}CO_2$ was absorbed in 5 ml of absorption medium[8] and analyzed after mixing with 15 ml of scintillation cocktail[9].

The calculation, in percent of the dose (Tables I and II), was based on these standard values.

The values listed in Table I, given in percent of the administered dose, were calculated for the whole organ; for the skin, bones, fatty tissue, lymph nodes, and spinal cord, these values refer to 1 g.

Scintillation Cocktail A—2,5-Diphenyloxazole[11] (10 g), 2,2′-p-phenylene-bis-5-phenyloxazole[11] (0.25 g), and naphthalene (100 g) were dissolved in 1 liter of dioxane.

Scintillation Cocktail B—2,5-Diphenyloxazole[11] (55 g) and 2,2′-p-phenylene-bis-5-phenyloxazole[11] (4 g) were dissolved in 5 liters of toluene.

RESULTS

Macroautoradiography after Intravenous Administration of Nanoparticles to Rats—The distribution of the ^{14}C-activity in the rat bodies after 0.5, 6, and 24 hr and after 7 days is shown in Figs. 1–4. One-half hour after intravenous administration, most activity was found in the lungs and in the liver; minor activity was found in the spleen, kidneys, and bone marrow. After 6 hr, activity also was observed in parts of the intestines, which points to biliary excretion (Fig. 2). All radioactivity had disappeared from the kidneys and intestines 24 hr after administration (Fig. 3). Strong radioactivity still could be found in the liver, lungs, and spleen 7 days after administration. The radioactivity in the bone marrow did not change significantly.

Macroautoradiography after Intramuscular Adminstration of Nanoparticles to Mice—Figures 5–7 show the distribution of ^{14}C-radioactivity in mice 7, 35, and 70 days after intramuscular administration of labeled nanoparticles. All radioactivity was found at the injection site.

Elimination Kinetics in Urine, Feces, and Breath—The kinetics of the ^{14}C-elimination in urine, feces, and breath after intravenous administration were followed for 7 days (Table II). The exhaled ^{14}C-activity (Table II) corresponded to a daily determination period of 2 hr. One percent of the administered dose was excreted in the urine, 3.5% in the

[2] Varian 3700, Varian AG, Zug, Switzerland.
[3] 60 F 254, E. Merck, Darmstadt, West Germany.
[4] Berthold LB 2722, Berthold, Wildbad, West Germany.
[5] Brünger, 4801 Bokel, West Germany.
[6] Jung/Dittes, Heidelberg, West Germany.
[7] Tritium carbon oxidizer type 306, Packard Instrument Co., Downers Grove, Ill.
[8] Chromosorb, Packard Instrument Co., Downers Grove, Ill.
[9] Permafluor, Packard Instrument Co., Downers Grove, Ill.
[10] Liquid scintillation spectrometer type 3380, Packard Instrument Co., Downers Grove, Ill.
[11] Fa. Zinsser, Frankfurt, West Germany.

Figure 1—*Macroautoradiography of a rat 30 min after intravenous administration of poly(methyl-2-^{14}C-methacrylate) nanoparticles.*

Figure 2—*Macroautoradiography of a rat 6 hr after intravenous administration of poly(methyl-2-^{14}C-methacrylate) nanoparticles.*

Figure 3—*Macroautoradiography of a rat 24 hr after intravenous administration of poly(methyl-2-^{14}C-methacrylate) nanoparticles.*

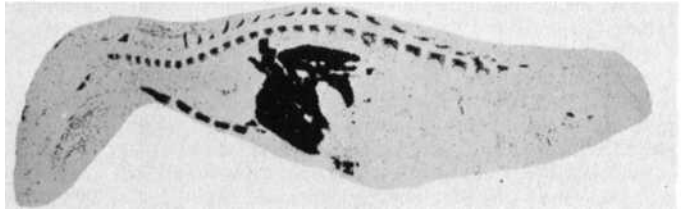

Figure 4—*Macroautoradiography of a rat 7 days after intravenous administration of poly(methyl-2-^{14}C-methacrylate) nanoparticles.*

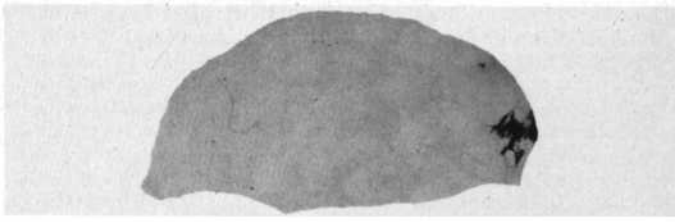

Figure 5—*Macroautoradiography of a mouse 7 days after intramuscular administration of poly(methyl-2-^{14}C-methacrylate) nanoparticles.*

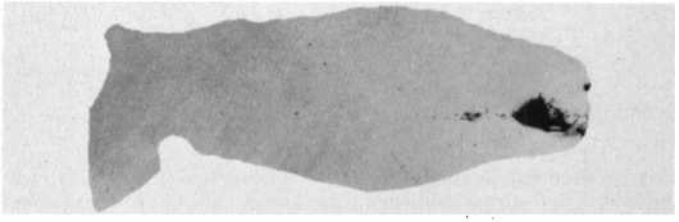

Figure 6—*Macroautoradiography of a mouse 35 days after intramuscular administration of poly(methyl-2-^{14}C-methacrylate) nanoparticles.*

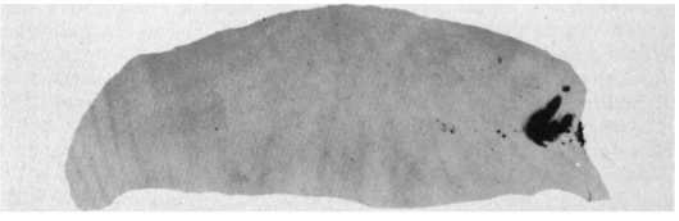

Figure 7—*Macroautoradiography of a mouse 70 days after intramuscular administration of poly(methyl-2-^{14}C-methacrylate) nanoparticles.*

feces, and 1% in the breath within 7 days. The ^{14}C-activity in the breath was significantly higher than the blank for only 2 days. The excretion in urine and feces reached a maximum at 0.9 and 2.6%, respectively, on the 1st day and decreased rapidly until Day 7.

Organ Distribution of Radioactivity—Table I shows the distribution of ^{14}C-radioactivity in the organs and tissue after intravenous administration of the nanoparticles. The values are given in percent of the administered dose and in micrograms of nanoparticles per gram of fresh weight. After 30 min, the most activity (~60%) was found in the liver, 22% in the lungs, 1.2% in the spleen, 1.7% in the GI tract, 1.5% in the GI tract contents, 0.4% in the kidneys, and 0.6% in the testicles. All other tissue and organs had an activity significantly lower than 1%.

The radioactivity distribution changed with increasing time. The portion of radioactivity in the lungs was reduced to 13.2% after 7 days, while the portion of activity in the liver increased to 67.6%. The portion of activity was also reduced in the GI tract, kidneys, and testicles but increased slightly in the spleen and bones.

This change in distribution of activity in the organism was demonstrated clearly by the nanoparticle concentrations, given in micrograms per gram of fresh weight. In the lungs, the concentration dropped from 758 μg/g after administration to 284 μg/g after 7 days. The nanoparticle concentration in the liver increased from 261 to 372 μg/g; in the spleen, it increased from 33 to 131 μg/g; and in the bones, it increased from 3 to 6 μg/g. In all other organs, the concentration of nanoparticles or their degradation products decreased markedly.

DISCUSSION

The distribution and elimination of radioactivity after intravenous administration of ^{14}C-labeled nanometer-sized poly(methyl methacrylate) particles (nanoparticles) was followed in rats for 7 days. The results obtained by quantitative determination of radioactivity in organs, tissue, and excreta correlate well with the findings obtained by macroautoradiography. At first, possibly during the first pass through the lungs, the nanoparticles were filtered out by the lung capillaries. After 30 min, the concentration of nanoparticle radioactivity reached a maximum of 758 μg/g, the highest concentration of radioactivity in any organ during the whole observation period. The concentration in the liver after 30 min was 261 μg/g, which still represented ~60% of the administered dose.

^{14}C-Radioactivity in the kidneys and GI tract was detected by using quantitative distribution measurements as well as macroautoradiography. The ^{14}C-activity in the intestine points to certain biliary excretion.

Seven days after administration, the ^{14}C-activity in most organs and tissue had decreased markedly while the greatest amount was found in the liver (~68% ≅ 372 μg/g). Furthermore, the concentration of the nanoparticles or their degradation products had increased in the spleen and the bone marrow. Liver, spleen, and bone marrow belong to the immune system. The accumulation of the nanoparticles in these immunocompetent organs may be related to the adjuvant effect and the protection yielded by these particles (1, 4).

The elimination of radioactivity in urine, in feces, and by exhalation was maximal shortly after application. With increasing time, the ^{14}C-activity in the excreta decreased. This decrease was accompanied by an activity decrease in the GI tract as well as in the kidneys and may have been due to an elimination of lower molecular weight contents of the nanoparticles. Approximately 5.3% of the administered dose was excreted within 7 days. It is unlikely that all of this material was monomeric since it was shown previously with nanoparticles (15) that no more than 1% of fugitive material such as methacrylate monomers is present in the final product.

After intramuscular administration of the labeled nanoparticles, all residual ^{14}C-activity was found at the injection site. No transportation and distribution of particles occurred during 70 days.

The results after intravenous and intramuscular administrations show that poly(methyl methacrylate) nanoparticles are not rapidly biodegradable. Although macroautoradiographies give mainly qualitative information about ^{14}C-radioactivity, the strong blackening of the autora-

diographs shows that a considerable amount of carbon-14 persisted in the body. In addition, as shown previously (12), a lag period might exist before the elimination of the polymer.

The long period of nanoparticle accumulation at the injection site after intramuscular administration correlates well with the previously observed prolonged adjuvant activity of these particles (1, 6). This prolonged activity was especially pronounced after incorporation into larger amounts of polymer and in comparison to other adjuvants (1).

The attraction of the nanoparticles to the immune system has already been mentioned. However, transporation of the adjuvant from the administration site is not necessary for a good immune response. This is also the case with other adjuvants (16, 17). In contrast to these other adjuvants (16–18), poly(methyl methacrylate) nanoparticles did not induce granulomas in guinea pigs within 1 year (6). Histological reactions were the same as with the fluid control. In spite of the long-term persistence at the injection site, poly(methyl methacrylate) nanoparticles might be an improvement over the presently used adjuvants from a toxicity standpoint; the other adjuvants also persist at the site of injection, but they exhibit much stronger adverse histological reactions (16–19).

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Analysis of Cyclazocine in Plasma

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Abstract □ The analysis of plasma cyclazocine by two methods is described. The radioimmunoassay employed a ^{125}I-labeled radioligand, rabbit antiserum, and separation of bound from free cyclazocine with a second antibody. The radioimmunoassay was specific for cyclazocine and had a detection limit of ~20 pg/ml. The GLC method employed a mass spectrometer as the detector and had a detection limit of ~109 pg/ml. Both techniques had acceptable accuracy and precision when used to quantitate cyclazocine in dog and human plasma. The methods were used successfully to quantitate cyclazocine from beagle hounds receiving 0.5 mg of ^{3}H-cyclazocine/kg iv. The decline in plasma cyclazocine fitted a two-compartment body model with a mean plasma clearance rate of 39.2 liters/hr.

Keyphrases □ Cyclazocine—analysis, GLC–mass spectrometry, radioimmunoassay, human and dog plasma, pharmacokinetics □ Analgesics—cyclazocine, GLC–mass spectrometric analysis, radioimmunoassay, human and dog plasma, pharmacokinetics □ GLC–mass spectrometry—analysis, cyclazocine, human and dog plasma □ Radioimmunoassay—analysis, cyclazocine, human and dog plasma

Cyclazocine, (2α,6α,11R*) - (±) - 3 - (cyclopropylmethyl)-1,2,3,4,5,6-hexahydro -6,11- dimethyl-2,6-methano-3-benzazocin-8-ol (I), is a member of the benzomorphan series (1). It is a relatively long-acting, orally effective narcotic antagonist (2) and may find clinical usefulness in the treatment of opiate dependence (3). Doses as low as 0.1–0.25 mg po have provided effective pain relief in humans (4).

Previously reported analytical methods for cyclazocine include TLC (5) and GLC (6). These methods have a detection limit of ~10 ng/ml. From these laboratories, a radioimmunoassay using ^{3}H-cyclazocine was previously reported (7) with a sensitivity of ~3 ng/ml. The antibody dilution was 1:50, and the antiserum for this assay was soon exhausted.

To estimate accurately the cyclazocine concentration in plasma following low oral doses, a sensitive measurement method is needed. This article reports the development of two new analytical methods: a radioimmunoassay using a ^{125}I-labeled cyclazocine derivative and a a GLC method using a mass spectrometer as the detector. Both techniques demonstrate sensitivity in the picogram range. These assays have been used to measure cyclazocine added to human and dog plasma. The techniques successfully measured cyclazocine in the plasma of dogs receiving 0.5 mg/kg iv. The pharmacokinetic parameters were determined from the radioimmunoassay data.

EXPERIMENTAL

Radioimmunoassay—*Solutions*—Phosphate-buffered saline was prepared by dissolving 8.17 g of NaCl, 0.50 g of $NaH_2PO_4 \cdot H_2O$, 1.07 g of Na_2HPO_4, and 0.095 g of thimerosal[1] in 1 liter of distilled water and adjusting to pH 7.0. The assay buffer contained 0.1% (w/v) gelatin dissolved in phosphate-buffered saline. Nonimmune normal rabbit serum was diluted 1:500 in phosphate-buffered saline containing 0.05 *M* edetate

[1] Sigma Chemical Co., St. Louis, Mo.

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