Chapter 10

Oral Delivery of Microencapsulated Proteins

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1. INTRODUCTION

While the neonatal mammalian small intestine is able to absorb macromolecules (Walker and Isselbacher, 1974; Walker, 1979), in the mature gut the oral bioavailability of polypeptide drugs is generally poor (Lee et al., 1991; Humphrey and Ringrose, 1986; Pusztai, 1989). This low bioavailability is not surprising given the large number of barriers in the gastrointestinal tract to the uptake of intact peptides and proteins. Proteolytic enzymes in the stomach, the intestinal lumen, and the brush border of the enterocytes digest proteins by hydrolysis. Most digestion in the small intestine is due to the proteolytic enzymes trypsin, chymotrypsin, and carboxypeptidase, all from the pancreas (Castro, 1981; Matthews et al., 1968). Any remaining peptides larger than three amino acids are further hydrolyzed in the brush border. Tripeptides are hydrolyzed extracellularly, by the brush border enzymes, or, if absorbed, by cytoplasmic amino peptidases (Nicholson and Peters, 1977). Besides enzymatic barriers, there are significant physical barriers to peptide drug absorption in the intestinal wall. Within the intestinal mucosa, the membrane of the microvilli consists of the typical trilaminar arrangement observed in biological membranes: two molecular layers of lipid with the hydrocarbon tails oriented inward and the hydrophilic heads on the outer part of the protein-coated membrane. The
outer membranes of adjacent cells are fused at the basal membrane, forming a tight junction—a significant barrier between the lumen and the intercellular space (Humphrey, 1986; Csaky, 1984). The glycocalyx, a uniform layer of filamentous glycoproteins on the surface of the microvilli (Egberts et al., 1984), possesses a negative charge at physiologic pH due to the presence of sialic acids at the terminal portion of the carbohydrate chain. The glycocalyx, furthermore, lies within a layer of mucus, about 1–5 µm thick, upon which lies an unstirred water layer about 100–400 µm in depth (Humphrey, 1986; Thomson and Dietschy, 1984). The unstirred water layer can act as a barrier for both water-soluble (Alpers, 1987) and hydrophobic substances (Humphrey, 1986), while the combination of mucus and glycocalyx may be a barrier to polar molecules due to both the viscosity and the electronegativity of that layer (Smithson et al., 1981; Esposito et al., 1983).

Despite all of the above obstacles to peptide and protein absorption, there is both clinical and experimental evidence that large molecules may penetrate the intestinal mucosa (Gruber et al., 1987). The degree of this macromolecular absorption is certainly not of any nutritional significance but still is often high enough to be of biological importance (Alpers and Isselbacher, 1967; Bernstein and Ovary, 1968; Bockman and Winborn, 1966; Casley-Smith, 1967; Chisui, 1968; Cornell et al., 1971; Danforth and Moore, 1959; Walker et al., 1972; Warshaw et al., 1971), and such macromolecular absorption has been observed to occur under normal physiologic conditions (Gruskay and Cooke, 1955; Korenblat et al., 1968; Wilson and Walzer, 1935). Adults, for example, develop precipitins in serum following ingestion of milk proteins (Korenblat et al., 1968). The oral absorption of β-lactam antibiotics, angiotensin-converting enzyme inhibitors, and cyclosporins further reveals that enteral uptake of peptides or peptide analogs is possible. In experimental systems, even large proteins such as insulin (Danforth and Moore, 1959) and horseradish peroxidase (Warshaw et al., 1971) in rats and large-molecular-weight antigens in guinea pigs (Bernstein and Ovary, 1968) have been noted to cross the intestinal wall. The permeability, reactivity (chemical or enzymatic), transit time (Amidon et al., 1988; Sinko et al., 1991), and solubility and dissolution rates (Oh et al., 1993) will affect the degree of oral absorption for each peptide or protein. Factors such as molecular weight (Bernstein and Ovary, 1968), charge, structure, and configuration all will determine those characteristics which will influence intestinal permeability.

One way to potentially enhance the uptake of proteins and peptides is to microencapsulate them prior to administration. Besides providing protection from intestinal enzymes, microcapsules could be formulated with characteristics such as charge and hydrophobicity, which might exploit the
above-summarized intestinal wall features and result in increased systemic uptake as compared to that of the native, nonencapsulated peptide. This chapter is intended to provide, first, an overview of the specific mechanisms that result in the transport of proteins and peptides across the intestinal wall. A summary of the observed and theorized mechanisms of microparticulate intestinal absorption will then be presented. The final section will focus on various case studies wherein microspheres have been used as oral protein and peptide drug delivery systems.

2. MECHANISMS OF INTESTINAL ABSORPTION OF PROTEINS AND PEPTIDES

The intestinal absorption of intact peptides or proteins occurs to varying extents (Gardner, 1984; Silk et al., 1985; Humphrey and Ringrose, 1986) and generally involves either a passive mechanism, some type of carrier-mediated transport, or receptor-mediated or non-receptor-mediated endocytotic transport. Each mechanism will contribute to the absorption of a peptide or protein to a different degree, depending upon such characteristics as the size, charge, or lipophilicity of the peptide or protein.

2.1. Passive Diffusion

Most peptide drugs—those larger than three amino acids—that are passively absorbed, are absorbed by diffusion through the lipid membrane of enterocytes. Although the diffusivity of the drug in the membrane and the membrane thickness are both important factors affecting permeability, the principal determinant of the degree of absorption of a drug across the lipid membrane is the partition coefficient between the membrane and the lumen (Matthews, 1991; Houston and Wood, 1980). Lipid-soluble molecules are taken up readily when compared to molecules taken up by other passive-transport mechanisms, such as diffusion through aqueous pores (Menzies, 1984). In one experimental system, the relatively nonpolar rat jejunum exhibits increased absorption of lipid molecules with decreasing polarity of those molecules (Westergaard, 1987). This phenomenon is also demonstrated in the absorption of endotoxins from gram-negative bacteria due to the interaction between the lipid A portion of the endotoxin and the apolar epithelial membrane (Kabir et al., 1978). With regard to drug design, increasing the lipophilicity of a drug can result in increased intestinal absorption by either passive mechanisms or the carrier pathways that will be discussed below (Clayton et al., 1975; Wyvratt and Patchett, 1985).
Increasing lipophilicity can, however, result in a decrease in solubility, which, in turn, can result in solubility/dissolution problems and ultimately adversely affect intestinal absorption (Oh, 1991).

Besides diffusion across the lipid bilayer of intestinal epithelial cells, another mechanism by which peptides and proteins can be absorbed is by diffusion through aqueous pores in the cell membrane. Aqueous pore diffusion in undamaged cells, though, is probably dominated by smaller-molecular-weight species, particularly those that are water-soluble and are not taken up by carrier-mediated transport mechanisms (Pusztai, 1989).

The effect of molecular weight on drug absorption by either of the diffusion pathways discussed above has not been fully explored. Cyclosporin, with a molecular weight of 1200, exhibits a low, but acceptable, extent of oral absorption of 23% (Wood et al., 1983). Drugs of molecular weight 2000–3000, therefore, might be expected to exhibit bioavailabilities of 10–20% (Amidon and Lee, 1994). A number of significant drugs, e.g. labetolol, morphine, and propanolol, exhibit low systemic availabilities (Benet and Williams, 1990). Even with an upper molecular-weight limit of 1000–2000 for peptide drugs which might exhibit some reasonable bioavailability, the number of possible peptide drugs that might be synthesized from natural amino acids is quite large (Amidon and Lee, 1994).

Passive diffusion can also occur by paracellular routes. Horseradish peroxidase (HRP) has been observed to cross the mucosal barrier intact in both normal rat jejunum (Heyman et al., 1982) and surgically traumatized guinea pig intestinal tissue (Rhodes and Karnovsky, 1971). The actual route of transport of the HRP in the normal intestinal tissue was not known but was concluded to be either by an intracellular pathway or intercellular, through tight junctions (Heyman et al., 1982). Transport of the HRP in the guinea pig intestine was observed to be through the epithelial tight junctions but required bovine serum albumin (BSA) as a macromolecular cofactor to alter the tight-junction barrier. While the use of BSA, which has sometimes been found to be contaminated with endotoxins or phospholipases (Dvorak and Bast, 1970), complicates analysis of these observations, the result still suggests the possibility of intercellular diffusion. Similarly, paracellular transport of macromolecules has been observed when the brush border has been damaged through disease or experimentally, as with hypertonic solutions (Menzies, 1984; Wheeler et al., 1978). Paracellular transport of macromolecular food proteins also has been observed, albeit under the more natural circumstance of passage across the villus tip, where epithelial cells are routinely shed into the lumen. In that extrusion zone, where the intercellular junctions have been naturally disrupted, macromolecular transport has been observed in both normal and diseased small intestine (Jackson et al., 1983).
2.2. Carrier-Mediated Transport

Carrier-mediated transport represents another pathway for intestinal absorption, particularly for di- and tripeptides (Adibi and Phillips, 1968; Matthews and Payne, 1980; Humphrey, 1986; Matthews, 1975, 1983, 1991) and their analogs (Kramer et al., 1990; Kimura et al., 1983; Nakishima et al., 1984; Allen et al., 1979; Yokohama et al., 1984a,b; Friedman and Amidon, 1989a; Hu and Amidon, 1988; Sinko and Amidon, 1988, 1989; Tsuji et al., 1987). Di- and tripeptides, as well as amino acids, are produced naturally by the enzymatic degradation of polypeptide and protein fragments remaining after gastric and luminal digestion of ingested macromolecules. Intraluminal hydrolysis accounts for only a small portion of the protein digestion (Newey and Smith, 1962), with the exo- and endopeptidases of the brush border of the microvilli being the principal producers of absorbable peptide fragments (Adibi and Kim, 1981).

Carrier-mediated transport involves cotransport of the absorbable species with a proton. The required proton gradient is hypothesized to be maintained by a Na⁺–H⁺ exchanger. The lumen of the intestine is acidic relative to the epithelial cell cytosol. The low cytosolic sodium concentration, required to produce the transporter driving force, is maintained by the Na⁺–K⁺ ATPase in the basolateral membrane. The sodium/proton exchanger working in concert with the sodium/potassium ATPase, therefore, results in a transport mechanism for the uptake of di- and tripeptides into the intestinal wall (Ganapthy and Leibach, 1985).

The carriers for free amino acids are not the same as those for di- and tripeptides, and the number of different carriers and the specificity of those pathways are not known (Matthews et al., 1968). The large number of natural di- and tripeptides, furthermore, limits the ability to completely describe all existing mechanisms of carrier transport (Matthews, 1975, 1983). Through extensive study of these mechanisms (Matthews, 1991), however, a number of general guidelines have been developed.

The carrier transport pathway is stereospecific (Matthews, 1975, 1983; Boyd and Ward, 1982; Asatoor et al., 1973; Cheeseman and Smyth, 1972); peptides of the D-configuration are handled by the transporter (Boyd and Ward, 1982; Asatoor et al., 1973; Cheeseman and Smyth, 1972) but are poorly taken up and slowly hydrolyzed (Matthews, 1975, 1983). An α-peptide bond is preferred (Matthews, 1975, 1983), though not required (Bai et al., 1991) for carrier transport, whereas methylation, acetylation, or other modification of the N-terminal α-amino group (Addison et al., 1974; Das and Radhakrishnan, 1975; Rubino et al., 1971; Addison et al., 1975), as well
as modification of the C-terminal carboxyl (Matthews, 1975, 1983), decreases or eliminates affinity for the transport carrier. The presence of a β-amino acid as part of a dipeptide is compatible with carrier transport (Addison et al., 1974, 1975; Tomita et al., 1990), but the presence of a γ-amino acid eliminates affinity for the carrier (Das and Radhakrishnan, 1975; Tomita et al., 1990). There is little evidence for carrier transport of tetrapeptides (Adibi and Morse, 1977; Burston et al., 1979; Kerchner and Geary, 1983); when observed, tetrapeptide uptake has been concluded to be by passive mechanisms (Matthews and Payne, 1980; Boyd and Ward, 1982; Addison et al., 1975). Similarly, penta- and hexapeptides are rarely absorbed intact and are generally digested by the brush border peptidases, down to molecules that can be absorbed by carrier pathways (Gruber, et al., 1987).

Peptide analogs such as β-lactam antibiotics (Kramer et al., 1990; Quay, 1972; Quay and Foster, 1970; Sinko and Amidon, 1988; Kimura et al., 1983; Nakashima et al., 1984), angiotensin-converting enzyme (ACE) inhibitors (Kramer et al., 1990; Friedman and Amidon, 1989a,b; Hu and Amidon, 1988; Yee and Amidon, 1990), alafosfalin (Allen et al., 1979), and thyrotropin releasing hormone (TRH) (Yokohama et al., 1984a, b) are also absorbed via the carrier transport system, as has been demonstrated in studies of competitive inhibition with di- and tripeptides, though no competitive inhibition is seen with free amino acids (Sinko and Amidon, 1988; Oh et al., 1989). The study of the transport of these peptide analogs has reflected some of the specificities of the carrier pathway that were outlined above and revealed other characteristics of this route of absorption. Work with TRH, which is absorbed by the carrier though it lacks both a free N-terminal α-amino group and a C-terminal carboxyl group (Yokohama et al., 1984b; Humphrey and Ringrose, 1986), has shown that the carrier pathway is saturable with increasing dose (Yokohama et al., 1984b). Substitution in TRH of a pyroglutamyl residue with carboxybutyrolactone, creating the analog DN 1417, results, however, in a molecule taken up only by passive mechanisms (Addison et al., 1975). The lack of requirement for a free N-terminal α-amino group for carrier transport also has been proven in studies of such β-lactam antibiotics as cefixime and ceftibuten (Tsuji et al., 1987; Oh et al., 1990; Tsuji et al., 1986) as well as with ACE inhibitors such as captopril, enalapril, and lisinopril (Hu and Amidon, 1988; Friedman and Amidon, 1989a, b; Yee and Amidon, 1990). Investigations of ACE inhibitors have shown that the ester prodrugs such as enalapril are absorbed by the transporter whereas the diacids, such as enalaprilat, are poorly absorbed. Lisinopril, which is somewhat similar in structure to enalaprilat, exhibits an affinity for the transporter, but with low carrier permeability (Friedman and Amidon, 1989a, b; Tsuji et al., 1986; Yee and Amidon, 1990). The underlying reasons for these specificities are not entirely understood.
2.3. Receptor-Mediated and Non-Receptor-Mediated Endocytosis

With the carrier transport pathway restricted almost exclusively to uptake of amino acids, dipeptides, and tripeptides, and passive diffusional uptake also limited mostly to smaller, and generally more lipophilic, species, the principal pathway for uptake of macromolecules in the intestine is by endocytosis. Similarly to passive absorption, endocytotic uptake is truly appreciable only in the immature intestine, decreasing significantly after gut closure (Pusztai, 1989). The capacity for uptake of macromolecules in the mature intestine, however, is still significant enough to allow antigen sampling for the development of mucosal immunity or permit appreciable absorption of various toxins.

Endocytotic uptake in the intestine can occur by either receptor-mediated or non-receptor-mediated pathways. For both pathways, endocytotic uptake follows the same general process, one that is similar to macrophage phagocytosis. Endocytotic uptake starts with either nonspecific adsorption or receptor binding of large molecules to the absorptive cell. Fluid-phase markers have also been observed to be internalized during receptor-mediated endocytosis (Doxsey et al., 1987; Sandvig et al., 1987). Once enough molecules are adsorbed or bound, invagination of the membrane causes the formation of a membrane-bound vesicle (endosome or phagosome). The phagosome will migrate across the cell and, in most cases, depending on the type of absorptive cell, fuse with a lysosome to form large vacuoles (phagolysosome). The majority, though not all, of the contents of the phagolysosome are then digested (Heyman et al., 1982), after which the phagolysosome fuses with the basal surface of the absorptive cell and releases the contents of the vacuole into the interstitium (O’Hagan et al., 1987). Since there is not always fusion of the endocytotic vesicle with a lysosome, and since lysosomal digestion is not always complete, macromolecules can be absorbed intact into the intestinal wall.

The efficiency of macromolecular uptake by endocytosis, as well as the pathway of transcellular transport of the resulting endosomes, is related to whether fluid-phase or absorptive, receptor-mediated or non-receptor-mediated, endocytosis is involved (Mayorga et al., 1989; Roederer et al., 1987; Wessling-Resnick and Braell, 1990; Abrahamson and Rodewald, 1981; Tartakoff, 1987). The ability of the endosomes to discriminate between soluble and membrane-associated species has been demonstrated in neonatal rodents with soluble HRP and immunoglobulin. Immunoglobulin is taken up in the neonatal rodent by receptor-mediated transport across the intestinal epithelial cells. When the apical side of the intestinal epithelial cells is exposed to both HRP and immunoglobulin, both are taken up within the
same vesicles and found within endosomes. The HRP, though, is directed toward lysosomes, whereas the immunoglobulin is found in more deeply situated tubules and vesicles (Abrahamson and Rodewald, 1981).

Factors such as charge (Shen and Ryser, 1978), or the specific characteristics of the ligands and receptors (Salzman and Maxfield, 1989; Dunn et al., 1989; Tartakoff, 1987) will also influence endocytosis and the fate of endosomes. The conjugation of poly(L-lysine) to HRP, for example, has been observed to result in a 200-fold increase in uptake of the HRP by cultured mouse fibroblasts. This was concluded to be a result of the addition of a strong cationic charge to the HRP (Shen and Ryser, 1978).

Intestinal absorption of macromolecules by endocytosis occurs primarily through two types of cells: the enterocytes in the epithelium of villi and the so-called M cells (membranous or microfold cells), which are scattered throughout the intestine but are mostly concentrated in the Peyer’s patches overlying the gut-associated lymphoid tissues (GALT). The principal function of the M cells is, in fact, to sample and present protein antigens (Fara, 1985; Bockman and Cooper, 1973; Owen, 1977; Owen and Jones, 1974) or microorganisms (Wolf et al., 1981; Owen et al., 1986b) to the underlying lymphoid tissue. That the M cells lack a well-developed lysosomal apparatus (Owen et al., 1986a) assists in this antigen-presenting function, because the low number of lysosomes improves the chances for presentation of intact absorbed proteins to the underlying lymphoid cells. When the concentration of an absorbable antigen is low, transport through the M cells dominates, whereas once concentrations are high, endocytotic uptake is seen in other epithelial cells, both in the Peyer’s patch and in the villi (Owen, 1977; Walker, 1982). Once an antigen binds to the apical membrane of an M cell, endocytosis, transcytosis, and exocytosis occur fairly rapidly (Bockman and Cooper, 1973; Owen, 1977; Danforth and Moore, 1959).

Much of the work on non-receptor-mediated endocytosis has involved the use of HRP as a tracer. In one study in rats (Cornell et al., 1971), HRP was injected into the lumen of ligated portions of the jejunum and ileum. Uptake of the HRP was comparable in the jejunum and ileum and was greatest near the apical portion of the villi, with less in the base and none in the crypts. Besides being absorbed at the apical surface membrane, HRP was also present within membrane-bound cytoplasmic cannicular vesicles and vacuolar structures. HRP was also absorbed into extracellular spaces between adjacent absorptive cells and in the lamina propria. In a study of HRP infusion into ileal segments of mice (Owen, 1977), HRP was observed to be taken up more readily by M cells than by columnar cells. HRP absorbed by M cells was released into the extracellular space, for uptake by lymphoid cells, rather than being sequestered into lysosomes, as was seen
with HRP absorption by epithelial cells in other locations (Straus, 1969). In a study in rabbits (Heyman et al., 1982), an estimated 67–97% of the HRP that was endocytosed by epithelium in villi was degraded during transcytosis.

A related study by Heyman (1990) with CaCo-2 cells confirmed HRP transport by endocytosis, primarily along a degradative pathway most likely associated with the lysosomal system. Other work with CaCo-2 cells and HRP (Hidalgo et al., 1989) found that HRP is endocytosed by a fluid-phase mechanism; this proposed uptake mechanism was supported by the Heyman study, in which monensin was found to have no effect on HRP transcytosis. The degree of HRP degradation also varied depending on whether the marker was presented at the apical or the basal membrane of the CaCo-2 cells. Different cell types of intestinal epithelium, therefore, could process endocytosed proteins in disparate ways (Heyman et al., 1990).

Within the small intestine, there are several types of ligand–receptor pairs for receptor-mediated endocytosis. Lectins are one such class of receptors or ligands that are often associated with receptor-mediated endocytosis and have been seriously considered as conjugates to enhance oral delivery of protein and peptide drugs. M-cell luminal membranes display a variety of lectin-binding specificities (Owen and Bhalla, 1983; Roy, 1987). Lectin–antigen conjugates that attach to M-cell apical membranes are absorbed more efficiently than conjugates that do not adhere (Neutra et al., 1987). Most microbial and plant lectins or toxins interact with the epithelium of the small intestines, though many of those lectins/toxins in binding, cause some damage to the cells, with subsequent endocytosis of the lectin/toxin (Pusztai, 1989). Lectins can be bound to toxins, and act to provide a mechanism for uptake of the toxin by the cell, though the lectin itself is not toxic (Blaustein et al., 1987; Pappenheimer, 1977; Gill, 1978; Dallas and Falkow, 1980; McDonel, 1980; Pusztai, 1986a). Alternatively, lectins may be unassociated with a toxin but simply consist of multiple, and varied, lectin subunits. Even with no associated toxin, these lectins still may have some toxic effect, though far less an effect than is seen with lectins conjugated to toxins. Once bound, these lectins can cause extensive morphological changes in the cell membrane and interfere with the cell metabolism. Often, these types of lectins can be found in dietary plants and microorganisms of the intestinal tract (Goldstein and Poretz, 1986; Pusztai, 1986b; Pusztai et al., 1986; Pusztai, 1988). PHA, from kidney beans (Phaseolus vulgaris), is one of the most studied lectins (Greer et al., 1985; King et al., 1980, 1982). When ingested, PHA is mostly excreted intact, but a relatively high percentage, 5–10%, is taken up systemically (Pusztai, 1988). This degree of uptake is particularly significant when compared to the 0.1%
uptake of tomato lectins, nontoxic lectins that also bind strongly to intestinal epithelium (Kilpatrick et al., 1985). Furthermore, though most of the endocytosed PHA interacts with lysosomes (King et al., 1986), most of the lectin is exocytosed intact.

Two other related systems of receptor-mediated transport are those associated with iron and vitamin B₁₂ uptake. In both systems, the specific binding protein is released into the intestine and binds to the ligand in the lumen of the gut. In the case of iron, transferrin is released in the stomach, and binds to the iron, and then this complex binds to receptors in the duodenal mucosa. Vitamin B₁₂ forms a stable complex (Grasback et al., 1959) with intrinsic factor (IF) released in the stomach, and this B₁₂–IF complex in turn interacts with enterocyte membrane-bound receptors for IF (Mathan et al., 1974), which are located in the terminal ileum. The actual uptake of the B₁₂–IF complex is not completely understood, though it is known to be slow, taking over 3 hr, is energy-dependent (Chanarin et al., 1978), and, like the uptake of the iron–transferrin complex, involves endocytosis (Russell-Jones and de Aizpurua, 1988; Castle, 1953; Fox and Castle, 1942; Allen and Majerus, 1972a, b; Jani et al., 1989).

Immunoglobulins, specifically IgA and IgG, have also been shown to adhere selectively to M-cell luminal membranes in several experimental models. Mouse monoclonal IgA antibodies were observed to adhere to the M-cell membranes and to be transported across the epithelium, independent of the antigen specificities of the antibodies. IgG was found to inhibit this binding of IgA by attaching to the M-cell surface. These results suggest that there exists a common immunoglobulin domain which, regardless of species specificity, mediates adherence to M cells (Weltzin et al., 1989).

3. MECHANISMS OF INTESTINAL ABSORPTION OF MICROPARTICULATES

Given the susceptibility of orally administered peptide and protein drugs to enzymatic degradation in the lumen and brush border of the intestine, there has been increasing interest in the microencapsulation of those drugs and in understanding the uptake of microparticulates in the intestine. Most particulate uptake involves transcellular pathways across the intestinal epithelium, though there have been some observations of paracellular transport of particles. The focus of the discussion below will be on these pathways and on how factors such as size, charge, and hydrophobicity affect particulate absorption in the intestine.
3.1. Transcellular Pathway

Based on the majority of observations of particulate uptake in the intestine, transcellular transport, specifically through the epithelium of the Peyer’s patch, appears to be the primary pathway of microparticulate absorption (LeFevre and Joel, 1984; Pappo et al., 1991; Eldridge et al., 1990; Damge et al., 1990; Pappo and Ermak, 1989). Furthermore, most of the transcellular transport involves M cells (Bockman and Cooper, 1973; Joel et al., 1978, 1970; LeFevre et al., 1978a), though there have been a few select instances in which other cell types appeared to be involved (Wells et al., 1988; Landsverk, 1988).

The size of a microparticle may be the most significant parameter with regard to absorption into the Peyer’s patch and the ultimate fate of that particle once inside the gut-associated lymphoid tissue (GALT). Particles ranging from 27-nm carbon (LeFevre and Joel, 1986; Joel et al., 1978; Hammer et al., 1983; LeFevre et al., 1985b) to 10-µm polymer microspheres (Eldridge et al., 1990) have been observed within Peyer’s patches following oral administration in test animals. Polymer microspheres greater than 10µm have specifically been observed not to be absorbed (Eldridge et al., 1990; LeFevre et al., 1980), so 10µm is often considered the cutoff for particulate absorption. Smaller particles also appear to be absorbed more avidly than larger ones. Particles 1µm in diameter have been observed to be taken up less efficiently than 100-nm and 500-nm particles (Jani et al., 1989), and 2.65µm latex particles are more readily absorbed than 9.13-µm particles in the Peyer’s patches of mice (Ebel, 1990). Even after transepithelial transport, size can still affect the destiny of an absorbed particle. In one study, polymer particles less than 5µm in diameter were observed to be transported through efferent lymphatics in macrophages, whereas most particles of the same polymer but greater than 5µm in diameter remained within the Peyer’s patch (Eldridge et al., 1990). In another study, latex particles less than 5.7µm in diameter were absorbed into the blood, whereas larger particles were not seen in the blood but were observed in the Peyer’s patch and mesenteric lymph node (LeFevre et al., 1980).

The hydrophobicity or charge of a particle has also been observed to affect uptake (LeFevre et al., 1978a, 1979, 1980, 1985b; Pappo and Ermak, 1989; Eldridge et al., 1990; Eldridge, 1988). Microspheres consisting of polymers exhibiting relatively high hydrophobicity have been demonstrated to be more readily absorbed into Peyer’s patches than those made of less hydrophobic polymers (Eldridge et al., 1990; LeFevre et al., 1985b). Carboxylated latex particles, carrying a negative charge, exhibited decreased uptake relative to uncharged latex microspheres (Jani et al., 1989).
There still are some general observations regarding this pathway for particulate absorption which are somewhat independent of the specific characteristics of a particle. Absorption of microparticulates into the Peyer’s patch is most likely via intracellular vesicles (O’Hagan et al., 1987; Jani et al., 1989), but generally without formation of a phagolysosome because of the relative deficiency of lysosomes in M cells (Owen et al., 1986a). This absorption process is quite rapid (Wolf et al., 1981; Landsverk, 1988; Pappo and Ermak, 1989; Damge et al., 1990), with transport of microspheres across the M cells occurring within 10 minutes (Pappo and Ermak, 1989; Damge et al., 1990). In one investigation, the rate of transport of 650- to 750-nm latex particles was comparable to that observed with soluble antigens (Bockman and Cooper, 1973; Owen, 1977), lectin–ferritin conjugates (Neutra et al., 1987), and microorganisms (Wolf et al., 1981; Owen et al., 1986). This rapid transport suggests that there is some form of membrane binding, based on studies showing that transport across M cells is generally faster with binding (Neutra et al., 1987). The transport of microparticulates through the M cells into the Peyer’s patch dome may be facilitated by fenestrations in the basal lamina supporting the follicle epithelium (McCluggage et al., 1986; Pappo et al., 1988).

Assuming a high concentration of microparticles in the intestinal lumen, as time progresses, an increasingly larger fraction of microparticles are transported across the follicle epithelium, with a distinct directionality of particles being taken up from the lumen and discharged into the M-cell pocket (Pappo and Ermak, 1989). This creates a concentration gradient from the lumen to the subepithelial dome, with localization of particles on the M-cell apical membrane, in the M-cell pocket, and in the subepithelial dome (Pappo et al., 1991). The uptake of particles, however, is not always continuous. One study revealed what appeared to be an 80-min pause in uptake, 10 min after infusion into an intestinal loop, despite a high concentration of latex microspheres in the lumen (Pappo and Ermak, 1989). Once absorbed into the GALT, microparticulates generally are transported into the lymphatic system, often after being taken up by macrophages (LeFevre and Joel, 1984; Jani et al., 1989; LeFevre et al., 1978b, 1989).

Though dependent upon the size of the microparticulates, and not large on an absolute scale, the fraction of microparticulates taken up into the Peyer’s patch following an intraluminally administered dose, estimated to be as high as 5%, is still quite significant (Pappo and Ermak, 1989). This can be compared to estimates of 0.01–0.02% for the fraction of antigen absorbed through non-Peyer’s patch and non-receptor-mediated pathways following similar dosing (Gruskay and Cooke, 1955; Warshaw et al., 1971). While the uptake of antigen into Peyer’s patches is known to be more efficient than absorption elsewhere in the gut (Keljo and Hamilton, 1983),
the observed high level of uptake for polystyrene nanoparticles (Pappo and Ermak, 1989) suggests that M-cell phagocytosis of those polymer particulates may be more proficient than that seen with biological particles (Wolf et al., 1981; Owen et al., 1986b). With regard to protein and peptide drug delivery, particulate uptake has been shown to be further enhanced when the particles are conjugated to anti-M-cell antibodies (Pappo et al., 1991).

3.2. Paracellular Transport

While the previous discussion reveals that a large number of investigations have reported transcellular transport of particulates, the number of studies demonstrating paracellular transport is far smaller. Some of the earliest work on paracellular transport of particulates was performed by Volkheimer and co-workers (Volkheimer and Schulz, 1968, 1969; Volkheimer, 1975, 1977; Volkheimer et al., 1968, 1969), who observed intestinal absorption into the blood, following oral administration in several animal models, including humans, of a wide variety of particles (e.g., starch grains, diatoms, pollens). Candida albicans has also been reported to be absorbed in the intestine by a paracellular pathway (Krause et al., 1969; Stone et al., 1974), and lipoidal loaded alkyl cyanoacrylate nanocapsules were observed in the intercellular space between enterocytes 10–15 min after injection into a jejunal loop (Damge et al., 1990).

While, as discussed above, the maximum particle size generally reported for transcellular transport is 10µm (Eldridge et al., 1990; LeFevre et al., 1980), Volkheimer observed the absorption of particles from 5 to 150µm into the blood following oral administration. According to Volkheimer, the optimal size for particles absorbed by this process, which he assumed to involve paracellular transport and which he labeled "persorption," was 5–70µm. The particle hardness was also reported to be a factor in the degree of absorption into the blood, with harder particles exhibiting more efficient absorption (Volkheimer and Schulz, 1968, 1969; Volkheimer et al., 1968, 1969; Volkheimer, 1975, 1977).

Some form of local desquamation of intestinal epithelium seems to be the most common explanation of how particles, of any size, might be absorbed via the usually tight junctions between epithelial cells (Luckey, 1974; Csaky, 1984). One hypothesized mechanism for the extrusion of epithelial cells is a combination of the pressure from reproducing epithelial cells, which loosens the surrounding cells, and variations in lymphatic pressure in the lacteals acting on the epithelium. A decrease in lymphatic pressure could then, in theory, draw particulate matter into the site through
this region of deep epithelialization (Luckey, 1974). Persorption also may involve the “kneading” of large particles through the intestinal epithelial layer, and increased gastrointestinal motility, therefore, has been surmised to be important for increasing persorption (Volkheimer et al., 1968). This hypothesis was supported by studies in which villi movement was either stimulated by drugs such as caffeine or neostigmine or deterred by atropine or barbituric acid and persorption rates of starch granules increased and decreased, respectively (Volkheimer, 1977; Volkheimer et al., 1968).

3.3. Liposome Absorption

The uptake of liposomes into the intestinal wall appears to parallel the transcellular transport of particulates. All reports of liposome absorption indicate that the Peyer’s patches are the principal pathway for transepithelial transport (Aramaki et al., 1993; Michalek et al., 1992; Childers et al., 1990; Alpar et al., 1992; Rowland and Woodley, 1980, 1981), though the specifics of this process are poorly characterized (Aramaki et al., 1993). Similar to the observed uptake of latex particles by Peyer’s patches (LeFevre et al., 1985a), distearoylphosphatidylcholine, phosphatidylcholine, phosphatidylserine, and cholesterol liposomes (DSPC-liposomes), 374 and 855 nm in diameter, were preferentially taken up by Peyer’s patches in the lower ileum of rats (Aramaki et al., 1993). Notably, these DSPC-liposomes have been found to be very stable in the acidity of the gastrointestinal tract (Alpar et al., 1992; Rowland and Woodley, 1980, 1981), a characteristic considered critical for the potential transport of any encapsulated drug (Chiang and Weiener, 1987a, b). A negative charge may enhance uptake of liposomes, as was reported for somewhat larger, negatively charged phosphatidylserine liposomes in rat Peyer’s patches (Tomizawa et al., 1993). Also, similarly to polymer particulates, liposomes have been observed to be transported across the epithelium by endocytosis (Childers et al., 1990; Aramaki et al., 1993) and then exocytosed into the intercellular space of underlying lymphoid cells (Childers et al., 1990). The ultimate fate of liposomes, once absorbed into the intestinal wall, is in some dispute. Some studies report uptake of intact liposomes into the portal vein (Das et al., 1984) or even the systemic blood circulation (Dapergolas and Gregoriadis, 1976a). Other investigations dispute the possibility of intact liposome uptake into the systemic blood circulation (Patel and Ryman, 1977; Deshmukh et al., 1981).
4. CASE STUDIES

4.1. Introduction

From the recognition and verification that labile therapeutic compounds can be protected from the harsh environment of the gastrointestinal system and that particles in the $< 10\mu m$ range can cross the barrier of the intestinal mucosa have arisen many potential applications of microencapsulation for drug or vaccine delivery. Requirements for biocompatibility and stability have excluded many potential matrices for use although the potential for microspheres (monolithic systems) or microcapsules (reservoir systems) both to protect labile compounds and to aid in systemic delivery has inspired numerous companies and academic institutions to investigate such approaches for the oral delivery of peptides and proteins. Research and development in this area has progressed along many different pathways. Certain institutions have applied expert knowledge of microencapsulation techniques, whereas others have initiated projects based on an understanding of the physiological factors involved. Compounds under investigation for delivery range from established or novel systemic or targeted therapeutics to antigens for vaccine delivery. The systems themselves range from conventional aqueous insoluble microspheres of polymers with distinct properties to lipid systems, microencapsulated cells, and aqueous soluble bioadhesive microspheres. Although it is recognized that many of these systems will protect peptides and proteins from endogenous pH and enzymes, the main drive for the development of these types of systems is the panacea of significantly improving absorption and achieving systemic therapy, by oral administration, of compounds that are conventionally administered by the parenteral route.

The following case histories will elucidate the interest in not only therapeutic peptide and protein administration but also microencapsulated antigens for oral vaccine delivery. Oral immunization offers many practical advantages over parenteral. Not only is this route of administration more acceptable to patients, but the reduction in the need for highly trained personnel and refrigerated storage results in simpler logistics for mass immunization. In addition, oral immunization has been shown, in various systems, to induce a vigorous immune response at mucosal surfaces, the most common sites of entry of infectious agents.
4.2. Polyester Microspheres

Poly(lactic/glycolic acid) (PLGA) polymers and closely related analogs have been used for biomedical applications for many years, both as surgical sutures and as bone plates for internal fixation. In addition, PLGA polymers have been used for the preparation of parenteral controlled release drug delivery systems. Long experience with these compounds shows that they are completely biodegradable by hydrolysis of the ester linkages, to toxicologically acceptable products that are eliminated from the body. The polymer degradation rate is determined primarily by the ratio of lactide to glycolide present in the copolymer. Microspheres of PLGA are manufactured by solvent extraction and solvent evaporation techniques generally involving three inter-related processes: droplet formation, droplet stabilization, and droplet hardening (Arshady, 1991). Because of their well-documented history and their hydrophobicity, PLGA microspheres have also been investigated as potential antigen delivery systems for oral vaccines. Antigens in particulate form are generally effective oral immunogens, while soluble antigens are not (Cox and Taubman, 1984), possibly due to increased survival of the particulate antigen in the gastrointestinal tract and its enhanced adsorption and recognition in the immune inductive environment of the Peyer’s patches. PLGA microspheres, with various ratios of lactide to glycolide, have been shown to be specifically taken up into the Peyer’s patch lymphoid tissue of the gut. As previously discussed, the majority of the microspheres <5 µm in diameter are shown to leave the Peyer’s patches and enter the mesenteric lymph nodes, whereas the majority of particles >5 µm in diameter remain in the Peyer’s patches until digested, thereby stimulating a purely mucosal response (Eldridge et al., 1990,1989a). A number of preclinical studies on various antigens encapsulated into PLGA microspheres for oral delivery have been completed. The use of PLGA microspheres as an oral antigen delivery system for staphylococcal enterotoxin B (SEB) in mice has been described (Eldridge et al., 1990,1989b). The microparticles induced circulating toxin-specific antibodies and a concurrent secretory IgA anti-toxin response in saliva, gut wash fluid, and bronchial-alveolar wash. Induction of the pulmonary antibody response by oral immunization with microencapsulated SEB has potential implications for the development of oral vaccines against respiratory tract pathogens (Eldridge et al., 1991). PLGA microspheres have also been investigated as oral delivery systems for entrapped influenza virus (Moldoveanu et al., 1993), parainfluenza virus (Ray et al., 1993), simian immunodeficiency virus, and purified enterotoxigenic Escherichia coli colonization factor antigens (Edelman et al., 1993; Reid et al., 1993).
Although it appears that the majority of work with PLGA microspheres for oral delivery is directed toward vaccine administration, PLGA nanospheres have also been investigated as a potential alternate oral delivery system for cyclosporin (Sanchez et al., 1993).

4.3. Zein Microspheres

Prolamine is the name given to a characteristic class of proteins occurring specifically in cereals. Proteins of this class are rich in hydrophobic amino acids and soluble in aqueous alcohol. Zein, one of the typical prolamines and a major storage protein of corn (Larkins et al., 1984; Swallen, 1941), possesses many applicable characteristics for designing an oral drug delivery matrix: zein is insoluble in aqueous media yet is degraded over time by protease enzymes to constituent peptides and amino acids; it is hydrophobic and exhibits mucoadhesive properties; it is also GRAS, is covered by a United States Pharmacopoeia monograph, and has been previously used in pharmaceutical coating methodology. Zein has also been investigated as a potential sustained release, direct compression tablet excipient (Katayama and Kanke, 1992).

Alkermes Inc. (Cambridge, Massachusetts) has developed a patented drug delivery system (Mathiowitz et al., 1993) using zein as a microencapsulation matrix for therapeutic compounds, including peptides and proteins, for enteral delivery. The microspheres are formed by phase separation in a nonsolvent followed by solvent removal by extraction. The manufacturing process uses all GRAS materials and yields monolithic-type microspheres with greater than 90% therapeutic peptide or protein encapsulation efficiency and an average size of 1–5 \( \mu \)m. The size distribution of the microspheres is Gaussian and can be varied by modification of the manufacturing procedure.

The zein microsphere system (OraLease®) produced by Alkermes has been designed to protect drugs from the harsh environment of the stomach and the small intestine, increase the residence time of drugs targeted to the gastrointestinal tract, and enable or improve the transport of drugs from the lumen into the body. Oral peptide and protein delivery is one application of the microsphere system that is being investigated. Peptides and proteins successfully incorporated into the OraLease system include calcitonin, erythropoietin, desmopressin (dDAVP), vasopressin, and insulin. The company has also invested effort in the development of a solid oral dosage form for delivery of the microspheres as an appropriate pharmaceutical product. A Phase I clinical trial of an OraLease formulation of dDAVP delivered in
capsules in normal volunteers has been completed and demonstrated that the OraLease formulation of dDAVP is safe and well tolerated and showed a dose-dependent physiological effect in humans (Alkermes press release, November 22, 1993).

Because of their hydrophobicity and biocompatibility, zein microspheres are also being investigated as potential drug delivery systems for oral vaccines (Alkermes, 1994). Potential oral vaccine targets include several infectious diseases such as pneumonia, meningitis and diarrhea. Applications of OraLease as a drug delivery systems may also be further advanced by the reported bioadhesive properties of zein microspheres (Mathiowitz et al., 1994).

4.4. Proteinoid Microspheres

Proteinoids are man-made condensation polymers produced by random or directed assembly of natural or synthetic amino acids or small peptide chains. Following the discovery, in the late 1950s, that linear condensation polymers of mixed natural amino acids could interact with water to form hollow microspheres, proteinoids have been the subject of extensive investigations.

Emisphere Technologies Inc. (Hawthorne, New York) has developed a patented oral drug delivery system (Steiner et al., 1990), citing the ability to encapsulate therapeutic agents, including peptides, proteins, and antigens, in microspheres composed of proteinoids. The system was developed to allow drugs to be absorbed unchanged into the bloodstream while protecting them from the harsh environment of the gastrointestinal tract. Reservoir-type microspheres, capable of carrying a cargo of drug, are formed by linear thermal condensation of amino acids at elevated temperatures in acidic medium. A spray-drying process can also produce uniform proteinoid material. The proteinoid microspheres assemble and disassemble purely on the basis of pH. Inclusion in the polymer mix of a stoichiometric excess of acidic dicarboxylic or polycarboxylic amino acid results in an acidic proteinoid, and inclusion of an excess of basic diamino or polyamino monomer results in a basic proteinoid. Ability to modify the system as such allows manipulation of the solubility of the microspheres to be dependent on the pH of the environment. It is reported that acidic proteinoids remain intact in the stomach but, when discharged into the small intestine, where a higher pH is encountered, undergo spontaneous disassociation to release drug (Robinson, 1993). However, direct information as to whether or not the proteinoids actively increase absorption of entrapped drug is not
available at the time of writing. A variety of molecules have been incorporated into proteinoid microspheres, including insulin, heparin, glycoproteins, polio vaccine, monoclonal antibody IgG 2a, calcitonin, human growth hormone, and influenza vaccine (Milstein et al., 1992; Emisphere Technologies, 1993). The size distribution of the microspheres tends to be Gaussian and can be varied by varying the ionic strength and the choice of amino acids to be incorporated in the polymer [a library of over 600 proteinoid compounds had been synthesized at Emisphere as of 1993 (Emisphere Technologies, 1993)]. The encapsulation efficiency of the process is reported to vary according to the type of proteinoid and the characteristics of the compound being encapsulated. Heparin has been incorporated at 2.1% in a hydrophobic amino acid proteinoid and at 8.9% in a positively charged amino acid proteinoid whereas insulin was incorporated at 43% and 13%, respectively (Milstein et al., 1992).

The drug delivery system is reportedly nontoxic in rats, mice, guinea pigs, chickens, dogs, and monkeys (Milstein et al., 1992; Steiner et al., 1990). A potential for use of proteinoid microspheres for vaccine delivery has also been demonstrated by oral immunization of rats with proteinoid microspheres encapsulating HA-NA and M1 influenza virus antigens (Santiago et al., 1993). The study reports that a single enteric dose of M1 entrapped in proteinoid microspheres was able to induce a significant IgG response as early as two weeks post dosing, while rats dosed orally with the same M1 total dose (no microspheres) showed no detectable antibody response. A single enteric dose of HA-NA spheres induced a response up to eight times higher than that observed in rats dosed with unencapsulated antigen. Studies in cebus and cynomolgus monkeys indicate that the oral dosing of proteinoid encapsulating low-molecular-weight heparin (LMWH) elicits a consistent clinical response (Milstein et al., 1992). Initial clinical assessment of the system has also been conducted with a human safety and dose escalation study in the United Kingdom. A total of 14 subjects were fasted overnight and dosed with a microsphere suspension containing LMWH. Study results show that the system is effective and nontoxic in humans and that all doses administered were well tolerated. Emisphere plans to develop a proteinoid microsphere pharmaceutical product as an oral suspension, capsule, or tablet formulation.

4.5. Polycyanoacrylate Microspheres

The alkylcyanoacrylates are biodegradable polymers which have been used as tissue adhesives in surgery (Woodward et al., 1965) and have been
investigated as nanoparticles for controlled release of adsorbed drugs by parenteral administration in humans (Verdun et al., 1986). Distribution of the polymer in the body is reported to be dependent on physicochemical properties of the particles such as particle size, surface charge, and rate of degradation. The degradation rate has been shown to be dependent on the molecular weight of the polymer. Polyalkylcyanoacrylate nanospheres have been thoroughly studied for applications ranging from ophthalmic delivery to use as carriers in cancer chemotherapy. They are generally prepared by polymerization of alkylcyanoacrylate in an acidic aqueous medium containing the drug (Chouinard et al., 1994) although nanocapsules of polyisobutylcyanoacrylate, composed of an oily core surrounded by a polymeric film, have been developed to enable better delivery of lipophilic compounds (Chouinard et al., 1991). It has been reported that polyalkylcyanoacrylate nanocapsules, defined as spherical vesicles less than 300 nm in diameter, can improve the intestinal absorption of lipophilic drugs and do pass through the intestinal epithelium (Damge et al., 1988).

Insulin polybutylcyanoacrylate nanocapsules can be prepared by interfacial emulsion polymerization of isobutyl 2-cyanoacrylate. Insulin is added to a lipid phase containing miglyol and isobutyl 2-cyanoacrylate dissolved in ethanol. This lipid phase is then added to an aqueous phase containing nonionic surfactant. The suspension is concentrated by evaporation and then purified (Damge et al., 1990). In corroborating studies, insulin nanocapsules, with a mean diameter of 0.22 \( \mu m \) and an encapsulation efficiency of 54.9%, administered orally to diabetic rats, induced a significant decrease in glycemia after 2 days, and the effect was maintained for up to 20 days (Damge et al., 1988). Although an earlier study reported that alkylcyanoacrylate nanoparticles with adsorbed insulin were not effective following oral administration, the hypoglycemic effect of insulin nanocapsules was again reported in a further study in which the effect of site of administration in the gastrointestinal tract was assessed (O’Hagan, 1994). The hypoglycemic effect lasted from 11 to 16 days, depending on the site of administration.

Although insulin-loaded polybutylcyanoacrylate nanocapsules have been demonstrated to be effective at reducing the glucose-induced peak of hyperglycemia in both rats and dogs, other peptides investigated in this system, including secretin, cholecystokinin, and somatostatin, did not exert a prolonged biological effect after oral administration (Damge et al., 1990).

Polybutylcyanoacrylate nanoparticles have also been investigated as potential antigen delivery systems. Particles with adsorbed ovalbumin and mean particles sses of 0.1 and 3\( \mu m \) were administered to rats by gastric intubation (O’Hagan et al., 1989). Both groups of rats showed enhanced salivary IgA antibody responses in comparison to those shown by rats
administered soluble ovalbumin. However, only the group receiving 0.1-µm particles showed an enhanced IgG antibody response. The authors of the study suggested that the particles may gain access to lymphoid tissue through the M cells of the Peyer’s patches.

4.6. Lipid-Based Systems

In the following, lipid-based systems for oral drug delivery of peptides and proteins have been classified into liposomes and emulsions, although the distinction between these classifications is somewhat vague.

4.6.1. EMULSIONS

Cortecs International Limited (Middlesex, U.K.) has been developing a patented oral drug delivery system for peptides and proteins since 1987. The system, Macromo®, involves loosely associating the peptides or proteins to be delivered with two types of lipid which can cross the cell membranes in the gut and are taken into the systemic circulation through the lymphatic system. The emulsion system comprises a water-in-oil microemulsion in which the aqueous phase contains the therapeutic and the oil phase contains lecithin, nonesterified fatty acids, and cholesterol in critical proportions equivalent to those required by the cell for optimal secretion of chylomicrons (Cho and Flynn, 1989). Protease inhibitors have also been incorporated into the system to protect the therapeutic compound. The emulsion is converted to a solid oral dosage form by coating onto a solid core; during this process, the water is driven off, leaving the protein embedded in the oil. The delivery system is administered in hard gelatin capsules. Cortecs is focusing on the oral delivery of insulin and calcitonin by this method. Macromol® has been tested directly in preclinical studies in pigs with both insulin and calcitonin (New et al., 1993); the oral availability of calcitonin was reported to be increased by an order of magnitude as judged by fall in plasma calcium levels (New et al., 1994a). Human trials have also been completed, and results have been reported that demonstrate oral uptake of calcitonin at commercially viable doses as measured by appearance of collagen cross-links in the urine (New et al., 1994b). A Phase II study of oral calcitonin is in progress at the time of writing (New et al., 1994c).

Affinity Biotech. Inc. (Aston, Pennsylvania) is also investigating the oral delivery of peptides and proteins using a microemulsion delivery system. The company is reported to be running preclinical studies of the formulation with calcitonin and human growth hormone (Anonymous, 1994).
4.6.2. LIPOSOMES

Liposomes are microscopic closed vesicles composed of a bilayered phospholipid membrane. The ability of liposomes to transport drugs or other bioactive molecules to various tissues has been a subject of extensive investigation for some time. Among various routes of administration, oral dosing is the simplest and has obvious advantages, and a number of studies have been performed to investigate uptake and biodistribution of liposomes following oral delivery (Das et al., 1984; Aramaki et al., 1993).

Liposomes have been investigated for oral delivery of both peptide and protein therapeutics and vaccines. Liposomes have been used in rats and dogs as a means of preventing insulin degradation in the upper gastrointestinal tract and enhancing insulin absorption (Patel and Ryman, 1976; Patel et al. 1982; Dapergolas and Gregoriadis, 1976b). Liposomes of various fluid (e.g., phosphatidylcholine, cholesterol, and dicetyl phosphate) and solid (e.g., dipalmitoyl phosphatidylcholine, cholesterol, and dicetyl phosphate) formulations were produced in these studies by sonication processes. However, although liposomes do exhibit some ability to protect insulin and enhance absorption, massive amounts of peroral liposomal insulin are required to achieve modest reductions in blood glucose, and a significant amount of further study will likely be required before feasible dosing levels are attained (Spangler, 1990). Liposomes have also been investigated for their ability to deliver blood coagulation factor VIII. Oral administration of factor VIII in liposomes to a hemophiliac patient led to a higher rise in blood levels than did that of the free component (Hemker et al., 1980).

Investigations to support the use of oral liposomes as delivery systems for vaccines have included Peyer’s patch uptake studies in rats (Michalek et al., 1992); homogeneous unilamellar liposomes of \(~100\) nm were reported to be taken up into M cells. In vivo studies in rats to develop a liposomal vaccine against Streptococcus mutans were also reported in the study; oral administration of antigen in liposomes resulted in a mucosal response that was higher than that obtained when the oral administration consisted of antigen alone. Studies on the effectiveness of liposomes as adjuvants of orally and nasally administered tetanus toxoid in guinea pigs (Alpar et al., 1992) and soluble proteins in mice (Clarke and Stokes, 1992) have been reported. The distearoyl phosphatidylcholine and cholesterol tetanus toxoid liposome formulation significantly improved the immune response as compared to that obtained with the free antigen. However, Clarke and Stokes (1992) concluded from their in vivo studies that liposomes containing ovalbumin or keyhole limpet hemocyanin were ineffective at eliciting any significant increase in serum or intestinal antibody response as compared with the free antigen. In further investigations, in vitro studies performed by
Clarke and Stokes reported that the addition of bile caused a rapid and profound release of protein marker from the liposomes. These results led to the overall conclusion of the study that liposomes may be useful as carriers for orally administered compounds but are ineffective as adjuvants for the nonparticulate, naturally weak immunogens used in the investigation.

5. CONCLUSION

With the comprehensive research base that has developed in this area, if therapeutic peptides or proteins can be successfully and, to some extent, economically microencapsulated to produce a homogeneous and stable drug delivery system, the potential for a successful system is unchallengeable. However, although many systems are in the process of preclinical and even early clinical trials, there are still many issues in this field that need to be addressed and resolved. Certain studies are performed with compounds that, for a number of reasons, are not viable commercially for oral delivery, usually because of related issues such as dose, cost, and encapsulation efficiency. Although many such studies are cited as “proof of concept,” it is extremely difficult to define a model peptide or protein with which to optimize a delivery system. Detailed scientific information on mechanisms of action is also lacking in many circumstances and, if available, would allow logical approaches to system optimization and ultimately potential widespread system application.

REFERENCES


transport in the small intestine available for FK089, a new cephalosporin antibiotic without an amino group, *J. Antibiot.* **39:**1592–1597.


Wolf, J. L., Rubin, D. H., Finberg, R., Kauffman, R. S., Sharpe, A. H., Trier, J. S., and Fields,


