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The Uptake and Translocation of Latex Nanospheres and Microspheres after Oral Administration to Rats

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Abstract—Non-ionic and carboxylated fluorescent polystyrene microspheres (100, 500 nm, 1 and 3 μm in diameter), were fed by gavage (2·5% w/v; 1·25 mg kg^{-1}) daily for 10 days to female Sprague Dawley rats. Peyer's patches, villi, liver, lymph nodes and spleen of animals fed the non-ionic microspheres from 100 nm to 1 μm showed unequivocal evidence of uptake and translocation of the particles. Heart, kidney and lung showed no evidence of the presence of microspheres. Carboxylated microspheres were taken up to a lesser degree than the non-ionised particles. Experiments with ^{125}I radiolabelled 100 nm and 1 μm particles showed a higher uptake of the smaller particles, which were concentrated in GI tissue and liver. Particles were not distributed randomly in the tissues, but were concentrated at the serosal side of the Peyer's patches and could be seen traversing the mesentery lymph vessels towards the lymph nodes. The results demonstrate a need to re-examine the possibilities of particulate oral delivery, as well as the potential toxicity of ingested particulates.

The wall of the mammalian gastrointestinal tract is commonly assumed to be an impenetrable barrier to the passage of inert particulates. O'Mullane et al (1987) have concluded that "the transport of intact carriers across the gastrointestinal tract is restricted to exceptional and unusual circumstances". However, a number of investigators have shown that passage of colloidal particles across the intestinal mucosa is possible (Thompson et al 1960; Sanders & Ashworth 1961; Volkheimer 1968, 1975; Pontefract & Cunningham 1973; Le Fevre et al 1978). Most recently, Alpar et al (1989) found 1·1 μm particles to reach the circulation after oral administration, and evidence for transcellular passage of 200 nm diameter latex particles was provided over 25 years ago (Sanders & Ashworth 1961).

The neonatal mammalian small intestine, like the reticuloendothelial (RES) system in man, has a capacity to ingest macromolecules by endocytotic mechanisms (Daniels 1972; Walker et al 1974) allowing transmission of immunity to the young.

Membraneous epithelial (M) cells are specialized cells overlying the lymphoid follicles in the gastrointestinal and respiratory tracts; a review by Wolf & Bye (1984) has outlined their nature and structure. While solitary lymphoid nodules may occur along the entire intestine, they tend to be more numerous in the ileum, where they are grossly recognizable in aggregates as Peyer's patches. These are generally oval and usually located in the antimesometrial wall of the intestine. Their number changes with age, being maximal (circa 300) at puberty. Viral and bacterial particles can penetrate the mucosal barrier via membranous epithelial cells which endocytose and transport macromolecules, including antigens, and microorganisms into the Peyer's patches or associated lymphoid tissues (gut-associated lymphoid tissue).

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The ability of the intestinal mucosa of the adult rat to transport macromolecules from the lumen to the lymphatics and other organs of the RES was demonstrated by Hemmings (1978) and Barbour & Hopwood (1983) who clearly showed the involvement of pinocytotic vacuoles each containing several molecules of ferritin (mol. wt 750 000 and mol. diameter 10 nm). The intestinal transport of macromolecules in food has been discussed by Weiner (1988).

Absorption of Ru 41740 (a glycoprotein extract from *Klebsiella pneumonia*) from the gastrointestinal tract of the rabbit was greater from the duodenum than from Peyer's patches (Heyman et al 1987), a result similar to that obtained by Owen (1977) for glycoprotein horseradish peroxidase. However, most of the Ru 41740 crossing Peyer's patches was still in high molecular weight form. Le Fevre et al (1978, 1989) have shown that macrophages of Peyer's patches may be responsible for the uptake of particulate matter in the 2 μm size range, which then reaches the lymph nodes via the lymphatic circulation.

Patel & Ryman (1976) and Hemker et al (1980) have demonstrated successful oral delivery of insulin and Factor VIII, respectively, in liposomal carriers. Oral uptake of methotrexate administered in non-ionic surfactant vesicles of about 120 nm diameter and elevated liver levels of methotrexate suggested the uptake of intact vesicles and their subsequent sequestration by the RES (Azmin et al 1985).

The products of biotechnology in the form of peptide and protein drugs now present new challenges in the pharmaceutical areas of specific drug targeting and for delivery by the oral route.

Successful oral therapy with labile peptides, proteins and drug molecules has largely eluded solution. Immunization via the oral route would have many obvious advantages (Mestecky & McGhee 1989). The potential for the delivery of labile drugs in carrier systems was the primary motivation for the present work.

In this work fluorescent polystyrene nanoparticles and microparticles in the size range 100 nm–3 μm with varied

Table 1. Characteristics of the fluorescent microspheres.

No. of particles mL^{-1} (2·5% w/v)	Polystyrene microspheres	Size in μm (±s.d.)	Lot Number (Polysciences) (Northampton)	Excitation maxima (nm)	Emission maxima (nm)
4×10^{13}	Carboxylated	0·1±0·003	55171	530	590
$3{\cdot}8 \times 10^{10}$	(negative)	1·1±0·03	64381	530	590
2×10^{13}	Non-ionic	0·13±0·01	61034	458	540
$2{\cdot}8 \times 10^{11}$	(plain)	0·54±0·005	84102	458	540
$6{\cdot}25 \times 10^{10}$		0·95±0·01	71825	458	540
$1{\cdot}6 \times 10^{9}$		3·06±0·17	74352	458	540

surface characteristics were administered orally to rats. The location of the microspheres was determined histologically by light and UV microscopy, and confirmed by the use of radiolabelled microspheres. Polystyrene is not biodegradable but its use here avoids complications of interpretation which would ensue from degradation. Most importantly, the particles are non-immunogenic so there is no opportunity for them to enhance their own uptake or influence uptake by the lymphoid tissue.

Materials and Methods

Microspheres

Monodisperse negatively charged carboxylated polystyrene microspheres (nominally 100 nm, 1 μm in diameter) with covalently linked rhodamine, and non-ionized polystyrene microspheres with covalently linked fluorescein (nominally 100 nm, 500 nm, 1 μm, 3 μm in diameter) were used as received from Polysciences Ltd (Northampton). Particle sizes were confirmed using photon correlation spectroscopy and are shown in Table 1 along with a summary of the data on the systems employed.

Animals

Female Sprague Dawley adult rats (average weight 200 g; 15–20 weeks) were used. Each group of treated and untreated animals contained at least 3 rats.

The microspheres were administered by gavage. A dose of 1·25 mg kg^{-1} (=0·1 mL volume) was administered daily for 10 days. The animals were given free access to water, but food was removed overnight, about 8–10 h before the morning dose of microspheres. Urine and faeces were collected daily, and, occasionally, at random; the urine was freeze dried and examined for the fluorescent beads. The animals were weighed daily and were kept in individual metabolic cages to ease the collection of urine and faeces and to prevent coprophagia. After the final dose was administered, the animals were kept for two days in a microsphere-free environment to clear the gastrointestinal tract of any unabsorbed microspheres. Before being killed with ether, the animals were fasted for 15 h to clear the gut of food particles. Stomach, intestine (with mesentery network), colon, liver, spleen, kidney, heart and lungs were carefully removed to avoid cross contamination of microspheres, weighed and stored at −70°C before the preparation of frozen sections using a cryostat. This was preferred since some methods of traditional sectioning, fixing and cleaning of the tissue in absolute ethanol and chloroform would destroy the polystyrene microspheres. Ten sections were prepared from each tissue for each animal.

Intravenous administration

Two sizes of polystyrene microspheres were administered intravenously to compare the results with those obtained from oral administration. 100 nm beads (volume 0·2 mL, containing 5×10^8 particles) and 1 μm beads (volume 0·2 mL, containing 5×10^6 beads) were injected into the exposed femoral vein of each of the two groups of two rats anaesthetized lightly with ether, the skin then being clipped back. The animals were killed with ether 40–50 min after recovering from anaesthetic and organs weighed and stored as before.

Histology

The organs were maintained at −70°C using a dry ice-ethanol (90%) mixture. Samples (0·5 to 1 cm square) were taken for sectioning. To prevent cross contamination samples from each group were sectioned and mounted on separate days. Throughout the sectioning and mounting procedures, the temperature was maintained at −30° to −20°C. Sample tissues were embedded in a cryostat medium [OTC (TEKII) 4583 compound], and 3 μm thick sections were prepared except for the mesentery lymph nodes which were sectioned 6 μm thick because of difficulty in thin sectioning of frozen fatty tissue. The sections were viewed by fluorescence microscopy.

Results and Discussion

There has always been strong circumstantial evidence of the uptake of particulate matter, and our results clearly show the uptake and the subsequent translocation from the gastrointestinal tract (or GI mucosal surfaces) of the non-ionic fluorescent polystyrene microspheres. Table 2 summarises the data from the histological slides. These showed the presence of 100 nm particles clearly by the fluorescence of the tissue, but the resolution of individual particles was not possible under the light microscope, hence the concentration of our commentary on the 500 nm size particles.

Not surprisingly, we found that the 1 μm diameter microspheres were taken up less efficiently than smaller particles. The 100 and 500 nm non-ionic microspheres producing unequivocal evidence of their presence after oral administration (Fig. 1A–F). Viewed under the fluorescence microscope the liver, the tissues of Peyer's patches, villi, lymph nodes and spleen showed evidence of non-ionic microspheres while sections of heart, kidney and lung tissues showed none.

Animals fed carboxylated (negatively charged) fluorescent microspheres presented less clear evidence of the uptake (Table 2). Even the 100 nm particles appear not to be

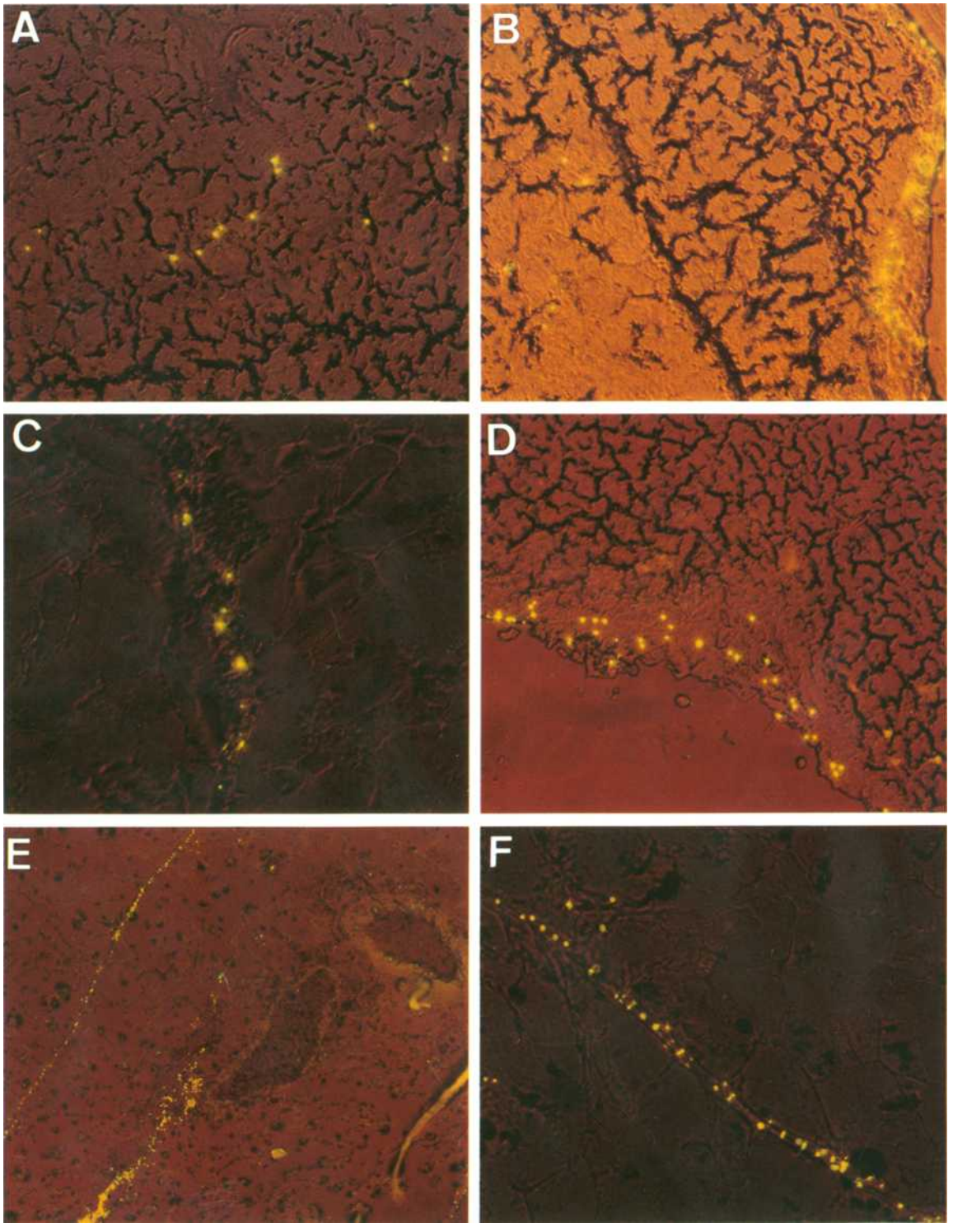
A
B
C
D
E
F

Table 2. Uptake in various tissues after oral feeding of microspheres.

Particle type	Stomach	Peyer's patches	Small intestine	Mesentery node	Colon	Liver	Spleen	Kidney (1)	Heart	Lungs (2)
YG	++	++++	++	++++	+++	+++	++	O	O	O
YO	+	++	+	+	++	++	O	O	O	O

Key: YG = Fluorescein dye non-ionized (monodispersed) microspheres of size 0·13 μm and 0·95 μm. YO = Rhodamine dye carboxylated (negative) (monodispersed) microspheres of size 0·1 μm and 1·1 μm. O = no evidence of latex particles, + = very low uptake, ++ = low uptake, +++ = moderate uptake, ++++ = high uptake.

absorbed, although the evidence is not unequivocal because of the difficulty in distinguishing the rhodamine fluorescence and the auto-fluorescence of the tissue.

The Peyer's patches and the mesentery nodes comprise the most obvious part of the gut-associated lymphoid tissue involved in the uptake of these particles. Fig. 1A–F show 500 nm non-ionic microspheres being translocated in the submucosa and serosal layer of the Peyer's patches and traversing the lymphatic vessels from the gut to lymph node. Most importantly, there is substantial evidence of these microspheres in the Peyer's patches, confirming Le Fevre's findings (Le Fevre et al 1978, 1989) although we have more clearly defined the location and subsequent fate of the particles.

Le Fevre et al have provided the most convincing evidence to date for a mechanism for the uptake of micrometre size particles via the gut-associated lymphoid tissue system, but their choice of 2 μm polystyrene beads may be considered to be suboptimal for uptake in mice.

The Peyer's patches are another major site of recirculation of small lymphocytes, i.e. the process whereby small lymphocytes continually extravasate and are then returned to the blood via the lymph. This recirculation gives the peripheral lymph a uniquely high content of lymphocytes and other macrophage cells. Peripheral lymph from other organs and tissue contains very few white cells. In addition, intestinal lymph, be it peripheral or post-nodal, contains the chylomicra and any other macromolecular materials taken up from the gut lumen (Weiss & Greep 1977).

Our experiments showed that no 3 μm microspheres were located in the major target organs, e.g. lymph nodes, after administration for 10 days. None was taken up by the gut or was found in the serosal layer of the Peyer's patches, unlike the 500 nm particles, but this may well be the result of the difference in number of administered particles in each size range. Le Fevre et al (1978, 1989) after chronically feeding microspheres for up to 60 days, found a greater uptake than we have shown. Duration of exposure (in our case 10 days) of the GI surfaces to particles will obviously affect the probability of uptake. Now that the potential for oral uptake has been confirmed, there is a need to determine the effect of single dose administration and the extent of uptake.

As early as 1961, Sanders & Ashworth used electron microscopy to demonstrate the uptake of 200 nm latex particles into the rat intestinal and hepatic cells after oral administration. These particles were also observed in the lamina propria and the lymphatics of the mucosa. Sections of the intestinal epithelial cells revealed that the particles were contained in the membrane-bound vesicles suggesting an endocytotic mechanism of uptake. We also believe that the 100 nm diameter microspheres are also taken up by endocytosis. We are now investigating the extent and uptake of 50 nm particles and will endeavour to quantify the "absorption" data by chemical analysis of the polystyrene in various tissues.

FIG. 1. A. Photomicrograph (×400) of a Peyer's patch showing a collection of 500 nm non-ionic fluorescent polystyrene microspheres in the submucosal layer of a frozen section. Amongst the lymphoid nodules in the Peyer's patch the surrounding villi, some of which overhang the follicular surfaces, should be noted. The Peyer's patches, which make up the most obvious part of the gut-associated lymphoid tissue system in the intestine contain fibroblasts, macrophages as well as masses of lymphocytes. There are on average 20 such patches in the rat principally in the lower part of the ileum on the side opposite the mesenteric attachment. B. Photomicrograph (×400) of a Peyer's patch showing a collection of 500 nm non-ionic fluorescent polystyrene microspheres arranged uniformly in the serosal layer, with some microspheres in the submucosal layers. More microspheres accrete in the Peyer's patches than in the villi and the microspheres in the villi occur mainly in the granular cells. The serosal layer, composed mainly of connective tissue, contains phagocytic macrophages as resident cells and is served well with a lymphatic supply which goes directly to the lymph node. As indicated in the text, the microspheres will be translocated from the Peyer's patches to the mesenteric lymph node by the lymphatic system. C. A higher magnification (×400) of part of the section showing an afferent lymphatic vessel, at the convex surface, discharging the 500 nm non-ionic fluorescent microspheres into the lymph capsule and then into the subcapsular sinuses. D. Photomicrograph (×400) showing a collection of 500 nm non-ionic fluorescent microspheres in a lymph node. The microspheres appear to be in the subcapsular sinus, and amongst the interstitial cells. The interstitial cells contain lymphocytic and other free cells especially the full lineage of phagocytic plasma cells, relevant to the fate of colloidal particles. The trabecula can be seen making the characteristic appearance in this frozen section.E. A low power photomicrograph (×100) of the mesentery lymph node (frozen section) showing lymph vessels containing 500 nm non-ionic fluorescent microspheres, taken up during the feeding studies and moving towards the lymph nodes. Unlike lymphatic vessels in many other parts of the body, those in the mesenteric network possess muscular walls and are thus able to propel their contents towards and through the mesenteric lymph nodes. F. A higher magnification (×400) of part of this section (E) showing a lymph vessel cut in a horizontal plane translocating the 500 nm microspheres.

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