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Nanoparticle Uptake by the Rat Gastrointestinal Mucosa: Quantitation and Particle Size Dependency

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Abstract—Polystyrene microspheres in the size range 50 nm to 3 μm were fed by gavage to female Sprague Dawley rats daily for 10 days at a dose of 1·25 mg kg^{-1}. Previous histological evidence of the uptake of these particles and their absorption across the gastrointestinal tract and passage via the mesentery lymph supply and lymph nodes to the liver and spleen was confirmed by analysis of tissues for the presence of polystyrene by gel permeation chromatography. Measurement of radioactivity of tissues following administration of 100 nm and 1 μm I^{125}-labelled polystyrene latex particles for 8 days was corroborative although less secure because of the potential lability of the labelled particles. The extent of absorption of 50 nm particles under the conditions of these experiments was 34% and of the 100 nm particles 26% (as measured by determination of polystyrene content), of which total, about 7% (50 nm) and 4% (100 nm), was in the liver, spleen, blood and bone marrow. Particles larger than 100 nm did not reach the bone marrow, and those larger than 300 nm were absent from blood. No particles were detected in heart or lung tissue.

Oral immunization and delivery by mouth of molecules which presently must be given parenterally has obvious advantages, and might be possible if absorption of colloidal carriers for vaccines and drugs could be achieved in useful quantities. Carriers can protect labile molecules from degradation in the gastrointestinal tract, and, if absorbed intact, might transport labile and non-absorbable molecules into the systemic circulation. The possibility of uptake and absorption of nanoparticles and microparticles by the gastrointestinal tract has been a controversial issue, although there is now accumulated evidence that it can and does occur (Thompson et al 1960; Sanders & Ashworth 1961; Volkheimer 1968, 1975; LeFevre et al 1978, 1989; Aprahamian et al 1987; Alpar et al 1989; Jani et al 1989; Kreuter et al 1989), although reliable quantitative estimates of uptake are largely lacking. Alpar et al (1989) claimed that about 39% of administered 1·1 μm polystyrene latex particles appear in the circulation after 45 min in the rat. We (Jani et al 1989) showed that polystyrene latex particles in the size range of 100 nm to 3 μm were taken up by the rat gastrointestinal tract via the gut-associated lymphoid tissue (GALT) and particles, with the exception of the 3 μm sample, were subsequently transported to the liver, albeit in low numbers. Polystyrene particles were seen in discrete anatomical compartments and structures such as Peyer's patches, the mesenteric lymph vessels and lymph nodes and, in lesser amounts, in the liver and spleen. The evidence from our laboratories (Jani et al 1989), suggests that the sites of latex uptake are the lymphoid aggregates of the Peyer's patches in the gastrointestinal tract. Absorbed microspheres then traverse the mesentery via the mesentery lymph vessels towards the mesenteric lymph nodes and are transported from the lymphatic circulation into the venous circulation and subsequently into the liver.

LeFevre et al (1978, 1989) have similarly shown uptake of latex microspheres by the lymphoid aggregates of the Peyer's patches. Many particulate materials find their way into the gut, from inhaled and swallowed dusts, to insoluble drugs and materials in pharmaceutical formulations such as titanium dioxide, aluminium and bismuth salts, and many others. Without quantifying the extent of uptake, the question whether uptake of colloidal carriers implies anything of potential therapeutic, or indeed, toxicological significance cannot be answered.

The "porosity" of the epithelial membrane to particulates could be due to the macrophagic and pinocytotic activity of the M-cells, overlying the Peyer's patches, as discussed by McClugage et al (1986) and Wolf & Bye (1984). Aprahamian et al (1987) reported transmucosal absorption of 100–200 nm diameter polyalkylcyanoacrylate nanoparticles; insulin administered in these particles (220 nm diam.) resulted in decreased glucose levels allowing the authors to conclude that polyalkylcyanoacrylate nanoparticles could be useful carriers for oral administration of peptides and other labile drugs (Damge et al 1987, 1988).

What factors affect uptake of particles? In a recent article, Eldridge et al (1990) found that the nature and surface characteristics of the polymer microsphere affects uptake, some cellulosic nanoparticles being unabsorbed. In our work (Jani et al 1989) there was an obvious qualitative difference in the absorption and uptake of particles of different size but the results obviously required quantification. The present paper describes both the use of radiolabelled nanospheres and microspheres and quantitative analysis of uptake following oral administration of cold particles by gel permeation chromatographic determination of extracted polystyrene, to establish the extent of uptake as a percentage of administered dose.

Materials and Methods

Microspheres

Monodispersed non-ionized polystyrene microspheres with covalently linked fluorescein nominally 50, 100, 300, 500 nm, 1 and 3 μm in diameter were used as received from Polysciences Ltd (Northampton, UK). Particle sizes were

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Table 1. Characteristics of the polystyrene microspheres.

No. of particles mL^{-1} (2·5% w/v)	Nominal size (nm)	Size* (±s.d.) (nm)	Lot number†	Apparent mol. wt**
$4·11 \times 10^{14}$	50	48±2	85470	410,000
2×10^{13}	100	130±10	61034	220,000
$1·68 \times 10^{12}$	300	298±6	70125	800,000
$2·8 \times 10^{11}$	500	540±5	84102	380,000
$6·25 \times 10^{10}$	1000	950±10	71825	410,000
$1·6 \times 10^{9}$	3000	3060±170	74352	700,000

* Measured by PCS. ** From gel permeation chromatography (see Fig. 1) † Polysciences (Northampton).

confirmed using photon correlation spectroscopy (Malvern Instruments) with the results shown in Table 1.

I^{125} labelled polystyrene spheres

Two mL of 100 nm (2×10^{13} particles mL^{-1}) and 1 μm (6×10^{10} particles mL^{-1}) non-ionized fluorescein-labelled particles (Polysciences, UK) were added to distilled water (2 mL) and 0·01 M NaI (1 mL). To this 10 μL (500 μCi) of Na ^{125}I was added, each addition preceded by bubbling nitrogen to remove air/oxygen. This solution was sealed in a 10–15 mL amber glass vial, under nitrogen. A duplicate vial was processed exactly as above.

The ampoules were gamma-irradiated from a ^{60}Co source (5M rad over 20 h) (Huh et al 1974). The contents of the ampoules were dialysed in Visking tubing to remove free (unbound) radioactive iodide, the Visking tubing having been boiled in double distilled water for 3 h and left to stand overnight in distilled water. The radiolabelled latex was transferred from the ampoule to the Visking tubing using a syringe, and the tube sealed. The dialysis bag was then placed in 800 mL of continuously stirred double distilled water. After 1 h a sample (2 mL) was placed in a glass vial and counted for 100 s. The conical flask was washed and a further 800 mL of water added; after a further 1 h, a second sample was removed and counted. This procedure was repeated until the radioactivity in the dialysate water had reached a constant low value, usually after 100–120 h. All counts were corrected for background.

Oral administration of polystyrene suspension

Female Sprague Dawley adult rats (average wt 200 g; 15–20 weeks old) were used, each group of treated and untreated animals comprising at least 3 rats. The microspheres were administered by gavage, a dose of 1·25 mg kg^{-1} (0·1 mL volume) being administered daily for 10 days, as described by Jani et al (1989). Animals were given free access to water, but fasted for 8–10 h before each dose. After the final dose, the animals were kept for two days in a microsphere free environment to clear the gastrointestinal tract of unabsorbed microspheres.

Oral administration of ^{125}I polystyrene beads

Female Sprague Dawley adult rats (average wt 200 g; 15–20 weeks) were used as above. Each treated group (n=3) received 0·4 mL ($3·2 \times 10^{12}$ particles) (dose 2·00 mg kg^{-1}) of radioactive latex beads daily by gavage for 8 days. The animals were given free access to water, but were fasted for about 8–10 h before the morning dose of the microspheres, food being restored at least 1–2 h after the dosing. The animals were weighed daily and kept in individual metabolic cages to facilitate the daily collection of urine and faeces (for measurement of radioactivity) and to prevent coprophagia. On days 9–10 the animals were kept in a particle-free environment with limited food supply but access to drinking water. On day 11 the animals received water only and again urine and faeces were collected.

Animals in both sets of experiments were killed by the excess ether method.

Extraction of polystyrene from tissues

Stomach, small intestine (with mesentery lymphatic network and lymph nodes), colon, liver, spleen, heart, kidney and lungs were analysed. Stomach, small intestine and colon were longitudinally dissected and washed gently to remove any unabsorbed microspheres, avoiding damage. In selected groups of animals, blood samples (2 mL) were also taken.

Dissected organs were clamped with freeze-clamping tongs and the resulting "wafers" of organs were subjected to further freeze drying for 60 h, ensuring total removal of water from the samples, which were then transferred to a mortar (porous pot) pre-cooled to −70°C and containing liquid nitrogen. The organ was ground to a fine powder, which was transferred to a stoppered flask and 15–20 mL chloroform added. This was mechanically shaken overnight in a water bath at 30°C. After two further washes with chloroform, the slurry of the ground organ with chloroform was first filtered through a glass wool wedge in a glass funnel. The chloroform was evaporated to dryness on a rotary evaporator (Rotavapor-R, Buchi) under reduced pressure. The dry residue was resuspended in an accurately measured volume (up to 6 mL) of tetrahydrofuran (THF), filtered through a solvent resistant filter (Millex-SR, Millipore, UK, 0·5 μm) and a sample was analysed using gel permeation chromatography. At all times, steps were taken to prevent cross contamination of the latex polymer between samples.

Gel permeation chromatography

THF, stabilized with 0·1% quinol, was used as solvent and mobile phase; all work was carried out using glass containers: 2 × 300 × 7·5 mm, 10 μm mixed pore highly cross-linked spherical macroporous polystyrene-divinylbenzene matrix (PLGel) columns (Polymer Laboratories Ltd 1112-6100) preceded by a 1 × 50 × 7·5 mm 10 μm mixed pore guard column (PLGel; Polymer Laboratories Ltd 1110-1120) a Rheodyne injector valve (Waters USA 7125) fitted with a 100 μL sample loop, formed the gel permeation components. A Waters 484 tunable UV absorbance detector, was used in conjunction with a chart recorder/integrator (Hewlett Packard 3396A). The polystyrene calibration mixture was a gift from Polymer Laboratories UK. A flow rate of 1 mL min^{-1} was maintained. The total retention time calibrated using a very dilute solution of toluene in THF was 27 min.

Molecular weight calibration

A polystyrene calibration mixture containing polymers (about 5 mg) of mol. wt 4×10^6, 950×10^3, 165×10^3, $28·5 \times 10^3$, 7000 and 1250 was dissolved in 6 mL THF to give approximately 0·08% w/v of the polystyrene mixture. A

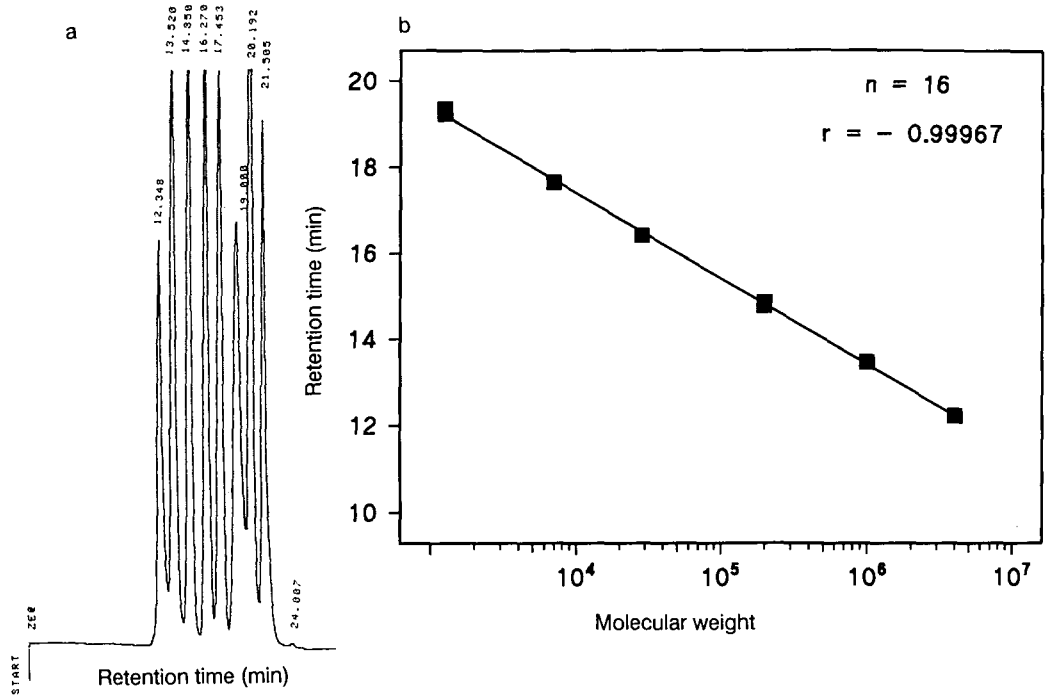

FIG. 1. A. Gel permeation chromatogram of the standard (Polymer Laboratories Ltd, UK) mixture of polystyrene of a range of molecular weights; B is derived from such plots (n = 16) and shows the molecular weight of polystyrene versus retention time, under the conditions of the experiment. These data were used to estimate the mean molecular weight of the polystyrene which constitutes the latex particles, the results being recorded in Table 1.

number of 30 μL samples of the polystyrene mixture were injected and the elution profile of a given molecular weight corresponding with its retention time was obtained for the system. A typical chromatogram and the relationship between the molecular weights and the retention time are shown in Fig. 1. Individual samples of polystyrene microspheres were dissolved in THF (1 mg/100 mL). Thirty μL samples were injected and the elution profile was obtained, from which the molecular weight distribution was obtained. The weight average mol. weight can be obtained from the chromatogram for the individual sample (Evans 1973; Yau et al 1979).

Recovery of polystyrene from tissue

This was assessed by perfusing a suspension of polystyrene latex beads into a rat liver (7·5 g) in-situ, ensuring that the exits were ligated so the total dose of latex remained in the liver. The liver was removed after 30 min, freeze-dried as above and extracted with chloroform and THF. This was repeated for 300, 500 nm and 1 μm latex particles. The tissue samples obtained were treated as described above and the percentage recovery obtained. A mean value of 74% was obtained; extraction values were corrected by a constant factor throughout.

Sensitivity

For latex of 50, 100, 500 nm and 1·0 μm size it was found that the lowest limit of detection was $6{\cdot}25 \times 10^{-5}$% w/v polystyrene.

Measurement of radioactivity

For measurement of radioactivity, organs were separately homogenized in 5% w/v potassium hydroxide solution and small samples assayed. A control group, also with 3 animals, was treated in the same manner and received 0·4 mL of 0·9% NaCl (saline) by gavage daily. The urine and faeces were collected and weighed, and homogenized in 5% w/v potassium hydroxide and activity measured as background. On the last day of the experiment, the organs were removed and homogenized in 5% w/v KOH, the gamma-emission measured and used for background correction.

Results and Discussion

In this work we have endeavoured to quantitate the oral absorption and the location of polystyrene latices of a range of sizes. Of the two techniques used, gel permeation chromatography is more reliable than the technique involving the radiolabelled latex. We have included the results of the ^{125}I studies as confirmatory, but consider the direct measurements of the polystyrene to be the definitive results.

Results are shown in Table 2 and graphically in Figs 2–8. Fig. 2 suggests that total uptake ranges from about 33% (50 nm latex) to about 7% (1 μm latex); this figure also demonstrates the size-dependency of cumulative uptake in all tissues examined. While these data and those which include gastrointestinal tissue (Figs 3, 4) may be inflated because of the possible inclusion of particles adsorbed, but that did not penetrate the epithelial barrier, there is no doubt that particles found in tissues physically separate are conclusive proof of transport. The results show the presence in such organs (Figs 5, 6) of polystyrene particles in the size range 50 nm–1 μm, although the largest particles studied, which are 3·0 μm in diameter, do not apparently migrate to the liver

Table 2. Polystyrene latex uptake (per cent ± s.d.) as a function of size and tissue.

Organs (g) (n = 21)	Particle size 50 nm	100 nm	300 nm	500 nm	1 μm	3·0 μm
Stomach (1·76 ± 0·163)	1·1 ± 0·189	0·65 ± 0·15	0·45 ± 0·07	1·013 ± 0·06	0·27 ± 0·12	1·376 ± 0·08
Small intestine with Peyer's patches and mesentery (5·142 ± 0·386)	12 ± 0·47	3·4 ± 0·21	2·027 ± 0·14	4·28 ± 0·52	1·082 ± 0·12	3·627 ± 0·05
Colon with Peyer's patches, appendix and mesentery (3·621 ± 0·282)	14 ± 1·46	16 ± 1·61	4·32 ± 0·47	6·53 ± 0·75	2·43 ± 0·46	7·53 ± 0·5
Liver (8·335 ± 0·662)	3·3 ± 0·93	3·8 ± 0·73	1·38 ± 0·35	1·38 ± 0·34	0·54 ± 0·03	Not detected
Spleen (0·53 ± 0·083)	0·92 ± 0·22	0·69 ± 0·07	0·21 ± 0·03	0·507 ± 0·03	0·24 ± 0·01	Not detected
Blood 2 mL per animal (n = 3)	2·2 ± 0·39	1·255 ± 0·44	1·1 ± 0·19	Not detected	Not detected	Not detected
Bone marrow 2 mL per animal (n = 3)	NE	0·1 ± 0·011	Not detected	Not detected	Not detected	Not detected
Kidney (2) (1·7 ± 0·282)	0·2 ± 0·05	Not detected	Not detected	Not detected	Not detected	Not detected
Lungs (2) (1·302 ± 0·061)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Heart (0·682 ± 0·063)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Total	33·72 ± 3·71	25·95 ± 3·21	9·487 ± 1·25	13·71 ± 1·2	4·562 ± 0·73	*

NE = not examined. * see text

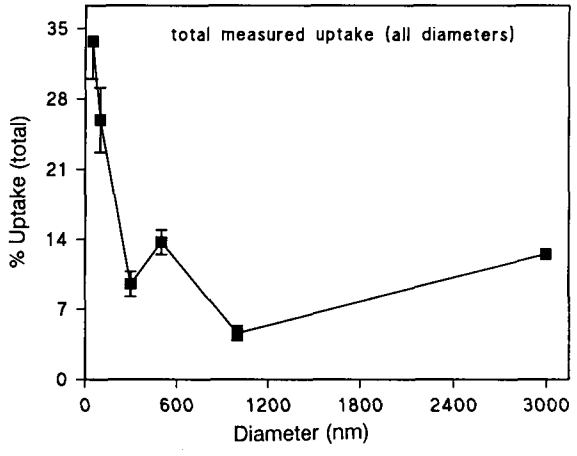

FIG. 2. The total uptake of polystyrene microspheres from the gastrointestinal tract of female Sprague Dawley rats (n = 3) following oral administration for 10 days at a dose of 1·25 mg kg^{-1} as measured by extraction of polystyrene from the tissues mentioned in the text, the data being plotted as a function of the particle size of the latex.

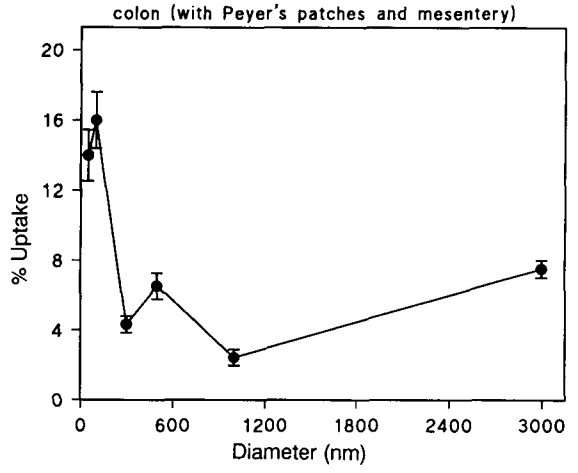

FIG. 4. Uptake of orally administered polystyrene latex particles by the colon, as a function of latex size. Histological evidence points to the accumulation of particles in the lymphoid aggregates present in the colon.

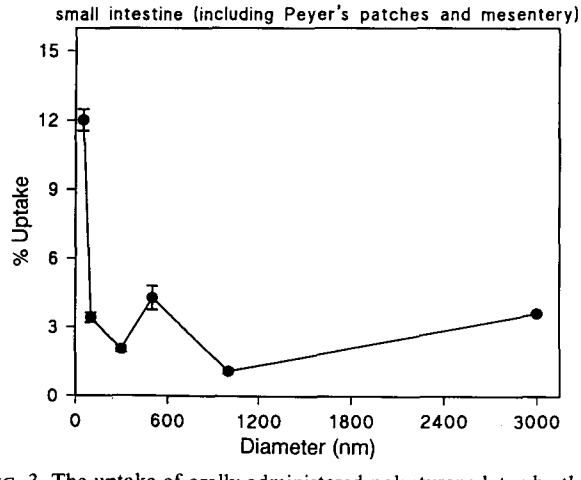

FIG. 3. The uptake of orally administered polystyrene latex by the small intestine as analysed by gel permeation chromatography, as a function of particle diameter. Histological evidence showed that the microspheres are present mainly in the Peyer's patches and the mesentery network of the small intestine.

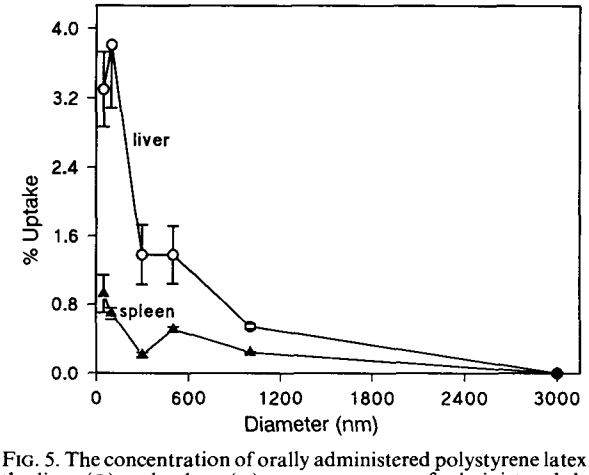

FIG. 5. The concentration of orally administered polystyrene latex in the liver (○) and spleen (▲) as a percentage of administered dose plotted as a function of particle size. The particles are present mainly in the macrophage cells of the liver and the granular follicles of the spleen.

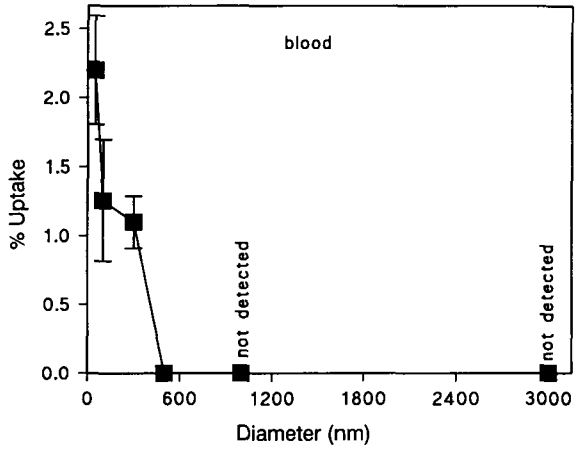

FIG. 6. The presence of polystyrene latex particles of varying size in the blood. No evidence of polystyrene was found in blood after administration of particles in the size range 500 nm to 3 μm; the limit of detection of $6{\cdot}25 \times 10^{-5}$% w/v does not allow a conclusion that there are no particles in the blood, but the probability is reduced at these larger diameters.

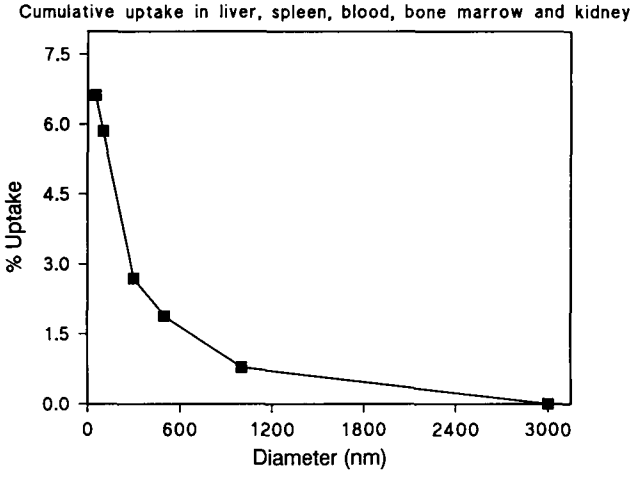

FIG. 7. The cumulative uptake of polystyrene, orally administered to female Sprague Dawley rats for 10 days at a dose of 1·25 mg kg^{-1}, as a function of particle diameter in the liver, spleen, blood, bone marrow and kidney. In the case of particles of 500 nm and 1 μm these data refer only to liver and spleen as no microspheres were detected in blood, bone marrow and kidney. For the 300 nm latex the data refer to liver, spleen and blood.

and spleen and blood. The total polystyrene in liver, spleen, bone marrow and blood possibly show the truest extent of translocation, falling from 6% for 50 nm latex to around 1% for 1 μm and zero for 3 μm particles (Fig. 7).

Although 3·0 μm latex beads were not detected either in the systemic circulation or in the liver and the spleen, the histological evidence was that these particles were adsorbed and immobile within the submucosal layer of the thicker mucosa and the Peyer's patches. Eldridge et al (1990) have also reported similar findings. The smaller beads are quickly translocated into the serosal layer and thence into the systemic circulation (Fig. 6). The apparently high uptake of the 3 μm particles may be explained in part by adsorption. However, adsorption of smaller particles is undoubtedly a prelude to absorption.

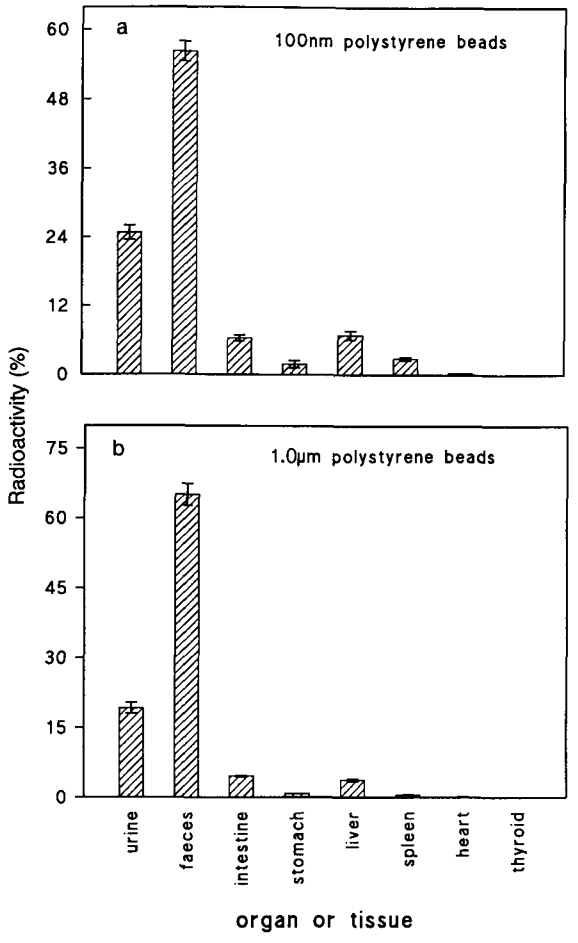

FIG. 8. Measurement of radioactivity following administration a) of 100 nm and b) of 1 μm I^{125}-labelled polystyrene (see text) at a dose of 2 mg kg^{-1} for 8 days to rats; the extent of uptake in the liver and spleen is comparable to that estimated by determination of polystyrene levels.

Results from the measurement of radioactivity for 100 nm and 1·0 μm particles are shown in Fig. 8. The high levels of radioactivity in the urine cast doubt on the stability of the labelled latex; levels in the tissues of the intestine, stomach, liver and spleen are comparable with those discussed above. The urinary levels are thought to result from the undialysed free iodine.

After the exposure to ^{60}Co the particle suspensions were examined and it was found that they were stable with regard to the external surfaces and covalent bonding and to coagulation, the particles undergoing no detectable change, as seen both by electron microscopy and light scattering. In-vitro studies with liver and gastrointestinal enzyme tissue homogenate prepared in Tyrode solution gave little (1%) evidence of cleavage; however, the data indicate high levels of activity in the urine which suggests breakdown. Chronologically these were the first experiments carried out; the inexplicable urinary data led to the histological work and subsequently what we consider to be the definitive work on the analytical determination of the polystyrene content of tissues and organs.

Our histological work (Jani et al 1989) indicated that the particles in the liver were mainly present in the Kupffer cells

and the endothelial cells of the sinusoids. The Kupffer cells have active fixed macrophages which line the sinusoids alongside regular endothelial cells. Some investigators (Weiss & Greep 1977) believe that the endothelial cells and Kupffer cells belong to the same cell lines, and their different appearances reflect different functional states; both cell types have a capacity for phagocytosis.

Heart and lungs revealed no latex particles, and, except for the 50 nm particles, no polystyrene was detected in the kidney, while 100 nm latex particles seem to represent the upper limit for transport into the bone marrow.

We have so far shown that particulate uptake in the gastrointestinal tract takes place mainly at the Peyer's patches, which are rich in lymphatic supply and mononuclear phagocytic cells; the beads then are translocated to the mesentery network especially to the mesentery nodes. The microspheres, within the size constraints discussed, while circulating in the lymph, would eventually enter the liver and general circulation. The venous circulation would transfer the microspheres to the liver, the blood circulation of which transports the beads to the liver sinusoids where they are phagocytosed by the Kupffer cells and the epithelial cells of the sinusoid spaces. It is pertinent that there is a direct lymphatic portal circulation connection from the mesometrial wall of the Peyer's patches and the mesentery network and the other parts of the gastrointestinal tract.

Conclusions

The translocation of intact particles in measurable quantities across the gastrointestinal wall has several toxicological and pharmaceutical consequences. We have shown that there is an uptake of polystyrene particles of 50 nm (to the extent of 7%) in systemic organs, after oral administration of microspheres daily for 10 days.

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