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Review

The Oral Absorption of Micro- and Nanoparticulates: Neither Exceptional Nor Unusual

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This mini-review covers some of the historical and recent arguments over the experimental evidence on the uptake by and translocation from the intestinal mucosa of microparticulates after oral administration. It is concluded that there is now no dispute over the fact that this is a normal occurrence. Particulate uptake does take place, not only via the M-cells in the Peyer's patches and the isolated follicles of the gut-associated lymphoid tissue, but also via the normal intestinal enterocytes. Factors affecting uptake include particle size, surface charge and hydrophobicity and the presence or absence of surface ligands. The covalent attachment of lectin or invasin molecules to the surface of carrier particles leads to greater systemic uptake. Whether or not the route can be utilized for the routine administration of therapeutic agents which are not normally absorbed from the gut is not yet proven. Many studies show that 2–3% of the ingested dose of submicron particles can be absorbed. The increasing diversity of carrier systems, which includes dendrimers and liposomes, needs to be exploited fully. More also must be learned about the inter- and intra-subject variability of lymphoid tissue so that appropriate selectivity can be achieved through the design of specific carriers.

KEY WORDS: microparticles; Peyer's patches; GALT; particle absorption; oral delivery; lymphatics.

> In Medicine one must pay attention not to plausible theorizing but to experience and reason together . . . . I agree that theorizing is to be approved, provided that it is based on facts, and systematically makes its deductions from what is observed. . .But conclusions drawn from unaided reason can hardly be serviceable; only those drawn from observed fact. Hippocrates, Precepts

BACKGROUND

Paradigm shifts are sometimes slow to be appreciated. The ability of intact microparticles and nanoparticles to be absorbed through the gut wall seems to have been more difficult to accept than other discoveries, even though the topic has a respectable history of at least a century. Verzar in 1936 in his book on Absorption from the Intestine (1) discusses the absorption of what he calls "corpuscular elements" citing papers dating back to 1854, including Hirsch's (1906) observation that raw starch fed to rats was absorbed across the intestinal mucosa. This was some sixty years before the work of Volkheimer (2) who also included starch[3] in the array of particulates fed to animals and medical students, which found their way into the systemic blood. Basset and Carné (1907) stated definitely that a perfectly normal intestinal epithelium is impermeable to particles if these "do not wound the cells", and that only an "inflamed mucosa" allows micro-organisms to pass. But after feeding Indian ink to rabbits Kumagai in 1923 found greater amounts in the epithelium which covers the lymph follicles and less between the epithelial cells, suggesting some phagocytic activity. More recently the debate not only about whether particles are taken up by the gut but whether this occurs by natural or pathological processes has been revived. In our 1989 paper (3) on polystyrene latex uptake we cited a paper published two years previously which concluded that "the transport of intact carriers across the gastro-intestinal tract is restricted to exceptional and unusual circumstances" (4). Recently one could read that "the topic of particle uptake from the gastrointestinal tract has [also] been an area of recent discussion and controversy . . ." and that . . "the quantities of particulate material that can be taken up into the general circulation from the gastrointestinal tract is extremely small" (5). A review of a book on biological barriers to protein delivery complained of an unnecessary emphasis on membrane traffic in intestinal M cells which is an important topic for vaccine delivery "but of little importance for most therapeutic peptides and proteins" (6).

The uptake of macromolecules by intestinal M cells is well established as the source of immunity in the newborn (7), although excessive exposure of antigens to the immune system may lead to gastrointestinal disease (8). It is possibly true that there is a low percentage uptake into the circulation of most unengineered nanoparticles in the size range from 50 nm to 1000 nm, but the uptake of particles into the lymphatics can

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[3] Starch granule diameters: corn starch 3–25 μm; potato starch, 5–110 μm; and rice starch, 3–10 μm.

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reach 2–3% of the administered dose, and much more if specific ligands are attached to the particle surface.

Gut Associated Lymphoid Tissue (GALT)

Figure 1 is a diagrammatic representation of the potential modes of entry of macromolecules and particles from the undamaged intestine. Three possible routes are identified, 1) through M-cells, 2) through normal epithelial cells (enterocytes) and 3) by paracellular means. It is now clear that the GALT plays an important but not an exclusive role in particle uptake mechanisms. These specialized regions of the intestine are still the subject of intensive study to understand their origins, differences throughout the gastrointestinal tract, and their roles.

Observations that inert particles are taken up by the GALT and translocated by the lymphatics throughout the body apparently went against the grain of the teaching that solutes must be in solution before they are absorbed. Yet the contrary evidence of nature was there. Bacteria and reoviruses have the ability to target to cells in the gut and be absorbed and maternal antigens from milk are absorbed by the M-cells of the neonatal GALT causing immunity to develop, but absorption of particles? That Volkheimer must have been wrong was a widely held view, even though he proposed that the phenomenon he observed was one of "persorption" where relatively large particles are absorbed after the sloughing off of villus cells to create a greater access to the lymphatic system.

PARTICLE, PRION AND BACTERIAL UPTAKE

The M-cells are specialised for endocytosis and transport into intraepithelial spaces and the adjacent lymphoid tissue. Table 1 surveys the microorganisms and particles which adhere to M-cell apical membranes and translocate into the lymphoid tissue. Quantitative models have shown that particle binding to M-cell apical membranes is followed by rapid internalization and "shuttling" to lymphocytes and to mucosal immune inductive regions (9).

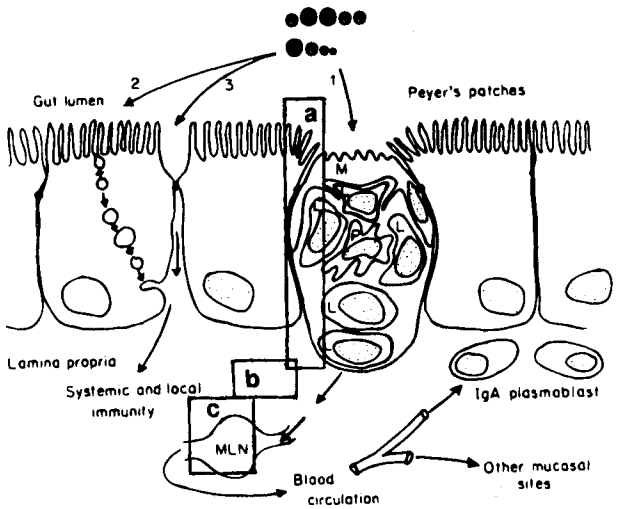

Fig. 1. Representation of the three possible routes of entry of micrioparticulates into the lympathic system or blood supply. Key: (1) By way of the M-cells of the Peyer's patches of the GALT; (2) By transcellular routes involving intestinal enterocytes; and (3) By paracellular avenues through the tight junctions between cells. The boxes a, b and c approximate to the histological sections illustrated in Fig. 2.

Table 1. Microorganisms and Particles Which Adhere to M-cell Apical Membranes

Bacteria
Vibrio cholera
Salmonella typhi
Yersinia enterocolitica
BCG
Campylobacter jejuni
Shigella flexneri
Escherichia coli (R-DEC 1 strain)
Viruses
Reovirus
Poliovirus
HIV-1
Protozoa
Cryptosporidium
Inert particles
Carbon particles
Latex beads
Copolymer microspheres
Hydroxyapatite
Titanium dioxide

Note: Modified from H. M. Amerogen et al., *Ann. N. Y. Acad. Sci.*, **664**:18–26 (1992).

Given the complex milieu in which microparticles find themselves after oral administration, and the small target area represented by lymphoid tissue, their uptake is counterintuitive. However, so too, perhaps, is particle-mediated delivery of recombinant expression vectors to the skin (10), absorption of intact liposomes through dermal barriers (11), penetration of asbestos into the blood through the digestive tract (12) and the oral absorption of prions or prion proteins allowing their oral transmission. The last is still conjecture but is relevant to the subject of this review, as it demonstrates the need for research into phenomena for their own sake whether or not there is an obvious endpoint at the time of commencement of the research. Collee has written (13) : ". . the chemical resistance of the prions . . . . . would protect against the defence mechanism of the stomach; and the relative proteinase resistance of the prions would allow substantial amounts to be available for uptake by the lymphoreticular system in Peyer's patches throughout the intestine." We should be prepared for anything in science and not dismiss possibilities based on what Hippocrates called "unaided reason". Collee provides a possible scenario for prions, largely trapped within the immune system, reaching the brain by a neurotropic tracking mechanism as with poliomyelitis. The ability of particulates to reach distant parts of the body after administration has been known for some time. Casley-Smith's work on the permeability of lymphatic vessels to particulates of 10 μm in diameter, provides a clue as to the mode of particle dispersal following absorption (14).

PARTICLE UPTAKE

Figure 2 shows the evidence of transport of 500 nm polystyrene latex particles in the regions a), b), and c) delineated in Fig. 1.

Micro- and nanoparticles absorption has many pharmaceutical, biological and toxicological consequences. Indeed there has been speculation that particles from the atmosphere or insoluble agents as in toothpaste and other products when taken up by lymphoid tissue are involved in the etiology of Crohn's disease (15) and other inflammatory diseases. GALT tissue in normal individuals contains exogenous pigments and minerals (16,17) from food, the environment or pharmaceuticals. The finding that titanium dioxide (rutile) particles (nominal size 500 nm) are absorbed in rats suggests that insoluble particulates used in pharmaceutical formulations may not be entirely free of long term consequences of their accumulation in lymphoid tissue (18). Although lymphoid tissue is not present in normal gastric mucosa, lymphomas can form in the stomach which assume the features of MALT (19). Local infection by *Helicobacter pylori* is known to stimulate the acquisition of gastric lymphoid tissue resembling PP tissue, although whether or not it is functionally the same is not known. The number and nature of PP in the intestine seem to reflect the diversity of the flora of individual species and sites. Colonic lymphoid tissue differs morphologically from lymphoid tissue in the small intestine (20). Rabbit caecal lymphoid patches also have distinctive surface characteristics (21).

Gut translocation by bacteria (22)—the passage of viable enteric bacteria across the intact mucosa of gastrointestinal tract into normally sterile extra-intestinal tissue—was first described at the turn of the century. It was recently reported (23) that bacterial translocation occurred in 10% of general surgical patients. It was more common in cases where there was obstructive bowel disease, a result also obtained by Deitch (24) and by Ambrose (25). Damage to the intestinal epithelium has been reported to facilitate bacterial movement, although bacterial lipopolysaccharide (LPS)—induced bacterial translocation does not appear to involve loss of epithelial viability (26), perhaps in conflict with the claim (27) that "physical disruption of the gut mucosal barrier appears to be the primary mechanism whereby endotoxin promotes bacterial translocation." While this suggestion that disorders of the epithelium affect uptake, absorption of macromolecules across the PP epithelium was not affected in piglets infected with transmissible gastroenteritis, but transport in areas adjacent to lymphoid rose to the level seen in PP tissue (28). These are important issues in considering the possibility of delivery of drugs via GALT and intestinal tissue, as abnormal and unusual circumstances must be borne in mind in determining the safety of the route, which includes its variability.

One of the most prolific growth areas in research, the development of oral vaccines, has been a direct consequence of the better understanding of the interaction between particulates and the GALT. It is from an understanding of uptake and translocation of naturally occurring particles that we can design synthetic or semi-synthetic carrier particles to be more selec-

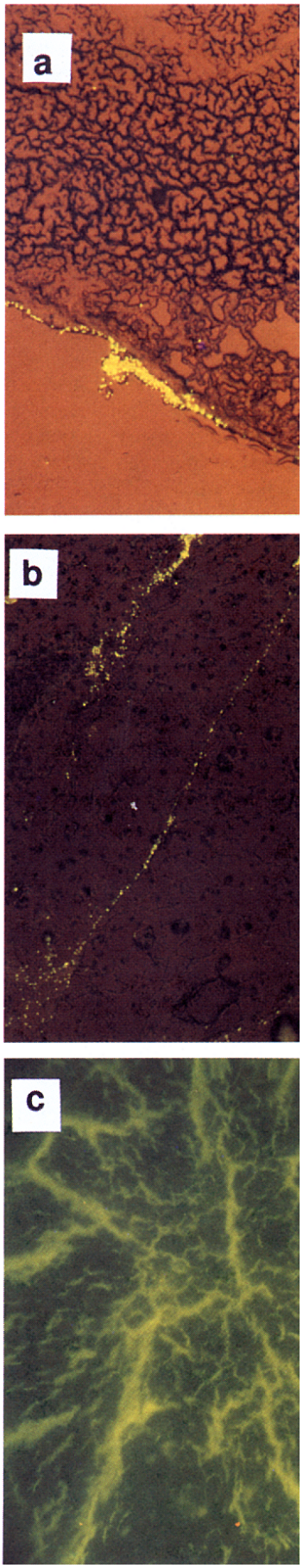

Fig. 2. Histological evidence of particulate uptake after oral administration. The particles in question are 500 nm polystyrene latex particles with covalent fluorophore. The regions approximate to the areas marked a, b and c in Fig. 1 and show a: passage of particles across a Peyer's patch region from the lumen to the serosal side of the gut; b: passage of particles in the mesenteric lymph vessels and c: collection of particles in a mesenteric lymph node.

tively targeted, adsorbed, then absorbed and translocated, to allow not only efficient oral vaccination, but to make a reality of the therapeutic administration of potent peptides and proteins.

EXTENT OF PARTICLE UPTAKE

The fate of nanoparticles after oral administration has been the subject of several reviews (29–34). There is no doubt that uptake occurs as a natural process and not as a result of damage, that is, the circumstances are not exceptional. Establishing a consensus on the extent of uptake from the published data has proved more difficult and is confounded by differences in the chemical nature and surface characteristics of the particles used, their dosage and dosage volumes, as well as assay methods and the actual duration of each study. Data from our laboratories on the absorption of polystyrene particles in the size range 50 nm to 3000 nm, where tissues were assayed by GPC, showed levels of interaction, adhesion and uptake to be 30% of the dose for the smallest particles (35). We have seen no reason to modify our view on the accuracy of this data, although it has been referred to as "extraordinary." The data have also been misquoted, as the figure of 30% includes both adsorbed and absorbed particles. The test of absorption is translocation from the lymphoid tissue and the subsequent appearance of particles in organs such as the liver and spleen and in the blood. The data of 1990 is shown in Fig. 3, determining that 6–7% of the 50 nm particles accumulate in the liver, spleen, blood, bone marrow and kidneys. One key to the future is the investigation of particles below 50 nm in diameter.

Further histology has been taken as confirmation of the extent of distribution (36). Uptake of polystyrene occurs through both the GALT and, to a lesser extent, normal intestinal tissue (37). The markedly increased uptake attained after covalent attachment to particles of tomato lectin molecules is not achieved solely by uptake through lymph tissue as normal enterocyte involvement in the process is strongly indicated. This reinforces the view that uptake can not only be significant, but can be improved upon by using specific surface ligands such as tomato lectin and invasin molecules (38,39). Uptake is reduced when polystyrene latex surfaces are coated with hydrophilic poloxamers (40) (Fig. 4), emphasizing that the absorption process is dictated by surface features. Table 2 summarizes the factors which so far have been identified as critical in determining uptake. More research on each parameter is still required.

Particle size is a key factor, as seen in Fig. 3. Jenkins and co-workers have also reported the size-dependency of uptake over the range 150 to 1000 nm (41). They measured uptake by a sensitive flow cytometric assay of cannulated lymph, the number of particles in the mesenteric lymph nodes being determined 90 min after dosing. Increasing uptake with increasing size up to 3 μm was observed but the low amounts determined led the authors to conclude that "the magnitude of microparticulate absorption is several orders of magnitude lower than supposed previously". However, integration of the data over the whole intestine rather than over the relatively meager amounts of tissue investigated, brings it more in line with our own and other's data, even though the particle-size dependency appears, at first sight, strange. However, Pratten and Lloyd (42) found the rate of clearance of 1100 nm polystyrene to be 10-times greater than for 100 nm polystyrene and over 60 times that of

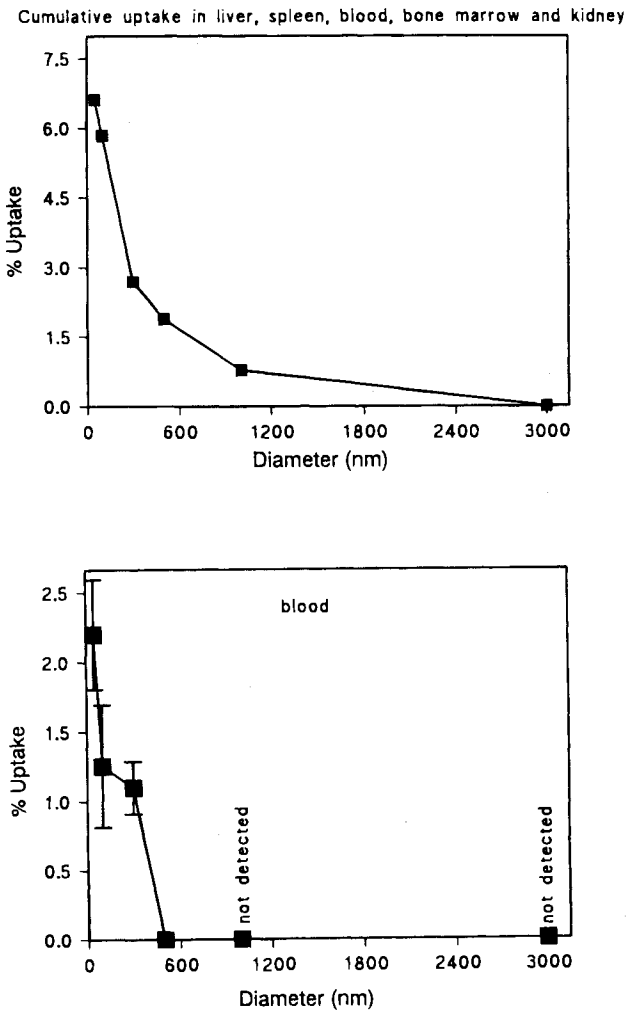

Fig. 3. The cumulative uptake of polystyrene particles in the size range 50 to 3000 nm after feeding by oral gavage for 10 days to male rats: upper plot: in the liver, spleen, blood, bone marrow and kidney, as measured by gel permeation chromatography; lower plot: levels of polystyrene found in blood as a percentage of the dose. Both plots from P. U. Jani, G. W. Halbert, J. Langridge, and A. T. Florence, *J. Pharm. Pharmacol.* **42**:821–826 (1990).

30 nm Percoll particles[4] at similar concentrations to cultured rat peritoneal macrophages. In these experiments the smaller polystyrene particles were present in 1000 fold greater numbers than the larger particles. The data indicated, according to the authors "that there is a size of particle that is too large for uptake by pinocytosis but too small to initiate phagocytosis," but there was no evidence for a radical discontinuity between the two processes. In the range 0.9 μm to 6 μm there was a decrease in phagocytosis by lung and peritoneal macrophages and by neutrophils and monocytes (43); adsorbed BSA effectively reduced uptake as did a negative surface, while a positive charge aided uptake in these experiments. Adsorption of poloxamer surfactants onto particles also reduces phagocytic uptake,

[4] Percoll particles are nearly spherical polyvinylpyrrolidone-coated silica particles, from Pharmacia, Uppsala.

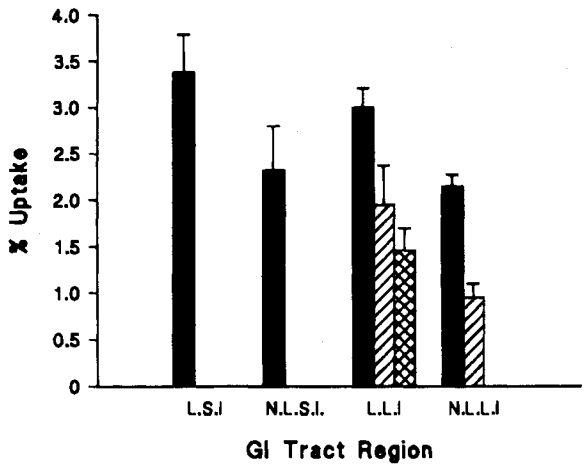

Fig. 4. Data on the influence of coating particles with hydrophilic block copolymers on the percentage uptake of polystyrene latex particles (nominal diameter 60 nm) in the lymphoid and non-lymphoid regions (L and NL) of the small intestine (SI) and large intestine (LI). Filled in bars: uncoated particles; hatched bars: poloxamer 407 coated particles, and cross hatched bars: poloxamer 188 coated particles. Data from Hillery, Jani and Florence (37).

Table 2. Factors Enhancing Particle Uptake by the GALT

Stability of the particle in the gut lumen
Submicron particle diameter (<5 μm and preferably in the submicron range)
Lack of surface charge (charged particle less well absorbed)
Surface hydrophobicity (adsorbed hydrophilic materials eg poloxamers reduce uptake)
Presence of specific ligands (lectins, invasins increase adhesion and internalization)

proportionally to the thickness of the adsorbed hydrophilic layer (44).

Recent studies include those of Desai *et al.* (45) on PLGA particles from 100 nm to 10 μm, showing that the former diffused throughout the submucosal layers while the latter were "predominantly localized in the epithelial lining of the tissue." It seems that particles in the range 3–10 μm are sequestered within the Peyer's patches and do not migrate into the mesenteric lymph nodes (46–48). Eldridge and coworkers' contributions to our understanding of the processing of microparticles by PP tissue has been significant (49). Observing the effect of size and the nature of particles on the rate and extent of movement of particles through the PP into the MLN region and illuminates the choice of systems for vaccine or drug delivery.

Regardless of the controversy of whether sufficient carrier particles are taken up to produce therapeutically worthwhile levels of drug (how can one predict what potent entities will be available to deliver?) considerable effort has been expended, rightly, on the development of nanoparticles for the delivery of proteins and peptides (45). Others have reviewed the possibilities of oral vaccination which is outside the scope of this review. Recent confirmation of uptake of 133 nm polylactide-glycolide particles (assisted by administration of milk) to the extent of about 2% after 1 hr (50) is encouraging. Kreuter's extensive data on the uptake of radiolabelled nanoparticles, such as those on PMMA, cannot be ignored (51). 4% of the dose of a lyophilized preparation of methylmethacrylate-^{14}-C-2-hydroxyethylmethacrylate, butylacrylate nanoparticles were absorbed after a single oral dose in male Wistar rats. Seven days after administration 0.15% of the dose was found in lung, spleen and liver (52). Dyan's (53) and Carr's groups have also contributed evidence. The percentage of 2 μm polystyrene observed in rat proximal PP 0.5 hr after administration was 1.35% and 0.38% in the so-called middle PP region as measured by confocal fluorescence microscopy (54). Although more of the 2 μm particles were taken up, the 6 μm size delivered a greater volume to the lymph nodes. It is clear that the most appropriate size range for a particular end purpose must be chosen carefully. It may be that a mixture of sizes will produce the most beneficial effect. Particles which target immune cells within PP may be optimal for initiating IgA responses, but particles which can escape from the PP region to peripheral lymphoid organs might be chosen for the generation of systemic IgG responses (55). While the very smallest sizes possible might well be taken up in greater quantities, the technology of manufacturing small particles below 100 nm diameter it is not straightforward: encapsulation efficiencies may be low, and the large surface area can mean that labile encapsulated molecules can be exposed to degradation.

ALTERNATIVE CARRIER TECHNOLOGIES

The range of polymeric nanoparticles which have been found to be taken up by PP offers a reasonable range to allow formulation of a range of antigens and drugs for delivery. The problems of preparation of homogeneous microspheres or nanospheres of a defined size range and achieving an appropriate release rate (systems which resist release in the GI tract but dispense drug or antigen at a rate *in vivo* which maximizes response) will not be rehearsed here, but the problems can be formidable. Hence the need to look to other technologies.

We have synthesized an LHRH copolymeric compound using n-butylcyanoacrylate and the peptide vinyl acetate as comonomers. The resulting polymer (56,57) and fed to male Wistar rats, as particles of around 100 nm in diameter produced significant reduction in testosterone and in the weight of the seminal vesicles and prostate after 14 days feeding compared with a solution which produced no detectable LHRH levels in blood or significant biological effect (58). Liposomes have been found to be taken up by GALT (59,60). Polymerized liposomes are absorbed after oral administration to the extent of 2.7% by PP, with 0.06% retained in the patches and the remainder in the circulation at 2 hr (61). Protein cochleates—stable protein-phospholipid-calcium precipitates are apparently taken up by Peyer's patches (62). Exploring new chemical architectures such as clathrate complexes and dendrimers might also be fruitful in the search for carriers which are more extensively taken up than larger polymeric structures. The value of dendrimers is that they can be synthesized and formed to very precise molecular dimensions with a great variety of external surface groups, hydrophilic, ionic or hydrophobic (63,64).

ENHANCING UPTAKE: ENGINEERING PARTICLES

The prospects for using the Peyer's patches as means of delivering peptides and proteins has been said to be "severely

compromised" (65) by (a) the limited efficiency and capacity of the absorption pathway, (b) the time to onset of pharmacological response, due both to the kinetics of processing of the particles and the slow flow rate of the lymph; and (c) the potential loss of drug to local lymphocytes and macrophages. The first can be tackled by targeting to absorptive tissues, presently assumed (perhaps mistakenly as hinted at above) to be principally Peyer's patches.

As shown both *in vitro* and *in vivo*, the surface of the carrier microparticles can be crucial in determining association with lymphoid and non-lymphoid tissue and consequently can determine the extent of uptake. This led us to covalently attach to carboxylated microspheres both tomato lectin and invasin molecules, exemplifying two different approaches, the former to take advantage of the possibility of specific interactions between cell surface carbohydrate moieties and the carrier bound lectin, and the latter to take advantage of bacterial mechanisms of entry to intestinal tissue. The invasin proteins of *Yersinia* spp gain entry to cells by interactions with the β1 integrin family of receptors, the intracellular domains of which interact with the cytoskeleton. Bacterial invasion is accompanied by cytoskeletal changes, necessary to allow the movement of the relatively large bacterial particles through the cell structures (66).

LECTINS AND PARTICLE UPTAKE: TARGETING TO M-CELLS?

Targeting systems to M-cells is an attractive proposition. Pappo and colleagues (67) showed that using a monoclonal antibody (MAb) with specificity for rabbit M cells attached to 1 μm polystyrene particles increased the particle count in rabbit PP, whereas a non specific MAb had no effect. The lectin *Ulex europaeus* Agglutinin I (UEA I), has been suggested to be mouse M-cell specific, leading Chen *et al.* (68) to incorporate modified UEA I and wheat germ agglutinin (WGA) into polymerized liposomes and to measure GI uptake. About 11% and 6% of the oral dose was, respectively, absorbed, a higher percentage than the lectin-free liposomes previously studied. The work of Naisbett and Woodley (69) and of Lehr *et al.* (70) laid the ground work for such *in vivo* studies. Reviews of the use of "carbohydrate handles", as Palamino (71) terms them, have appeared (72).

Our earlier work showed that uptake of polystyrene latex in the small and large intestine takes place through Peyer's patch and non-PP regions of the epithelium. In suckling mice, uptake of Percoll particles is through enterocytes, the epithelial cells covering PP regions containing significantly fewer particles (73). Tomato-lectin bound to microspheres induced marked increases in systemic uptake which could only be accounted for by induction of uptake through enterocytes as well as lymphoid tissue (74). The bioadhesion and absorption of lactose-conjugated microspheres was found to be low throughout the intestine, which indicates either competitive inhibition by food components such as carbohydrates (75). Lactose conjugated microspheres which were partially blocked by the lectin from *Erythrina cristagalli*, were absorbed into the blood to the extent of 3.4%—nearly double that for plain polystyrene of the same 500 nm diameter.

Tomato-lectin coupled microspheres show unusual absorption tendencies. Single dosing of animals produced no evidence of uptake, whereas 5 or 10 days daily dosing caused uptake of 18% of the dose (76), not due to tissue damage, but perhaps to the induction of receptor expression by the lectin. Many questions remain unanswered, because inter alia, the tissue distribution of lectin-bound particles differs considerably from experience with plain polystyrene. Very recently, Irache et al. (77) have found that tomato lectin conjugates bind to mucus gel, whereas, asparagus pea lectin and *Mycoplasma gallisepticum* lectin have a specificity for the PP region of rats. Jepson et al. (78) attributed the difference in uptake of polystyrene particle and polylactide-coglycolide particles to the fact that the former exhibited selective binding to (rabbit) M cells. Variations in lectin binding properties of intestinal M cells have been studied by that group (79); there is marked species and regional variation in affinities. Peanut agglutinin (PNA), wheat germ agglutinin (WGA) and *Bandeiraea simplicifolia* agglutinin II (BSA-II) did not distinguish between enterocytes or M cells, while the highly selective binding of UEA-I to mouse PP M-cells was not seen in the rabbit. Such complexity makes extrapolation from animal data difficult and hence the design of systems problematical, particularly if these are to be based on putative selective binding ligands. Nonetheless, research to prove concepts is vital, and the use of a human intestinal xenograft model may be promising in this regard (80).

Utilizing Bacterial Modes of Entry

In attempting to mimic bacterial modes of epithelial uptake in the gut we have coupled an invasin molecule, derived from the outer membrane protein of *Yersinia tuberculosis* and *Y. enterocolitica* to polystyrene particles of 500 nm diameter (81). The interaction of the invasin with cell integrin receptors involve subsequent cytoskeletal changes, thus the microparticles are truly bioactive. After a single oral dose of maltose-binding protein (MBP)-invasin-192 polystyrene conjugates, 13% was found in the systemic circulation of rats. Controls (invasin conjugates blocked with mucin) which exhibited low levels of uptake. The results obtained are in line with those reports in which *E. coli* expressing invasin on its outer surface (82), inert particles coated with invasin containing membranes (83) or MBP-invasin-192 fusion protein (84) efficiently entered human cell lines. Our data represent an early *in vivo* demonstration of the effectiveness of the approach to enhancing uptake, although not exclusively by way of the Peyer's patches.

Polystyrene has served as a good model because it is not degradable, but it is unacceptable as a carrier. It is one of a now very large number of particles which have been found unequivocally to be absorbed from the gut by way of lymphoid and non-lymphoid tissues. Sometimes the differences in published data on the fate of such particles reflect where investigators have looked, rather than the extremities the particles have reached. Differences in the levels of uptake sometimes reflect differences in the methodology, and whether or not the whole gut, or a small part of it, was examined, and whether or not the animal was anesthetized. Blood and lymph flow in the region of uptake can be crucial. There is evidence of saturation of the uptake process, and of biliary re-excretion into the lumen. Particles which have been absorbed from the GI lumen are

taken into the biliary system (85): levels in the bile for 50 nm, 500 nm and 1 μm polystyrene particles were 18, 8 and 1 percent respectively.

Can Drug Particles be Absorbed Directly?

There seems to be no *a priori* reason why drug suspensions of suitably small size and appropriate hydrophobic surface characteristics should not be taken up by PP tissue. Recent interest in the methodology of forming particles, say with supercritical fluid technology (86) or wet milling technology such as used to produce 200 nm crystals of piposulfan, camptothecin, etoposide and paclitaxel (87) might lead to interesting studies on direct absorption.

CONCLUSIONS

The constraints of a mini-review mean that this survey has been selective. However, the range of submicron colloidal particles available now as potential oral carriers is formidable, especially as the dividing line between macromolecules, polymer aggregates, micelles and submicron particles is blurred. The story is far from over. Utilizing bacterial and viral mechanisms of uptake and selectivity it should be possible to engineer carriers which not only have a specificity for particular intestinal tissue sites, but also have the optimal characteristics for both maximizing the load and maximizing the stability of carried drug or other therapeutic entity.

The example provided by Newton (88) in which a synthetic oligonucleotide specifying an epitope of cholera toxin subunit B has been inserted into a cloned *Salmonella* flagellin gene, illustrates what is possible and how far we have come from starch and polystyrene. One concludes that uptake and translocation are neither exceptional nor unusual; whether the process will be mastered to achieve therapeutic drug delivery has yet to be shown conclusively. The next 5 years should be exciting. If, as suggested by the data now available, particulate uptake can be induced by way of non lymphoid intestinal tissue, then the possibility of delivering "difficult" molecules in carriers by the oral route will no longer be conjecture. The more that is learned about viral and bacterial modes of entry from the gut, the more we will be able to harness these mechanisms. Increasingly the lymphoid tissue may itself become a target for delivery of antiviral and antibacterial agents.

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