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Some biological issues in oral, controlled drug delivery

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Contents

Abbreviations: GI, gastrointestinal; GALT, gut-associated lymphoid tissue; HRP, horseradish peroxidase; BSA, bovine serum albumin.

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Summary

Some of the significant biological issues relating to the oral, controlled delivery of drugs have been discussed. Examination of the problem has largely concentrated on macromolecular drugs such as polypeptides and proteins. The prime considerations highlighted concern the presence of both normal and pathological physiological responses and various anatomical (epithelial) barriers which combine to present modern effective drug delivery with formidable problems. Various strategies for overcoming these barriers have been suggested, including (a) localization of dosage form in regions of the GI tract where protease activity is minimal; (b) inhibition of protease activity; (c) reduction in macromolecule potential complexation with immunoglobulins; (d) masking of the antigenicity of protein drugs; and (e) promotion of lymphatic uptake by selected mechanisms.

I. Introduction

The preferred and most commonly used route for delivery of drugs is the gastrointestinal (GI) tract, an anatomic area that is not well characterized in terms of biological responses and barriers responsible for interaction with and exclusion of drug intended for either local or systemic effects. As a result of an inadequate understanding of physiologic events under normal or pathologic conditions, and the lack of a detailed description at the cellular/molecular level of how drugs are processed, it is common to approach the design of oral controlled-release* products on an empirical basis.

The present review has as its primary aim the description of some of the areas of uncertainty relative to oral drug delivery with the intent of attempting to identify areas of inquiry, and hence areas of opportunity, for new or improved strategies in oral drug delivery. As a review it will necessarily be subjective and will present a particular point of view. Any oversight of pertinent literature is inadvertent.

Location and extent of oral absorption of a drug is dependent on:
(1) physico-chemical properties of the drug;
(2) physiologic responses and anatomic barriers;
(3) dosage form effects.

This, of course, ignores patient characteristics and pathology of the condition. Of these three issues, all of of which are dependent on each other, the least understood are the physiologic responses and anatomic barriers. This is somewhat ironic in that, in some senses, these present the most significant constraints and offer the greatest opportunity for strategies to improve control over oral drug absorption.

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* The term 'controlled release' will be used interchangeable with 'sustained release', since control is a subjective term in the absence of a suitable standard. Moreover, the ability to place spatially a drug conveys the concept of control, as does the ability to regulate the rate of drug availability. Controlled-release systems wherein both spatial placement and a sustaining effect is achieved have not yet been defined, but will presumably be referred to as 'absolute' control.

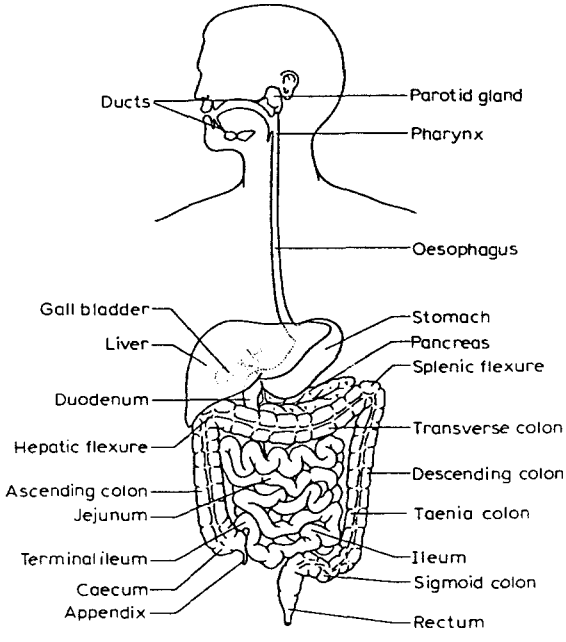

Fig. 1. Gross anatomy of the gastrointestinal tract.

TABLE I

GROSS CHARACTERISTICS OF THE HUMAN GASTROINTESTINAL TRACT

Characteristic and location	Measured value	Refs.
Length[a] (in cm)		
Entire GI tract	500–700	4,5
Duodenum	20–30	
Jejunum	150–250	
Ileum	200–350	
Colon	90–150	
Surface area (in cm^2)		
GI tract	$2 \cdot 10^6$	6
pH		
Stomach[b]	1–3.5	7–9
Duodenum[c]	5–7	
Jejunum	6–7	
Ileum, rectum	≈7	
Colon[d]	5.5–7	
Resting volume (in ml)		
Stomach	25–50	10,11
Bacterial concentration (per ml)		
Stomach, duodenum, jejenum, ileum	$\leq 10^3$	12
Distal ileum	10^5–10^7	
Colon	10^{10}–10^{13}	

[a]Based on autopsy, thus the various sections may have elongated post mortem.
[b]Can be as high as 7.
[c]pH of chyme entering duodenum can be as high as 6.
[d]Depends on type of food ingested.

Necessarily then, efforts should be focused in this area.

Numerous reviews [1–3] exist on selected subjects pertaining to physiologic responses and anatomic barriers relative to controlled drug delivery, and it is not the intent of this review to provide an additional discussion of this subject. Rather, our approach will be to raise questions regarding certain aspects of the anatomy and physiologic responses.

II. Overview of relevant anatomy

A pictorial presentation of the GI tract is shown in Fig. 1. While a broad definition of this system of organs includes the mouth, esophagus, stomach, small intestine, large intestine and rectal vault, the more important areas, in terms of frequency of use, center on the stomach, small intestine and large intestine. The mouth and rectal vault have recently received attention as accessible and relatively controllable areas for drug delivery and these will be discussed as appropriate.

Some characteristics of the stomach and intestines are shown in Table I [4–12]. The specialized nature of these organs for their roles in digestion is apparent from this table in terms of surface area and length, but will become more apparent with subsequent detailed descriptions.

One of the major problems associated with oral drug delivery is the uncertainty of the location, and hence environment, of the dosage form/drug at any point in time following ingestion. GI motility causes drugs and dosage forms to move away from the upper small intestine, the optimal site for absorption. It is regulated primarily by the presence or absence of food in the stomach; the intestine seems to play a minor role in this regard.

TABLE II

ANIMAL MODELS FOR PROCESSING DRUGS AND DRUG-DELIVERY SYSTEMS IN THE GI TRACT

Variable	Animal model
Transit	The rat and mouse have a rather long GI transit of 20–30 h, whereas the beagle dog is approximately 6–8 h.
pH	The dog has a higher stomach pH than man, i.e., pH 3.5–4.0. The cynomolgous monkey appears to have a stomach pH closer to humans' pH.
Fasted stomach content	Mice, rats and rodents eat their feces, and it is common to find large hard spheres of feces, hair and other debris in the stomachs of these animals. One can lavage the stomach, but to prevent further build-up of this debris requires fitting of the test animals with a substantial collar and housing the animals in a metabolism cage.
Mucin	Rank-ordering test animals on the basis of soluble stomach mucin would be: dog > human > rat.
Bacterial flora	The rat has approximately 10^3–10^6 organisms/ml in its stomach and intestine as contrasted to 1–1000 for humans.
Peyer's patches	Rodents have a considerable number of Peyer's patches as compared to humans.

III. Animal models

Finding a suitable animal model is troublesome for any route of drug administration, but it is particularly troublesome for the gastrointestinal tract, since dietary and genetic differences can lead to substantial variation from animal to animal. Table II compares a small number of variables in experimental animals relative to man.

The authors have had no experience with pigs, but a number of pharmaceutical firms employ the minipig for development of oral controlled-release dosage forms. Undoubtedly, the most commonly employed test animal is the dog, despite anatomical and physiological differences. A substantial data base has been built up for the dog, although the relationship of the dog to the human is generally uncertain.

Individual drugs with specific properties are commonly studied in a test animal that appears to mimic the human, e.g., an acid-sensitive drug might be tested in the cynomolgous monkey rather than the dog.

IV. Transit

The effective mouth-to-colon transit time, for purposes of controlled drug-delivery design, has historically been assumed to be 8–12 h, and thus twice daily

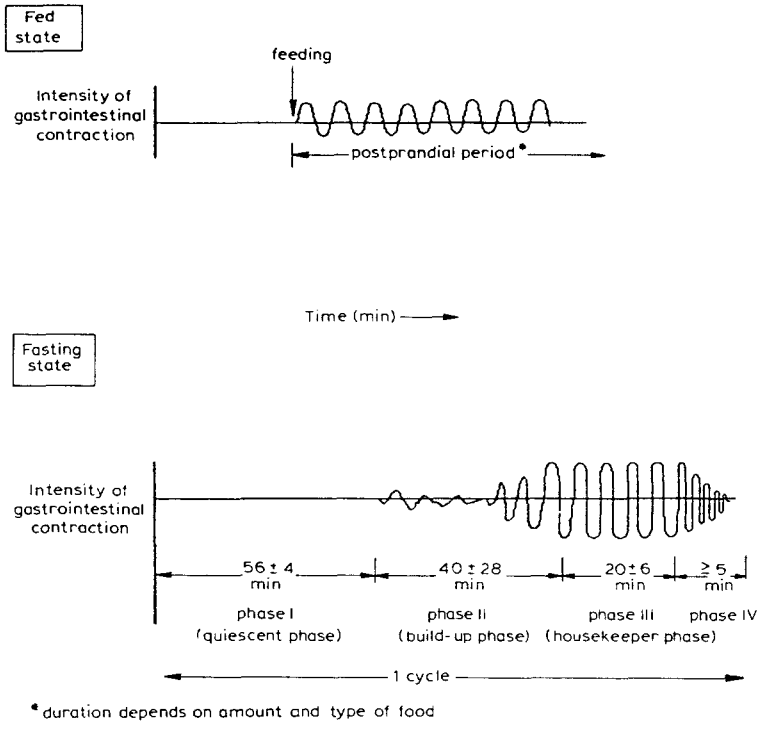

Fig. 2. Gastrointestinal motility patterns for the fed and fasting states in the dog (adapted from Ref. 14).

administration would appear to be the maximum frequency achievable. Several events, in the absence of specific approaches to extend transit time, challenge this assumption. Firstly, the assumption that little or no drug absorption occurs in the colon because of limited surface area for absorption appears to be overstated. In the case of Theo-24®, it is clear that sufficient absorption of drug occurs at later times post-dosing, so that therapeutic drug levels are maintained for 18–24 h. The issue of colonic absorption has been explored further by Fara, J.W. et al. (The Alza Corporation, Palo Alto, CA; personal communication) and they found that of 15 drugs studied, 12 showed reasonably good colonic absorption with the added implication that perhaps a larger number of drugs could be put in sustained form for 12–24 h drug dosing. It is unlikely that variability in the extent of colonic absorption can be explained on the basis of a limited anatomic surface area. It is possible, however, that colonic contents affect the surface area accessible to the drug and thereby its extent of absorption.

The second factor bearing on transit deals with the presence of food. One can anticipate a several-fold extension of stomach-emptying time for dosage forms larger than 2 mm and an overall mouth-to-colon extension of a few hours in the presence of food [13].

Starting with the fasted state, and using a dog as a model for human performance, one sees a series of discrete stages of motility, and associated time frames for these stages as shown in Fig. 2. The intensity of contractions is almost zero in phase I, intermittent in phase II, and very high in phase III, so that during phase II some, and during phase III all, of the contents of the stomach are discharged into the intestine.

Studies in dogs in this laboratory have shown that after ingestion of particles or pellets of varying size and density, the particles can be discharged at any time during a 2-h period of time, which constitutes the cycle. Thus, the particles can be discharged as short as 5–10 min after ingestion and as long as 120–140 min after ingestion, mostly dependent on proximity of ingestion to the phase III cycle. During phases I and II relatively little particle discharge occurs. Interestingly, the apparent randomness of discharge (both intra- and intersubject) can be controlled by simply knowing when phase III is going to occur. A 2–3 h difference in discharge from the fasted stomach can have a significant effect on such dosage forms as enteric coated products, sustained release products and those drugs sensitive to prolonged contact with gastric milieu.

It would be highly desirable to maintain the stomach in phase I or in a fed-like state for the purpose of localizing a dosage form in the stomach or upper small intestine or increasing its transit time. We already are aware that phase III can be postponed by administering food. Thus, the housekeeper wave does not usually occur until 90–120 min after the stomach is empty. Bass, P. and Russell, J. (University of Wisconsin-Madison, WI, personal communication) found that large doses of the polymer, polycarbophil, induce a fed-like state in the dog for a considerable period of time. It would be attractive therefore to search for other polymers or dosage forms that could artificially induce a phase I or fed-like pattern to delay gastric emptying, and hence increase dosage-form transit time.

Indeed, this raises issues relative to non-traditional dosage forms, and it is reasonable to suggest that drugs impregnated in fibers are attractive, as are forms other than capsules and tablets. Perhaps a sizeable 30–60 g 'dosage bar' containing substances to modify physiologic processes such as motility are appropriate.

One of the emotional issues that is commonly discussed is whether it is better to administer a collection of discrete particles or a single unit, with the assumption that discrete particles are well mixed in the stomach and hence randomly distributed in the entire GI tract. This, if true, would minimize GI irritation for those drugs prone to do so, and presumably optimize the likelihood of getting some drug in the blood. Our experience with the fasted dog shows some interesting results. Firstly, there is considerable luminal mucin in the stomach at all times in the fasted state. Secondly, irrespective of size, density or composition of test particles, the particles were routinely maintained in a 'slug' form due to this mucin. Indeed, these clots of mucin and test particles move as an intact entity to the ileo-cecal junction. Hence, in the fasted state in dogs particles are not randomly distributed, but are maintained as a uniform plug and this plug travels in relatively intact form. This raises interesting issues relative to controlled release dosage forms that are administered in the fasted state.

Transit through the small bowel appears to be fairly constant, about 3 h in humans, followed by residence at the ileo-cecal junction for some period of time, before discharge into the colon. Transit through the colon varies from a few hours to a few days, depending on the individual.

Of some consequence to the issue of transit through the small bowel, and particularly residence time at the ileo-cecal junction, is the role of Peyer's patches in protein absorption. There are small animal data suggesting that certain adjuvants, when added to vaccines, not only encourage lymphatic uptake via Peyer's patches, but may also encourage antigen uptake by endocytosis in the distal portion of the small bowel. If these data are transferable to humans, it would be desirable to show transit or localize the drug-delivery system near or at the ileo-cecal junction.

In a similar fashion, it would be desirable to maintain drug-delivery systems in the upper portion of the colon for those systems requiring local delivery to the colon or whose delivery is based on the local environment of the colon.

V. Localization

A recurring theme in recent years is the desirability of placing a drug or drug-delivery system in a specified region for an extended period of time. This not only has the advantage of delaying removal, a particularly attractive feature for the stomach and small and large bowel where both drug and delivery system continue in their movement, but if the contact is intimate, there is the potential to modify membrane permeability, to inhibit specific enzymes in a localized area and perhaps to mask antigenicity of antigenic substances.

Several strategies for localization of drug-delivery systems in the gastrointestinal tract are apparent – those based on lumenal substances, particularly as they pertain to longitudinal differences, and those based on membrane-associated mate-

rials. In the case of longitudinal lumenal differences such approaches as mechanisms based on pH, bile salts, mucus and bacteria come to mind, whereas for the transverse approach, mucin, enzymes, bacteria and the colon and a variety of receptors, e.g., sugars and other substances absorbed by either an active transport or a receptor-mediated endocytic process, can be utilized.

An early approach to extend duration in the stomach and intestine was to vary densities of the administered particles. Low-density substances such as swelling hydrocolloids were expected to float on chyme and thus be retained in the stomach for extended times, whereas high density particles were expected to be trapped in the mucosal coating near the tissue and be delayed during propulsive movement.

Unfortunately, the low-density approach does not work well in subjects. In the fasted state, particles are removed by phase III activity regardless of density, and there is no noticeable difference in dogs between high- and low-density particles, i.e., densities in the range of 0.5–3.0 g/cm^3. In the fed state, gastric emptying of particles larger than 2–4 mm will be delayed due to retropulsion, but this effect is largely independent of particle density. Limited data in humans confirm this observation.

Small bowel transit, as a function of particle size and density, seems reasonably consistent in the fasted state with little difference due to size and density. In dogs this may be because the particles are commonly trapped in a mucus plug or clot, and it is the clot that moves through the intestine.

It would appear that, in general, particle size and particle density have only limited influence in delaying transit and/or localizing drugs and drug-delivery systems. An alternative approach is to employ bioadhesives or, if the substrate of attachment is mucus, mucoadhesives. Nagai and Machida [17] have shown the clinical advantages of a mucoadhesive system in the mouth and cervix, two areas that are not as constrained as the stomach and intestines. Thus, with a mucoadhesive polymer containing a steroid that was attached to the mucus lining of the mouth, they found a significant therapeutic improvement in treating aphtha, a local inflammation in the mouth. In a similar manner, they demonstrated that cervical cancer could be treated more effectively by placement of the drug in a bioadhesive polymer and locally administering the drug-delivery system. Improvements in ocular drug delivery have also been noted [18].

In the GI tract mucoadhesives offer the potential advantages of attachment and specificity, given the structural variations of mucin in the various regions. Attachment is limited in duration by mucin turnover, and specificity requires an understanding of structure-activity. Thus, using bacterial attachment as a model for this specificity, it is reasonable to expect to achieve colonic attachment but not small bowel attachment. Alternatively, a variation in mucus structure in various parts of the GI tract can also serve as a model.

However, to employ mucoadhesives in the GI tract, it is necessary to overcome two limiting constraints: (1) eliminating the rather extensive unattached mucin that exists in the stomach and which contaminates the mucoadhesive surfaces prior to their attachment to the mucosal membrane surface; and (2) insuring that the mucoadhesive is sufficiently well attached to overcome the magnitude of the phase III activity.

In the case of eliminating soluble mucin, this is a substantial but not prohibitive task, given that mucins are secreted during phases II and III of the digestive phase. Once attachment occurs, it is a firm attachment, as judged by animal studies. Interestingly, rats are poor models for this phenomenon, since they have little mucin in their stomachs, and a portion of their stomachs is keratinized. Dogs, on the other hand, are equally troublesome, since they generally have excess soluble mucin to contend with.

An alternative possibility to mucoadhesives is to employ fibronectin or fibronectin fragments that are expected to bind directly to the cell surface. Fibronectins are the 'glue' that hold epithelial cells together, and recent work has established that a small peptide fragment possesses the attachment properties of the parent compound. Thus, the fibronectin fragment is able to inhibit platelet aggregation, i.e., there is a fibronectin binding site on platelets. Given that the fibronectin fragment is small it should readily diffuse to the cell surface, provided it is not metabolized or bound in the local environment of the site of application. Chemical linking drugs to this fragment offers a potential additional mode of localization. It is clear that localizing a drug or drug-delivery system in the GI tract is a formidable challenge, given that a primary characteristic of the GI tract is to maintain clearance of all exogenous material.

VI. Colonic drug delivery

One of the seemingly easiest areas to target drugs is the colon, given that it is the terminus of the GI tract. There are a number of local pathologies warranting direct release of drug in the colon, and perhaps some opportunities for systemic drug delivery.

At present, only antimicrobial agents, employing resident bacteria for the drug-release mechanism, appear to be specifically targeted to the colon; namely sulfasalazine, which relies on azo-reduction, and conversion to 5-amino salicylic acid, and steroid d-glycosides, which rely on local glycosidases. The most frequently described approach for delivering other drugs to the colon is through the use of pH-sensitive polymers. Because a pH in the neighborhood of 8–8.4 is assumed to exist in the colon; a polymer that dissolves only in this pH range would therefore be useful. Unfortunately, the pH elsewhere in the GI tract can rise to these levels, and indeed studies in dogs in this laboratory have shown pyloris and ileo-cecal pH values in this range. The tentative conclusion would appear to be that luminal pH in the colon may be unreliable to routinely release drug only in the colon.

However, there appear to be other strategies that have not been examined with respect to drug delivery. For example, the level of bacteria in the colon is several orders of magnitude higher than in the small bowel and a rich range of bacterial types for specific chemical reactivity is also available.

It is well known that certain dietary fibers such as guar bean fiber are acted upon by bacteria, and are thus biodegradable. Therefore it should be a relatively trivial task to impregnate these fibers with steroids, antibiotics, and other drugs of interest, and administer the drug-laden fiber to the patient in a variety of forms. In-

deed, the bacterially eroded polymer concept can be generalized to a variety of other polymers and drugs to create systems that will release only in the colon. In this regard, natural substances such as chitin and chitosan may have appeal.

Indeed, it is surprising, given the relative constancy of bacteria type and level in the colon, that strategies based on these bacteria have not been prominent in the literature. Thus, polymers with drugs covalently linked, or selected cases of drugs made into polymer forms, should be amenable to bacterial degradation.

Recent work by Saffron et al. [58] has employed an azopolymer to deliver insulin via the oral route. The mechanism of release is based on azoreductase bacteria which cause release of insulin, and this should occur at the ileo-cecal junction in humans. The work was conducted in rats, which have high bacterial levels in their stomachs and small bowel, and have large numbers of Peyer's patches as compared to humans.

To date, use of known sugar and other receptors in the bowel has not been used to deliver drugs, despite its potential. For proteins and peptides, the presence of high levels of bacteria, which can degrade the protein, is a substantial barrier that needs to be addressed.

VII. Macromolecular drug absorption

It is generally assumed that all ingested macromolecules undergo digestion prior to being absorbed, and that there is no nutritionally significant uptake of intact macromolecules. However, clinical and experimental evidence exists to suggest that large molecules may penetrate intestinal mucosa in quantities that may be of biological rather than nutritional importance [19–22]. This observation implies that the intestine may be a site for absorption of a number of substances previously not considered absorbable via this route, including intact proteins, enzymes, antigens, and bacterial toxins. The potential utility of such a phenomenon in the area of oral drug delivery is quite desirable, considering the increasing number of peptide, polypeptide, and protein drugs (hereafter generally referred to as 'macromolecular drugs') being made available for use in drug therapy [23,24]. As a prelude to a description of the factors that must be addressed in attempting to orally deliver macromolecular drugs, it would be instructive to examine some of the salient features of the digestion and absorption of normal dietary protein. For purposes of

TABLE III

APPROXIMATE MOLECULAR WEIGHTS AND NUMBERS OF AMINO ACID RESIDUES FOR PEPTIDES, POLYPEPTIDES AND PROTEINS

Name	Approximate molecular weight	Approximate number of amino-acid residues
Peptide	200–1000	2–10
Polypeptide	1000–5000	10–50
Protein	more than 5000	50–more than 100 000

the discussion, peptides, polypeptides, and protein will be defined as amino-acid polymers with approximate molecular weights and numbers of amino-acid residues as shown in Table III.

VIIA. Normal protein digestion and absorption: relevance to controlled drug delivery (Refs. 25 and 26)

Digestion of proteins involves the hydrolysis of the peptides bonds linking its individual amino acid residues. The enzymes responsible for this hydrolysis are generally referred to as proteolytic enzymes, proteases, peptide hydrolases, or peptidases, and occur in three main locations: the stomach, the intestine, and the brush border of the enterocyte. Digestion in the stomach and intestine is termed 'intralumenal digestion', while digestion at the brush border is termed 'membrane digestion'. Protein digestion begins in the stomach by the action of pepsin, an endopeptidase with specificity for peptide bonds involving aromatic, L-amino acids. The inactive enzyme precursor (termed a proenzyme or zymogen) is pepsinogen, secreted primarily by peptic (chief) cells and mucus cells in response to a meal and low pH. Pepsin provides as much as 10–30% of the total protein digestion, producing mainly polypeptides as hydrolysis products. It is capable of digesting all of

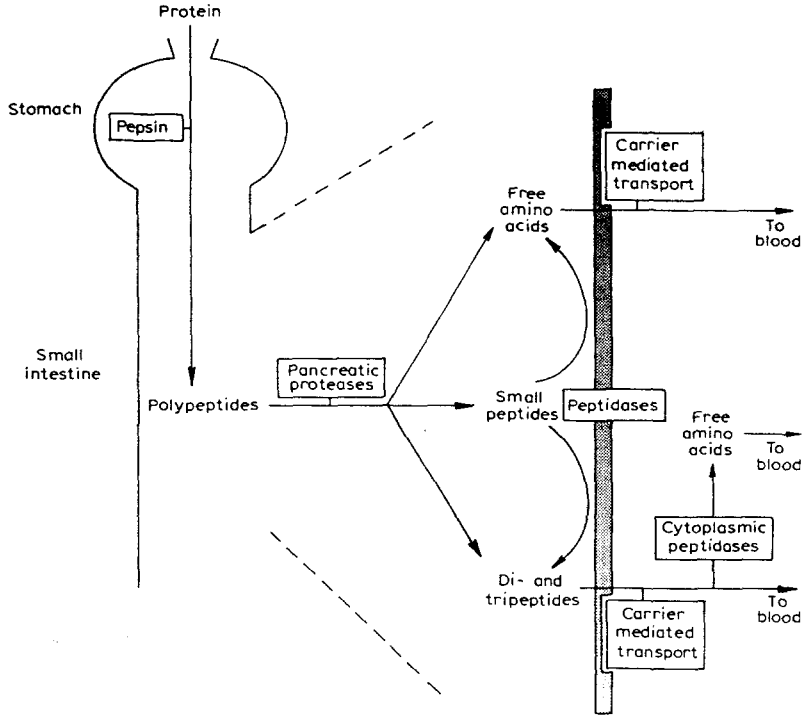

Fig. 3. Summary of digestion and absorption of dietary protein. Enzymes and transport systems are in boxes; the shaded area represents an absorptive cell brush-border membrane.

the various dietary proteins, including collagen, which is resistant to the other digestive enzymes. Its activity is maximal at a pH of 2–3, and is inactive above pH 5. The majority of protein digestion occurs in the small intestine by proteolytic enzymes from pancreatic secretions, namely trypsin, chymotrypsin and carboxypeptidase. Both trypsin and chymotrypsin are endopeptidases; carboxypeptidase is an exopeptidase that cleaves individual amino acids from the carboxyl ends of the polypeptides. The result of pancreatic enzyme digestion is a mixture of free amino acids and small peptides having from 2 to 6 residues; less than 1/3 of the total amino-acid content exists in the free form. This mixture is presented to the intestinal mucous membrane, where an active, sodium-dependent process transports the free amino acids into the cell. The remaining peptides are further hydrolyzed by brush-border peptidases, the most abundant of which is aminopeptidase.

For many years it was assumed that all protein was hydrolyzed to free amino acids prior to transport across the epithelial membrane. This assumption was disproved in the early 1960's by the work of Newey and Smith [27], who showed that (a) intralumenal hydrolysis accounts for only a minority of the protein hydrolyzed, and (b) small amount of dipeptides could be absorbed intact. In 1968, Matthews et al. [28] made the important observation that dipeptides could be absorbed more rapidly than free amino acids, thus demonstrating that there are independent mechanisms of transport for these two types of compound.

While an in-depth discussion of all of the work that has been done in the field of protein digestion and absorption is beyond the scope of this article, the current concepts regarding protein assimilation may be summarized as follows (Fig. 3). Intralumenal digestion of a protein meal results in a mixture of free amino acids and small peptides (2–6 residues). Of these products, the free acids, di- and tripeptides can be absorbed intact by active transport processes. Tetra-, penta- and hexapeptides are less well absorbed intact, and succumb to membrane digestion by brush-border peptidases. While it is known that the carriers for free amino acids are different than those for di- and tripeptides, the number and degree of specificity of the peptide carriers in unclear. Once inside the cell, peptides are further hydrolyzed to free amino acids by cytoplasmic peptidases; a small percentage of pep-

TABLE IV

PHYSIOLOGIC FACTORS THAT REDUCE TRANSPORT OF INTACT MACROMOLECULAR DRUGS IN THE INTESTINE

A Nonimmunologic factors
– Indigenous intestinal flora
– Secretions (pancreatic enzymes, mucins, gastric acid)
– Membrane-bound enzymes
– Mucus layer thickness
– Intestinal motility
– Hepatic filtration
B Immunologic factors
– Secretory IgA (sIgA)
– Cell-mediated immunity
– Other immunoglobulins (IgG, IgM, IgE)

tides, however, do escape this hydrolysis and enter the blood intact.

It would seem that, based upon the preceding discussion, a macromolecular drug has very little chance of being absorbed intact from the intestine, primarily because of the highly effective protease activity. However, if a protein molecule were able to escape digestion (and many do, as indicated earlier), what other factors must that molecule contend with in order to be absorbed intact? At least two general issues can be identified: (1) factors arising from the physico-chemical properties of the protein molecule itself; and (2) factors presented by the normal physiology of the GI tract. The physico-chemical property of greatest importance is that of molecular size. The size of the molecule will affect, for example, its diffusivity, membrane permeability, and potential for antigenicity. In the case of macromolecular drugs, however, a physico-chemical property such as size cannot be significantly altered without interfering with the biological activity of the compound. A better opportunity for influencing oral macromolecular drug absorption lies in manipulation of the physiologic factors that are capable of reducing the transport of these compounds. These factors include both nonimmunologic and immunologic processes, as shown in Table IV.

VIIB. Nonimmunologic factors

Most of the nonimmunologic factors are shared by drugs of all types, regardless of size, and these factors will not be elaborated upon here. The exception to this is the contribution from secretory and membrane-bound enzymes, which, as described earlier, are responsible for the degradation of orally administered proteins. Opportunities for the manipulation of these enzyme systems have been demonstrated. Studies have shown that inhibitors of pancreatic enzymes can increase the absorption of biologically active molecules such as insulin [20], as can pancreatic duct ligation [29]. Aminopeptidase accounts for up to 3.5–8% of the total protein content of the brush-border membrane [30], and, as mentioned previously, has been shown to be primarily responsible for completing the hydrolysis of orally administered proteins [31]. However, its activity varies depending upon its location in the intestine. The lowest activity is found in the duodenum, followed by intermediate activity in the jejunum, and a high level of activity in the distal ileum [32,33]. This implies that, based on enzyme activity alone, an orally administered macromolecular drug has the highest probability of being absorbed intact in the upper portion of the small intestine, with a continually diminishing probability as it moves further down the length of the gut.

VIIC. Immunologic factors

Of equal importance as the nonimmunologic factors in regulating macromolecular transport in the intestine are the immunologic factors. Approximately 25% of the intestinal mucosa is composed of lymphoid tissue, commonly referred to as gut-associated lymphoid tissue (GALT), that is capable of mounting a local im-

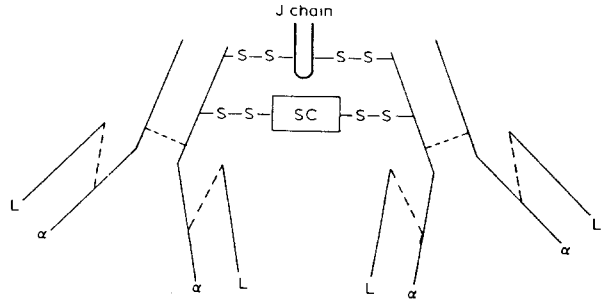

Fig. 4. Diagrammatic sketch of the structure of human secretory IgA (sIgA). Heavy chains are designated with α, light chains with L. The secretory component (SC) is joined to the α-chains by strong noncovalent bonds as well as by disulfide (–S–S–) bonds.

munologic response to protect against the penetration of antigens across the epithelium. This local immunologic response is independent from the systemic immunologic response and is controlled by antigenic stimulation at the epithelial surface [34,35]. Antibodies in mucosal secretions, particularly secretory IgA (sIgA), may exclude the antigenic macromolecule from contact with, and penetration into, the mucosa.

GALT consists of the intrinsic lymphoid tissue in the gut wall and the lymph nodes that receive intestinal lymph. The intrinsic components are the lymphoid cells within the epithelium (intraepithelial lymphocytes), the lymphoid and reticuloendothelial elements in the lamina propria, and the organized lymphoid aggregates in the mucosa. The organized lymphoid tissue includes isolated, or solitary, lymphatic nodules (follicles), and groups of nodules known as Peyer's patches.

The predominant class of immunoglobulins present in mucous secretions, synthesized by lymphoblasts and plasma cells (i.e., B cells) of the GALT, is sIgA [36–38]. The structure of sIgA is shown in Fig. 4. The molecule has a molecular weight of 400000 and comprises two four-chain units; a polypeptide chain called secretory component (SC; molecular weight, 70000) and a small polypeptide (J, or joining, chain; molecular weight, 15000). It is a dimer, $(IgA)_2$, in contrast to serum IgA which is primarily monomeric (IgA). This antibody is uniquely equipped to function in the harsh conditions of the intestinal lumen, which contains the variety of proteolytic enzymes discussed earlier [39]. Histologically, it can be found in close association with the mucosal surface of epithelial cells, especially in the crypt region, forming a barrier between the intestinal lumen and the tissues of the intestine.

The dimeric form of IgA is transported into intestinal secretions by two distinct mechanisms. One involves diffusion of $(IgA)_2$ from its site of synthesis to the basolateral membrane of columnar epithelial cells, followed by complexation to the secretory component, vesicular transport across the columnar cell, and eventual exocytosis into the lumen [40,41]. The second mechanism is by selective transport into the bile by hepatic parenchymal cells [42–44].

There are several possible functions of IgA on mucosal surfaces [45]. Briefly,

they include the inhibition of bacterial adherence, the neutralization of toxins and viruses, the prevention of absorption of antigens (immune exclusion), and possibly the regulation of mucus secretion. The process of immune exclusion [46] was one of the first functions suggested for sIgA. Heremans et al. [47] demonstrated that orally immunized mice absorbed less ovalbumin than did control animals, and that this effect could be transferred in mucosal scrapings. Additional studies have confirmed these results [48]. Evidence that IgA can mediate this effect was shown in experiments where myeloma IgA, administered intratracheally to rats, reduced the absorption of specific antigen from the trachea compared with control rats [49]. The reduction in absorption is presumably due to the complexing of antigen with IgA and its subsequent removal or inactivation via one or more of the nonimmunologic factors shown in Table III.

Others researchers have studied the process of immune exclusion in vivo. Isselbacher and co-workers [50,51] examined the effect of oral immunization on the uptake of horseradish peroxidase (HRP) and bovine serum albumin (BSA), and showed that it can reduce the amount absorbed by up to 50%, with no effect on non-crossreacting proteins. They found, however, that antibody belonging to the IgG_1, but not the IgA class, had antigen binding capacity. In subsequent experiments [52] it was found that parenteral immunization, which stimulated high levels of IgG antibody in serum that later appeared in intestinal secretions, also reduced antigen absorption, although the effect was not as dramatic as that after oral immunization. It appears that, in light of these results, more studies on the relative roles of IgA and IgG in immune exclusion need to be carried out.

Although the process of immune exclusion does significantly reduce antigen absorption, it does not eliminate it entirely. Another property of IgA, namely its ability to be selectively transported into the bile by hepatic parenchymal cells, may aid in the removal of systematic antigen from the circulation. Several experiments have shown that the liver will take up and transport into the bile antigen complexed with dimeric IgA [43,53,54].

It is clear from the above discussion that secretory IgA responses generated at the mucosal surface of the intestine may present a significant barrier to absorption of orally administered peptide or protein drugs, and that this barrier may operate at two different levels. The first is at the level of the mucosal surface itself, by complexation and blockage of adsorption to the enterocyte membrane, subsequently reducing endocytotic uptake and potentiating the effect of proteolytic enzymes. The second is at the level of the liver, where an already absorbed antigen, complexed to IgA, is rapidly cleared from the serum via the bile back into the gut lumen.

Up to this point in the discussion, the emphasis has been focused primarily on those factors which the protein drug encounters prior to actually penetrating the mucosal epithelium. The mechanism by which this penetration occurs, however, may play a critical role in the ultimate fate of the compound. It would be profitable once again to digress momentarily from our interest in drug delivery to review what is known about the routes of penetration and fate of a macromolecule after it has crossed the epithelium. In reference to this subject, analogy is drawn

to the study of antigen absorption, which has received considerable attention due to the potential pathologic conditions resulting from such absorption.

VIID. Mechanisms of antigen uptake

There are two primary cell types in the intestine where macromolecules appear to gain entrance to the lamina propria underlying the single epithelial cell layer of a villus. One is the enterocyte (also referred to as the absorptive cell) and the other is the epithelial cells(s) overlying Peyer's patches. Electron microscopic studies of HRP uptake suggest that it is first adsorbed on the enterocyte surface and is then taken up into the cell by pinocytosis [55]. This mechanism may account for the observed energy dependency associated with HRP absorption, i.e., it can be reduced by inhibitors of oxidative phosphorylation and glycolysis. After adsorption and endocytosis, the pinocytotic vesicle coalesces with intracellular lysosomes to form phagolysomes, which then digest a majority of the macromolecules. Those remaining intact are discharged into the lateral intercellular spaces by exocytosis. Whether these intact macromolecules are taken up by capillaries or lymphatics is not entirely clear. For example, after infusing HRP into the rat jejunum, it can be found in both mesenteric lymph and portal blood [56]. Quantitative measurements of ^{131}I-elastase absorption showed that 36% of the absorbed material entered the general circulation from the lymphatics and the remainder from the portal blood [57].

The second site of absorption for antigens is via Peyer's patches [15]. The epithelium overlying these aggregated lymphoid nodules is unique in that it lacks villi and contains a specialized cell type, known as an 'M' cell. M cells appear to have pinocytotic abilities, and may act as sites of antigen 'sampling', providing antigen to the underlying lymphatic tissue for processing and subsequent induction of an immune response. HRP has been shown to be taken up by M cells [13] and even particles as large as 2 μm in diameter have been found in the Peyer's patch, villi, and mesenteric lymph node [16]. Questions concerning the nature of the antigen and how it is presented to the lymphocytes in the Peyer's patch are presently not able to be fully addressed. This represents a very active area of research in the field of gastrointestinal immunology.

VIIE. Potential strategies to promote macromolecular drug absorption

Several key points concerning the nature and problems associated with oral absorption of peptide and protein drugs have been exposed in this discussion. The central issue encompassing all of these points is the ubiquitous existence of physiologic responses and anatomic barriers that have been specifically designed to decrease the probability of a macromolecule gaining entrance into the circulation. It is the task of the pharmaceutical scientist to evaluate each of these responses and barriers, and to devise methods to alter or relax them in such a fashion that will increase this probability. Examples of some possible strategies to complete this task are as follows: (a) localization in regions of the GI tract where protease activity is

minimal; (b) inhibition of protease activity; (c) reduction of IgA complexation; (d) masking of antigenicity; (e) promotion of lymphatic uptake by a selected mechanism.

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