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Further histological evidence of the gastrointestinal absorption of polystyrene nanospheres in the rat

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Summary

Further histological evidence of the oral absorption of fluorescent polystyrene nanospheres is presented. Fluorescent polystyrene microspheres of diameter ranging from 50 nm to 3 μm were administered daily for 10 days by oral gavage (2.5% w/v; 12.5 mg kg^{-1}) to female Sprague Dawley rats. All except the 3 μm non-ionic fluorescent polystyrene nanospheres and microspheres were concentrated in the serosal layer of Peyer's patches and could be seen thereafter traversing the mesentery lymph vessels. The migrating particles were subsequently found in the lymph nodes and liver tissues. No particles were found in the lung or heart. We have previously shown that uptake and translocation is size dependent, increasing with decreasing size. Special emphasis is placed on the fate of the smallest particles investigated – 50 nm non-ionic polystyrene microspheres – which were seen in kidney and were also present in the villi and GI crypts.

Introduction

The controversy over the uptake and translocation from the gastrointestinal tract of solid particles in the colloidal size range has continued for over 30 years since the early reports of Thompson et al. (1960) and Sanders and Ashworth (1961). The discussion of the phenomenon has been complicated by the claims of Volkheimer (1968, 1975) that the uptake of colloidal and larger particles from the gastrointestinal tract occurs as a paracellular process. Starch granules were found by Volkheimer (1968) to be transported intact across the intestinal barrier, described as a result of muscular activity in the muscularis mucosa and the 'kneading' of the particles with some regularity between cells at the desquamation zones of the intestinal villi, allowing movement from the intestinal lumen into the subepithelial region. Volkheimer (1968, 1975) termed this process 'persorption'.

The literature now contains substantial independent evidence of uptake and facts on the nature of the uptake of particulate matter from the gastrointestinal tract (LeFevre 1978a,b, 1980,

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1989; Gerhart et al., 1981; Urbanski et al., 1989) and includes work from this laboratory (Jani et al., 1989, 1990). The evidence shows that the uptake of both microparticulate matter and macromolecules takes place by endocytic mechanisms, at the M-cells of Peyer's patches. Reovirus type 1 adheres to the surface of M cells and appears in the intracellular vesicles (Wolf et al., 1983; Wolf and Bey, 1984; Bass et al., 1988; Sicinski et al., 1990) and occasionally larger microorganisms such as *Mycobacteria* spp. are found in the extracellular spaces between M cells and columnar cells and in macrophages enfolded by M cells (Fujimura, 1986). Thus, the gut associated lymphoid tissue plays an important role in particulate uptake and transport.

Uptake from the gastrointestinal tract occurs via the specialised epithelium overlaying the Peyer's patches, consisting of epithelial cells with a reduced number of goblet cells, restricting mucus production which otherwise might impede the passage of particles. Uptake also takes place from the microfold cells (M cells) (Owen, 1977; Wolf et al., 1983; Wolf and Bey, 1984; Sneller and Strober, 1986) and, to a lesser extent, the epithelial cells surrounding the Peyer's patches. These have profligate phagocytic activity having been shown to carry macromolecules and viruses across the gut wall to the systemic circulation (Owen, 1977; Wolf et al., 1983; Heyman et al., 1987; Neutra et al., 1987) and to regions of the gastrointestinal tract such as the lamina propria, isolated lymphoid follicles and colon (Faulk et al., 1971; Owen and Nemanic, 1978; Owen and Ermak, 1990; Owen et al., 1991).

Work from this laboratory has provided evidence of the size-dependent uptake of non-ionic polystyrene microspheres of 50 nm, 100 nm, 500 nm, 1 μm and 3 μm diameters in female Sprague Dawley rats (Jani et al., 1989, 1990). We showed that the polystyrene spheres were deposited in the serosal layer and then translocated to the mesentery lymphatic tissues. After 10 days' oral feeding of polystyrene microspheres, levels reaching the liver and spleen – a good indication of the amount having been absorbed *and* translocated – ranged from 5% for the smallest particles to around 0.75% for the largest polystyrene microspheres (Jani et al., 1990). Recent work (unpublished) suggests that uptake in Peyer's patches occurs after 1–2 h following a single oral dose. In this paper, we present further histological evidence of oral uptake and the destination of 50 nm and 3 μm diameter polystyrene fluorescent nanospheres and microspheres.

Materials and Methods

Microspheres

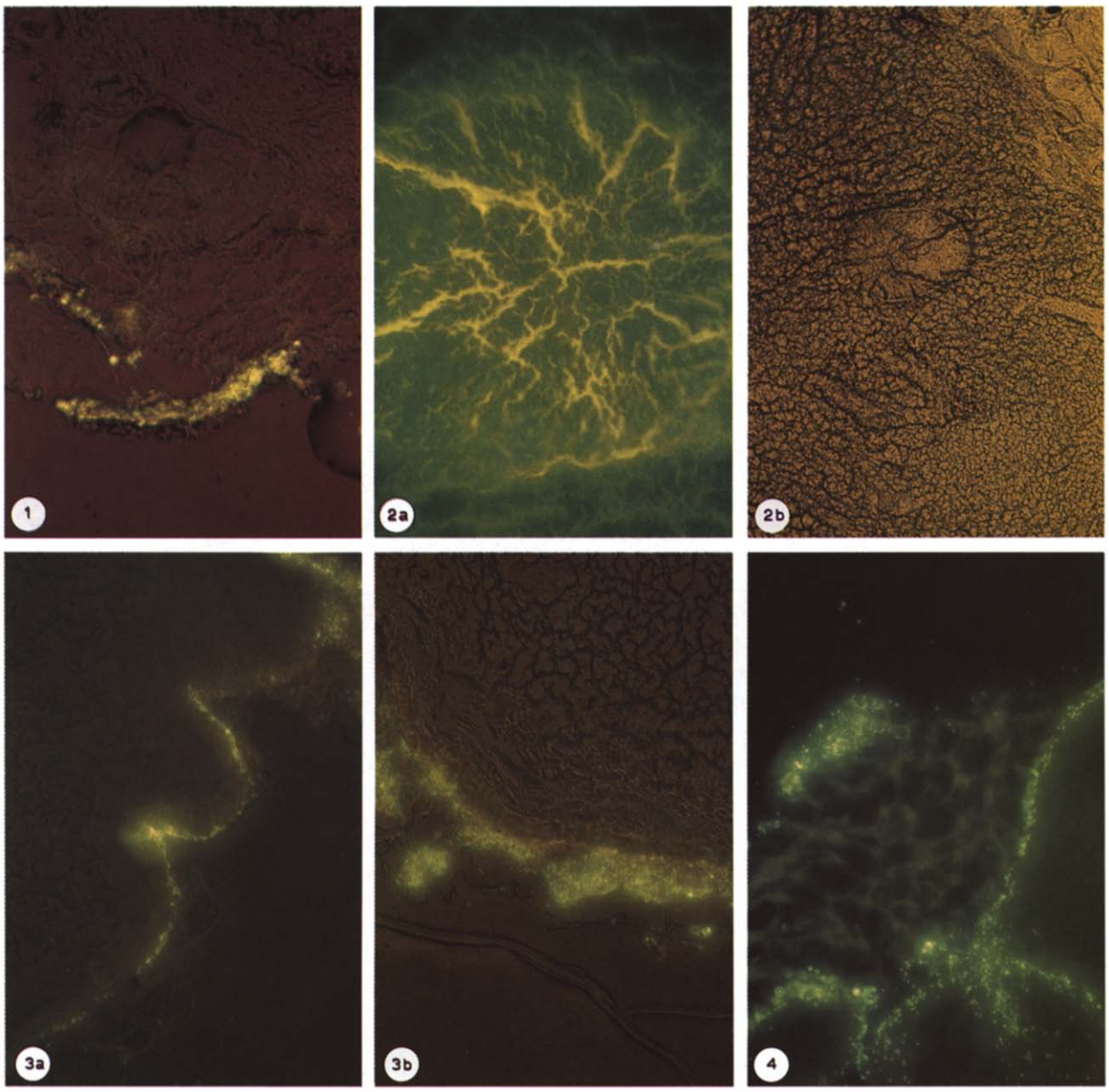
Monodisperse non-ionised polystyrene microspheres with covalently linked fluorescein of nominal size 50 nm, 300 nm, 1 μm and 3 μm in diameter were used as received from Polysciences

Fig. 1. Photomicrograph (×130) of Peyer's patch (frozen section) showing a collection of 50 nm non-ionic fluorescent latex particles arranged uniformly in the serosal layer.

Fig. 2. (a) A magnified (×325) frozen section of lymph node showing 50 nm non-ionic fluorescent spheres percolating through a lymphoid follicle. (b) A low-power photomicrograph (×65) of the above section with bright field background clearly showing the lymphoid follicle with surrounding interstitial cells. The lymph node consists of the capsule and trabeculae, which are composed of fibrous connective tissue. The subcapsular sinuses and trabeculae sinusoid are lined with macrophages and some lymphocytes amongst the interstitial cells that surround the lymphoid follicles.

Fig. 3. (a) Photomicrograph (×130) of a folded section of the serosal layer of a Peyer's patch with a collection of 300 nm non-ionic fluorescent beads, emphasising that the collection of the microspheres in this area is exceptionally specific and consistent. (b) A magnified (×260) section of the serosal layer of a Peyer's patch with collection of 300 nm non-ionic fluorescent beads.

Fig. 4. Photomicrograph (×130) of mesentery network neighbouring the gastrointestinal tract observed to be translocating the 300 nm non-ionic fluorescent microspheres.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Ltd (Northampton). Particle sizes were confirmed by photon correlation spectroscopy as outlined in Jani et al. (1989, 1990).

Animals

Female Sprague Dawley adult rats (average weight 150 g; 12–14 weeks) were used, each group of treated and untreated animals containing at least three rats.

Administration of microspheres

Microspheres were administered by oral gavage after 10 h fasting which lasted until 1–2 h after dosing; the animals were then given free access to food and water. A dose of 12.5 mg kg^{-1} (= 0.1 ml volume) was given daily for 10 days, as described in Jani et al. (1989). After the final dose was administered, the animals were kept for 2 days in a microsphere-free environment to clear the gastrointestinal tract of any unabsorbed microspheres. Before killing, the animals were fasted for 15 h to clear the gut of food particles, but to further facilitate the removal of the unabsorbed microspheres, access to water was allowed.

The animals were killed by the excess ether method and then carefully dissected, avoiding cross-contamination at all times. The various organs were carefully removed, weighed and placed in a plastic bag and frozen in liquid nitrogen. Some tissues were used immediately for preparing frozen sections.

Histology

Since some methods of conventional fixing and sectioning the tissue in absolute alcohol and clearing by using chloroform destroy the polystyrene microspheres, sectioning and mounting by the frozen method was preferred. In total, 10 microscopic slides were prepared from each animal and each tissue. Photomicrographs (at least 200) were prepared. A Reichert Polyvar I microscope (Reichert-Jung) with photographic attachment was used.

Organs removed from the rats were maintained below −70°C using a dry ice-ethyl alcohol (90%) mixture. Small samples (0.5–1 cm^2) were used for sectioning. To prevent any cross-contamination each group was sectioned and mounted on separate days and the laboratory area cleaned, the instruments being cleaned and changed between experiments. Throughout the sectioning and mounting procedures, the temperature was maintained at −30 to −20°C. An object holder (a small metallic cylindrical chuck) was used to mount the tissue. The tissue was held solid by surrounding the tissue with a cryostat embedding medium [OTC (TEK 11) 4583 compound]. The OTC compound supports and protects the tissue whilst sectioning. When the tissue is transferred on the slide the OTC compound melts away and does not interfere with the tissue, as outlined in Jani et al. (1989).

Results and Discussion

Previously (Jani et al., 1989) we have discussed results obtained both from photomicrographs and those based on quantitative determination of the latex. Although not strictly quantitative, photomicrographic results have been shown to correlate well with chemical analysis of the tissues for polystyrene by gel permeation chromatography (Jani et al., 1990). Examination of tissue such as the Peyer's patches, villi, mesentery network and mesentery nodes showed the presence of the polystyrene spheres. The presence of the 50 nm polystyrene microspheres under the optical microscope was judged by the intense fluorescence of the tissues. Polystyrene particles of 50 nm diameter have been located in the kidney, mostly in the capsular region. 50 nm microspheres were abundant in all organs. Unlike the larger 300 nm to 1 μm microspheres, 50 nm spheres were found in both Peyer's patches and also in the villi (Fig. 1), as well as in the crypts and intracellular junctions along the GI tract. Kataoka et al. (1989) have studied and demonstrated the presence of large gap junctions (over 500 nm in diameter), while numerous smaller gap junctions were found to be present on the lateral cell surfaces of the absorptive cells. None of the 300 nm or other larger microspheres were found in organs such as villi or junctional area. The 50 nm polystyrene spheres were found in secretory glands which extend into the subserosal layer, and, like those

of 300 nm, 500 nm, and 1 μm size these microspheres were also found in the mesentery network and mesentery lymph nodes (see Fig. 2a,b).

The 300 nm diameter size polystyrene spheres, like those of 500 nm, were found arranged in the serosal layer of the Peyer's patches (Fig. 3) and their presence was also significant in the mesentery network (Fig. 4) and in the liver (Fig. 5) and spleen. As before (Jani et al., 1989), our histological findings led us to an emphatic conclusion that the 1 μm diameter polystyrene microspheres, although found in the serosal layer of the Peyer's patches (Fig. 6) and most of the internal organs such as the liver and spleen, were taken up to lesser extent than the smaller sizes. Therefore, 1 μm should be regarded as the largest size of microspheres considered as carriers for oral drug or antigen delivery.

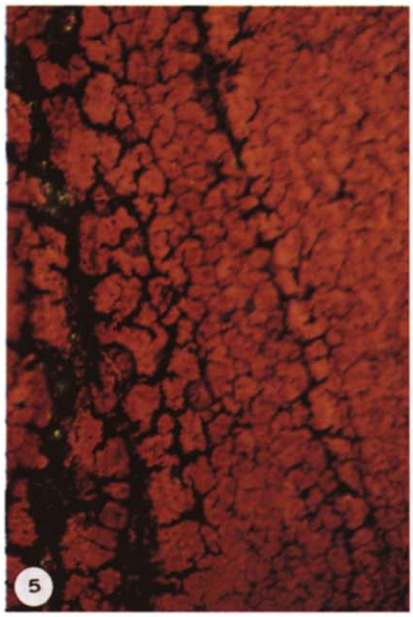
The presence and the alignment of 1 μm polystyrene microspheres in the spleen was unique compared to the smaller beads, since they are clearly seen in uniform arrangement in the trabeculae (Fig. 7), suggesting that most of the dose is accumulated here rather than as with the smaller sizes which are subsequently transported for excretion. We believe this may be due to the size of the fenestrations between the trabeculae and the reticular spaces (Weiss, 1988). Thus, the 1 μm microspheres are not removed and hence are trapped here. This observation is important in understanding the fate of colloidal drug carrier systems.

Work by Eldridge et al. (1990) showed that, following administration of particles below 10 μm in diameter, they were taken up by Peyer's patches; particles below 5 μm in diameter were transported to the mesentery lymph nodes while particles above 5 μm in diameter were found adsorbed to the mucosal and submucosal layer of the Peyer's patches. In our work we found that 1 μm polystyrene microspheres were the largest which were taken up by the Peyer's patches and subsequently found in the lymph nodes and other internal organs (liver and spleen) while 3 μm polystyrene microspheres were not found in the serosal layer or any internal structure such as the lymph nodes but were adsorbed to the submucosal layer of the Peyer's patches (see Fig. 8). We have thus strong evidence of the limit in the size of the microspheres taken up by Peyer's patches for systemic translocation.

Not surprisingly, like the 500 nm non-ionic polystyrene spheres, the 300 nm diameter polystyrene particles were taken up avidly by the Peyer's patches (Figs 3 and 4). Viewed under the fluorescence microscope, the polystyrene spheres accumulate in the serosal layer prior to subsequent transportation to the lymph nodes and the liver via the mesentery lymphatic network. The 300 nm polystyrene beads were observed both in the liver and the spleen.

Conclusions

In human subjects, particles in the size range of 2–6 μm have been observed in granular structures of the small intestine of patients with Crohn's disease, most adsorbed to the mucosal and submucosal surfaces (Urbanski et al., 1989; Roge, et al., 1991). The uptake of 50 nm particles may take place equally by endocytic mechanisms, by pinocytic or by phagocytic processes, but it is possible that some uptake could also occur by the paracellular route, as the presence of the 50 nm nanospheres was observed in the area of the zones of epithelial desquamation of the villi and between the villi. Subsequently, these small particles are found in the liver, mainly between the endothelial lining and the hepatic cells which represent the Space of Disse (Fig. 9). This type of uptake possibly operates for the 100 nm nanospheres and since few, if any, 300 nm particles were observed in abundance unlike the 50 nm and to some extent the 100 nm nanospheres, the upper size limit of uptake by the intestinal enterocytes and hence by paracellular processes, is possibly around 100 nm. Paracellular transport of 100 nm polyalkylcyanoacrylate nanocapsules has been illustrated by Aprahamian et al. (1987); Sanders and Ashworth (1961) noted PVC particles, when administered orally to rats, to be absorbed by the jejunal absorptive cells. Uptake of cationised ferritin by colonic epithelium has also been shown to take place by the paracellular route and by intestinal enterocytes (Barbour and Hopwood, 1983). The proposal for this mode of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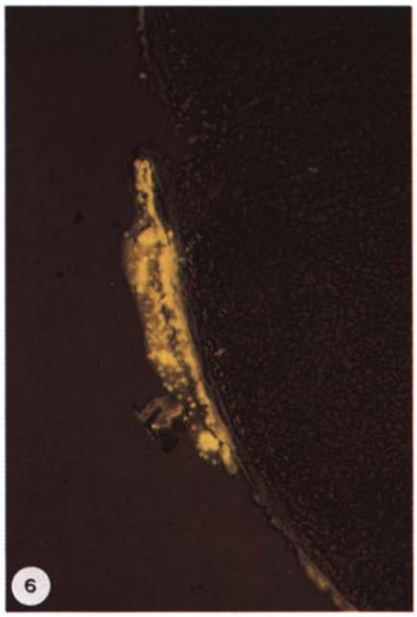
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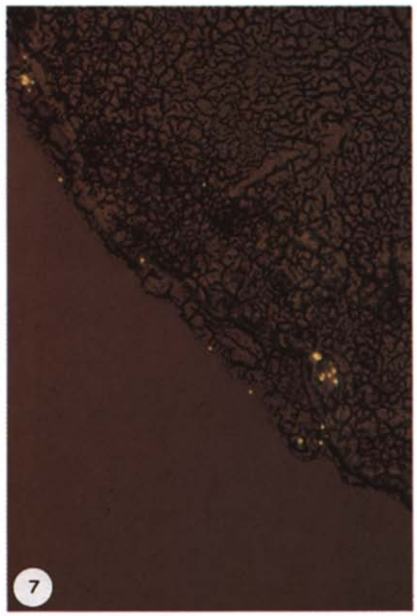
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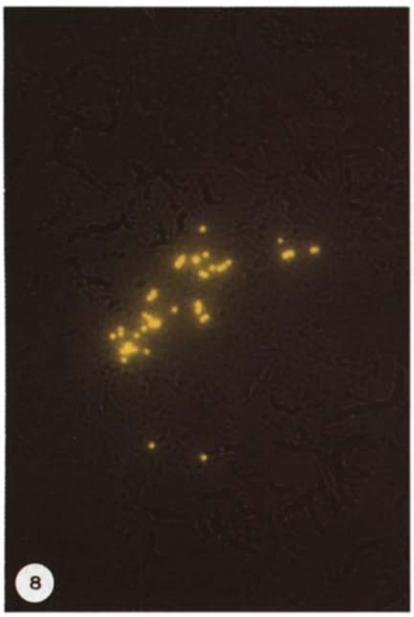
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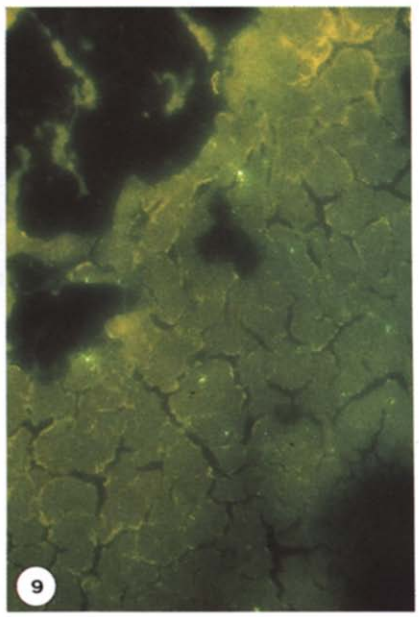
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uptake of 50 nm and 100 nm nanospheres is based on incidental evidence. The acceptance of the translocation of orally administered microspheres depends mainly on the further undeniable evidence of the uptake occurring in normal circumstances. Our findings here give us sufficient additional evidence to suggest that there is predictability and reproducibility of the intestinal uptake of these microspheres after oral administration in the rat. In conclusion, we feel that there is now substantial evidence of the oral uptake of particulate matter from the gastrointestinal tract. Now other questions more concerned with mechanisms and control need to be addressed, such as the route of excretion of these particles and their residence time in the body, before the approach to the oral administration of labile drugs via such carrier systems.

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Fig. 5. Photomicrograph (×130) of frozen section of liver showing, explicitly, 300 nm non-ionic fluorescent microspheres in the sinusoid spaces. The sinusoid spaces appear to be collapsed, a frequent occurrence with frozen sectioning. The endothelial and Kupffer cells sit astride the sinusoid canal, being in the strategic position to pick up microspheres or any foreign particulate matter.

Fig. 6. Photomicrograph (×130) of serosal layer of Peyer's patches of rat orally administered 1 μm non-ionic fluorescent microspheres. Note the discrete large 1 μm beads in the serosal layer tissue in this section.

Fig. 7. A frozen section of a spleen (×130) exhibiting the presence of 1 μm non-ionic fluorescent microspheres in the trabecular area, separated by the interstitial cells which are abundant with macrophages.

Fig. 8. Photomicrograph (×130) of the mucosal/submucosal region of a Peyer's patch with 3 μm non-ionic fluorescent microspheres which appear to be *adsorbed* in this region of the section. None were found to translocate to the serosal layer or to any major target organ.

Fig. 9. A frozen section of liver tissues (×130) exhibiting the presence of 50 nm non-ionic fluorescent polystyrene spheres between the endothelial lining and hepatic cells which represents the Space of Disse.

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