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Nanosphere and microsphere uptake via Peyer's patches: observation of the rate of uptake in the rat after a single oral dose

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Summary

Polystyrene latex particles of diameter 50 nm, 500 nm and 1 μm were fed by oral gavage as a single dose to female Sprague-Dawley rats. At intervals following administration, histological examination of tissues allowed the uptake and movement of the particles to be monitored, at least semiquantitatively. Uptake is relatively rapid. At 6 h, 50 nm particles were seen in considerable numbers in Peyer's patches. The rate of uptake is particle-size dependent, smaller particles gaining access to all tissues more rapidly. At 6 h, 50 nm particles can also be detected in the mesentery, while 1 μm particles cannot be observed until about 12 h after administration. Peak concentrations of 500 nm and 1 μm particles occur in the Peyer's patches at 18 h and in the lymph nodes at 12 and 24 h, respectively. Particles of 500 nm and 1 μm appear in the liver after 18 h, 50 nm particles reaching this organ in small quantities in 12 h.

Introduction

Following oral administration, particles as diverse as 1–5 μm resin particles (Payne et al., 1960) and 50 nm viral particles (Sicinski et al., 1990) pass through the gut mucosa by way of organised structures such as the Peyer's patches and their specialised absorptive M-cells. In previous papers we have demonstrated (Jani et al., 1989; 1990) the uptake and subsequent translocation of polystyrene latex colloids in the size range 50 nm to 3 μm to organs such as the lymph nodes, liver and spleen after 10 days of oral administration of microspheres, confirming the data of many other workers (for reviews, see O'Hagan, 1987; Gilligan and Li Wan Po, 1991).

Volkheimer (1968, 1975) claimed uptake of orally administered starch granules, ranging in diameter from 7 μm to 100 μm (12×10^6 granules g^{-1}) these being subsequently observed in the venous blood within 10–30 min of administration. He speculated that the passage of particulate matter across the intestinal barrier was a paracellular event, for which he used the term 'persorption'. This results from the activity of the

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muscularis mucosa layer of the gut, kneading the particles between cells at the desquamation zones of the intestinal villi.

Quantitation of uptake has been attempted in several previous investigations by employing various methods (Pappo and Ermak, 1989; Ebel, 1990; Eldridge et al., 1990). We analysed polystyrene in all target organs, after oral administration of polystyrene nanospheres for 10 days by gel permeation chromatography (Florence et al., 1990; Jani et al., 1990). It is important to investigate the rate of uptake of colloidal particles as estimates of rates of absorption and translocation are few. Alpar et al. (1989) detected 1 μm albumin microspheres in the venous circulation within 10 min of their oral administration, appearing maximally at 45 min. LeFevre et al. (1980), comparing single and continuous oral dosing of 5–15 μm particles, showed that the total intake was virtually unchanged. No microspheres were found in 0.5 ml of heart blood after 4 h of sampling. However, tissue samples showed the presence of some beads 4 h following a single dose but none of the larger 15 μm particles were found in any tissue. Eldridge et al. (1989) observed the migration of coumarin-labelled poly(DL-lactide-co-glycolide) microspheres over a period of 35 days.

Our work (Jani et al., 1989, 1990; Florence et al., 1990), showed uptake to be size dependent in the range 50 nm to 3 μm. In this paper we have observed the disposition of particles of 50, 500 and 1000 nm after a single oral dose, delivered by gavage, to female rats and have determined the rate of uptake histologically.

Materials and Methods

Microspheres

Monodispersed non-ionised polystyrene microspheres with covalently linked fluorescein (nominally 50 nm, 500 nm and 1 μm) were used as received from Polysciences Ltd (Northampton). Particle sizes were confirmed using photon correlation spectroscopy (Jani et al., 1989).

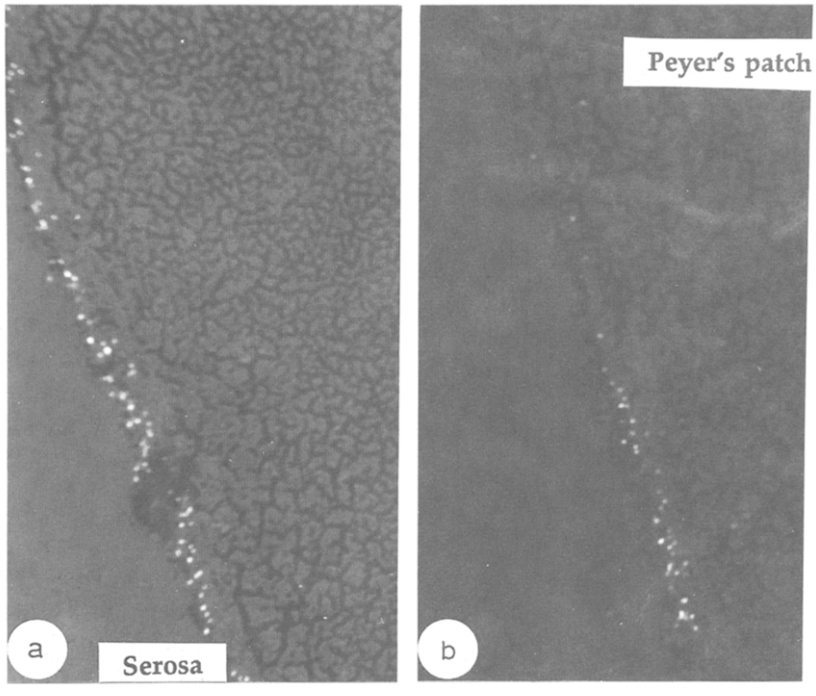

Fig. 1. Fluorescent polystyrene particles (500 nm diameter) (a) 12 h and (b) 18 h after oral administration already collected at the serosal side of a Peyer's patch.

Animals

Female Sprague-Dawley adult rats (average weight, 150 g; age, 12 weeks) were used. Kept under normal conditions, the animals were divided into three groups, each group being further divided into five groups containing four rats each. 12 female rats were treated as controls.

The animals were kept under standard conditions, with free access to water and food for 1 day after removal from the animal house. The food was removed 10 h prior to administration of the single dose. The microspheres were administered in a dose of 12.5 mg kg^{-1} (= 0.1 ml) by oral gavage. The animals were given free access to water, and were kept in individual metabolic cages to prevent coprophagia. Four animals in each of the main group were killed at 6, 12, 18, 24 and 36 h, by excess ether. Stomach, intestine (with mesentery network), colon, liver, spleen, kidney, heart, and lungs were carefully removed to avoid contamination of microspheres, the tissues weighed and stored in liquid nitrogen before the preparation of frozen sections using a cryostat. Sections were rapidly prepared for each tissue stated above from two animals in each group and viewed under a fluorescence microscope. The remaining tissues of other animals were stored at −70°C to be used later for quantitative analysis.

Histological analysis of each section was carried out as reported before (Jani et al., 1989), using fluorescence microscopy.

Results and Discussion

In earlier work, we (Jani et al., 1989) showed histologically the uptake of the polystyrene microspheres and their subsequent translocation from the gastrointestinal tract, and demonstrated that uptake took place mainly from the lymphoid aggregates of the gut-associated lymphoid tissue (GALT), specifically the Peyer's patches. The subsequent translocation of the microspheres was unequivocally towards the mesentery node by way of the mesentery blood and lymph vessels. Our previous data were obtained after 10 days daily dosing. In this paper, a single oral dose was administered, after which the presence of non-ionic fluorescent polystyrene microspheres could be detected (Figs 1–3) at various short time intervals in the Peyer's patches and mesentery network and vessels, respectively, establishing that there is a time-dependent increase in particle concentration, maximal in the mesentery network at about 18 h and reducing at 24 h.

In the present study, uptake of the polystyrene spheres is shown to be very rapid for the smaller 50 nm spheres, moderately rapid for those of 500 nm size and slow for the 1 μm beads. The findings are summarised in Table 1, which lists the rise and fall of levels of polystyrene nanospheres in various tissues as a function of time. Fig. 3a shows the presence of large numbers of microspheres in the lymph vessels at 12 h; Fig. 3b, on the other hand, depicts a similar section at 18 h where the vessel, cut in a horizontal plane, displays the microspheres as free particles, emphasising that these may be brought into the network both by phagocytes and by the blind open lymphatic tubules present in the Peyer's patches.

Studies of the rate of uptake of the latex beads have been published previously by Sass et al. (1990), Pappo and Ermak (1989) and LeFevre et al. (1980). The work of Sass and colleagues (1990) on the rate of uptake refers to the administration of the latex particles directly to Peyer's patches, thus avoiding the problem of the spontaneous transit of microspheres through the stomach and small intestine, avoiding also entrapment of the microspheres in the viscous mucus layer. Their use of 0.5 ml quantities of 500 nm and 1 μm particles provided rapid and equal uptake. Administration of such a large volume in a closed loop of about 5 cm might cause damage to the delicate epithelial structures. Nevertheless, the work clearly shows evidence of 500 nm and 1 μm particles in the vital sites of the Peyer's patches.

Pappo and Ermak (1989) administered fluorescent microspheres (average size: 670 nm) into intestinal loops of rabbits, observing that after presentation to the membraneous follicular absorptive sites, it took 90 min to translocate sufficient particles to the domes of the Peyer's patches. Our results show the presence of spheres at the serosal layer of the Peyer's patches 6 h after oral administration. 50 nm spheres also accumulated

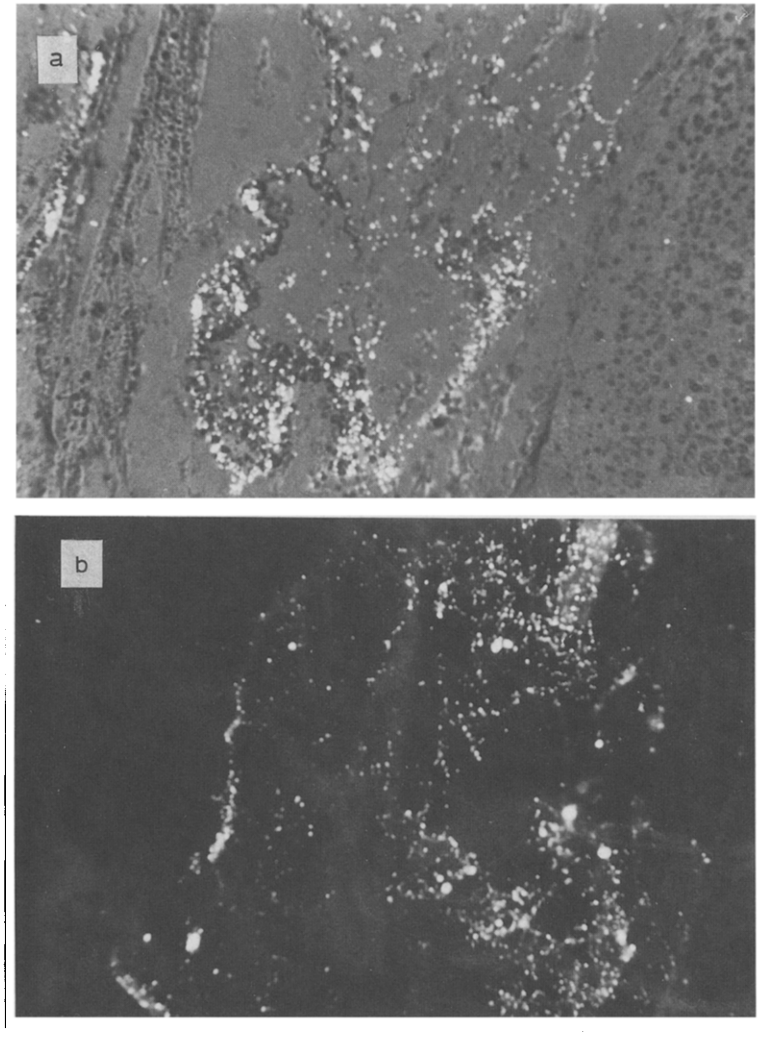

Fig. 2. (a) Photomicrograph (×200) of a frozen section of mesentery lymph network near the lymph node, depicting non-ionic fluorescent 500 nm diameter polystyrene microspheres at 6 h after single dose experiments in female Sprague Dawley rats. (b,c) Photomicrographs of a similar area of the mesentery network at 12 h (b) and 24 h (c) intervals after dosing as explained in the text.

in sufficient quantity to be visible in the mesentery network within this time, the amount peaking at 12 h in the mesentery nodes. Particles were evident in tissues such as liver and spleen after 18 h.

After a single oral gavage dose, the rate of uptake of 500 nm latex was not as rapid as that of the 50 nm spheres. For example, the uptake through the Peyer's patches was maximal at 12 h, while the presence of the 500 nm spheres in the mesentery network peaked between 12 and 18 h, particles beginning to concentrate in the nodes at the same time. The presence of the 500 nm latex spheres in liver and spleen commenced at 18 h, persisting in these tissues after 36 h. The translocation of the 50 nm and 500 nm latex spheres and their subsequent concentration in the various tissues of the reticuloendothelial system are clearly a function of size.

The larger 1 μm latex spheres from a single dose were poorly absorbed. Their presence in Peyer's patches, mesentery network and nodes,

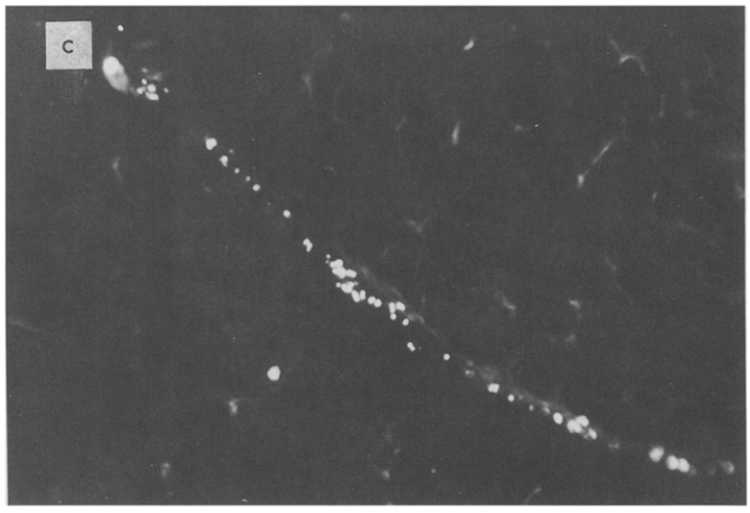

Fig. 2 (c).

liver and spleen was less obvious than that of smaller sizes, but particles persisted up to 36 h. Because of onward transit, the presence of 50 nm latex spheres in the mesentery nodes and Peyer's patches at 24 h was negligible, confirming that they are rapidly taken up and translocated to the liver and spleen; the uptake and presence of 500 nm latex spheres in the tissues of mesentery was abundant up to 24 h after oral administration. It was also observed that the mucus over the absorptive layers, however thin (Bye et al., 1984; Wolf and Bye, 1984; Neutra et al., 1987; Owen et al., 1988), did act as a microsphere trap (Fig. 4). Fig. 4 shows the 500 nm size diameter micro-

TABLE 1

Size and time dependence of latex uptake observed microscopically

Organs examined	6 h	12 h	18 h	24 h	36 h
Sizes of latex:	50 nm 500 nm 1 μm	50 nm 500 nm 1 μm	50 nm 500 nm 1 μm	50 nm 500 nm 1 μm	50 nm 500 nm 1 μm
Peyer's patches	+ + + + +	+ + + + + +	+ + + + + + +	− + + +	− + +
Mesentery lymph network and nodes	+ + + + + −	+ + + + + + +	+ + + + +	+ + + + +	− + +
Liver	− − −	+ − −	+ + + +	+ + + + +	+ + +
Spleen	− − −	− − −	+ + −	+ + + +	+ + +

(−) No evidence of uptake or presence of the latex; (+) very little uptake or presence of the latex; (+ +) evident uptake or presence of the latex; (+ + +) significant uptake or presence of the latex.

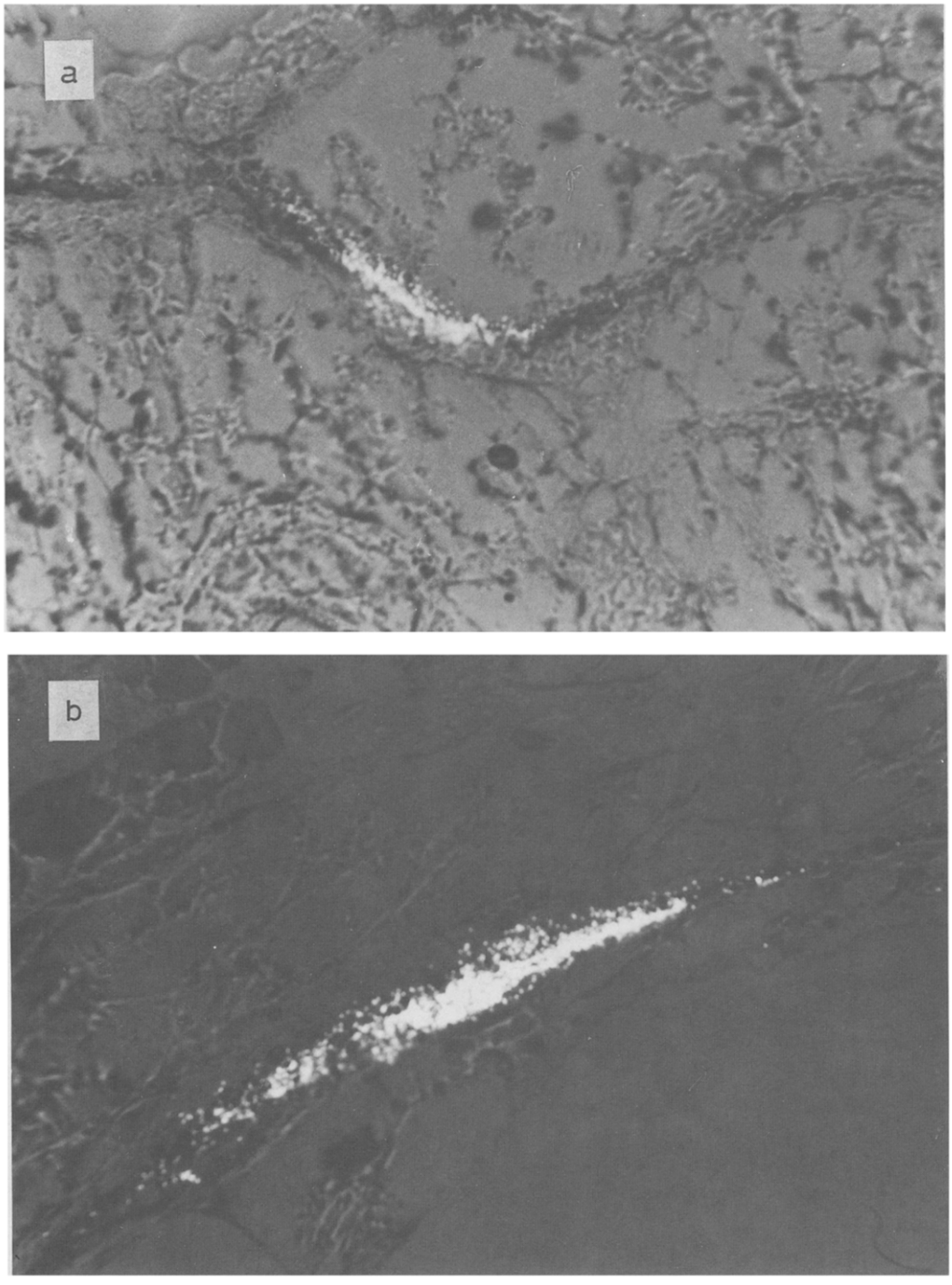

Fig. 3. Photomicrographs (×200) of a frozen section of a major mesentery lymph vessel containing 500 nm non-ionic fluorescent microspheres translocating towards the lymph nodes, 12 h after a single oral dose. (b) 18 h after dosing.

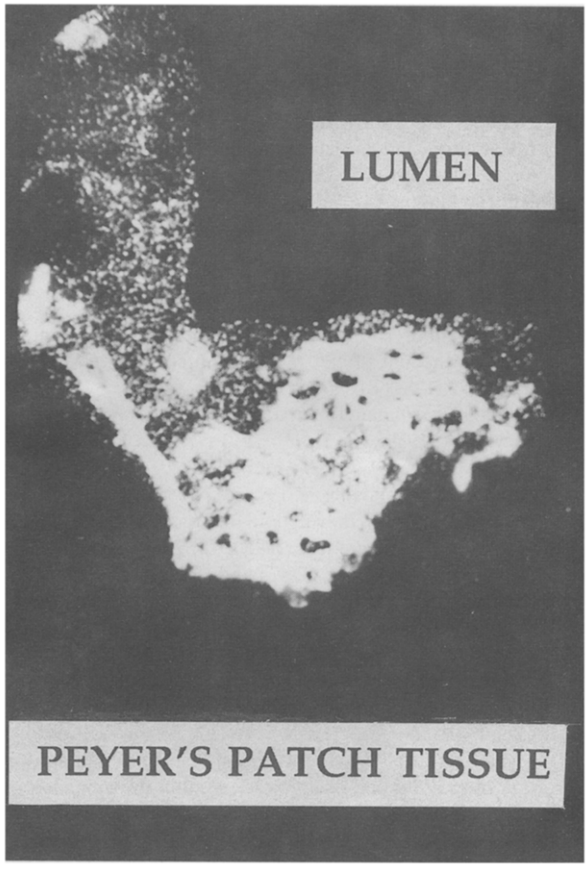

Fig. 4. Highly fluorescent region, resulting from the entanglement of 500 nm fluorescent polystyrene beads in the mucus in the small intestine, 6 h after administration by oral gavage.

spheres are entrapped by the mucus blanket in the vicinity of a Peyer's patch. Since uptake is also achieved in the distal parts of the gut such as the colon, a mucus 'trap' in the small intestine may act as a particle repository for the colon, particularly as the stability of the mucus structure is around 24 h in duration (Lebenthal, 1989).

Microspheres that are absorbed first have to contact the absorbing tissue. This follows transit down the alimentary tract and potential entanglement in the mucus layer. Contact time between the particles and the mucus will vary as the thickness and density of the mucus change. The transit of microspheres as a bolus or as individual particles is under study. Eldridge's work (Eldridge et al., 1989) shows microsphere concentration in tissues such as Peyer's patches continuing to rise after 48–60 h.

Since our chronic feeding experiment with 3 μm size diameter microspheres showed no uptake to internal organs such as mesentery nodes or liver, but their immobilisation in Peyer's patches' submucosal layer (Jani et al., 1992), we did not examine single doses of 3 μm microspheres. Eldridge et al. (1989) report that the uptake of microspheres such as poly(DL-lactide) and poly(DL-lactide-co-glycolide) (50:50) peaks at 7 days in organs such the mesentery nodes. For oral vaccination the initial release and subsequent secondary immunisation are important and for this a new approach is needed. The pattern of secretion of the antigen is as important as the persistence of the microspheres in the mesentery circulation. The interaction, penetration and the subsequent transit through the intestinal mucus must be important such that eventual presentation of microspheres to the absorptive sites such as enterocytes, lamina propria and above all the M-cells of Peyer's patches for eventual systemic uptake should be addressed.

Smaller, 50 nm size diameter microspheres, can be taken up not only by the Peyer's patches but also by enterocytes in the villous part of the gastrointestinal (GI) tract as illustrated by Kataoka et al. (1989) and Jani et al. (1992). Smaller spheres are observed in vacuoles as well as macrophages and in the epithelial layer of the crypts especially in the gastric area.

It may be assumed that the only route of excretion for microspheres absorbed orally must be via the bile duct, therefore there may be an opportunity for these microspheres to be re-introduced to the absorptive cells in the lower segments of the GI tract, gaining re-entry.

Conclusion

We have shown that micro- and nano-particulate uptake occurs through the gastrointestinal tract by means of the lymphoid tissue (Jani et al., 1989), albeit in small quantities and in a size-dependent manner (Jani et al., 1990). In this paper, we have shown that the uptake after a

single oral dose is rapid, the smallest particles being taken up most rapidly. While these data have yet to be quantified it is clear from the evidence provided here that the rate of uptake is such that the possibility of drug delivery or oral vaccination with such particles as carriers may be realised. The behaviour of a single dose of particles and its subsequent concentration in the lymphatic tissues – mainly of the mesentery lymphatic nodes – is important in the design of vaccine carriers. Much depends on the capacity of biodegradable carriers and their survival intact before uptake whether or not the route is viable for the delivery of potent but labile peptides and proteins.

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