11) for assaying and identifying I and III in bulk drug form or pharmaceutical preparations.

REFERENCES

(1) "The United States Pharmacopeia" 20th rev., U.S. Pharmacopeial Convention, Rockville, Md., 1980, pp. 155 and 156.

(2) M. P. Gruber, R. W. Klein, M. E. Foxx, and J. Campisi, *J. Pharm. Sci.*, **61**, 1147 (1972).

(3) R. E. Graham, C. T. Kenner, and E. R. Biehl, *J. Pharm. Sci.*, **64**, 1013 (1975).

(4) E. J. Kubiak and J. W. Munson, *J. Pharm. Sci.*, **71**, 872 (1982).

(5) K. Hayakawa, K. Kitada, M. Hamaki, and M. Miyazaki, *J. Chromatogr.*, **229**, 159 (1982).

(6) K. Miuro, H. Nakamura, H. Tanaka, and Z. Tamura, *J. Chromatogr.* **210**, 536 (1981).

(7) K. W. Phoon and C. Stubley, *J. Chromatogr.*, **246**, 297 (1982).

(8) F. W. Ezzedeen, A. N. Masoud, S. J. Stohs, and S. J. Lerman, *J. Pharm. Sci.*, **70**, 889 (1981).

(9) L. A. Sternson and W. J. DeWitte, *J. Chromatogr.*, **137**, 305 (1977).

(10) B. L. Karger, J. N. LePage, and N. Tanaka, in "High Performance Liquid Chromatography, Advances and Perspectives," vol. 1, C. Horvath, Ed., Academic, New York, N.Y., 1980, pp. 113-206.

(11) "The United States Pharmacopeia," 20th rev., U.S. Pharmacopeial Convention, Rockville, Md., 1980, p. 413.

Distribution and Elimination of Coated Polymethyl [2-^{14}C]Methacrylate Nanoparticles After Intravenous Injection in Rats

DANIEL LEU *§, BARBARA MANTHEY ‡, JORG KREUTER §, PETER SPEISER §, and PATRICK P. DeLUCA *x

Received March 11, 1983, from **The University of Kentucky College of Pharmacy, Lexington, KY 40536* and the §*School of Pharmacy, Swiss Federal Institute of Technology, CH-8603 Schwerzenbach, Switzerland.* Accepted for publication October 28, 1983. ‡Present address: Institute of Toxicology, Swiss Federal Institute of Technology, CH-8092 Zurich, Switzerland.

Abstract □ Surfactant-coated polymethyl [2-^{14}C]methacrylate nanoparticles had significantly different time-course distribution patterns in rats than noncoated and albumin-coated particles. Blood concentrations of poloxamer 188-coated particles were 70-fold higher after 30 min, and the particles persisted at higher levels in the circulation for up to 2 h. The initial and final liver levels were significantly lower (38% after 30 min, 51% after 7 d) and spleen levels were significantly higher (21% after 30 min, 23% after 7 d) than noncoated particles (74% in the liver and 5% in the spleen after 7 d) and the albumin-coated particles (84% in the liver and 5% in the spleen after 7 d). Specific activity was somewhat higher for the surfactant-coated particles in other organs such as the lungs, kidneys, testicles, ovaries, and lymph nodes. The bovine serum albumin sorption behavior of polymethyl methacrylate nanoparticles was followed under various conditions, and adsorption was found to increase with increasing protein concentration and increasing temperature, reaching a maximum at the isoelectric point of pH 4.9 after ~12 h of incubation. The zeta potential of the particles decreased with increasing pH, and the change was more pronounced with the albumin-coated particles.

Keyphrases □ Polymethyl methacrylate—^{14}C-labeled nanoparticles, distribution and elimination in rats, intravenous injection □ Distribution—^{14}C-labeled polymethyl methacrylate nanoparticles, intravenous injection, rats □ Elimination—^{14}C-labeled polymethyl methacrylate nanoparticles, intravenous injection, rats

There is growing interest in polymeric drug-delivery systems for parenteral administration to provide sustained release and action, and to increase the stability of easily metabolized drugs (1, 2). Nanoparticles (10 nm–1 μm) have been suggested as new drug-delivery systems (3–6). The goal is to develop a system to direct potent drug molecules to specific organs or cells, thereby limiting the systemic levels and potential toxicity to healthy or normal tissues and cells.

Polymethyl methacrylate has been used in surgery for over 30 years as a material for artificial bones (7). Implanted methyl methacrylate polymers appear to be well tolerated if the implants are monomer-free and under a certain threshold size (8). However, little is known about the biodegradability and the elimination of polymethyl methacrylate particles from the body. Nanometer-size particles exhibit a much larger surface area than the implants used in previous studies (9, 10) and are expected to degrade much faster. Information on the long-term effects of acrylic polymer particles on the living cell, however, is scarce (11).

It is generally accepted that polymers in the form of nanometer-size particles are mainly taken up by the cells of the reticuloendothelial system, predominantly in the liver and spleen. It was found that 70% of polymethyl [2-^{14}C]methacrylate nanoparticle radioactivity localized in the liver of rats 7 d after intravenous administration in phosphate-buffered saline (12). The clearance of labeled polystyrene divinylbenzene microspheres (3–25 μm) from the blood was size-dependent in beagle dogs (13, 14). Microspheres of ≥8 μm were mechanically filtered and retained in the lungs, while smaller spheres cleared the lungs and localized in the liver and spleen. Although the effect of physicochemical surface properties on distribution has been recently reported (15), the possible long-term effects associated with retention of particulate matter in the body tissues have not been studied.

Emulsifying agents have been found to affect the fractional removal rate of lipid emulsions from the blood stream (16, 17). Since emulsifying agents appear to play a major role in the removal of lipid substances from the blood stream, it was anticipated that adsorbed surfactants also influence the clearance of polymethyl methacrylate nanoparticles from the blood. Other workers have demonstrated the significance of plasma components with respect to the uptake of particulate polymeric matter by cells of the reticuloendothelial system and, accordingly, in the clearance from the blood and subsequent organ distribution (18, 19). Distribution in various organs has been reported to be influenced by the surface charge of the particles. Microspheres with adsorbed positively charged proteins (18) and nonionic surfactant (15) show a decreased uptake by the liver.

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Table I—Adsorption of Bovine Serum Albumin to Polymethyl Methacrylate Nanoparticles

Parameter Examined	Bovine Serum Albumin Adsorbed[a]	Conditions
Particle conc. (w/v)		
0.1%	3.3 ± 9.1%	pH: 4.9
0.3%	1.2 ± 25%	Incubation time: 4 h
		Albumin conc.: 2% (w/v)
		Temp.: 37°C
Protein conc. (w/v)		
3.0%	32.2 ± 5.0%	pH: 7.4
2.5%	20.0 ± 5.0%	Incubation time: 4 hr
1.5%	11.8 ± 9.3%	Particle conc.: 0.1%
0.5%	9.9 ± 9.1%	Temp.: 37°C
0.2%	4.6 ± 4.3%	
Temperature		
37°C	20.5 ± 7.8%	pH: 4.9
25°C	17.4 ± 8.0%	Incubation time: 4 h
4°C	16.9 ± 10%	Albumin conc.: 2%
		Particle conc.: 0.1%
pH		
3.9	15.8 ± 7.0%	Incubation time: 4 h
4.9	34.8 ± 5.7%	Albumin conc.: 2%
7.4	22.1 ● 5.4%	Particle conc.: 0.1%
		Temp.: 37°C
Time		
1 h	3.1 ± 13%	pH: 4.9
2 h	7.7 ± 9.1%	Albumin conc.: 2%
4 h	19.6 ± 11%	Particle conc.: 0.1%
12 h	24.9 ± 8.0%	Temp.: 37°C
22 h	24.0 ± 8.6%	

[a] Mean of four determinations. The statistical dispersion is given as the *SD* in percent of the mean value. The values are percent of albumin adsorbed from solution, except particle concentration (mg of albumin/mg of particles).

In a previous study, polymethyl methacrylate nanoparticles of ~150 nm were found to accumulate in the lungs in relatively high concentrations 30 min following intravenous administration (12), presumably due to agglomeration of the particles. This paper presents information on the fate of surface-coated polymethyl [2-^{14}C]methacrylate nanoparticles after intravenous injection in rats. Before the animal experiments, the adsorption of the nanoparticles to bovine serum albumin and the resulting surface charge (zeta potential) were determined.

EXPERIMENTAL SECTION

Preparation of ^{14}C-Labeled Nanoparticles—The nanoparticles were prepared as previously described (12) and freeze-dried from a suspension in 0.15 M phosphate-buffered saline. The polymer had a specific activity of 4 mCi/g[1]. The freeze-dried powder contained 56.3% of buffer salts (dibasic sodium phosphate dihydrate-monobasic potassium phosphate-sodium chloride (7.6:1.45:4.8, w/w/w) and 43.7% of polymethyl [2-^{14}C]methacrylate. Particle size determination by photon correlation spectrometry[2] (20) revealed an average diameter of 131 ± 30 nm.

***Preparation of the* Suspension**—*Reference Group*—Nanoparticle-buffer preparation (220 mg) was suspended in 11 mL of distilled water and 33 mL of phosphate-buffered saline, to give a suspension of 2.185 mg of nanoparticles/mL and ultrasonicated for 5 min at 20 kHz in a sonicator cell disrupter with a micro tip[3].

Surfactant Group—The nanoparticle preparation (220 mg) was suspended in 11 mL of 0.5% (w/v) poloxamer 188[4] in water and 33 mL of 0.5% (w/v) poloxamer 188 in phosphate-buffered saline and ultrasonicated.

Protein Group—The nanoparticle preparation (220 mg) was suspended in 76 mL of a 3% (w/v) solution of rat serum albumin[5] in 0.145 M phosphate buffer (pH 4.9), incubated in an end-over-end shaker (22 rpm) at 37°C for 12 h, and then centrifuged at 30,000×g_{ave}[6]. The protein-coated particles were washed twice with 0.145 M phosphate buffer (pH 4.9) and then suspended in 44 mL of phosphate-buffered saline and sonicated. The protein adsorbed was 8.2% from the solution, measured by UV absorbance at 280 nm. This corresponds to 1.92 mg of albumin/mg of nanoparticles. The adsorption of protein was irreversible since protein was not detected in the washings or phosphate-buffered saline suspensions.

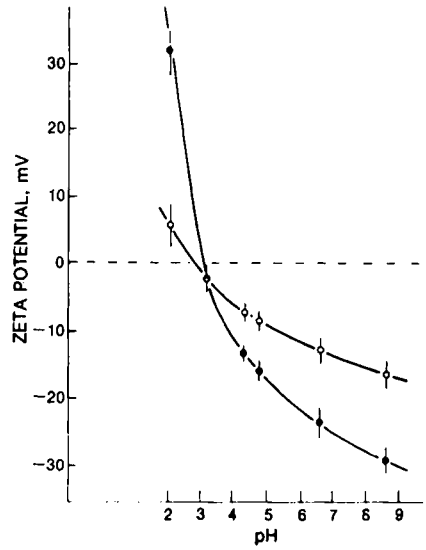

Figure 1—*The influence of pH on the zeta potential of noncoated (○) and protein-coated (●) polymethyl methacrylate nanoparticles. Each value is the mean of 10 measurements; the vertical lines represent* SD.

Specific Activity of the Suspensions—Five 1:1000 dilutions of each suspension and 0.175 mL of a combustion aid[7] were combusted in an oxidizer[8]. The resulting carbon dioxide was absorbed in 7 mL of an absorption medium[9]. This medium was then mixed with 15 mL of scintillation cocktail[10] and the ^{14}C-radioactivity was measured in a liquid scintillation counter[11]. The specific activities of the suspensions were calculated using a quench curve (19) and the following values were obtained: (*a*) reference group: 7.76 ± 0.39 μCi/mL; (*b*) surfactant group: 8.59 ± 0.48 μCi/mL; (*c*) protein group: 6.85 ± 0.16 μCi/mL.

Protein Binding Studies—Experiments were conducted to determine suitable conditions for the adsorption of albumin to the nanoparticles. The parameters assessed were particle concentration, protein concentration, temperature, time of incubation, and pH of the incubation solution. The nanoparticle suspensions were incubated in a solution of bovine serum albumin[12] in 0.145 M phosphate-buffered saline at a constant temperature in glass tubes which were rotated end over end. The suspensions were centrifuged, and the protein concentration in the supernatant was determined by measuring the absorbance at 280 nm. This method was verified by the Lowry test (21).

Surface Charge—The zeta potential of nanoparticle suspensions at different pH values (before and after the adsorption of bovine serum albumin) was calculated after measuring the microelectrophoretic mobility of small particle-agglomerations in a phosphate buffer (ionic strength 0.01 M) with a zeta meter[13]. An optimal particle concentration for tracking in the electrophoretic cell was 25 ppm. Ten particles (or small particle-agglomerations) were counted for each value at a voltage of 100 V. Measurements were not made of particles suspended in different solutions of two surfactants, poloxamer 188 and polysorbate 80[14], because these preparations did not show any agglomerations in a zeta-meter-suitable microscope and, therefore, were not detectable.

Injection—Groups of four (two male and two female) randomly-selected Wistar rats (203–335 g) were used for each suspension and for each time point (0.5, 2, 24 h, and 7 d). The rats were anesthetized with ether and then restrained in a supine position on a hard board. After exposing the femoral vein with sterile instruments, the animal was injected with the nanoparticle suspension using a 26-gauge needle attached to a plastic syringe at a rate of 1 mL/min. Immediately before injection the suspensions were ultrasonicated at 20 kHz for 20 s. The injection dose was 60 μCi/kg of bodyweight (~2

[1] Amersham Radiochemical Centre, Buckinghamshire, U.K.
[2] Model K7025 with 64 channels; Malvern Instruments, Malvern, U.K.
[3] Model W 185 F; Heat Systems Ultrasonic Inc., Plainview, N.Y.
[4] Pluronic F-68; Wyandotte Chemicals, Wyandotte, Mich.
[5] Pel-Freez Biologicals, Rogers, Ark.
[6] Beckman J 2-21, rotor JA 20 (20.000 rpm, 5°C); Beckman Instruments, Palo Alto, Calif.
[7] Packard Instrument Co., Downers Grove, Ill.
[8] Model B 306 Tri-Carb Sample Oxidizer; Packard Instrument Co.
[9] Carbo Sorb; Packard Instrument Co.
[10] Permafluor V; Packard Instrument Co.
[11] Model 3255 Tri-Carb Liquid Scintillation Counter; Packard Instrument Co.
[12] Cohn Fraction V; Sigma, St. Louis, Mo.
[13] Zeta Meter Inc., New York, N.Y.
[14] Tween 80; Atlas Chemie GmbH, Essen, West Germany.

Table II—Distribution of Carbon-14 after Intravenous Administration of Polymethyl [2-^{14}C]Methacrylate Nanoparticles to Rats [a]

Sample	Group[b]	Time after Injection: 30 min Percent of Dose	30 min Specific Activity	2 h Percent of Dose	2 h Specific Activity	24 h Percent of Dose	24 h Specific Activity	7 d Percent of Dose	7 d Specific Activity
Blood	Ref	0.3 ± 32%	0.02	0.1 ± 23%	0.01	0.1 ± 20%	0.00	0.0 ± 36%	0.00
	Sur	20.5 ± 26%	0.12	1.0 ± 34%	0.05	0.1 ± 39%	0.00	0.0 ± 18%	0.00
	RSA	0.3 ± 67%	0.02	0.2 ± 15%	0.01	0.0 ± 40%	0.00	0.0 ± 39%	0.00
Liver	Ref	85.3 ± 4%	7.78	79.2 ± 12%	7.89	80.9 ± 8%	8.07	73.8 ± 6%	6.78
	Sur	37.6 ± 19%	3.16	43.2 ± 14%	3.60	59.8 ± 8%	5.54	51.3 ± 5%	4.84
	RSA	84.7 ± 3%	8.31	83.7 ± 2%	6.81	85.4 ± 1%	7.91	84.2 ± 5%	8.42
Lungs[c]	Ref	1.4 ± 6%	1.16	2.2 ± 0%	2.05	3.3 ± 11%	2.82	0.2 ± 53%	0.18
	Sur	3.8 ± 20%	3.18	3.2 ± 19%	2.63	7.0 ± 32%	6.23	0.6 ± 38%	0.46
	RSA	2.3 ± 28%	2.07	2.9 ± 26%	2.55	2.1 ± 17%	1.90	0.2 ± 15%	0.17
Spleen	Ref	3.7 ± 25%	6.40	2.5 ± 16%	3.37	4.1 ± 6%	6.46	4.6 ± 13%	7.40
	Sur	20.9 ± 7%	34.21	30.6 ± 20%	49.34	13.3 ± 17%	21.45	22.6 ± 14%	32.20
	RSA	3.8 ± 35%	5.91	3.8 ± 15%	6.10	4.6 ± 2%	6.59	4.7 ± 4%	7.72
Kidneys	Ref	0.3 ± 15%	0.14	0.3 ± 48%	0.13	0.1 ± 29%	0.07	0.0 ± 34%	0.01
	Sur	0.9 ± 18%	0.47	0.3 ± 25%	0.13	0.2 ± 27%	0.09	0.0 ± 45%	0.02
	RSA	0.2 ± 27%	0.11	0.2 ± 32%	0.08	0.1 ± 17%	0.07	0.0 ± 19%	0.01
Testicles	Ref	0.01 ± 1%	0.00	0.00 ± 0%	0.00	0.00 ± 9%	0.00	0.00 ± 36%	0.00
	Sur	0.04 ± 11%	0.01	0.01 ± 22%	0.00	0.00 ± 8%	0.00	0.00 ± 44%	0.00
	RSA	0.01 ± 2%	0.00	0.00 ± 47%	0.00	0.00 ± 0%	0.00	0.00 ± 11%	0.00
Ovaries[d]	Ref	0.01 ± 4%	0.10	0.01 ± 0%	0.10	0.01 ± 4%	0.11	0.00 ± 12%	0.03
	Sur	0.10 ± 25%	1.11	0.01 ± 0%	0.73	0.01 ± 4%	0.14	0.01 ± 42%	0.22
	RSA	0.01 ± 0%	0.06	0.01 ± 20%	0.08	0.01 ± 27%	0.08	0.00 ± 35%	0.06
Lymph nodes[e]	Ref	0.02 ± 32%	0.02	0.02 ± 33%	0.02	0.02 ± 16%	0.02	0.02 ± 30%	0.02
	Sur	0.08 ± 21%	0.08	0.04 ± 1%	0.04	0.03 ± 8%	0.03	0.02 ± 22%	0.02
	RSA	0.01 ± 7%	0.01	0.01 ± 38%	0.01	0.01 ± 38%	0.01	0.01 ± 27%	0.01
Total	Ref	91.0 ± 4%	—	84.3 ± 10%	—	92.1 ± 6%	—	84.0 ± 4%	—
	Sur	83.9 ± 9%	—	78.4 ± 9%	—	83.1 ± 6%	—	79.4 ± 4%	—
	RSA	91.3 ± 3%	—	90.8 ± 2%	—	93.0 ± 1%	—	91.4 ± 4%	—

[a] Mean ± *RSD* percent of the administered dose; $n = 4$. The specific activity is the percent of administered dose per gram of tissue (wet weight) or milliliter of blood. The blood volume was assumed to be 64.1 ± 9 mL/kg (24). The 24-h and 7-d values include the elimination by urine and feces (see Table IV). [b] Key: (Ref) untreated particles; (Sur) particles treated with surfactant; (RSA) particles coated with rat serum albumin. [c] Without bronchia. [d] Without tubes. [e] One gram (wet weight).

mL/animal). The incision was sealed with aluminum wound clips and rinsed with an iodine solution.

Organ Distribution and Elimination of ^{14}C-Radioactivity—The animals were anesthetized with ether and injected with an overdose of sodium pentobarbital [100 mg/kg ip of a 2% aqueous solution (22)]. After ~5 min, the abdomen and chest were opened and a blood sample was taken from the vena cava. The vena cava was then cut, and the blood was drained. The liver, lungs, spleen, kidneys, testicles or ovaries, and lymph nodes (from the mesenterium and cervix) were removed. The organs were washed with distilled water, and the connective tissue and fat was removed. The organs were weighed and stored at −20°C for further examination.

After injection, a corresponding group of animals was housed in metabolism cages for 7 d (food and water *ad libitum*). Urine and feces were collected in separate containers and removed every 24 h. Weighed urine and freeze-dried fecal samples were stored at −20°C for further examination.

The organs were homogenized with water (1:10) and an aliquot (0.001–0.5 of an organ, depending on the expected radioactivity) was combusted in an oxidizer. The resulting $^{14}CO_2$ was absorbed in 7 mL of absorption medium, mixed with 15 mL of scintillation cocktail, and analyzed in the liquid scintillation counter. Urine (0.5 mL) was mixed with 15 mL of scintillation cocktail[15] and analyzed in the scintillation counter. Freeze-dried feces was homogenized with water, and a sample of 100 mg was combusted with several drops of a combustion aid. The resulting $^{14}CO_2$ was analyzed in the manner described above for the organs. The percentages of the administered dose of nanoparticles in the organs, blood, and excreta were calculated using the standard values and quench curves (23).

RESULTS

Protein Binding Studies—The adsorption of bovine serum albumin to polymethyl methacrylate nanoparticles as a function of the parameters studied is shown in Table I. From these experiments, the following conditions were selected for protein-coating prior to the zeta potential measurements and the animal studies: concentration of serum albumin, 3.0%; particle concentration, 0.1%; time of incubation, 12 h; buffer, 0.145 M potassium phosphate (pH 4.9); temperature of incubation, 37°C.

Surface Charge—The influence of pH on the zeta potential of noncoated and protein-coated nanoparticles is shown in Fig. 1. The zeta potential decreased with increasing pH for both particle types, but the influence was greater on the coated nanoparticles.

Organ Distribution of ^{14}C-Radioactivity—Table II shows the distribution of ^{14}C-radioactivity in several organs [selected using the findings of Kreuter *et al.* (12)] after intravenous administration of the nanoparticles. The distribution behavior in the reference and protein-coated groups was similar, but, in most of the organs examined, the surfactant group showed a very different accumulation of particles. One fifth of the administered dose of the surfactant

Table III—Comparison of the Distribution of Noncoated and Coated Nanoparticles in Organs [a]

Organ	Comparison	0.5 h	2 h	24 h	7 d
Blood	Ref/Sur	***	**	n.s.	n.s.
	Ref/RSA	n.s.	n.s.	*	n.s.
	Sur/RSA	***	**	n.s.	n.s.
Liver	Ref/Sur	***	***	•	***
	Ref/RSA	n.s.	n.s.	n.s.	*
	Sur/RSA	***	***	***	***
Lungs	Ref/Sur	***	*	*	*
	Ref/RSA	*	n.s.	**	n.s.
	Sur/RSA	*	n.s.	**	*
Spleen	Ref/Sur	***	***	***	***
	Ref/RSA	n.s.	**	*	n.s.
	Sur/RSA	***	***	***	***
Kidneys	Ref/Sur	***	n.s.	n.s.	n.s.
	Ref/RSA	n.s.	n.s.	n.s.	n.s.
	Sur/RSA	***	*	n.s.	*
Testicles	Ref/Sur	**	*	n.s.	n.s.
	Ref/RSA	***	n.s.	*	n.s.
	Sur/RSA	**	n.s.	*	n.s.
Ovaries	Ref/Sur	*	***	n.s.	n.s.
	Ref/RSA	**	n.s.	n.s.	n.s.
	Sur/RSA	*	***	n.s.	n.s.
Lymph nodes	Ref/Sur	***	**	***	n.s.
	Ref/RSA	n.s.	*	n.s.	•
	Sur/RSA	***	***	***	**

[a] Calculated with Student's *t* test. Key: (n.s.) not significant; (*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$.

[15] Aquasol-2; Packard Instrument Co.

Table IV—Elimination of Carbon-14 after Intravenous Administration of Nanoparticles in Rats [a]

Time	Group[b]	Feces	Urine
Day 0	Ref	0.02 ± 144%	0.96 ± 18%
	Sur	0.02 ± 60%	0.73 ± 13%
	RSA	0.06 ± 0%	—
Day 1	Ref	3.72 ± 3%	0.26 ± 23%
	Sur	2.39 ± 25%	0.34 ± 40%
	RSA	0.68 ± 18%	0.12 ± 9%
Day 2	Ref	0.36 ± 32%	0.04 ± 57%
	Sur	0.83 ± 53%	0.05 ± 32%
	RSA	0.75 ± 7%	0.05 ± 13%
Day 3	Ref	0.15 ± 13%	0.03 ± 67%
	Sur	0.15 ± 21%	0.03 ± 36%
	RSA	0.32 ± 37%	0.03 ± 20%
Day 4	Ref	0.09 ± 68%	0.01 ± 29%
	Sur	0.11 ± 25%	0.01 ± 50%
	RSA	0.15 ± 16%	0.02 ± 29%
Day 5	Ref	0.08 ± 13%	0.01 ± 21%
	Sur	0.08 ± 36%	0.01 ± 50%
	RSA	0.09 ± 7%	0.01 ± 32%
Day 6	Ref	0.05 ± 15%	0.01 ± 31%
	Sur	0.05 ± 24%	0.00 ± 24%
	RSA	0.06 ± 5%	0.01 ± 9%
Day 7	Ref	0.04 ± 18%	0.01 ± 33%
	Sur	0.05 ± 21%	0.00 ± 39%
	RSA	0.06 ± 5%	0.01 ± 17%
Total	Ref	4.06 ± 4%	1.33 ± 14%
	Sur	3.65 ± 24%	1.17 ± 15%
	RSA	2.11 ± 8%	0.25 ± 6%

[a] All values are mean ± *RSD* percent of the administered dose. [b] Key: (Ref) noncoated particles; (Sur) particles treated with surfactant; (RSA) particles coated with rat serum albumin.

group remained in the blood after 0.5 h. This was ~60 times more than that of the other groups. After 2 h, however, the blood level of the surfactant group was of the same order as that of the other groups.

After 0.5 h, 85% of the radioactivity was found in the liver in the reference and the protein groups. This value decreased to ~74% in the reference group but remained constant in the protein group. In the surfactant group, only ~38% was found in the liver after 0.5 h. This value increased to ~60% after 24 h and dropped to 51% after 7 d.

In the lungs, the accumulation after 0.5 h was 1.4% of the administered dose for the reference group. About three times as much was detected in the surfactant group; the value for the protein group was intermediate. The lung radioactivity remained constant for 24 h in the protein group, but it increased almost twofold in the other groups. In all groups, the clearance was essentially complete after 7 d.

In the spleen, the accumulation for the reference and the protein groups showed a similar value of ~4% after 0.5 h, which increased slightly to ~5% after 7 d. In the surfactant group, 21% of the injected dose was localized in the spleen after 0.5 h. This value increased to ~31% after 2 h and dropped to ~23% after 7 d.

In the other organs (kidneys, ovaries, testicles, and lymph nodes) the amount of radioactivity was <1% after 0.5 h, and the values decreased with time. The surfactant group showed a higher accumulation than the reference and protein groups for most values (Tables II and III). Although the absolute quantity in the testicles and ovaries was < 0.1% of the administered dose, higher concentrations of all particle suspensions were found in the ovaries. This higher affinity can be clearly demonstrated using the nanoparticle concentration, given in terms of specific activity, *i.e.*, percent of the dose per gram of organ (wet weight) (Table II). The spleen also showed a very high affinity for the surfactant-treated nanoparticles (3–15 times higher than in the other groups), whereas the affinity of the liver for surfactant-treated particles was significantly lower than the other groups. In all other organs (except the blood after 0.5 h), the specific activity was of the same order of magnitude for all preparations.

Elimination of ^{14}C-Radioactivity—The ^{14}C-elimination in urine and feces was followed for 7 d (Tables IV and V). In all groups, ~1% of the administered dose was excreted in the urine and 2–4% in the feces within 7 d. The majority of the ^{14}C-excretion was found after the first day.

DISCUSSION

It has been suggested that colloidal materials interact with blood components after injection into systemic circulation (13, 25–28). This interaction and coating with blood proteins may influence the subsequent organ distribution of these particles (28, 29). In investigating the sorption behavior of the nanoparticles in aqueous bovine serum albumin solutions, the amount of adsorbed protein was found to be higher with low particle concentrations and with high protein concentrations, as expected. The pH of the incubation medium influenced the adsorption; maximum adsorption was at pH 4.9, the isoelectric point of bovine serum albumin. This finding seems to be a general characteristic of proteinaceous solutions (30). The temperature, in the examined range of 4–37°C, showed only a small influence on the adsorption. Protein adsorption increased with incubation time up to 12 h, when equilibrium apparently occurred.

The greater influence of the pH on the zeta potential of the albumin-coated particles, compared to the noncoated particles, is probably caused by the protonation/deprotonation of the albumin amino groups, but the potential of the noncoated particles is influenced only by surface adsorption of protons or hydroxyl ions. Although a 10-mV difference in zeta potential was observed at pH 7.4, it is not possible to infer that this seemingly insignificant difference would influence body distribution. The effect of charge on distribution warrants further study.

While differences in the organ distribution among the three preparations were observed, the reference group and the protein group show generally the same distribution pattern. The albumin-coating procedure was conducted because albumin has been shown to prevent platelet adhesion and to enhance the biocompatibility of diverse materials (31, 32) and, therefore, was expected to lead to a significant change in the distribution of the particles. However, albumin coating did not alter the organ distribution significantly when compared to the noncoated particles. This suggests that injected noncoated particles soon become coated with serum proteins. Almost 90% of the administered dose in the reference and the protein groups accumulated in the liver and spleen. Since both organs are part of the reticuloendothelial system, this finding suggests that nanoparticles with incorporated or adsorbed antigens hold promise as adjuvants (3, 12). The only difference in the distribution behavior between the reference group and the protein group is that accumulation in the liver remained constant at ~85% in the protein group; in the reference group it decreased from 85% after 30 min to 74% after 7 d.

A distinctively different distribution pattern was obtained in the surfactant group. Only 38% of the administered dose was found in the liver after 30 min, and the accumulation was only 51% after 7 d. This is similar to results reported with poloxamer-coated polystyrene microspheres (15). The apparent loss from the liver between 1 and 7 d indicates some clearance and/or degradation phenomenon. Accumulation was significantly greater in the spleen for the surfactant group (21%) than in the other groups (<4%). After 2 h, the spleen levels increased to 31%, ~12 times higher than the noncoated group and 8 times higher than the protein group. As in the liver, the level of particles in the spleen decreased after 24 h to 13% and increased again to 23% within 7 d. No satisfactory explanation can be given for this oscillation in spleen levels.

Another distinct difference of the surfactant group is an apparent slower

Table V—Comparison of Radioactivity Levels in the Excreta of Rats after Intravenous Administration of Noncoated and Coated Nanoparticles [a]

Comparison	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Total
				Urine					
Ref/Sur	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	n.s.	n.s.
Ref/RSA	—	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	***
Sur/RSA	—	*	n.s.	n.s.	n.s.	*	***	*	***
				Feces					
Ref/Sur	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ref/RSA	n.s.	***	***	*	n.s.	n.s.	n.s.	**	***
Sur/RSA	**	**	n.s.	*	n.s.	n.s.	n.s.	n.s.	**

[a] Calculated with Student's *t* test. Key: (n.s.) not significant; (*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$.

blood clearance. About 21% of the administered dose still remained in the blood after 30 min, *versus* 0.3% for the other groups. Since nanoparticles may be preferentially taken up by tissues exhibiting enhanced endocytotic activity, such as tumor tissue (33, 34), a longer persistence of the particles in the circulatory system may enhance their potential for accumulation in cancerous tissue. Therefore, surface-treated nanoparticles may possibly be useful as carriers for toxic anticancer drugs.

The accumulation of nanoparticles in the lung was lower than that reported previously (12), in which particles similar to the reference group were used. In that study ~22% of the particles were localized in the lungs after 30 min, a value that decreased continuously to ~13% after 7 d, in this study only 1.4% was detected in the lungs after 30 min. After 24 h and 7 d, 3.3 and 0.2%, respectively, of the administered dose was found in the lungs. The surfactant and the protein groups showed similar lung accumulations of 3.8 and 2.3% after 30 min, respectively, and both values decreased to <1% within 7 d. This finding confirms that if agglomeration occurred, the clusters were small enough to pass through the capillary network in the lungs.

Coagulated particle clusters have been reported to be mechanically trapped by the lung capillaries (13, 14). However, these were clusters of microspheres >3μm in diameter. According to a recent review by Kreuter (28), particles in the submicron range, *i.e.*, 125 nm–1 μm, do not show any major differences in body distribution. It has also been shown that gentle mixing of polymethyl methacrylate nanoparticles with plasma will cause disintegration of particle clusters (20). It is, therefore, believed that the difference in the distribution behavior between surfactant-coated and noncoated particles was due to a difference in surface properties. The difference in the distribution behavior between the noncoated particles in this study and those used previously (12) can be attributed to certain refinements in methodology which minimized agglomeration tendencies. These refinements include sonication by means of a microtip immediately before each injection, as opposed to sonication of the total suspension in a bath-type sonicator ~20 min before the experiment. Additionally, in this study the particles were suspended in 2 mL of phosphate-buffered saline and injected over a period of 2 min into the femoral vein of animals anesthetized with ether; in the earlier experiment the particles were suspended in 0.5 mL and injected over 5–10 s into the tail vein without anesthetization.

The results of this study clearly demonstrate that surfactant-coated nanoparticles result in a slower blood clearance and a significantly different organ distribution after intravenous administration. The targeting of incorporated or adsorbed drugs to specific organs may, therefore, be influenced by pretreatment of the carrier particles with surface-active agents. More experiments with other surfactants are required to gain systematic information about factors that influence the distribution behavior of nanoparticles after intravenous injection. These factors include the surface charge as well as the chemical nature of the surface and, consequently, the agglomeration tendency of the particles. Although scanning electron micrographs of the coated particles did not reveal aggregation, they were not discernibly different from micrographs of noncoated particles. This study has revealed extended residence in the blood and differences in organ distribution patterns with coated nanoparticles. Therefore, physicochemical characterization of the particles, including particle size distribution, is essential to further organ distribution studies.

REFERENCES

(1) T. M. S. Chang, *Enzyme*, **14,** 95 (1972/73).

(2) K. F. O'Driscoll, R. A. Korus, T. Ohnuma, and I. M. Walozack, *J. Pharmacol. Exp. Ther.*, **195,** 382 (1975).

(3) J. Kreuter and P. P. Speiser, *Infect. Immun.*, **13,** 204 (1976).

(4) G. Birrenbach and P. P. Speiser, *J. Pharm. Sci.*, **65,** 1763 (1976).

(5) J. J. Marty, R. C. Oppenheim, and P. P. Speiser, *Pharm. Acta Helv.*, **53,** 17 (1978).

(6) J. Kreuter, *Infect. Immun.*, **53,** 33 (1978).

(7) J. Charnley, "Acrylic Cement in Orthopaedic Surgery," Livingstone, Edinburgh, Scotland, 1970.

(8) F. Bischoff and G. Brynson, *Prog. Exp. Tumor Res.*, **5,** 85 (1964).

(9) B. S. Oppenheimer, E. T. Oppenheimer, I. Danishefsky, A. P. Stout, and F. R. Eirich, *Cancer Res.*, **15,** 333 (1955).

(10) L. Tomatis, *Tumori*, **52,** 165 (1966).

(11) I. Sjöholm and P. Edman, *J. Pharmacol. Exp. Ther.*, **211,** 656 (1979).

(12) J. Kreuter, U. Taeuber, and V. Illi, *J. Pharm. Sci.*, **68,** 1443 (1979).

(13) M. Kanke, G. H. Simmons, D. L. Weiss, B. A. Bivins, and P. P. DeLuca, *J. Pharm. Sci.*, **69,** 755 (1980).

(14) H. G. Schroeder, G. H. Simmons, and P. P. DeLuca, *J. Pharm. Sci.*, **67,** 504 (1978).

(15) L. Illum and S. S. Davis, *J. Pharm. Sci.* **72,** 1086 (1983).

(16) K. M. D'Amico, S. S. Davis, M. Frier, P. Hansrani, J. G. Hardy, and C. G. Wilson, *Br. J. Pharmacol.*, **72,** 83 (1981).

(17) R. Jeppson and S. Roessner, *Acta Pharmacol. Toxicol.*, **37,** 134 (1975).

(18) D. J. Wilkins and P. A. Meyers, *Br. J. Exp. Pathol.*, **47,** 568 (1966).

(19) D. J. Wilkins and P. A. Meyers, in "Surface Chemistry of Biological Systems," N. Blank, Ed., Academic, New York, N.Y., 1970, p. 217.

(20) J. Krueter, *Int. J. Pharm.*, **14,** 43 (1983).

(21) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193,** 265 (1951).

(22) C. D. Barnes and L. G. Etherington, "Drug Dosage in Laboratorium Animals," University of California Press, Berkeley, Calif., 1973.

(23) Tri-Carb Sample Oxidizer Operation Manual, Packard Instrument Co., Downers Grove, Ill., 1980.

(24) P. L. Altman and D. S. Dittmer, "Biological Data Book," Fed. Am. Soc. Exp. Biol., Rockville, Md., 1964, p. 264.

(25) M. E. Soderquist and A. G. Walton, *J. Colloid Interface Sci.*, **75,** 386 (1980).

(26) J. Slack, M. Kanke, G. Simmons, and P. P. DeLuca, *J. Pharm. Sci.*, **70,** 660 (1981).

(27) J. Kreuter, *Pharm. Acta. Helv.*, **58,** 217 (1983).

(28) M. Kanke, I. S. Sniecinski, and P. P. DeLuca, *J. Parenteral Sci. Tech.*, **37**(6) (1983).

(29) T. M. Saba, *Arch. Intern. Med.*, **126,** 1031 (1970).

(30) W. Norde and J. Lyklema, *J. Colloid Interface Sci.*, **66,** 257 (1978).

(31) R. E. Baier and R. T. Dutton, *J. Biomed. Mater. Res.*, **3,** 191 (1978).

(32) T. M. S. Chang, "Artificial Cells," Thomas, Springfield, Ill., 1972.

(33) P. Couvreur, P. Tulkens, M. Roland, A. Trouet, and P. P. Speiser, *FEBS Lett.*, **84,** 323 (1977).

(34) F. Brasseur, P. Couvreur, B. Kante, L. Deckers-Passau, M. Roland, C. Deckers, and P. P. Speiser, *Eur. J. Cancer*, **16,** 1441 (1980).

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